Quality of red tuna (Yellowfin tuna) fishball, white oyster mushroom (Pleurotus ostreatus) on different types of packaging and storage time

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Abstract. This study aims to determine the effect of packaging type and storage time on the quality of red tuna (*Yellowfin tuna*) white oyster mushroom (*Pleurotus ostreatus*) meatball. This research was conducted at the Laboratory of Agricultural Product Technology, Ekasakti University, Padang, Laboratory of Instrumentation and Laboratory of Microbiology, Faculty of Agricultural Technology, Andalas University. The study was conducted for 2 months, from April to May 2019, using a Factorial Completely Randomized Design (CRD) consisting of 2 factors with 3 replications. The first factor is the type of packaging (without packaging, styrofoam packaging, wrap packaging), and the second factor is the storage time (0 hours, 12 hours, 24 hours). Observation data were analyzed using ANOVA with the F test followed by the Duncan Multiple Range Test (DMRT) at the 5% real level. The results showed that the type of packaging with storage time had a very significant effect on moisture content and protein content and had no significant effect on ash content. The type of packaging and storage time for the best white oyster mushroom tuna red tetelan meatball based on water content, ash content, protein content, ALT, Salmonella sp and Organoleptic bacterial contamination is the best use of Wrap packaging with a storage time of 12 hours.
Key words: packaging, storage, oyster mushrooms, meatballs.

1. Introduction

Seafood, especially fish, is a very potential food source as a source of protein, fat and several vitamins. Fish is a perishable food commodity. Decay is caused by enzymes, either from the fish itself or from microbes. The proportion of collagen protein in fish ranges from 3-5% of the total fish protein. The high water content can cause microorganisms to easily grow and reproduce [1].

Fish fat contains a lot of unsaturated fatty acids which are very easily oxidized which can cause a rancid odor [2], so it is necessary to handle, process, and preserve fishery products which aim in addition to preventing damage to fish so that it can extend the shelf life as well as to diversify processed fishery products [3]. Processing can be done by processing whole fish without changing its shape, such as smoked fish, pindang fish, and salted fish. In addition, it can also be processed by changing shapes such as fish meal, fish chips, surimi, fish balls, crackers, shredded and sausage [4].

Fish meatballs are processed fishery products with raw materials for whole fish or fish meat (minced) or surimi, added with starchy fillers or tapioca flour and spices, which are formed in round shapes and boiled in hot water. Good quality fish balls are pure white in color, compact and chewy texture, not brittle or mushy [5].

One of the substitute ingredients for fish balls is white oyster mushroom (*Pleurotus ostreatus*) with the aim of increasing the nutritional value. Oyster mushroom is a type of mushroom that contains high fiber by 11.5%. Oyster mushrooms contain 39.8% lignocellulose fiber, which is insoluble fiber which is very good for digestion, the rest is soluble fiber which is good for cholesterol. In addition, mushrooms also

contain beta glucan which can boost the immune system. Regular consumption of 6-7 grams of mushrooms per day can increase endurance [6].

The addition of oyster mushrooms to fish balls is an effort to diversify food, increase the nutritional value, especially fiber and vegetable protein and to get a chewy texture. Based on research by Hermalena [7], the use of oyster mushrooms and tuna red tetelan in the manufacture of fish balls resulted in a protein content of 5.40% and 18.69% carbohydrates in the ratio of red tuna to oyster mushroom (90:10). Meanwhile, the organoleptic value (color, aroma, taste, and texture) can be accepted by consumers in the ratio of red tuna and oyster mushrooms (70:30) with the level of preference, are color 5.30%, aroma 4.65%, taste 5.5%, and texture 5.14%

Many studies with the aim of extending the shelf life of meatballs have been carried out. The results of [8] study to extend the shelf life of beef meatballs with preservation methods using the antimicrobial substrate *Lactobacillus plantarum* 1A5 at room temperature, showed that the activity of the antimicrobial substrate was able to inhibit *E. coli* and *S. aureus* during storage for 9 hours at room temperature. The microbiological quality of beef meatballs with the addition of antimicrobial substrates is better than those without antimicrobial substrates stored for 9 hours at room temperature.

According to Hidayati [9], the deterioration of the quality of meatballs is the appearance of mucus, a distorted aroma and the emergence of gas. During storage, the meatball will experience syneresis, the release of water from the gel, changes in flavor, taste and decreased nutrients due to fat oxidation. Damage during storage can occur due to the presence of microbes, enzymatic reactions and the influence of temperature. Marketing of meatballs in the community generally takes place with less sanitary storage conditions at room temperature.

One method of preserving meatballs that can be done is preserving meatballs by packaging. Packaging conditions can affect the shelf life of a product during storage. At present there are many packaging materials used for foodstuffs, including paper and cardboard packaging, glass, cans or metal, plastic and composite packaging. Of the several packages commonly used for storage of meatballs are packaging made of plastic materials such as LDPE (Low Density Polyethylene), PP (Polypropylene) plastic, HDPE (High Density Polyethylene) and PS (Polystyrene).

