Research Article

Enhancing Tuna Fish Meatball Quality Through The Incorporation Of Tannin Extract From *Tomi-Tomi* fruit (*Flacourtia inermis*)

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Abstract | The quality of fish balls served as a key indicator in the food industry, and this study aimed to analyze the optimal quality of tuna fish balls by incorporating *Tomi-tomi* fruit (*Flacourtia inermis*) tannin extract. The study employed four control treatments (C0), treatment 1 (C1), treatment 2 (C2), and treatment 3 (C3) with sequential levels of *Tomi-tomi* fruit extract, namely 0, 1, 1.5, and 2% of the total weight of fish meatball. Data analysis revealed that the addition of *Tomi-tomi* fruit tannin extract concentration was effective in enhancing the quality of tuna fish balls. The results of the analysis of variance indicated a significant impact (p >0.05) of *Tomi-tomi* fruit tannin extract treatments at various concentrations on the water content and protein of tuna fish meatballs However, there is no significant difference on the sensory and total bacteria (p > 0.05) of tuna fish meat balls. The best treatment, determined to be the addition of 2% *Tomi-tomi* fruit tannin extract concentration (C3), resulted in round and well-formed meatballs with a resilient texture and a distinct taste favored by the panelists. The water content measured at 52.63%, protein content at 15.37%, and total bacteria at 2.68 x 10^2 CFU/g. In conclusion, the application of tannin extract from *Tomi-tomi* fruit in tuna fish meatballs proved to be effective during a 14-hour storage period.

Keywords | Extract, Fish tuna, Meatball, Preservation, *Tomi-tomi* fruit

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INTRODUCTION

Tuna fish trimmings, the byproduct of tuna loin processing, comprised both red and white meat. Fish meal poses severe environmental issues every year through Indonesia (Adli, 2021). Abundant in marine resources, trimmings are rich in nutrients like protein, vitamins, minerals, omega-3, and omega-6 (Mesias *et al*., 2015). Among the various products, fish balls stand out as a form of fisheries product diversification with high economic value, being a popular choice among consumers.

In addition, meatballs, a widely favored convenient meat product, are small balls of crushed or ground meat mixed with spices and other ingredients (Islam *et al*., 2018). To enhance the sensory quality of low-fat-comminuted meat products like meatballs, plant starches and flours are commonly used as binders and/or fillers. Conventional meat is associated with protein availability (Siddiqui *et al*., 2022). Bengal gram flour, a good source of proteins, carbohydrates, and vitamins Choudary *et al* (2019), acts as a binding and thickening agent, improving texture and extending shelf life. Meatballs (*bakso*) are enjoyed by the community for their delicious taste and nutritional value. Being perishable with more than 50% meat and other additives, meatballs, especially fish meatballs, are prone to spoilage due to the perishable nature of fish meat and its high water content of 80%, leading to susceptibility to microbiological contamination and a short shelf life. In an attempt to extend shelf life and improve quality, synthetic preservatives like formalin, borax, benzoic acid, nitrate/nitrite, and ethyl p-hydroxybenzoate are commonly used. However, the use of these synthetic preservatives has proven to have side effects, including liver dysfunction, carcinogenicity, and potent estrogenic effects (Alakolanga *et al*., 2015). Natural preservatives derived from plants are considered safer, more affordable, easily accessible, and environmentally friendly. Therefore, it becomes imperative to process fish balls using natural plant-based preservatives such as *Tomi-tomi*-fruit (*Flacourtia inermis Roxb*). This fruit contains tannins, phenols, flavonoids, saponins, terpenoids, and alkaloids (Alakolanga *et al*., 2015). These secondary metabolites also possess antibacterial, antibiotic, antifungal, and preservative properties (George *et al*., 2010; Pylori *et al*., 2015). It is hypotheses the addition of Tomi-tomi fruit could extend the preservation of the Tuna meatballs. Recognizing the potential and benefits of *Tomi-tomi*fruit, the author conducted research aimed at analyzing the best quality of tuna fish balls with the addition of *Tomi-tomi*fruit tannin extract during storage.

