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Combination of Chitosan Nanoparticles and Modified Tapioca-Whey Protein in The Preparation of Edible Coating on Antibacterial Activity and Organoleptic of Tenggiri Fish Products

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ABSTRACT

Product otak-otak ikan tenggiri is a promising fishery diversification product but is prone to spoilage. Edible coatings can help slow down spoilage. The purpose of this study was to determine the effect of different edible coating formulations on the characteristics of edible coating and to determine the protective potential of edible coatings against bacterial growth and organoleptic product otak-otak ikan tenggiri during storage. This research is an experimental study that is divided into two stages, the first stage is the characteristics of edible coatings using a completely randomized design (CRD) with testing including antimicrobial activity, water absorption, and viscosity with 5 treatments and 4 replicates, and the second stage is coating applications on the length of storage of product otak-otak ikan tenggiri testing including TPC and organoleptic using two-factor CRD 3×6 treatments and 3 replicates. Results indicated a significant impact ($P < 0.05$) of coating formulations on these characteristics. The best formulation, modified tapioca-WPI with CSNP (P4), showed antibacterial activity with a 9.16 mm inhibition zone, 3.46% water absorption, and 15.33 cP viscosity. This formulation maintained microbial stability and organoleptic quality for up to 3 days at room temperature and 12 days under cold temperature.

Keyword: Coating; nanopartikel; innovation; responsible production

INTRODUCTION

Otak-otak ikan tenggiri is a distinctive fishery product from Sumatra, Indonesia, renowned for its unique flavor and ease of preparation (Sipahutar & Siregar, 2021). The processing of fish into this product not only enhances its economic value but also diversifies the range of fishery offerings available for consumers (Astuti et al., 2023). According to the Indonesian National Standard (SNI) (2022), it must contain at least 30% fish meat, combined with flour, and shaped into flat or oval pieces with a chewy texture. While rich in protein and moisture, the high water content makes it susceptible to spoilage during storage. Padli (2015), indicates that it has a shelf life of only two days at room temperature. One effective technology for reducing product damage is using edible coatings to inhibit bacterial growth in otak-otak ikan tenggiri. As a popular processed fishery product, this item presents a valuable opportunity for the application of innovative technologies like edible coatings. These coatings can help maintain product stability during storage, enhancing its appeal to consumers (Narto, 2020). Currently, the use of edible coatings as food packaging technology has been widely practiced in preserving food (Farsanipour et al., 2020). However, these edible coatings typically possess a fragile structure and exhibit suboptimal antibacterial activity, making them insufficient to effectively prevent product spoilage.

The limited effectiveness of commercial edible coatings often stems from their composition, which usually consists of a single polymer. Zhao et al. (2022) noted that coatings made from a single material demonstrate unsatisfactory physical performance and thermal stability. Therefore, it is essential to combine polymeric materials to develop superior and multifunctional edible coatings. Polysaccharide materials, such as tapioca, are biopolymers that can serve as edible coating materials due to their affordability as a food source (Nair et al., 2023). However, the coating properties of starch alone are insufficient to maintain the moisture content of packaged food. Modifying tapioca can enhance its performance (Subroto et al., 2021).

Despite this, polysaccharides are water-soluble, leading to high water vapor permeability in polysaccharide-based edible coatings. Some studies have found that combining polysaccharides with proteins can effectively reduce water vapor permeability (Utama et al., 2022). Whey protein, including whey protein isolate and whey protein concentrate, is a by-product of cheese processing and is widely used as an edible coating material (Ananey-Obiri et al., 2018). Whey protein can protect food products from gases and aroma compounds (Alizadeh-Sani et al., 2018). However, its poor mechanical properties and high moisture permeability limit its application (Feng et al., 2018). Blending starch with proteins is known to improve the mechanical properties of films due to their complex structure and binding capacity (Izzi et al., 2023).

