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ANALYSIS OF MICROBIAL AND NUTRITIONAL CONTENT OF GOHU FISH USING CUILE JUICE (Citrus microcarpa) AND LIME (Citrus aurantifolia) WITH VARIOUS CONCENTRATIONS AND DURATION OF ACIDING

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ABSTRACT

North Sulawesi is one of the provinces that has a long coastline. Tuna is a potential product in North Sulawesi. Tuna as a commodity with high nutritional value but perishable because it contains high protein content with free amino acid content which is used for the metabolism of microorganisms, production of ammonia, biogenicamines, organic acids, ketones and sulfur components. Fish gohu is a type of traditional food produced from fresh (raw) skipjack/tuna without going through a cooking process. Processing with acidification, usually adding the juice of Citrus Microcarpa Bunge (Citrus aurantifolia) or lime (Citrus aurantifolia).Types of research was an experimental research with a pre-experimental design, namely post-test only design (One Shot Case Study). Samples were fresh raw tuna filleted as much as 100 grams for each treatment group. The treatments consisted of 8 groups, namely group 1 fish gohu with 25 ml of citrus cui for 15 minutes, group 2 fish gohu with 50 ml of citrus cui for 15 minutes, group 3 fish gohu with 25 ml of citrus cui for 30 minutes, group 4 fish gohu with 50 ml of citrus cui for 30 minutes, group of 5 fish gohu with 25 ml of lime for 15 minutes, group of 6 fish gohu with 50 ml of lime for 15 minutes, group of 7 fish gohu with 25 ml of lime for 30 minutes, group 8 gohu fish with lime 50 ml for 30 minutesAnalysis of the data used wa the ANOVA test The results of this research was The number of microbial gohu fish was below the threshold (<500,000 colonies/gram of food (5,105 colonies/gram), the protein contentof gohu tunawas below 21%. And the fat was 0.1 grams. Gohu tuna can be consumed as traditional food because it has a safety limit for its microbial content and stable nutritional value compared to the nutritional value of raw fresh tuna. The higher the concentration of acidification used both cui oranges and limes will cause a decrease in the number of microbes.

Keywords: Microbial analysis, Nutritional content, Cui citrus, Lime citrus, Gohu Fish

BACKGROUND

North Sulawesi is one of the provinces that has a long coastline. Most people in North Sulawesi have a livelihood as fishermen. Tuna / skipjack is one of the marine biological resources in North Sulawesi, which is mostly obtained by fishermen. The people of North Sulawesi, especially the people of Manado city, often consume the tuna/skip fish, because this fish tastes good, is nutritious and is often found in traditional markets and modern markets such as supermarkets. Tuna is a potential product in North Sulawesi that has high economic value, where one type of small tuna, namely skipjack tuna (skypjack tuna/Katsuwonus pelamis, is widely used by the community and entrepreneurs as raw material for various types of processing industries such as skipjack fufu, wood fish, canned fish, shredded skipjack tuna, and many other processed products that use fish as raw material (Lumi, 2013).

Tuna is known as a commodity that has high nutritional value but is perishable because it contains high levels of protein with free amino acids that are used for microorganism metabolism, production of ammonia, biogenic amines, organic acids, ketones and sulfur components (Radjawani et al. 2016).) and is classified as an important pelagic fishery resource and is one of the non-oil and gas export commodities (Kekenusa et al., 2012). Fresh fish has a high water content (80%), the pH of the fish body is close to neutral, and fish meat is very easily digested by autolysis enzymes so that it becomes a good medium for the growth of spoilage bacteria.

Fish is a food that undergoes a rapid decay process compared to other foodstuffs. Bacteria and chemical changes in dead fish can cause spoilage. Fish if left at room temperature, it will immediately begin the process of decay. The high content of water, protein and fat in the fish body is a suitable medium for the growth of spoilage bacteria or other microorganisms. Environmental conditions also affect the growth of spoilage microbes. These environmental conditions include temperature, pH, oxygen, storage time and the condition of the cleanliness of infrastructure (Ndahwali D, 2016).

