The effect of coconut sap amount and salt texture on the protein content and total bacterial number in *ina sua*

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**Abstract.** *Ina sua* is a salted-fermented fish made traditionally by the Teon-Nila-Serua community in the Maluku islands, Indonesia. In addition to salt, ‘sageru’ (coconut sap) is often added in *Ina sua* production. This study aims to find out the effect of coconut sap amount and salt texture on the protein content and the number of bacteria in the skipjack-*Ina sua*. This study used a complete randomized design with 3 treatments and 3 replications for each experiment. For coconut sap effect experiment, the amount of coconut sap used were 10 ml/100g fish, 30 ml/100g fish, and 50 ml/100g fish with 30% salt (a mixture of fine and coarse salt). For the salt texture experiment, 30 % coarse salt, 30% fine salt, and a mixture of fine salt (15%) and coarse salt (15%) with 30 ml coconut sap /100g fish were used. The mixtures were incubated at room temperature for two weeks. Analysis of variance and Tukey test were used to analyse the data. The results show that the amount of coconut sap has a significant effect on the protein content, total bacterial number, and pH in *Ina sua*, while salt texture has significant effect only on the protein content in *Ina sua*.

1. **Introduction**

*Ina sua* is a fermented-salted fish made traditionally by the Teon Nila Sarua (TNS) community, Central Maluku, Indonesia. The fish is also called by the community as *Inmama* or *Ina skua*. It is made as a source of protein in time where fresh fish is not available in sufficient quantities. Many kinds of fish are used to make *Ina sua*, including skipjack (*Katsuwonus pelamis*).

In addition to salt, ‘sageru’ (coconut sap) is often added to improve the shelf life of *Ina sua* and to obtain *Ina sua* with a certain taste and aroma. The microbes which are responsible for the fermentation process are originated from the fish themselves, coconut sap, and salt. The quantity of both salt and coconut sap and also the incubation time used to make *Ina sua* are varied among the community, which leads to the different quality of *Inasua* produced by the community. *Ina sua*’s production is still carried out at a household level without any quality control. There is no standard procedure available so far to make *Ina sua*.

*Ina sua* has been studied by some researchers. Mahulette *et al* [1] isolated and characterized bacteria from *Ina sua* taken directly from TNS community and found that the *Ina sua* contained total bacterial numbers of $3.5 \times 10^5$ to $2.8 \times 10^7$ CFU/g. They also found that cocci-lactic acid bacteria (LAB) are the predominant bacteria in the *Ina sua*. LAB such a *Bacillus sp.*, *Propionibacterium sp.*, *Leuconostoc sp.*, and *Lactobacillus sp.*, *Lactococcus sp.*, and *Pediococcus sp.* were found in the products of fermented salt fish, including *Ina sua* [1][2][3][4]. Besides lactic acid bacteria, coliform bacteria were also found in *Ina sua* [5].
In our previous study [6], we found that salt concentrations affect the total bacterial numbers and protein content of *Ina sua* and from the salt concentrations used, the salt concentration of 30% produced *Ina sua* with the lowest total bacteria numbers (2.3 x 10^4 CFU/g) and the highest protein content (30.7%). Based on this previous result, in this study, we made *Ina sua* using 30% of salt with the addition of different amounts of coconut sap and examined their effect on the total bacterial numbers, protein content, and pH of the *Ina sua*. We also examined the effect of salt texture on the bacteria numbers, protein content, and pH of *Ina sua* prepared with 50 ml of coconut sap and 30% of salt.

2. Materials and methods

2.1. The preparation of fish sample

The fish (*Katsuwonus pelamis*) used in this study were bought from the fishermen who caught the fish in Ambon Island, Indonesia. The fish with uniform sizes and body weights were eviscerated and cleaned before sliced into pieces (100g/piece).