The incorporation of various fillers in edible coatings is recommended in the literature to enhance their characteristics. Chitosan, a widely used polysaccharide, is valued for its biodegradable and biocompatible properties (Hartawan et al., 2023) and exhibits excellent bacteriostatic properties (Xiao et al., 2021). Nanoscale modifications, specifically chitosan nanoparticles (CSNPs), demonstrate a greater ability to inhibit microbial growth compared to pure chitosan (Elahi et al., 2024).

However, their antibacterial activity is somewhat limited due to low polarity, which causes slow diffusion (Hosseini et al., 2022). To address these limitations, edible coatings can be modified by combining different materials to create bio-nanocomposite food coatings, where the distribution of nanoparticles within the polymer matrix can significantly improve mechanical properties, barrier function, and antimicrobial capabilities (Moulia, 2018). The use of such coatings in food packaging is believed to inhibit the decay of otak-otak ikan tenggiri products by suppressing microbial growth (Nagarajan et al., 2021).

Currently, no research has combined modified tapioca and whey protein biopolymers with nanosized fillers to create a coating for otak-otak ikan tenggiri. This study aims to investigate the effects of combining edible coatings with whey protein formulations and chitosan nanoparticles on antimicrobial activity, water absorption, and coating viscosity, as well as to determine the impact of the optimal formulation on bacterial growth and the organoleptic properties of otak-otak ikan tenggiri during storage.

MATERIALS AND METHODS

This research was conducted from January to August 2024 at, the Faculty of Fisheries and Marine Sciences Laboratory, Airlangga University. The equipment used during the research were magnetic stirrer, hotplate stirrer (Thermo Scientific Cimarec), freezer, particle size analyzer (PSA, BIOBASE BK-802N), digital scale (OHAUS), Erlenmeyer, Petri dish, incubator caliper, chopper, knife, spoon, basin micropipette, pot, par, spatula, iron tray, silicone mold and others.

The materials used in this study are chitosan whey protein isolate (WPI, Puro protein 97%), whey protein concentrates (WPC, ORIANA protein 80%) modified tapioca (Siam Modified Starch), acetic acid, tripoliphosphate (TPP), NaOH, glycerol, distilled water, *Escherichia coli* strain provided by the institute of life, sciences, engineering and technology/ (LIHTR) Universitas Airlangga. Moreover, tenggiri fish (*Scomberomorus commerson*) from the marketplace, eggs, shallots, garlic, pepper, sugar, salt, broth powder, pepper, tapioca starch, spring onions, NaCl, oxoid, *butterfield'sphosphate buffered*.

Research Design

Stage 1. Characterization of edible coating.

This stage uses a completely randomized design (CRD) with different edible coating (P) formulation treatments consisting of 5 treatments and 4 replicates. The treatment of edible coating material formulations can be seen in Table 1 below.

Table 1. Edible coating formulation treatment

No.	Material	Composition				
		P1	P2	P3	P4	P5
1.	CSNP solution (ml)	Commercial edible coating (control)	-	-	10	10
2.	*Modified tapioca* (g)		4	4	4	4
3.	WPI (g)		1	-	1	-
4.	WPC (g)		-	1		1
5.	Gliserol (ml)		2	2	2	2
	Distilled water + Final volume (ml)	±100	±100	±100	±100	±100

Stage 2. Application Edible Coating on Otak-Otak Tenggiri

This stage is carried out with edible coating (C) against the length of storage (H) of otak-otak ikan tenggiri products using a completely randomized design (CRD) two factorial 3×6 three replications.

Factor I coating treatment;
CO: without coating (control)
C1: commercial coating
C2: coating with the best treatment in Stage 1
The second factor is storage duration; Room temperature (27 ˚C) = Days 0, 3, and 6 Cold temperature (5 ˚C) = Days 6,12, and 18

Research Implementation

Preparation of Chitosan Nanoparticles

To prepare the chitosan nanoparticle (CSNP) solution, 210 g of chitosan was dissolved in 70 ml of 4.5% acetic acid and stirred until homogeneous at room temperature. Once homogeneous, 29 ml of a 1.2% sodium tripolyphosphate (STPP) solution was added at a rate of 1 ml per minute. The mixture was then stirred using a magnetic stirrer at 1000 rpm for 3 hours (Wang et al., 2023a). Following this, the CSNP solution was analyzed using a particle size analyzer (PSA) with the BIOBASE BR 802N model (Amin *et al.*, 2019).