Fish damage occurs immediately after the fish comes out of the water, but the microbial activity that will damage the fish meat occurs only after the fish passes through the rigor mortis phase. Damage to fish can be caused by internal (intestinal) and external (environmental) factors, as well as the way of handling on board the ship, at the landing site or in the processing process. Food-destroying microbes can grow in the temperature range of 4-66 C, aw (Water activity) around 0.91 or more, pH 4.6-7 and the presence of oxygen. In these conditions food-destroying microbes in the form of bacteria, yeast, or molds can damage carbohydrates, fats and proteins. The most important microbes in causing damage to protein foods are bacteria. Various kinds of bacteria that destroy fish and food include Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Pseudomonas, Staphylococcus, Micrococcus, and Enterococcus (Ferdiaz, 1995). Gram-negative bacteria (eg Vibrionaceae, Pseudomonas spp., and Shewanella spp.) are the main contaminants that cause spoilage in fish. Gram-positive and gram-positive proteolytic and lipolytic microbes can reproduce to produce foul-smelling compounds. Proteolytic microbes are bacteria that produce extracellular proteinase enzymes, which are protein-breaking enzymes that are produced in the cell and then released out of the cell. Lipolytic microbes are bacteria that produce lipase, an enzyme that catalyzes the hydrolysis of fats into fatty acids and glycerol. Many bacteria are aerobic and proteolytic, also lipolytic, such as Pseudomonas, Alcaligenes Serratia and Micrococcus (Ndahawali D, 2016).

Improper handling or processing of fish will endanger the people who consume it. Moreover, many people consume tuna fresh or raw. People in North Sulawesi usually consume fresh skipjack tuna in processed products such as Gohu fish. Gohu fish a form of traditional food from the Ternate or Maluku region but has been widely made and consumed by the people of the city of Manado. Fish gohu is made from raw tuna / skipjack that is cut into small pieces and soaked in sour sauce. The sour sauce consists of a mixture of shallots, cayenne pepper, salt, and basil leaves and lime juice instead of vinegar to remove the fishy smell.

Gohu fish is a type of traditional food produced from fresh (raw) skipjack/tuna without going through a cooking process. Fish gohu only goes through a processing process with acidification, where in the manufacture of fish gohu the people of Manado city usually add lemon cui juice (Citrus Microcarpa Bunge), which is a kind of lemon. Lemon cui is a type of local lemon that only exists in North Sulawesi. The addition of orange juice can suppress microbes that are not resistant to acid. The use of raw fish is very worrying because raw fish still has the potential to contain unwanted microbes. Many microbes attack food that is still in the form of raw materials, including fish and its processed products. Food that has been infested with these microorganisms undergoes decomposition, so that it can reduce its nutritional value and delicacy, even food that has been in a decomposed state can cause illness to the death of someone who eats it (Dwijoseputro, 2005)..

Acidification in the manufacture of fish gohu has a relatively short time, therefore it is feared that there are still many microbes contained in the processed fish products. The threshold value for microbial contamination in processed fishery products with the TPC test issued by the POM RI in 2009 is 500,000 colonies/gram of food (5,105 colonies/gram). In addition, acidification can cause a decrease in the nutritional value of the processed fish products.

The purpose of this study was to determine the total number of microbes and nutritional value in fish gohu using cui orange and lime juice with different concentrations and duration of acidification.

RISET METHODS

The type of research used is experimental with a pre-experimental design, namely post-test only design (One Shot Case Study). The treatment group consisted of eight (8) groups, namely:

Group 1: fish gohu with a concentration of 25 ml of cui orange juice with an acidification time of 15 minutes ($25JC^{15}$).

Group 2: fish gohu with a concentration of 50 ml of cui orange juice with an acidification time of 15 minutes ($50JC^{15}$).

Group 3: gohu fish with a concentration of 25 ml of cui orange juice with an acidification time of 30 minutes ($25JC^{30}$).

Group 4: fish gohu with a concentration of 50 ml of cui orange juice with an acidification time of 30 minutes ($50JC^{30}$).