MATERIALS AND METHODS

Extraction and Tuna Fish Meatballs

Tomi-tomi fruits were obtained from Kaibobu Village, West Seram District, Maluku Province, Indonesia. After a thorough wash to remove impurities, the fruits were sliced and subjected to a drying oven at 60°C for three days. The resulting dried powder underwent fine grinding with a blender and was sifted through a 60-mesh sieve. Subsequently, 50g of *Tomi-tomi* fruit powder was soaked in 450 ml of 95% ethanol until 2 cm above the sample surface, allowing the mixture to stand for three days with stirring every 6 hours. The resultant liquid extract was obtained by filtration and concentrated using a rotary evaporator. Confirmation of tannin compounds in the *Tomi-tomi* fruit extract involved placing 1g of the extract in a reaction tube and mixing it with 15 mL of hot water. Boiling the mixture for 15 minutes, followed by filtration and the addition of 2-3 drops of 1% $FeCl_3$ solution, resulted in a dark green color, confirming the presence of tannin compounds. A test with 1% gelatin solution also produced a white precipitate, confirming the presence of tannin compounds. The tannin extract was then prepared in concentrations of 0% (control/C0), 1% (C1), 1.5% (C2), and 2% (C3).

The process of making tuna fish meatballs commenced with grinding 500 grams of tuna loin trimmings in a blender until smooth. Before blending, the tuna trimmings were cut into small pieces, mixed with 100 grams of ice, and combined with 7 grams of salt, seasoning, spices (3 grams of pepper, 15 grams of garlic), 2 eggs, tapioca (100 grams), and a bunch of celery leaves for further blending. Gradual addition of tapioca flour (50 grams) while stirring and kneading produced a homogeneous dough. The dough was then shaped into balls and boiled in water for 20 minutes until cooked. The boiled balls were lifted and drained, with one part remaining without the addition of *Tomi-tomi* fruit tannin extract (0% control), and the other part treated with *Tomi-tomi* fruit tannin extract at concentrations of 1% (C1), 1.5% (C2), and 2% (C3). The fish balls were immersed for 30 minutes, lifted, drained, and were then ready for analysis.

Nutrient content evaluation

The cup was subjected to drying in an oven at 100-105°C for 30 minutes or until a consistent weight was achieved. Following this, the cup underwent cooling in a desiccator for 30 minutes and was then weighed. A 5 g sample (B1) was placed in the cup and dried in the oven at a temperature of 100-105°C until a constant weight was attained (8-12 hours). Subsequently, the sample underwent another round of cooling in the desiccator for 30 minutes and was reweighed (B2).

Next, 0.51g of the sample was placed into a 100 ml Kjeldahl flask. To this sample, two grams of a selenium mixture and 25 ml of concentrated H_2SO_4 were added. The mixture was heated on an electric heater until boiling, and the solution became clear greenish (approximately 2 hours at 420ºC). After cooling, the sample was diluted, transferred to a 100 ml volumetric flask, and filled up to the mark. A 5 ml solution was pipetted and placed into a distillation apparatus. Subsequently, 5 ml of 30% of sodium hydroxide (NaOH) and a few drops of phenolphthalein (phph) indicator were added, and the mixture was distilled for 10 minutes. A 10 ml solution of 2% boric acid with an indicator served as the receiving flask. The condenser tip was rinsed with distilled water and titrated with 0.01 N hydrochloric acid (HCl) (AOAC, 2005).

Sensory and Total Bacterial Evaluation

The bacterial count calculation is carried out through the pour plate method. The sample undergoes dilution in ranges from 10^{-1} to 10^{-5}. In each dilution, 1 ml of the sample is pipetted and poured into a sterile petri dish. Subsequently, 15 ml of Plate Count Agar medium at 37°C is added, followed by homogenization. The petri dishes containing the samples are then placed in an incubator set at 37°C for 24 to 48 hours. The observation and counting of bacterial colonies that develop are performed.