Based on the type of plastic, it is possible to use Styrofoam and plastic wrap. Styrofoam is polystyrene, a type of plastic that is very light, stiff, translucent and cheap but brittle quickly. The advantages of Styrofoam packaging are practical and durable. Plastic Wrap or Wrapping is a type of thin plastic film, with a thickness of 0.010 to 0.020 microns. Its characteristics are transparent (translucent), not easily torn even though it is made of limp material, so it is easy to shape, resistant to acids to avoid dirt or bacteria, impermeable to water, steam or hot enough oil. The objectives of this study were: 1. To determine the effect of the type of packaging on the quality of white oyster mushroom tuna. 2. To determine the effect of storage time on the quality of white oyster mushroom tuna red tetelan meatballs.

2. Materials and methods

2.1. Place and Time

This research has been carried out in the Agricultural Product Technology Laboratory of Ekasakti University, Padang, the Instrumentation Laboratory and the Microbiology Laboratory of the Faculty of Agricultural Technology, Andalas University. The research was conducted for 2 months, namely April to May 2019.

2.2 Material

The main raw material used was yellowfin tuna red tetelan obtained from the by-product of making tuna fish fillets at PT. Dempo Andalas Samudera, Bungus Teluk Kabung Subdistrict, Padang City. Other raw materials used in this study were white oyster mushrooms obtained from the Kongsi soil market in Padang, chicken eggs, Pak Tani Gunung brand tapioca flour, garlic spices, shallots, Dolphin brand salt, ice cubes, and water.

The materials used in chemical analysis are 1) Protein content: selenium 0.4 grams, concentrated H_2SO_4 (PT Smart Lab Indonesia) 10 ml, 10 ml distilled water, 10 ml 40% NaOH, 50 ml H_3BO_3 (Merck), 2

drops indicator , HCl 0.1N. 2) Total Plate Figures (ALT): 70% Alcohol, Nutrient Agar (NA), 0.9% NaCl. 3) Identification of *Salmonella* sp: Mac Conkey Agar (MCA) and Salmonella Shigella Agar (SSA) media, sterile distilled water, 70% alcohol, label paper, aluminum foil, cotton, and plastic bags.

2.3 Tools

The tools used for making meatballs include; sliced knife-Slicing knife, basin, bowl, scissors, portable gas stove with one furnace, Rinnai RI-511C brand, cauldron, steamer pan, bucket, blender, spoon and scale. Chemical analysis tools are: 1) Moisture content: digital scale, oven with Memmert UN 55 53L, vacuum desiccator, dryer jar, clamp, scissors, and aluminum cup. 2) Ash content: Carbolite High Temperature Box Furnace type furnaces, analytical scales, desiccators, tongs, porcelain dishes, Maspion S300 electric stove stoves. 3) Protein content: 500 ml kjeldahl flask, distillation device, 50 ml burette, 5 ml measuring pipette, 50 ml Erlenmeyer, dropper pipette, 250 ml beaker, and fume hood.

The total plate count (ALT) testing tools are autoclave, erlenmeyer, petridish, 10 ml measuring pipette, test tube, test tube rack, water bath, incubator cabinet, colony counter, vortex, tissue , bunsen, and petri dishes. While the tools used to identify Salmonella sp. are ice flasks, knives, scissors, test tubes and racks, petri dishes, erlenmeyers, analytical scales, bunsen heaters, incubators, autoclaves, bent glass rods and magnetic stirrers.

2.4. Experimental Design

The research design used was factorial completely randomized design with 2 factors, namely the type of packaging used consisting of 3 levels and storage time consisting of 3 levels. Each treatment was repeated 3 times. The treatment factors can be seen as follows: 1. Factor A: The type of packaging used consists of 3 levels. A1 = without packaging; A2 = packing with Styrofoam; A3 = packaging with plastic Wrap. 2. Factor B: Duration of storage at room temperature with 3 levels. B1 = 0 hours, B2 = 12 hours, B3 = 24 hours.

2.5. Supply of Raw Materials

The raw materials in this study were fresh yellowfin tuna fillets taken directly from PT. Dempo Andalas Samudera in Bungus Teluk Kabung District, Padang City. The red tuna droplets used have been cleaned first. The binder used is tapioca flour and the cooking spices used in the manufacture of tuna meatballs are fried shallots, garlic, pepper, salt.

2.6. Procedure for making meatballs [7] modified

Seven hundred grams of yellowfin tuna and 300 grams of white oyster mushrooms are washed, added with ice cubes, then finely ground, added with spices, binder, and emulsifier. Then mix well until the dough becomes homogeneous for about 5 minutes and is printed as desired (in this study 20 grams / seed were made). Furthermore, the dough that has been processed is boiled in boiling water for 45 minutes until the meatballs float, cooled at room temperature for 30 minutes.

2.7. Meatball Packaging Process

Freshly cooked meatballs are cooled to room temperature ± 37 °C then put into packages according to the treatment, all treatments are stored at room temperature.

2.8. Observations

Observations were made on meatballs including moisture content, ash content, protein content, determination of Total Plate Number (ALT), *Salmonella* sp test and organoleptic test, with, namely the initial storage (0 hours) as a control, 12 hours and 24 hours.