Group 5: fish gohu with lime juice concentration of 25 ml with acidification time of 15 minutes ($25JN^{15}$).

Group 6: gohu fish with a concentration of 50 ml of cui orange juice with an acidification time of 15 minutes ($50JN^{15}$).

Group 7: fish gohu with a concentration of 25 ml of cui orange juice with an acidification time of 30 minutes ($25JN^{30}$).

Group 8: fish gohu with a concentration of 50 ml of cui orange juice with an acidification time of 30 minutes ($50JN^{30}$).

The population is tuna which is the basic ingredient for making fish gohu. The sample is a lot of fresh raw tuna filleted as much as 100 grams in each treatment group.

Materials and Tools

The Basic Ingredients for Making Fish Gohu (in 100 grams) consist of 100 grams of fresh tuna fillet, 300 ml of cui orange juice, 300 ml of lime juice, 10 pieces of cayenne pepper, 10 red onions, 50 grams of peanuts 50 grams, two basil handful, salt and seasoning to taste
The tools used consisted of tools for processing fish gohu, namely knives, cutting boards, spoons, basins, plates, orange juice and tools to analyze the number of microbes and nutritional value of fish gohu, namely Total Plate Count (TPC) and Micro Kjeldal.

RESULTS AND DISCUSSION

1. Characteristics of the research sample

The sample was fresh yellowfin tuna, which was obtained directly from the fish auction. The tuna obtained weighs in the range of 5-6 kg. From the weight of the fish, the net weight obtained is about 50%, which is about 2.8 kg. The tuna obtained is then filleted, which is removed from the skin and bones, then washed and cut into cubes.

2. Microbes in tuna fish gohu.

Microbes or microorganisms or micro-organisms are living organisms that are small in size. Every food ingredient, both raw and processed, contains microbes. The number of microbes in a food shows the quality of the food. The presence of excessive microorganisms in a food indicates that the quality of the food has decreased, including in fish. Fresh/raw fish containing microorganisms that exceed the safe limit indicates that the fish is no longer suitable for consumption because it can cause health problems

In gohu, tuna fish use fresh or raw tuna, so it is feared that there are still microbes in the processed product ingredients. Microbial calculation using Total Plate Count (TPC). The microbial content in fish gohu can be seen in table 1.

Table 1. Analysis of Microbial Content in Gohu Tuna Fish with Total Plate Count (TPC)

Treatment group	Number of Microbes **(CFU/g)**
Gohu tuna with squeezed Citrus Cui 25 ml with 15 minutes ($_{25}JC^{15}$)	400
Gohu tuna with squeezed Citrus Cui 50 ml with 15 minutes ($_{50}JC^{15}$)	300
Gohu tuna with squeezed Citrus Cui 25 ml with 30 minutes ($_{25}JC^{30}$)	700
Gohu tuna with squeezed Citrus Cui 50 ml with 30 minutes ($_{50}JC^{30}$)	300
Gohu tuna with lime juices 25 ml with 15 minutes ($_{25}JN^{15}$)	800
Gohu tuna with lime juices 50 ml with 15 minutes ($_{50}JN^{15}$)	100
Gohu tuna with lime juices 25 ml with 30 minutes ($_{25}JN^{30}$)	800
Gohu tuna with lime juices 50 ml with 30 minutes ($_{50}JN^{30}$)	200

Table 1 shows that gohu fish with various treatment groups had microbial counts below the threshold. The threshold value of microbial contamination in processed fishery products with the TPC test issued by the POM RI in 2009 is 500,000 colonies/gram of food (5,105 colonies/gram).

3. Nutritional value of fish gohu

Tuna is a fish that is very rich in nutrients, especially protein. Where the nutritional value of fresh tuna protein ranges from 22.6-26.2/100 grams of meat and low fat ranges from 0.2-2.7 grams/100 grams of meat. The nutritional value of fish gohu (protein) was tested using Micro Kjeldhal. The results of the analysis of the nutritional value of fish gohu can be seen in table 2.