To evaluate sensory attributes such as color, aroma, taste, and texture of tuna fish balls, a hedonic scale is employed for preference assessment. A questionnaire is utilized, assigning scores from 1 to 7, where 1 denotes 'dislike very much,' 2 is 'dislike,' 3 is 'neither like nor dislike,' 4 is 'like,' 5 is 'like moderately,' 6 is 'like very much,' and 7 is 'like extremely.' This evaluation involves 25 semi-trained panelists.

Statistical analysis

The research data analysis utilized a Completely Randomized Design (CRD) with three applications, and the outcomes were subjected to analysis of variance (ANOVA) at a 95% significance level. The following model was used (Adli *et al*., 2023; Sholikin *et al*., 2023).

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where Yij was parameters observed, μ was the overall mean, Ti the effect different the effect level of *Tomi-tomi*-fruit, and eij the amount of error number. All analysis was carried out in six replications and significant of difference was defined as the 5% level ($P<0.05$). At the end, probabilities values were subjected in Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Nutrient Content

The results of the analysis of variance indicated a significant impact ($p > 0.05$) of *Tomi-tomi*fruit tannin extract treatments at various concentrations on the water content of tuna fish meatballs. Subsequently, Duncan's Multiple Range Test (DMRT) was applied with a confidence level of 0.05% to evaluate water content at hours 0, 7, and 14, as illustrated in Table 1. The results presented in the table suggest that higher concentrations of *Tomi-tomi* fruit tannin extract result in decreased water content. This reduction in water content is linked to a decline in total microbes, as water constitutes a major component of microbes. The decrease is attributed to water evaporation, where the amount evaporated exceeds the amount absorbed. Furthermore, the water-soluble and polar nature of tannins in the *Tomi-tomi*fruit extract contributes to this reduction by binding to water. Immersing tuna fish meatballs in *Tomi-tomi* fruit tannin extract is shown to decrease water content as the meatballs absorb the solution.

Concerning storage conditions, no significant differences ($p < 0.05$) were observed among all treatments. The highest water content was recorded in C0 storage at 60.22%, while C3 exhibited the lowest at 54.73%, followed by C2 at 56.85%, and C1 at 58.64%. After 7 hours of storage, C0 showed the highest value at 59.56%, C3 had the lowest at 53.54%, followed by C2 at 55.55%, and C1 at 57.45%. Meanwhile, at the 14th hour of storage, all treatments experienced a decline in water content. C0 had the highest value at 58.45%, C3 had the lowest at 52.63%, followed by C2 at 54.75%, and C1 at 56.44%. This suggests that the water content of tuna fish meatballs decreases during storage with an increasing concentration of *Tomi-tomi* fruit tannin extract. This reduction is influenced by various factors, including active compounds from *Tomi-tomi* fruit, such as tannin, which has water-binding properties. The inhibition of total microbes is attributed to the decreasing microbial count influenced by air humidity, resulting in water absorption from the surrounding environment during food storage. The water content in fish balls is also influenced by chemical compounds, temperature, consistency, and interactions with food components like proteins, fats, vitamins, free fatty acids, and other constituents (Divekar *et al*., 2022).