2.9. Analysis and Testing

2.9.1. Water content [10]

Determination of water content is a way to measure the amount of water contained in a food ingredient. The drying method with the oven method is based on measuring the weight loss due to evaporation of water from the dried material at a temperature of about 100°C – 105° C. This method is used for all foodstuffs, unless the product contains volatile components or if the product will decompose on heating at 100 °C-105°C.

2.9.2. Ash content [10]

Ash is an inorganic residue obtained by ashing or heating at high temperatures > 450 °C and / or digesting organic components with strong acids. This inorganic residue consists of various minerals whose composition and amount depend on the type of foodstuff and the analytical method used. Ash in foodstuffs is determined by weighing the remaining minerals as a result of combustion of organic matter at a temperature of about 550 °C.

2.9.3. Protein Content [10]

Determination of protein content to determine the protein content in food. Measurements based on the total nitrogen content present in the sample, protein can be calculated by assuming a certain ratio of protein to nitrogen for the sample being analyzed. The determination of protein is based on the oxidation of carbonaceous materials and the conversion of nitrogen to ammonia. Then ammonia reacts with excess acid to form ammonium sulfate. The solution is made alkaline, and ammonia is evaporated and then absorbed, the amount is determined by titration using 0.02 N HCl.

2.9.4. Testing of Total Plate Numbers [11]

This method of determining the total plate number is used to determine the total number of aerobic and anaerobic microorganisms in meatball products. Before the incubation process, which is carried out the first time is homogenization of the material, homogenization is a way of preparing samples to obtain the best possible distribution of bacteria in the specified sample. The next process after homogenization is sample dilution.

2.9.5. Identification of Salmonella sp. [12]

Salmonella growth on selective media with pre-enrichment, and enrichment followed by biochemical tests and serological tests. Bacteria were isolated by wiping the entire surface of the meatball using a sterile cotton swab then put into a test tube containing *Selenite Cystein Broth* (*SCB*) and incubated at 37°C for 24 hours. Then the bacterial culture was cultured on *Salmonella Shigella* Agar (SSA) selective media. Next, Gram stain was carried out on separate colonies that grew on SSA media. *Salmonella sp* bacteria identification was carried out by inoculating the bacteria on IMViC (Indol, Methyl Red Voges-Proskauer (MR-VP), Simmons Citrate Agar, Sulfite Indol Motility (SIM), Triple Sugar Iron Agar (TSIA) media.

2.9.6. Organoleptic test [13]

Organoleptic testing is carried out on the resulting product. Samples are presented in a uniform form. This test includes tests based on parameters on appearance, smell (aroma) and texture carried out by 15 panelists. This test is carried out to determine the value of product specifications starting from the highest to the lowest product specifications. The panelist test results are then tabulated by entering the panelist assessment numbers into the table. The numbers in the table are the values selected by the panelists against the parameters that have been tested. The parameters to be tested are appearance, smell and texture values.

3. Results and discussion

3.1 Moisture Content

The results of the analysis of diversity showed that variations in the type of packaging, storage time, and interactions had very significant effects on the moisture content of the meatballs produced. The average moisture content of the meatball results of the study is presented in Table 1.

Table 1. Average moisture content (%) of research meatballs

Factor A (Packing Type)	Factor B (Storage Time)		
	B_1 (0 hour)	B_2 (12 hour)	B_3 (24 hour)
A_1 (Non Package)	71,99 a A	54,93 a B	43,50 a C
A_2 (*Styrofoam*)	72,03 b A	63,10 b B	57,38 b C
A_3 (*Wrap*)	73,45 c A	69,99 c B	66,61 c C
KK		0,10	

The numbers followed by the same letter have an insignificantly different effect at the 5% level according to the DMRT follow-up test. Where uppercase is read horizontally and lowercase is read vertically.

Based on Table 1 above, it can be seen that the treatment without packaging with a storage time zero zero hours is very significantly different at 12 hours and 24 hours of storage. The use of Styrofoam packaging was very significantly different for each storage duration of 0 hours, 12 hours and 24 hours. The use of wrap packaging was also significantly different at the storage time of 0 hours, 12 hours and 24 hours. The highest water content was found in the treatment of type of wrap packaging and storage time of 0 hours (A3B1), namely 73.45%. Plastic wrap has mechanical properties that are transparent, strong, flexible, has good resistance to chemical solvents, and low water vapor permeability, thus providing microclimate conditions for the materials it is packed with. The low permeability of wrap packaging can control the hydration process, namely the entry and exit of water and water vapor. While the lowest water content was found in the treatment without packaging and storage time of 24 hours (A1B3), namely 43.50%. The low water content is related to the influence of environmental factors that are directly contaminated with the material, the volatile nature of water results in a decrease in water content. When the temperature of the water increases, the average number of water molecules decreases and hydrogen bonds break and form again rapidly [14].