Table 2. Analysis of the Nutritional Value of Fish Gohu

Treatment group	Protein (%)	Fat (gram)
Gohu tuna with squeezed Citrus Cui 25 ml with 15 minutes ($_{25}JC^{15}$)	18.7	0.1
Gohu tuna with squeezed Citrus Cui 50 ml with 15 minutes ($_{50}JC^{15}$)	17.6	0.1
Gohu tuna with squeezed Citrus Cui 25 ml with 30 minutes ($_{25}JC^{30}$)	20.1	0.1
Gohu tuna with squeezed Citrus Cui 50 ml with 30 minutes ($_{50}JC^{30}$)	17.8	0.1
Gohu tuna with lime juices 25 ml with 15 minutes ($_{25}JN^{15}$)	18.9	0.1
Gohu tuna with lime juices 50 ml with 15 minutes ($_{50}JN^{15}$)	18.4	0.1
Gohu tuna with lime juices 25 ml with 30 minutes ($_{25}JN^{30}$)	19.2	0.1
Gohu tuna with lime juices 50 ml with 15 minutes ($_{50}JN^{30}$)	17.8	0.1

Table 2 shows the nutritional value of fish gohu protein is below 21%. The protein value of fresh yellowfin tuna is 24.1%, meaning that in gohu fish there is a change in the nutritional value of the protein caused by the acidification treatment of both cui oranges and limes. This table also shows a tendency to decrease the nutritional value of protein in the application of acid from both lime and lime, the more acid given will affect the nutritional value of the protein produced. If it is seen that a higher concentration of protein content will show a significant decrease in nutritional value and the highest decrease in nutritional value is more in cui oranges. While the nutritional value of fat did not differ in each treatment group, where the nutritional value of fat was stable.

4. Differences in the number of microbes and the nutritional value of fish gohu

To see the difference in the microbial count and the nutritional value of gohu fish, an ANOVA test was carried out

Table 3.Microbial count and Nutritional value

	p-value
Microbial count	0.047
Protein content	0.048
Fat content	0.075

Anova test

There are differences in the amount of micro and protein nutritional content in gohu given different concentrations of cui orange and lime juice with different acidification times.

5. Effect of Acidification on Microbes and Nutritional Value of Fish Gohu

To see the effect of acidification, the Pearson regression test was carried out. For details, see table 4.

Table 4. Effect of Acidification on the microbial value and Nutritional Value

		Microbial value	Nutritional value
Treatement group	*p*	0.776	0,166
	r	0.126	0,120

Table 4 shows that there is no significant effect of acidification and duration of acidification on the number of microbes and nutritional value. That is, basically the acidification of both cui oranges and limes with different concentrations and different acidification times has been shown to provide different numbers of microbes and nutritional values, where the higher the concentration of acidification given both cui oranges and limes will affect the number of microbes and nutritional value. but the change in value did not prove statistically that there was a significant effect of various acidifications with different concentrations on the number of microbes and nutritional value.

B. DISCUSSION

Acidification of cui oranges and limes with the number of microbes in fish gohu.

Microbes will always be present in every food ingredient, both raw and processed food. The growth of microbes in foodstuffs can change the composition of foodstuffs, by hydrolyzing starch and cellulose into smaller fractions, causing rancidity and digesting proteins and producing foul odors and ammonia. Some microbes can form mucus, gas, foam, color, acid, toxin and others. Microbes like warm and humid conditions (Supardi and Sukamto, 2004).

Fish is a food that is easily contaminated by microorganisms because of its nutritional content. In addition to the nutritional value of the environment where fish are obtained and the method of processing fish can affect the number of microbes they contain. Fish from coastal waters are often contaminated by Vibrio parahaemolyticus bacteria which can be transmitted during transportation and marketing. Bacteria that often contaminate fishery products are generally Vibrio vulnificus and F. cholerae bacteria.