Protein serves as a foundational material for new tissues, regulates metabolic processes, and acts as fuel when the body's energy needs are not met by fats and carbohydrates. According to the analysis of variance, all concentrations of *Tomi-tomi* fruit tannin extract treatments significantly impact the protein content of tuna fish balls ($P>0.005$). Subsequently, a Duncan test (DMRT) was conducted at a confidence level of 0.05%, and the protein content observed at 0 hours, 7 hours, and 14 hours can be found in Table 2. As per Table 2, the results indicate that the average protein content in tuna fish balls under various treatments has the highest value in treatment C3 (at 14 hours) at 15.37%, and the lowest value in treatment C0 (at 0 hours) at 10.57%. At 14 hours, all concentrations of *Tomi-tomi* fruit tannin extract treatments experienced an increase. The higher the concentration of *Tomi-tomi* fruit tannin extract given to tuna fish meatballs, the higher the protein content. The increase in protein content is attributed to the decrease in water content, where *Tomi-tomi* fruit tannin aids in enhancing protein levels by improving nutrient absorption and binding compounds that interfere with nutrient absorption, such as heavy metals. Additionally, functional groups in tannins cause protein precipitation, leading to the formation of protein complexes in food ingredients. Tannins also bind with proteins, influencing absorption and enhancing the nutritional value of proteins (Li *et al*., 2020).

The protein content in tuna fish meatballs can be influenced by the primary ingredient, which is the tuna fish itself. However, the addition of varying amounts of flour and tapioca flour in each formulation resulted in different protein levels. In this study, the use of tuna fish in all formulations remained consistent, and the differentiating factor was the addition of *Tomi-tomi* fruit extract. Lower amounts of flour and tapioca flour added led to higher protein content in tuna fish meatballs. The protein content is presumed to be influenced by the reduction in water con

Table 1: Average results of tuna fish meatballs moisture analysis (%).

Treatment	Observation (Hours -)		
	0	**7**	**14**
C0	60.22 ± 0.13^{a}	59.56 ± 0.12^{a}	58.45 ± 0.07^{a}
C1	58.64 ± 0.12^{b}	57.45 ± 0.03^{b}	56.44 ± 0.12^{b}
C2	56.85 ± 0.13^{c}	55.55 ± 0.11^{c}	54.75 ± 0.10^{c}
C3	54.73 ± 0.12^{d}	53.54 ± 0.12^{d}	52.63 ± 0.12^{d}

* Different superscript letters in the same column indicate a significant difference in water content between treatments ($p > 0.05$).

Table 2: Average results of tuna fish meatballs protein analysis (%).

Perlakuan	Observation (Hours -)		
	0	**7**	**14**
C0	10.57 ± 0.25^{d}	10.76 ± 0.19^{d}	11.73 ± 0.21^{d}
C1	12.34 ± 0.11^{c}	12.57 ± 0.08^{c}	13.52 ± 0.09^{c}
C2	13.64 ± 0.10^{b}	13.67 ± 0.23^{b}	14.41 ± 0.19^{b}
C3	14.46 ± 0.11^{a}	14.53 ± 0.10^{a}	15.37 ± 0.15^{a}

*Different superscript letters in the same column showed a marked difference in protein levels between treatments ($p>0.05$)

Table 3: Average result of total tuna fish meatballs total bacterial analysis (log CFU/g)

Treatment	Observation (Hours-)		
	0	**7**	**14**
C0	5.53 ± 0.10^{a}	5.41 ± 0.13^{d}	4.27 ± 0.14^{d}
C1	5.53 ± 0.10^{a}	4.40 ± 0.10^{c}	3.56 ± 0.04^{c}
C2	5.53 ± 0.10^{a}	4.24 ± 0.12^{b}	3.25 ± 0.13^{b}
C3	5.53 ± 0.10^{a}	4.17 ± 0.14^{a}	2.68 ± 0.26^{a}

* Different superscript letters in the same column show a marked difference in the total number of bacteria between treatments ($p>0.05$)

tent in tuna fish meatballs. The primary raw material, tuna fish meatballs used in the processing process, also affects the protein content. For storage, all treatments did not show significant differences ($p > 0.05$).