Storage time has an effect on the water content of white oyster mushroom tuna red tetelan meatballs, the longer the storage of each type of packaging used, the lower the water content. This decrease in water content is due to dehydration, namely the transfer of product moisture to the storage room. The use of plastic wrap with low permeability in meatball packaging can prevent moisture absorption because the pores in the packaging are very small, causing the ability to pass gas particles and water vapor as well. This is in line with the opinion of Gunardi [15] which states that changes in water content during storage can be influenced by the permeability of the packaging used, water absorption properties, and environmental humidity.

The use of styrofoam packaging with a higher moisture permeability than wrap packaging resulted in a decrease in moisture content higher than that of wrap packaging. Styrofoam or polystyrene (PS) is a monomer that is clear rigid but high water vapor and gas permeability with a density level (0.915 - 0.939 g / cm3), while "Wrapping plastic" has a density level (<0.915 g / cm3) is one type of plastic LDPE (Low Density Polyethylene) with water vapor and low water permeability [16].

The decrease in water content in the packaged material is related to the hydration characteristics. The hydration factor can be expressed by water activity (aw), water content (KA), and relative humidity (RH). This water vapor transfer takes place from a product that has a higher water vapor pressure to a product with a lower pressure. According to Syarief and Halid [17], if the humidity in the room is smaller than the food ingredients, some of the water will evaporate. In drying food, there are 2 levels of water removal rate (dehydration). At the beginning of drying, the rate of water loss per unit time is fixed, then a decrease in water removal rate per unit time will occur. This relates to the type of water bound in the material [14].

In food packaging, hydration characteristics are very important, especially with regard to water vapor. Product packaging affects the rate of water evaporation that takes place during storage depending on the type and nature of the packaging itself in controlling the hydration process. When food products are stored

at relative humidity below the monolayer area, there will be less water available to form hydration from trace metals so that the catalytic reaction is active [18].

The use of wrap packaging in food packaging is better at preventing the absorption of steam by packaged products and the release of moisture from excessively packed products during storage. Changes in water content at 12 and 24 hours storage are not a measure of the quality of meatballs. The water content of a good meatball is determined by the composition of the ingredients and the processing process, while the amount of water content that is affected by storage is indicated by external factors. The main function of packaging is to control the interaction between food products and the external environment [19].

3.2. Ash content

The results of the diversity analysis showed that the type of packaging treatment and storage time had no significant effect on the ash content of the resulting meatball. The average ash content of the meatball research results is presented in Table 2.

Table 2. Average ash content (%) of the research results of meatballs

Factor A (Type package)	Factor B (Storage time)		
	B_1 (0 jam)	B_2 (12 jam)	B_3 (24 jam)
A1 (Non package)	1,56	1,54	1,48
A2 (*Styrofoam*)	1,56	1,55	1,51
A3 (*Wrap*)	1,57	1,56	1,54
KK		0,16	

Table 2 shows that the type of packaging treatment, storage time and Interaction of treatment have no significant effect on the ash content of red oyster mushroom tuna. From these data, there is actually a decrease in ash content with insignificant values. Table 2 shows that the ash content of the red tetelan of tuna, white oyster mushroom, decreased in each treatment. The use of the type of packaging and storage time does not significantly affect the increase or decrease of mineral substances in the materials they are packed with. It is assumed that the physical and chemical qualities of food are able to maintain the contents in it, including ash. That is, the different treatment factors for the type of packaging and storage time did not affect the ash content of red oyster mushroom tuna.

Research by Hutapea [20] on the storage of red tilapia fish balls with plastic packaging at room temperature showed no significant changes in ash content. This research is confirmed by Hamdani [21], regarding the packaging of banana heart and catfish meatballs in vacuum and non-vacuum methods at cold temperatures, there was no significant change in ash content.

Ash content is a mixture of inorganic or mineral components found in non-volatile foodstuffs, which remains in the combustion and annealing of organic compounds [22]. In general, ash consists of sodium (Na), calcium (Ca), potassium (K), and silicate (Si) compounds. Ash content is the residual inorganic substance from combustion. Ash content is related to mineral elements contained in the material [14].

The ash content of the red oyster mushroom tuna can be based on the flour used in the dough. The mineral content in flour is not large but it is very important. The mineral material will be found in flour residue which has been completely burned to white ash [23]. According to the National Standard Agency (2014) the limit of ash parameters on fish meatballs is a maximum of 2%, so that the red tetelan tuna, white oyster mushroom meatballs on the type of packaging and storage time differ by below 2%, so that it still meets the Indonesian National Standardization.

3.3. Protein Content

The results of the analysis of diversity showed that variations in the type of packaging, storage time, and their interactions were significantly different to the moisture content of the meatballs produced. The average protein content of the research results is presented in Table 3.

Table 3. Average protein content (%) of research results

Factor A (Type Package)	Factor B (Storage time)		
	B_1 (0 hour)	B_2 (12 Hour)	B_3 (24 Hour)
A_1 (Non Package)	3,67 a A	4,28 a A	3,67 a A
A_2 (Styrofoam)	3,88 a A	4,16 a A	5,47 b B
A_3 (Wrap)	4,79 a A	4,45 a A	4,25 a A
KK			

The numbers followed by the same letter have an insignificantly different effect at the 5% level according to the DMRT follow-up test. Where uppercase is read horizontally and lowercase is read vertically.