Preparation Edible Coating

The edible coating was prepared by dissolving 4 g modified tapioca in 93 ml of distilled water for 35 minutes at 160 °C and 550 rpm. Next, 2 ml of glycerol and lg of WPI or WPC were added according to the treatment and stirred for 30 minutes at 90 °C and 1000 rpm (Izzi *et al.*, 2023). In the treatment with the addition of CSNP, after the first stirring, 10 ml of CSNP was added (Shen *et al.*, 2022), and then stirred for 30 minutes at 1000 rpm.

Antibacterial Activity Testing

Antibacterial Activity Testing Bacterial activity testing was carried out using the disc diffusion method using the gram-negative

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bacterial strain *Escherichia coli*. Mueller Hinton agar (MHA) media on a petri dish that contained bacteria made two holes with a diameter of 5 mm in each plate, and then 30 µl of coating suspension was dripped into the sterile blank disc. The plates were incubated and examined and the clear area around the holes (zone of inhibition) (Algarni et al., 2022).

Water Absorption Testing

Water absorption testing was carried out with 2×2 cm film prepared and weighed until it reached a constant weight for 4 days. Calculation of water absorption (Moulia, 2018).

$$Water\ absorption = \frac{W2 - W1}{W1} \times 100$$

Note:
W_1: Initial sample weight (g)
W_2: Final sample weight (g)

Viscosity Testing of Solution

Testing the viscosity of the solution using a Brookfield viscometer by preparing a test solution, then the spindle is inserted into the solution. The viscometer is set at 60 rpm. The viscosity measurement results are then recorded and analyzed (Rofikoh et al., 2021).

Making Products

The making of otak-otak ikan tenggiri products in this study refers to the formulation of Giovani et al. (2023) with several modifications. The initial stage of the process of making otak-otak ikan tenggiri products is done by preparing cleaned tenggiri fish meat mixed with other ingredients such as shallots, garlic, salt sugar, eggs, and tapioca flour. The dough is stirred until well mixed and forms an oval. The molded dough is boiled until the otak-otak ikan tenggiri products float. The cooked otak-otak ikan tenggiri products are then drained and cooled.

Coating Products

Coating application was done by immersion method for 3 minutes and drained to dry, except for the control treatment (Hosseini *et al.*, 2016). Otak-otak ikan tenggiri products were then stored at room temperature storage (27 °C) and observations were made on days 0, 3, and 6. Meanwhile, otak-otak ikan tenggiri products stored at cold temperatures (5 ºC) were observed on days 0, 6, 12, and 18.

Total Microbial Testing

A total of 25 g of sample was weighed and put into 225 ml of Butterfield's phosphate buffered solution, then homogenized to obtain dilution 10^{-1}. From this homogenate, 1 ml was taken and diluted in 9 ml of diluent solution to reach dilution 10^{-2}, and so on up to 10^{-5}. Next, 1 ml of each dilution 10^{-1}, 10^{-3}, and 10^{-5} was pipetted into a sterile petri dish and 12-15 ml of PCA was added, then mixed until homogeneous. The dishes were then incubated upside down for 48 hours at 35 °C. After incubation, colony growth was observed and recorded (BSN, 2015). The formula for calculating total bacteria (BSN, 2015) includes:

$$N = \frac{\Sigma C}{[(1 \times n1) + (0{,}1 \times n2] \times (d)}$$

Note:
ΣC : Total number of colonies counted
N : Number of colonies per ml/g
n1 : Number of first dilution cup
n2 : Number of second dilution cup
d : The dilution rate obtained from the first cup is calculated

Organoleptic Testing

Organoleptic testing was carried out by 6 trained people using a scoresheet with a 5-9 test scale, the sensory test parameters icluded appearance, odor, taste, and texture of otak-otak ikan tenggiri products (BSN, 2022).