At 0 hours of storage, C3 obtained the highest value at 14.46%, while C0 obtained the lowest value at 10.57%, followed by C2 at 13.64%, and C1 at 12.34%. At 7 hours of storage, C3 obtained the highest value at 14.53%, and C0 obtained the lowest value at 10.76%, followed by C2 with a value of 13.67%, and C1 with a value of 12.57%. Meanwhile, at 14 hours of storage, all treatments experienced an increased protein content. C3 obtained the highest value at 15.37%, and C0 obtained the lowest value at 11.73%, followed by C2 with a value of 14.41%, and C1 with a value of 13.52%. This suggests that the longer the storage of tuna fish meatballs, the higher the protein content. The higher protein content during storage enhances its function as a binder for meat crumbing during cooking, forming a compact structure.

Sensory evaluation

The total bacterial analysis aimed to assess the overall microorganism count in treated samples at 0, 7, and 14 hours. The analysis of variance indicated that the administration of *Tomi-tomi* fruit tannin extract at 0 hours did not yield a significant effect ($p>0.05$). However, at 7 and 14 hours, it notably influenced ($p < 0.05$) the total bacteria in tuna fish meatballs. Subsequently, the Duncan test (DMRT) was conducted with a confidence level of 0.05% for the total bacteria in tuna fish meatballs obtained at 7 and 14 hours, as outlined in Table 3.

At 0 hours of storage, the bacterial count was 5.53×10^5 CFU/g for all treatments, and thus, C0 did not significantly differ from C1, C2, and C3. The bacterial count adhered to the limits set by (BSN, 2014) at 7 hours of storage, all treatments exhibited significant differences. C0 had the highest value at 5.41×10^5 CFU/g, while C3 had the lowest value at 4.17 CFU/g, followed by C2 and C1, both obtaining a value of 4.40×10^4 CFU/g. Similarly, at 14 hours of storage, all treatments showed significant differences in total bacterial count. C0 had the highest value at 4.27×10^4 CFU/g, C3 had the lowest value at 2.68×10^2 CFU/g, followed by C2 with a value of 3.25×10^3 CFU/g, and C1 with a value of 3.56×10^3 CFU/g. This indicates that the use of *Tomi-tomi* fruit tannin extract can inhibit microbial growth.

The bacteriostatic mechanism of *Tomi-tomi* fruit tannin extract involves inhibiting the metabolic processes of bacterial cells, slowing down microbial growth without necessarily killing them. Bacteriostatic compounds often inhibit protein synthesis or bind to ribosomes. Plant-derived tannins, like those found in *Tomi-tomi* fruit, contain bioactive compounds that damage cell walls, deactivate enzymes, disrupt extracellular and soluble proteins, inhibit bacterial growth and protease enzyme activities, and interfere with the peptidoglycan component in bacterial cell walls. Tannin compounds exhibit antibacterial activity by reducing bacterial cell wall size, disrupting permeability, and preventing the bacterial cell wall from carrying out normal metabolic processes (Das *et al*., 2020).

The antibacterial properties of *Tomi-tomi* fruit tannin compounds were demonstrated when treated with iron (III) chloride solution, producing a characteristic dark green solution. This indicates that tannins can cause bacterial cells to undergo lysis, targeting the polypeptide cell wall of bacteria, leading to imperfect cell wall formation and eventual bacterial cell death. The mechanism of action involves precipitating proteins, enzyme inactivation, and the destruction or inactivation of genetic material, collectively contributing to bacterial inhibition. Tannin compounds play a role in contracting cell walls, disrupting permeability processes that can result in bacterial cell death. Based on the research results, the tannin extract from *Tomi-tomi* fruit exhibits antibacterial activity, evident through the power of tannins to precipitate proteins (Kunyanga *et al*., 2011).

The results in Figure 1 indicate that the higher the concentration of *Tomi-tomi* fruit tannin extract, the higher the texture values of tuna fish meatballs produced. The texture values of tuna fish meatballs increase, correlating with the decrease in water content and microbial count to reduce environmental contamination, thereby extending the shelf life. Being familiar with the product, gave a highly favorable value to 2% (C3) as the highest average value, which is 5.92, and the lowest average value was for treatment 0% (C0) at 5.77. The characteristic texture of tuna fish meatballs is described as dense, compact, elastic, and not brittle. The panelists' assessment of the texture characteristics produced is considered the best, as they are familiar with the texture of the fish meatballs.