Table 3 shows that the highest protein content is found in the Styrofoam packaging type with a storage time of 24 hours (A_2B_3), while the lowest is in the unpackaged treatment with a storage time of 0 hours and 24 hours (A_1B_1, A_1B_3). The effect of the type of packaging and storage time had an inconsistent effect on changes in the protein content of the white oyster mushroom tuna. Table 3 shows that the protein content of unpackaged meatballs has increased at 12 hours of storage, while 24 hours of storage have decreased. This condition is related to the growth and development of bacteria which is influenced by high water content and contamination with the environment. A decrease in water content can lead to an increase in protein levels. Furthermore, at 24 hours of storage there was a decrease in protein levels, presumably the effect of the exudation in the form of mucus out of the meatballs due to microbial activity. The discharge of mucus causes several nutrients such as salts, polypeptides, amino acids, lactic acids, purines, etc. which dissolve in water to be carried away with the water that comes out of the meatballs from microbial activity.

During storage, when using Styrofoam packaging, there is an increase in protein content. The type of Styrofoam packaging with a storage time of 24 hours (A_2B_3) is the highest protein content of all treatments, namely 5.47%. The increase in protein content is influenced by the decreasing water content of the meatballs. This condition cannot be separated from the nature of the Styrofoam packaging material which is able to maintain hot and cold temperatures. The ability of Styrofoam packaging to store heat results in reduced moisture content in the ingredients resulting in drying. According to Adawyah [3] cit Riansyah [24], decreasing water content will result in increased protein content in the material. In the type of wrap packaging, the protein content decreased during storage but it was not significant because the wrap packaging on the red tuna, white oyster mushroom tuna meatballs was better at preventing protein denaturation by bacteria. The decrease in protein content in the wrap packaging is due to the effect of reactive group bonds on the polypeptide chain of the material itself. If the bonds between the reactive groups hold the entire liquid, a gel will form, whereas if the liquid is separated from the coagulated protein, the protein will precipitate [14].

Wrap packaging during storage can reduce contamination by environmental factors so that protein denaturation caused by bacteria can be minimized. This is because the packaging used has low water vapor permeability and moderate gas permeability so that bacterial growth can be inhibited [18]. According to Winarno [25] protein content is closely related to water content and total microbes in food. The activity of microorganisms and enzymatic activity can affect protein levels in meatballs. Bacterial growth will accelerate protein denaturation so that protein levels will decrease. Microorganisms that grow generally will damage proteins turning them into peptides and amino acids [26].

Protein is a great source of amino acids which contain large organic compounds. Based on the atomic arrangement, proteins contain 50-55% carbon atoms (C), 20-23% oxygen atoms (O), 12-19% nitrogen atoms (N), 6-7% hydrogen atoms (H), and 0.2 -0.3% sulfur atom (S) [27]. Protein is an important substance for the body, because in addition to functioning as fuel in the body, it also functions as a building and regulatory substance [23].

Bacteria can break down complex molecules and organic substances such as polysaccharides, fats and proteins into simpler units. This initial breakdown can occur due to the excretion of extracellular enzymes, which are closely related to the process of food spoilage [28].

3.4. Total Plate Count

In this study, an examination of the Total Plate Count was carried out, namely calculating the number of colonies that grew on the media from sample dilution. Dilution aims to reduce the number of microorganism populations. The calculation of the total plate count of microorganisms is selected from petri dishes with a colony of 30-300. This is because the media so that the number of colonies is high (> 300 colonies) is not validly counted so it is likely that the calculation error is very large while the number for small colonies (<30 colonies) is not statistically valid.

The results of the observation / calculation of the Total Plate Number (ALT) using the Plate Count Agar (PCA) method in the calculation results are presented in Table 4.

Table 4. Calculation results of meatball TPC

Factor A (Type Package)	Faktor B (Storage time)		
	B_1 (0 Hour)	B_2 (12 Hour)	B_3 (24 Hour)
A_1 (Non package)	9,2 x 10^3 cfu/g	8,6 x 10^4 cfu/g	3,0 x 10^5 cfu/g
A_2 (Styrofoam)	1,0 x 10^3 cfu/g	2,1 x 10^4 cfu/g	2,8 x10^5 cfu/g
A_3 (Wrap)	4,3 x 10^2 cfu/g	1,2 x 10^4 cfu/g	1,2 x 10^4 cfu/g

Table 4 shows the lowest total number of microbes found in wrap packaging and 0 hour storage time (A_3B_1), while the highest was found in unpackaged meatballs and 24 hours storage time (A_1B_3). The total number of meatball microorganisms that did not meet the SNI requirements for fish balls was found in the A_1B_3 and A_2B_3 treatments. According to SNI 7266: 2014 fish meatballs have a maximum limit of Total Plate Count (TPC) or a total plate number of 1.0x10^5 colonies / gram (5 log CFU / gram). The higher number of microbes was caused by the type of packaging that was unable to control the conditions of moisture, temperature and air during storage. According to Atma [29], the number of microorganisms in processed food is influenced by water content, water activity, pH, temperature, and length of time. High microbial growth is due to the availability of oxygen, free water and sufficient air to support optimal microbial growth [30].