In coconut sap effect experiment, where we used 30% of salt concentration and varied the amount of coconut sap, four fish were used. From each fish, four pieces were used, so in total 12 pieces were used. Nine pieces were smeared evenly with 30% salt (a mixture of coarse salt and fine salt with a ratio 1:1). Nine clean jars were prepared and inside of each jar, a piece of salt-treated fish meat was put. Three jars contained fish meats (each from different fish) were poured with 10 ml of coconut sap/jar. Another 3 jars were poured with 30 ml of coconut sap/jar. The last 3 jars were poured with 50 ml of coconut sap/jar. The coconut sap used was coconut sap that had been stored for 8 hours after taken from the tree. Before used, the coconut sap was checked for its pH with a digital pH meter. The jars were closed with their lids and stored for 2 weeks at room temperature. The last three pieces were analysed immediately for their protein contents and bacterial numbers without any treatments. After the incubation time, each *Ina sua* was analyzed for its bacterial number and protein content. The pH of the *Ina sua* was also checked using a digital pH meter.

In salt texture experiment, we used 50 ml of coconut sap and varied salt textures. The procedures for fish preparation were the same as the first experiment explained above. Nine fish pieces and nine clean jars were prepared. Three pieces of fish were smeared evenly with 30% of coarse salt, another three pieces were smeared with 30% of fine salt, and the last three pieces were smeared with 30% of a mixture of coarse salt and fine salt with a ratio 1:1. Each fish was put inside each jar and each jar was poured with 50 ml of coconut sap. The jars were closed with their lids and stored for 2 weeks at room temperature. After the incubation time, each *Ina sua* was analyzed for its bacterial number and protein content. The pH of the *Ina sua* was also checked with a digital pH meter.

2.2. Protein content analysis

The protein content was analyzed by the Kjeldahl method. The crushed sample was weighed as much as 1g, put into the digestion flask, and 5 g of concentrated sulfuric acid is added to the flask, and the flask was heated at 400°C until the solution became clear. Into the flask, 45% of NaOH solution was added until the solution became alkaline tested with litmus paper. The flask was then placed on the cooling end of the distillation device with the position of the condenser end were immersed in a boric acid solution (5%). Titration was carried out with 0.1 N HCL until it reached an equivalent point (gray color).

2.3. Total bacteria number analysis

About 4 grams of the crushed sample was weighed and put into a sterile centrifuge tube (50 ml) containing 36 ml of sterile distilled water. The tube was homogenized using a vortex. From this solution, a serial dilution was then done. From each dilution, 0.1 ml of bacterial suspension was pipetted and dropped onto the middle surface of a marine agar plate. The bacterial suspension was then spread evenly on the surface of the agar using a sterile spreader. The plates were incubated in an incubator at 35°C
for 1-2 days. A colony counter was used to count the number of bacteria grown on the agar plates. Only plates with colonies between 30 – 300 colonies were recorded.

2.4. Data analysis
Data were analysed with one way-analysis of varians (Anova) and Tuckey test with a significant level of 5 %.

3. Results

3.1. The effect of coconut sap amount
The protein content of the fresh fish and Ina sua treated with 30 % of salt (a mixture of coarse salt and fine salt with a ratio 1:1) and different amounts of coconut sap for 2 weeks of incubation time at room temperature can be seen in Figure 1. There is a significant decrease in the protein content (p <0.05) in the Ina sua treated with 50 ml of coconut sap compared to that in fresh fish and other Ina sua.

![Figure 1](image_url)

**Figure 1.** Protein content in fresh skipjack and Ina sua treated with 30 % of salt ((a mixture of coarse salt and fine salt with ratio 1:1) and a different amount of coconut sap for 2 weeks at room temperature. The vertical line shows the standard deviation of each data. The same letters indicate no significant difference, while different letters indicate a significant difference. The 0 ml of coconut sap indicates the protein contents of the fresh fish without any treatments.

The log total bacteria number of fresh fish and Ina sua treated with 30 % of salt and different amounts of coconut sap for 2 weeks of incubation time can be seen in Fig. 2. The total number of bacteria in the Ina sua treated with 50 ml of coconut sap is significantly lower than that in fresh fish and the two other Ina sua (p <0.05).

The pH of the fresh fish and Ina sua treated with 30 % of salt and different amounts of coconut sap for 2 weeks of incubation time can be seen in Fig. 1. The pH of Ina sua treated with coconut sap of 50 ml/100 g fish is significantly lower than that of fresh fish and Ina sua with the addition of 10 ml coconut sap. The pH of coconut sap used in this experiment is 5.
Figure 2. Total bacteria numbers in fresh skipjack and Ina sua treated with 30% of salt (a mixture of coarse salt and fine salt with ratio 1:1) and a varied amount of coconut sap for 2 weeks at room temperature. The vertical line shows the standard deviation of each data. The same letters indicate no significant difference while different letters indicate a significant difference. The 0 ml of coconut sap indicates the bacteria number of fresh fish without any treatments.