Moreover, considering the texture characteristics of the tuna fish meatballs in this study, there are specific contents of protein, amylose, amylopectin, and gluten in the added flour. The Duncan test results show that the texture of tuna fish balls with a concentration of 2% (C3) is significantly different from all other treatments; in comparison, the 1.5% (C2) concentration is not significantly different from the control concentration (C0). Similarly, the treatment with a concentration of 1% (C1) produces a texture that is not significantly different from the control concentration. Several factors, including protein, water, and fat content from the constituent ingredients, can cause a difference in the elasticity of the texture of tuna fish meatballs. Tuna fish tendons have a relatively high protein content, allowing the production of fish meatballs with a chewy texture with proper and appropriate mixing (Ravendran *et al*., 2018). Microbial activity in food ingredients can degrade protein and carbohydrate structures, resulting in a softer texture. According to Barrett *et al* (1998) microorganisms such as bacteria, yeast, and fungi, along with their enzymes, are widely used in various food processing methods to enhance flavor and achieve a soft texture, such as in meat tenderizers and the improvement of egg quality (Yuliati *et al*., 2021).

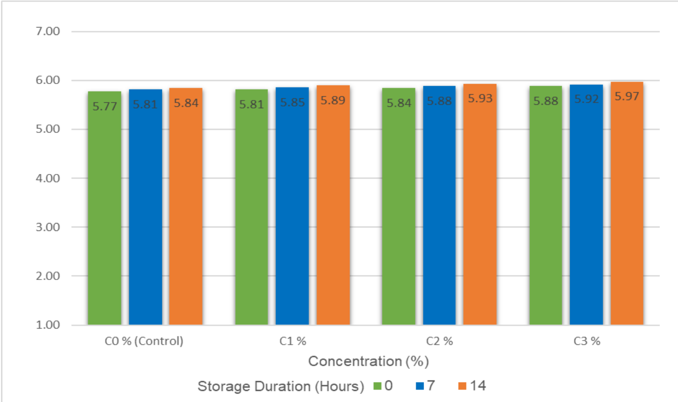

Figure 1: The Panelist's preference on tuna fish meatballs texture

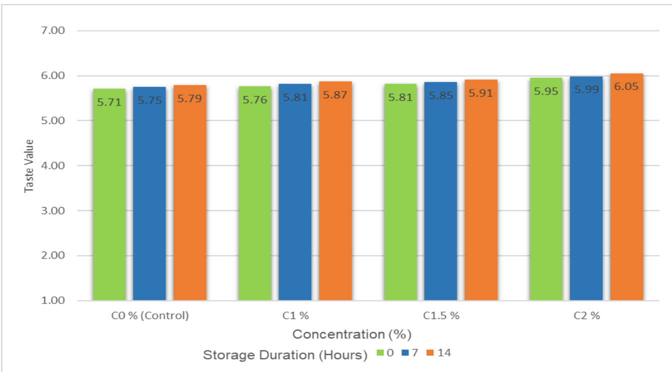

Figure 2: The Panelist's preference on tuna fish meatballs taste

Flavor is a critical sensory aspect in the acceptance of a food product. The variety analysis results revealed a significant impact ($p < 0.05$) on the taste value of tuna fish meatballs for all treatment concentrations of *Tomi-tomi* fruit tannin extract. Figure 2 results demonstrate that the taste value of tuna fish meatballs increases with the higher concentration of added *Tomi-tomi* fruit tannin extract. The top average taste value for tuna fish balls is observed at a

2% concentration (C3) with a value of 6.05, while the lowest is at a 0% concentration (C0) with a value of 5.71. The panelists' evaluation of the taste of tuna fish meatballs, with the incorporation of *Tomi-tomi* fruit tannin extract, ranges from 5.71 to 6.05 (very likable), indicating that the taste of all treatments is acceptable to the panelists.