Meatball as a processed meat product is an ideal culture growth medium for microorganisms because of its high water content, pH that is close to neutral and rich in nutrients. The cause of microbial contamination in foodstuffs can be due to the initial number of microbes in fish affecting the number of microbes in the future so that it will increase the number of microbial contamination in fishery products [31].

Contamination from spoilage organisms is difficult to avoid. The total number of microbes in food products is an indicator of food safety [32]. Microbiological analysis needs to be done as an evaluation of the number of microbes in a food ingredient. The raw materials used contain high nutrients, high moisture content, and packaging characteristics and properties. In accordance with the quality requirements of fish balls, this type of wrap packaging still meets the food safety threshold because it can slow down the growth rate of microorganisms and the enzymatic changes that occur after processing and during 24 hours of storage.

3.5. Identification of Salmonella sp

Salmonella sp. is a type of rod-shaped bacteria with a size of 1-3.5 µm x 0.5-0.8 µm. *Salmonella sp.* is a pathogenic bacteria that can cause food poisoning. This identification is done to determine the presence or absence of *Salmonella sp.* on the red oyster mushroom tuna red meatball after different packaging and storage. SNI 7266: 2014 stipulates that fish meatballs should not contain *Salmonella* (negative *Salmonella).*

From the results of identification that was carried out starting from the pre-enrichment stage, enrichment, selective media to biochemical testing of the red oyster mushroom tuna red meatball samples, the identification results of *Salmonella sp*. Bacteria were obtained. presented in Table 5.

Table 5. Identification results of *Salmonella* sp. on the meatball

Treatment	10^1	10^2	SNI	Information
A_1B_1	Negative	Negative	Negative Per 25 g	Accordance with sni
A_2B_1	Negative	Negative		Accordance with sni
A_3B_1	Negative	Negative		Accordance with sni
A_1B_2	Positive	Positive		Not accordance with sni
A_2B_2	Positive	Positive		Not Accordance with sni
A_3B_2	Negative	Negative		Accordance with sni
A_1B_3	Positive	Positive		Not Accordance with sni
A_2B_3	Positive	Positive		Not Accordance with sni
A_3B_3	Negative	Negative		Accordance with sni

Amount sample : 9
Amount positif (+)% : 44,44%
Amount negatif (-)% :55,56%
Total :100%

Based on Table 11, the isolation results on SSA media that were positive for *Salmonella sp* were shown by the formation of clear colonies with black in the middle. The formation of a colorless colony with a black center because *Salmonella* sp. can produce Hydrogen Sulfide (H_2S) which is characterized by the formation of black deposits on the AAS media. In the identification of *Salmonella sp.* In 9 samples of white oyster mushroom tuna red meatballs, samples A_1B_2 (without packaging with 12 hours storage time), A_2B_2 (styrofoam packaging with 12 hours storage time), A1B3 (without packaging) with a shelf life of 24 hours), A_2B_3 (styrofoam packaging with a shelf life of 24 hours) were positive for salmonella sp After the percentage, the results (44.44%) were obtained from the total of all samples. However, meatball with wrap packaging and storage time of 24 hours (A3B3) was not indicated that *Salmonella sp* was present, meaning that the use of wrap packaging with a storage time of up to 24 hours was still able to protect the meatball products from Salmonella sp bacteria.

Based on research by Susanti [33] the occurrence of bacterial contamination with *Salmonella sp*. in smoked fish is influenced by the hygiene practices of producers and sellers. Poor hygiene practices, for example unwashed hands, using dirty utensils, uncut nails and leaving food open are a means of spreading bacteria. In addition, dirty environmental conditions allow the spread of microbes and germ particles to be carried into smoked fish.

According to the theory of Arlita [34], which causes the sample to be contaminated by bacteria is caused by a knife used to cut raw materials such as raw meat that is contaminated by pathogenic bacteria if the equipment is used again without washing it first to cut cooked food that will be contaminated by the tool, by means of displacement of pathogens that can pose a risk to those who consume these foods. *Salmonella sp* in food in large enough quantities will not cause a change in appearance, smell or taste [35]. *Salmonella sp* can grow in a temperature range of 5−47 °C with the optimum growth temperature is 37 °C and the maximum temperature is 45.6 °C [36].

Food containing *Salmonella sp* in small amounts will not change the shape, taste and smell of the meal. However, if the food contains large amounts of bacteria, it will change the shape, taste, and distinctive smell of bacteria. The presence of *Salmonella sp.* According to SNI 7266: 2014 concerning fish meatballs, it must be negative for *Salmonella sp* microbes per 25 grams. In the research, tuna red tetelan meatball with wrapper was still protected from the growth of *Salmonella sp* bacteria for 24 hours.

3.6. Organoleptic Test

Organoleptic tests are carried out through sensory assessments, namely by observing appearance, observing odors, and observing texture. This test is carried out by means of the sample made in accordance with the treatment formulation. Furthermore, the test was carried out on 15 panelists. Panelists will provide a value based on the meatball specifications provided.