In this study using fresh tuna so that it allows the number of microbes contained in it, but in making fish gohu using citrus flavored water, namely cui oranges or limes. Making fish gohu, which is fish filleted and then washed in running water without blanching (referring to the way of making fish gohu in general), then cut into cubes and then mixed with sour sauce with cui and lime juice according to the acid concentration (25 ml and 50 ml).) at 15 minutes and 30 minutes. Stirred until smooth after reaching the immersion time limit (15 minutes and 30 minutes) then carried out the microbial analysis test with Total Plate Count (TPC). The results of microbial analysis showed that the number of microbes produced from gohu tuna with various types of acidification and concentration and acidification time was below the threshold (value < 500,000 colonies/gram of food ($5{,}10^5$ colonies/gram).

This is possible because in the manufacture of tuna gohu, although using fresh tuna, it is soaked using orange juice, namely oranges and limes. Acidification techniques in this case soaking in organic acid solutions found in cui oranges and limes in principle can reduce the total number of microbes in food because of their acidic nature. The use of acids in food processing according to Supardi and Sukamto (2004) has an important role that is antimicrobial. This property is because the addition of acid will affect the pH thereby reducing microbes that are not resistant to low pH.

The results of this study are in line with the research of Andriani (2013), who conducted microbial testing on Lawa Bale Makassar traditional food using anchovies with vinegar soaking, where the number of microbes produced was below the threshold. In theory, improper handling of fish and an unsterile working environment can affect the number of microbes in food. This means that if the food ingredients used to make processed products are

not in a clean state and the use of tools and cleanliness of food processors that are not up to standard will affect the final product. The lack of microbes in this study was influenced by the acidification technique provided as well as by the processing process. The tuna fish processing process started in this study from the preparation process to the manufacture of fish gohu carried out in a sterile state, especially on all the tools used ranging from knives, containers used to accommodate fish gohu and work tables to tools and materials used in microbial analysis. all are sterilized so as to allow the number of microbes contained in fish gohu to be minimized.

In processed foodstuffs, the number of bacteria and types of microbes that are dominant are influenced by poor processing processes, in this case, for example, adding or mixing food with other contaminated materials or the use of processing equipment that does not pay attention to hygiene aspects (Supardi and Sukamto). , 2004).

When viewed from the use of acidification, it turns out that limes suppress the number of microbes more than Cui oranges at high concentrations even though the value is not too far away. The results of research conducted by Namura, lime has a lower degree of acidity (pH) when compared to other oranges, namely kasturi oranges, kaffir limes and jungga oranges. This may also be the background so that the total microbial gohu of fish in lime is less than that of cui oranges because the degree of acidity (pH) is lower than that of cui oranges

Acidification of cui oranges and limes with the number of microbes in fish gohu

Protein is a nutrient that is easily denatured. This decrease in protein levels is caused by the occurrence of amino acid resemination, changes in the L form to D. This can cause a decrease in the nutritional value of protein due to the availability of essential amino acids (Asrullah et al, 2012). Acidification will affect the pH value of fish because acidic conditions will accelerate the breakdown of protein into short-chain peptide groups or amino acids that are easily soluble in water, causing protein levels in the ingredients to decrease. According to Campbell and Farrell (2016) protein levels can decrease at low pH due to weakening of ionic bonds between protein molecules which causes protein denaturation. While the nutritional value of fat can not be affected by acidification.

CONCLUSION AND RECOMMENDATION

A. Conclusion

1. Number of Microbes in Gohu tuna fish with various acidification treatments with different concentrations and duration of acidification are categorized as safe (value < 500,000 colonies/gram of food ($5{,}10^5$ colonies/gram)
2. The higher the concentration of acidification used both cui orange and lime will cause a decrease in the number of microbes.
3. The nutritional value of gohu tuna, namely protein in various acidification treatments with different concentrations and duration of acidification, was below 21%.
4. Gohu tuna can be consumed as traditional food because it has a safety limit for its microbial content and stable nutritional value compared to the nutritional value of raw fresh tuna.

B. Suggestion

1. In making fish gohu, you can use fish oranges, both cui oranges and limes because the use of different oranges still produces tuna gohu which is safe in terms of microbial content and nutritional value.
2. The process of making fish gohu must pay attention to the quality of the tuna used and the handling process starting from the preparation and cooking process must pay attention to sanitation and hygiene.

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