The highest panelist rating for the taste of tuna fish meatballs with 2% *Tomi-tomi* fruit tannin extract reflects a distinct fish flavor, savories, and a slight sour taste. Conversely, the lowest panelist score is found without *Tomi-tomi* fruit tannin extract at 0% (C0), exhibiting a relatively strong fish flavor. Numerous factors influence taste, including chemical compounds from kitchen spices/seasonings, temperature, concentration, and interaction with other flavor components. The taste is primarily determined by the formulation used and is significantly influenced by the processing of food products. This assertion is corroborated by research Ojo *et al* (2022) which underscores the significant influence of protein, fat, and carbohydrate content on the taste of food. Regarding storage, there are notable differences among all treatments ($p>0.05$). Taste scores for tuna fish balls indicate an upward trend during storage. At hour 0 of storage, C3 attains the highest value at 5.95, while C0 registers the lowest value of 5.71, followed by C2 at 5.81 and C1 with a score of 5.76. After 7 hours of storage, C3 once again secures the highest value at 5.99, while C0 records the lowest at 5.75, followed by C2 at 5.85 and C1 with a score of 5.81. At the 14-hour mark of storage, all treatments witness an increase in taste value, with C3 achieving the highest at 6.05, and C0 having the lowest at 5.79, followed by C2 at 5.91 and C1 at 5.87. The heightened taste of tuna fish meatballs during storage is influenced by the meat, seasonings, and additional fillings incorporated during processing. As highlighted in Damodaran, (2017), spices play a vital role in enhancing taste and prolonging the shelf life of tuna fish meatballs.

Total Bacterial

The total bacterial analysis aimed to determine the overall microorganism count in treated samples at 0, 7, and 14 hours. According to the analysis of variance, the administration of *Tomi-tomi* fruit tannin extract at 0 hours did not have a significant effect ($p>0.05$). However, at 7 and 14 hours, it significantly impacted ($p>0.05$) the total bacteria in tuna fish meatballs. Subsequently, the Duncan test (DMRT) was performed with a confidence level of 0.05% for the total bacteria in tuna fish meatballs obtained at 7 and 14 hours, as outlined in Table 3.

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The bacteriostatic mechanism of *Tomi-tomi* fruit tannin extract involves inhibiting the metabolic processes of bacterial cells, slowing down microbial growth without necessarily killing them. Bacteriostatic compounds often inhibit protein synthesis or bind to ribosomes. Plant-derived tannins, like those found in *Tomi-tomi* fruit, contain bioactive compounds that damage cell walls, deactivate enzymes, disrupt extracellular and soluble proteins, inhibit bacterial growth and protease enzyme activities, and interfere with the peptidoglycan component in bacterial cell walls. Tannin compounds exhibit antibacterial activity by reducing bacterial cell wall size, disrupting permeability and preventing the bacterial cell wall from carrying out normal metabolic processes (Alakolanga *et al*., 2015).

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CONCLUSIONS

The utilization of *Tomi-tomi* fruit tannin extract in tuna fish meatballs proves to be effective in enhancing various quality attributes during a 14-hour storage period. This improvement is particularly significant for water content, protein content, total bacteria count, texture, and taste, specifically at a 2% concentration of *Tomi-tomi* fruit tannin extract (C3).

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

NOVELTY STATEMENT

The Novelty of this research were used *Tomi-tomi*fruit inorder to enhanced quality of meatball.

AUTHOR'S CONTRIBUTION

Sandriana J Nendissa contributed to collecting data, analysis of nutrient, data analysis and preparing the manuscript. Meta Mahendradatta, Zainal, Februadi Bastian contributed to the research design, revised the manuscript and supervision. All authors read and approved the final version of the manuscript in the present journal.

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