3.6.1. Appearance

Appearance is the first characteristic that consumers can judge for the first time. This appearance assessment aims to determine the panelists' acceptance which is assessed from the surface appearance of the meatball. The average results of the assessment of the appearance of the meatballs are presented in Table 6.

Table 6. Average parameter values for the appearance of meatballs

Treatment	average
A_1B_1 (Without packaging with a long shelf of 0 hours)	8,60
A_2B_1 (Packaging *Styrofoam* a long shelf of 0 hours)	8,60
A_3B_1 (Packaging *wrap* a long self 0 hours)	8,60
A_1B_2 (Non packaging a long self 12 hours)	6,33
A_2B_2 (Packaging *Styrofoam* a long self 12 hour)	7,00
A_3B_2 (Packaging *wrap* a long self 12 hour)	7,53
A_1B_3 (Non Packaging a long self 24 hour)	4,47
A_2B_3 (Packaging *Styrofoam* a long self 24 hour)	5,53
A_3B_3 (Packaging *wrap* a long self 24 hour)	6,87

Test parameter description:
9: smooth surface, not hollow, bright., 7: surface less smooth, slightly hollow, less bright., 5: surface rough, hollow, dull., 3: surface slightly cracked, hollow a lot, dull, 1: surface lots of cracks, lots of cavities, very dull

Table 6 shows the average value of panelists' acceptance of the appearance of meatballs in different treatments which tends to decrease. The appearance of the red oyster mushroom tuna red meatballs has an average value ranging from 4.47 to 8.60. The treatments for meatballs without 0 hour storage packaging (A_1B_1), Styrofoam packaging for 0 hours storage (A_2B_1), and 0 hours storage wrap packaging (A_2B_1) both had the highest value reaching 8.60. Based on the panelists' values 7-9, the three treatments have specifications of smooth, non-hollow, bright surface. The high value of the appearance test is due to the test carried out before the product experiences storage and the type of packaging used.

While the lowest appearance value was found in the A_1B_3 treatment (without packaging for 24 hours storage time) with a value of 4.47 with a slightly cracked surface specification, a lot of hollow, dullness. In accordance with SNI 7266: 2014 concerning fish meatballs, the minimum sensory value of 7 (score 1-9) with the surface specifications is less smooth, slightly hollow, less bright. From this research, those that do not meet the requirements according to SNI for the appearance value are without 12-hour storage packaging (A_1B_2), without 24-hour storage packaging (A_1B_3) and 24-hour storage packaged Styrofoam meatballs (A_2B_3).

Table 6 shows the longer the retention of the panelists' acceptance of the appearance of the meatballs, the decreasing. The use of styrofoam packaging and wrap for 12 hours of storage still meets the minimum threshold for the appearance value of meatballs based on SNI 7266: 2014, which is a minimum of 7, meaning that it still has a surface specification that is less smooth, slightly hollow, less bright. Meanwhile, the 24-hour storage without Styrofoam packaging and packaging no longer meets the sensory quality requirements of meatballs.

Products or foodstuffs that experience storage result in quality degradation, both physically and chemically [37]. Meatballs are known to have decreased water content during storage resulting in the appearance of dull meatballs. According to Chamidah [38], explaining that the decrease in appearance value during storage is suspected because the water content of the product during storage has also decreased. In addition, during storage, there will be microorganism activity which results in the appearance of slimy

meatballs. This is in accordance with the opinion of Hidayati [9], said that the meatballs will look soft and slimy due to the activity of microorganisms. It can be seen that the red and white oyster mushroom tuna meatballs during storage for up to 24 hours have deteriorated quality.

3.6.2 Odor (aroma)

Aroma is a parameter that affects the quality of a processed product. The smell or smell of food can determine the delicacy of these food ingredients. In general, the smells received by the nose and brain is a combination of four main smells, namely fragrant, sour, rancid, and charred [25]. The average value of the red oyster mushroom tuna red meatball aroma is presented in Table 7.

Table 7. Average value of meatball odor parameters

Treatment	average
A_1B_1 (Without packaging with a long shelf of 0 hours)	8,33
A_2B_1 (Packaging *Styrofoam* a long shelf of 0 hours)	8,60
A_3B_1 (Packaging *wrap* a long self 0 hours)	8,73
A_1B_2 (Non packaging a long self 12 hours)	6,87
A_2B_2 (Packaging *Styrofoam* a long self 12 hour)	6,47
A_3B_2 (Packaging *wrap* a long self 12 hour)	7,93
A_1B_3 (Non Packaging a long self 24 hour)	4,87
A_2B_3 (Packaging *Styrofoam* a long self 24 hour)	4,47
A_3B_3 (Packaging *wrap* a long self 24 hour)	6,07

Test parameter description:
9 = specific product 7 = less specific product 5 = neutral 3 = slightly rotten, rancid 1 = rotten and very rancid

The results of the panelists' average assessment of the aroma parameters of red tetelan tuna, white oyster mushroom tuna on different treatments ranged from 4.47 to 8.33. The highest aroma value of the tested fish balls was achieved by the A_3B_1 treatment (0 hour storage wrap packaging), namely 8.73. The high value of this aroma is because it has not been influenced by the type of packaging and storage time so that the aroma is still maintained with product specifications. While the lowest value was achieved by the A_2B_3 treatment (Styrofoam packaging for 24 hours), namely 4.47.