Data Analysis

Data on antibacterial activity, water absorption, and viscosity in stage 1 research was analyzed using one-way ANOVA a = 5%. If the results were different, Duncan's further test was conducted. TPC data in stage 2 research was analyzed using two-way ANOVA univariate a = 5%. If the results obtained are different, the Duncan test is continued. The organoleptic data in stage 2 were analyzed using Kruskal Wallis and if the results were significantly different, the Mann-Whitney test was continued.

RESULT AND DISCUSSION

This research begins with particle measurement at CSNP and then characterizes the resulting edible coating on the parameters of antibacterial actīvity, water absorption, and viscosity. Testing the application of edible coatings on otak-otak ikan tenggiri products on shelf life includes TVN and organoleptic testing appearance, odor, taste, and texture) of otak-otak ikan tenggiri products.

Particle Size of CSNP

Measurement of CSNP particles was carried out using a PSA (BIOBASE BK-802N). Based on the results of ANOVA statıstical analysis (Table 2) on the measurement of CSNP particles, it shows that different lengths of stirring have a significant effect on CSNP particle size (P<0.05).

Table 2. Particle size CSNP

CSNP	Stirring Time	Particle Size (nm)
CSNP1	1 hour	$18.04^{c} \pm 1.31$
CSNP2	2 hours	$14.08^{b} \pm 1.53$
CSNP3	3 hours	$10.52^{a} \pm 1.26$

Continued on the next column

Note: different letter notations indicate differences in CSNP particle size have a significant effect (P<0.05).

Duncan's test results showed that the smallest particle size resulted from a 3-hour stiring period of 10.52 nm. The largest particle size resulted from 1 1-hour stirring time of 18.04 nm. Based on research by Nwankwo *et al*. (2023), the size of CSNP ranges from 1-100 nm. According to Wang (2023a), the smaller size of CSNPs in edible coatings provides a larger surface area for nanoparticle contact with the substrate thus increasing 1ts ability. The chitosan nanoparticles selected for use in this study are CSNPs with a stirring time of 3 hours (CSNP3) because they have a lower average particle size. Wang *et al*. (2023b) stated that CSNP chitosan with a longer stirring process will increase the electrostatic force from the interaction between chitosan ammonium groups and STPP phosphate groups so that more particles are split into nano-sized particles (Ayumi *et al*., 2018).

Characteristics of Edible Coating

Edible coatings made in this study use a combination formulation of different ingredients so that the characterization of edible coatings is carried out to obtain a formulation that produces the best edible coating properties. The results of testing the characteristics of edible coatings in this study obtained edible coating characteristics with a range of antibacterial activity of 8.20-9.16 mm, water absorption of 3.46-7.14%, and viscosity of 15.33-32.18 cP (Table 1).

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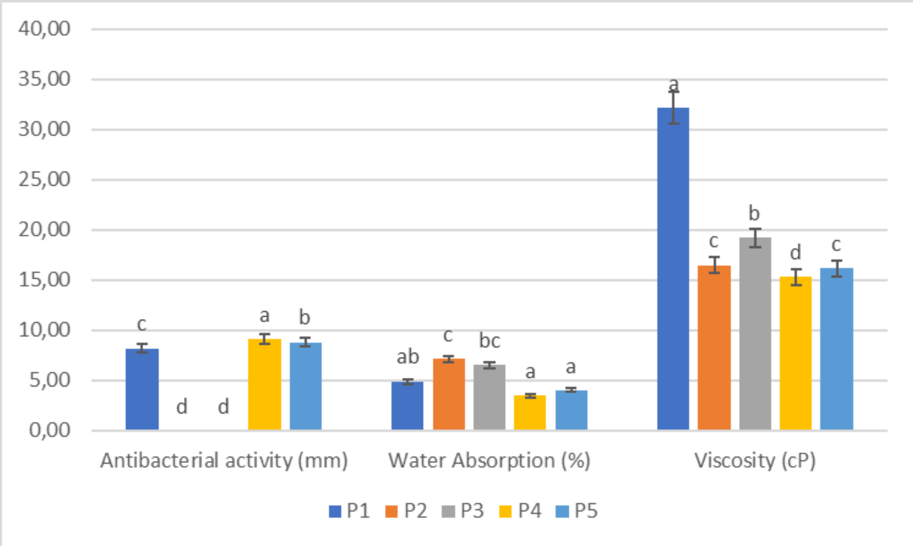