Figure 3. The pH value of fresh skipjack and Ina sua treated with 30% of salt ((a mixture of coarse salt and fine salt with ratio 1:1) and different amount of coconut sap (10ml/100g fish, 30 ml/100g fish, 50ml/100g fish) for 2 week-incubation times at room temperature. The same letters indicate no significant difference, while the different letters indicate a significant difference. The 0 ml of coconut sap indicates the pH of the fresh fish without any treatments.

3.2. The effect of salt texture
The protein content of the fresh fish and Ina sua treated with 50ml of coconut sap and 30% of salt with different texture (fine salt, coarse salt and a mixture of the two with the ratio 1:1) for 2 weeks of incubation time at room temperature can be seen in Figure 4. The results show that the salt texture significantly affects the protein content of Ina Sua (p <5%). Ina sua with the addition of a mixture of fine salt and coarse salt has the highest protein content, while Ina sua with the addition of only coarse
salt has the lowest protein content. *Ina Sua* with the addition of only fine salt has protein content between the two.

The log total bacteria number and pH of the fresh fish and *Ina sua* can be seen in Figure 5 and 6 respectively. The results show that the salt texture does not significantly affect the total number of bacteria in *Ina sua* ($p > 0.05$) (Figure 5). The pH of the three *Ina sua* is significantly different with the pH of fresh fish (Figure 6).

**Figure 4.** Protein content in fresh skipjack and *Ina sua* treated with coconut sap (50 ml) and different salt textures (fine salt, coarse salt, and a mixture of fine salt & coarse salt with the ratio of 1:1) for 2 weeks of incubation time at room temperature. The vertical line shows the standard deviation of each data. The same letters indicate no significant difference, while different letters indicate a significant difference. The fresh fish indicate fish without any treatments.

**Figure 5.** Log total bacteria number in fresh skipjack, and *Ina sua* treated with coconut sap (50 ml) and different salt texture, fine salt, coarse salt, and a mixture of fine salt & coarse salt with the ratio 1:1 for 2 weeks of incubation time at room temperature. The vertical line shows the standard deviation of each
data. The same letters indicate no significant difference, while different letters indicate a significant difference. The fresh fish indicates fish without any treatments.

![Figure 6](image)

**Figure 6.** The pH value of fresh skipjack and *Ina sua* treated with salt (30 %) and a different amount of coconut sap for 2 week-incubation times at room temperature. The same letters indicate no significant difference, while the different letters indicate a significant difference. The fresh fish indicates fish without any treatments.

4. **Discussion**

The penetration of salt and coconut sap into the fish occurs simultaneously with the release of water from the fish due to osmotic pressure. These occur until equal concentrations of salt and coconut sap between inside and outside of the fish are established. The microbes that can survive in this saline environment are then grown using available nutrients including the fish protein. The degradation of fish protein not only involves microbe-proteolytic enzymes but also fish-proteolytic enzymes. Amino acids resulted from protein degradation are used for microbe growth. They can also be degraded into compounds that evaporate like amines, ammonia, phenols, and alcohols [7][8]. These compounds are responsible for the typical aroma of fermented fish such as *Ina sua*. The amino acids and peptide may also be released together with the release of fluids from the fish due to differences in the concentration of salt between outside and inside of the fish. Abbey *et al.* [9] found about 12% of the peptide in the fluid. In addition to the processes that can cause the reduction of fish-protein content explained above, the processes that can contribute to the increase of protein content in the fish such as microbial growth and water release from the fish also occur in *Ina sua* processing. The combination of these two processes determines the final protein content of *Ina sua*.