The decrease in the average value of the odor specification for stored meatballs at 0 to 12 hours, the odor sensory value was still acceptable to the panelists, but after the 24th hour the value had been rejected because the aroma had changed. The production of aroma compounds is largely determined by the composition of the bacteria involved in these compounds [25]. Basically, the stored meatball does not experience a change in aroma to become rancid, but rather an increase in the aroma of fish in the meatball. The increase in the fishy aroma of meatballs can occur due to an overhaul of fish protein by enzyme activity and microorganisms. According to Rahmadana [39], a change in a sharp fishy odor is a sign of protein decomposition in fish.

The decrease in the average value of the odor specification of fish meatballs from the 0th hour to the 12^{th} hour of the odor sensory value of the unpackaged meatballs and wrap packaging was still acceptable to the panelists, but after the 24th hour the value was rejected for fish balls. Where the characteristic appears unpleasant odor is close to neutral until a sour smell arises. From the sensory value, it can be seen that the treatment of meatballs without Styrofoam packaging and packaging decreases the specification value faster than the packaging during storage. According to Saleh [40], during storage, the sensory value of aroma / smell tends to decrease, this is in line with the increase in the value of ammonia, TVB, TPC, fat oxidation. The increasing number of microorganisms causes the formation of compounds that produce unpleasant odors and tastes.

3.6.3. Texture

Texture is one of the factors that determine the acceptance of a product. Texture assessment aims to determine the panelist's acceptance of the level of elasticity or resilience of a product which can be assessed

using the sense of touch, namely through touch stimuli. The results of the scoring test regarding the texture of the red and white oyster mushroom tuna meatballs are presented in Table 8.

Table 8. Average value of meatball texture

Treatment	average
A_1B_1 (Without packaging with a long shelf of 0 hours)	8,47
A_2B_1 (Packaging *Styrofoam* a long shelf of 0 hours)	8,60
A_3B_1 (Packaging *wrap* a long self 0 hours)	8,60
A_1B_2 (Non packaging a long self 12 hours)	7,00
A_2B_2 (Packaging *Styrofoam* a long self 12 hour)	7,00
A_3B_2 (Packaging *wrap* a long self 12 hour)	7,13
A_1B_3 (Non Packaging a long self 24 hour)	6,07
A_2B_3 (Packaging *Styrofoam* a long self 24 hour)	6,07
A_3B_3 (Packaging *wrap* a long self 24 hour)	6,60

Description of test parameters: 9 = solid, compact, chewy 7 = compact, rather springy 5 = not solid, not compact, not chewy 3 = easy to break 1 = very easy to break

In Table 8, it can be seen that the average value of the panelists' acceptance rate of the texture of the white oyster mushroom tuna red tetelan meatball ranges from 6.7 to 8.60. The highest average value was found in the A_2B_1 treatment (Styrofoam packaging for 0 hours storage) and A_3B_1 (24 hours storage wrap packaging), which had the same value of 8.60 with the specifications of solid, compact, and thick. While the lowest was in the A_1B_3 treatment (without 24-hour storage packaging) and A_2B_3 (24-hour storage Styrofoam packaging) which had the same value of 6.07 with the specifications of solid, compact, rather chewy.

Based on the data above, it is known that the meatball product with storage for 12 hours has a value of 7, meaning that it still meets the product specifications for solid, compact, somewhat chewy. In accordance with SNI 7266: 2014 regarding fish meatballs with a minimum sensory value of 7 (score 1-9) with a compact, rather chewy solid specification. Storage at 0 to 24 hours texture parameters in all treatments decreased. However, the texture of the oyster mushroom tuna red tetelan meatball on the A_3B_3 treatment (24-hour storage wrap packaging) was still acceptable to the panelists with a value of 6.60, meaning that it still met the specifications for solid, compact, somewhat chewy.

One of the parameters that people like about meatballs is their chewy texture. There are several factors that can affect the texture of the meatballs such as the composition of the meatballs, the manufacturing process and heating time [41]. During storage, changes in the texture of food can occur due to changes in water content, temperature and microbiological activity which can lead to a decrease in food quality [42].

4. Conclusion

Based on research on "The Effect of Packaging Type and Storage Time on Quality of Red Tuna, White Oyster Mushroom Meatballs" can be drawn the following conclusions: 1. Type of red oyster mushroom tuna red meatball packaging based on water content, ash content, protein content, TPC, Salmonella sp and Organoleptic bacterial contamination is the best use of Wrap packaging. 2. The duration of storage of red oyster mushroom tuna red meatballs based on water content, ash content, protein content, TPC, Salmonella sp and Organoleptic bacterial contamination is the best storage for 12 hours. 3. Combination of treatment on the quality of white oyster mushroom tuna red meatballs is the use of wrap packaging with a storage time of 12 hours.

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