Figure 1. The result of edible coating characteristics
Note: Different letter notations indicate significant differences in Duncan test ($P<0.05$). P1 = commercial EC (control), P2 = EC modified tapioca-WPI, P3 = EC modified tapioca-WPC, P4 = EC modified tapioca-WPI and CSNP, P5 = EC modified tapioca-WPC and CSNP.

Based on the results of ANOVA analysis show that different edible coating material formulations have a significant effect on antibacterial activity, water absorption, and viscosity of edible coatings ($P<0.05$) (Figure 1). Duncan's test results showed the highest antibacterial activity was found in the edible coating formulation of modified tapioca-WPI and CSNP (P4) with a bacterial inhibition zone value of 9.16 mm. This inhibition zone value is higher than the results of Shojaei *et al.* (2019) who used hydroxypropyl methyl cellulose-WPI bio nanocomposite film with CSNP with an antibacterial activity value of 6-8 mm. The lowest antibacterial activity in this study belongs to edible coating formulations of modified tapioca-WPI (P2) and modified tapioca-WPC (P3) of 0 mm.

The results of Duncan's test showed that edible coating with modified tapioca-WPI and CSNP formulation (P4) had the lowest absorption value of 3.46%, while the highest water absorption value was owned by edible coating with modified tapioca-WPI formulation (P2) of 7.13%. The results of the Duncan test on viscosity testing resulted in commercial edible coating data having the highest viscosity value of 32.18 cP, while the lowest viscosity value was owned by edible coatings with modified tapioca-WPI formulation adding CSNP (P4) of 15.33 cP. Edible coatings made from whey protein, both WPI and WPC, did not show any antibacterial activity against *E. coli*. Brink *et al.* (2019) also reported that pure whey protein films did not show any antimicrobial activity.

The addition of CSNP in edible coating formulations can increase antibacterial activity. According to Pan *et al.* (2019), chitosan as an antibacterial work electrostatically, where the positive charge of protonated chitosan amino groups will bind to negatively charged molecules on the surface of bacterial cells, the bond causes leakage of intracellular cell walls resulting in cell death due to permeabilization of damaged cell surfaces. Chitosan that has penetrated the cell membrane will also be absorbed into deoxyribonucleic acid (DNA) molecules thus blocking ribonucleic acid (RNA) and DNA transcription in bacteria (Wu *et al.*, 2017).

Whey protein isolate (WPI) material is a good carrier of antimicrobial substances, although it does not have antibacterial activity (Farsanipour *et al*., 2020). The combination of WPI with CSNP can increase the ability of antibacterial activity in edible coatings because WPI helps distribute CSNP evenly throughout the surface of the edible coating thus providing optimal protection to otak-otak ikan tenggiri products (Zhou *et al*., 2022). The WPI matrix also plays a role in protecting CSNP to slow down its release and provide a longer antimicrobial effect (Owonubi *et al.,* 2018).

Edible coatings with modified tapioca-WPI formulation with CSNP (P4) and modified tapioca-WPC with CSNP (P5) have the lowest water absorption value. The water absorption value of this study is known to be lower than the research of Maghfirah *et al.* 2023) which ranges from 15.38-36.36%. Whey protein contains lysozyme which has hydrophobic amino acid side chains. The chitosan has a high water barrier ability because it is hydrophobic (Moulia, 2018). Combining the two materials can reduce water vapor permeability due to increasing the compact structure of chitosan by lysozyme (Ulbin-Figlewicz *et al*., 2014). The strong chemical interaction of WP-CSNP interaction can produce a denser and more water-resistant structure that reduces water absorption. This happens because CSNP can fill the space between protein molecules (Kumar *et al*., 2020).