The results show that the amount of coconut sap added affects the protein content in *Ina sua* (Figure 1). Coconut sap is reported to contain glucose, protein, lipid and vitamin [10][11]. It also contains microbes such as *Saccharomyces* and LAB (10). These two microorganisms are responsible for alcohol and organic acid formation in coconut sap. The more coconut sap added the more microbes are involved in Ina sua processing, which then may lead to more protein degradation carried out by microbe-proteolytic enzymes. The process that causes the reduction of protein content explained above seems more dominant in *Ina sua* treated with 50 ml of coconut sap, which results in the lowest total protein content (p< 0.05) compared with that in fresh fish and Ina *sua* treated with 30 ml or 10 ml of coconut sap.

The results also show that the amount of coconut sap added affects the total number of bacteria in Ina sua. The total log numbers of bacteria in *Ina sua* treated with 50 ml of coconut sap is significantly
lower than that in the two other Ina sua (Figure 2). The presence of more numbers of LAB and their products in 50 ml of Coconut sap may contribute to this result. These bacteria are known to produce organic acids such as lactic acid and acetic acid, and bacteriocin, which can inhibit the growth of spoilage and pathogenic bacteria [12]. The fact that there is a significant decrease in pH of Ina sua treated with 50 ml of coconut sap (Figure 3) indicates the presence of more organic acids produced by LAB in this Ina sua. It is suggested that the growth of spoilage bacteria is much more inhibited in Ina sua treated with 50 ml of coconut sap than that in other Ina sua, which results in a significantly low number of total bacteria in this Ina sua. The log number of total bacteria is reported to decrease with the increased log number of lactic acid bacteria in some fermented-salted fish [13][14]. Protein degradation by other microbes such as molds can also be the cause a decrease in protein content of the Ina sua. Mould has been found in salted fish [15].

Salt texture has a significant effect on the protein content in Ina sua (Figure 4). The results show that Ina sua with the addition of only coarse salt has the lowest protein content compared with Ina sua with the addition of fine salt and Ina sua with the mixture of the two salts. Because of the large texture of coarse salt, the penetration of the salt into fish meat is slower than that of fine salt. It takes a longer time for the coarse salt to dissolve in the solution inside the fish meat and distribute evenly throughout the fish. This causes more microbial growth at the beginning of incubation and more protein degradation by proteases from the fish themselves and proteases produced by microbes. Bacteria in fish are not stunted in growth if the salt concentration in fish meat is still below 5-6% [16]. On the other hand, the small size of fine salt causes the penetration of the salt into the fish to occur faster than coarse salt resulting in less degradation of the fish protein. Consequently, the protein content in Ina Sua with fine salt is higher than that in Ina Sua with coarse salt. Even so, due to its more hygroscopic nature, large amounts of fine salt can cause salt clumps to form in the fish, thus inhibiting the even distribution of salt throughout the fish [16]. A mixture of fine and coarse salt seems to cause a fast and even distribution of salt in the fish, which ultimately results in significantly higher protein content of the Ina sua compared to the other two Ina sua. To get efficient salting, it is suggested to use a mixture of salt with different textures (granulometry) [17][18].

The total log number of bacteria in fresh fish and Ina sua is not significantly different (Figure 5). However, it is assumed that the number of lactic acid bacteria in Ina sua is higher than that in fresh fish which caused the pH of Ina Sua to be significantly lower than the pH of the fresh fish (Figure 6). Desniar et al., (2009) reported that a decrease in pH occurs along with an increase in the number of LAB in peda, a fermented fish product. The log total number of bacteria in the three Ina sua after 2 weeks of incubation is not significantly different. Even so, it is suspected that the growth of bacteria and protease activities at the beginning of fermentation in Ina sua with the addition of fine or coarse salt occurred more than in Ina sua with a mixture of salt due to differences in the salt penetration process explained before. Protease activity in fish is reported to be influenced by salt levels and pH [20]. This causes the protein content of the two Ina sua to be lower than that of Ina sua with a mixture of salt.

5. Conclusion
Based on the results, it is concluded that:
1. The amount of the coconut sap significantly affects the protein content, total bacteria number, and pH of Ina sua made using 30 % of salt with 2 weeks of incubation time at room temperature. The use of 50 ml coconut sap results in Ina sua with the lowest protein content and total bacteria number.
2. The texture of the salt significantly affects only the protein content but not the total bacteria number or pH of Ina sua made using 30 % of salt and 50 ml of coconut sap with 2 weeks of incubation time at room temperature. The use of a mixture of fine and coarse salt results in Ina sua with the highest protein content.
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