Commercial edible coating (P1) has the highest viscosity value among other treatments. This is due to the main ingredient of commercial edible coating is chitosan. A higher concentration of chitosan can cause the edible coating solution to be thicker (Wati *et al*., 2023). The modified tapioca-WPI edible coating formulation with CSNP (P4) and modified tapioca-IPC with CSNP (PS) have the lowest viscosity values, but the viscosity values in all edible coating formulations meet the edible coating viscosity standard of at least eP (Vatria *et al*., 2021).

The use of CSNP can reduce viscosity at high concentrations. This happens because chitosan molecules combined with TPP turn into denser particles whose hydrodynamic volume is smaller than chitosan chains (Maryam *et al*., 2018). Chitosan nanoparticles (CSNP) with a small amount of free chitosan chains will increase the cross-linked chains resulting mn decrease in the total hydrodynamic volume of chitosan. Small hydrodynamic volume is more efficient in movement compared to larger particles Selection of the best edible coatine formulation can be done through weighting given to the criteria of antibacterial activity water absorption, and viscositv (Moulia, 2018). Based on the weighting of edible coating Characteristics, the moalfied tapioca-WPI and CSNP (P4) edible coating formulation produces the best edible coating.

Total Plate Count (TPC) Value of Otak-Otak Ikan Tenggiri Products

The calculation of TPC in this study was carried out based on SNI 2332.3-2015. The test results of the TPC value of otak-otak ikan tenggiri products with different coating during storage at room temperature and cold temperature can be seen in Table 3. The results of ANOVA analysis showed that the coating of modified tapioca-WPI and CNSP had a different effect on bacterial growth with different temperatures and lengths of storage time ($P<0.05$).

Table 3. TPC value of otak-otak ikan tenggiri products

Treatment	TPC (log CFU/g)							
	Room Temperature (27°C)			Cold Temperature (5°C)				Standard (Moulia et al., 2019)
	H0	H3	H6a	H0	H6b	H12	H18	
C0	$1.80^{c} \pm 0.08$	$5.31^{i} \pm 0.01$	$7.27^{n} \pm 0.02$	$1.80^{c} \pm 0.08$	$4.23^{f} \pm 0.02$	$5.82^{j} \pm 0.03$	$7.11^{m} \pm 0.01$	
C1	$1.67^{b} \pm 0.06$	$5.06^{h} \pm 0.01$	$7.27^{n} \pm 0.01$	$1.67^{b} \pm 0.06$	$3.90^{e} \pm 0.03$	$4.74^{g} \pm 0.04$	$6.29^{k} \pm 0.01$	5.69
C2	$1.49^{a} \pm 0.20$	$4.72^{g} \pm 0.01$	$6.54^{l} \pm 0.09$	$1.49^{a} \pm 0.20$	$2.96^{d} \pm 0.02$	$4.15^{f} \pm 0.02$	$5.74^{j} \pm 0.04$	

Note: Different letter notations in columns indicate significant differences in the Duncan test (P<0.05). P1 = commercial EC (control), P2 = EC modified tapioca-WPI, P3 = EC modified tapioca-WPC, P4 = EC modified tapioca-WPI and CSNP, P5 = EC modified tapioca-WPC and CSNP.

Duncan's test results showed that the lowest TPC value was found in otak-otak ikan tenggiri products with modified tapioca-WPI and CSNP coating (C2) at 1.49 log CFU/g. This shows that the coating of modified tapioca-WPI and CSNP is better able to inhibit bacterial activity than commercial edible coatings and without coating on the storage of otak-otak ikan tenggiri products. Chitosan is a biopolymer material that has antimicrobial activity, modification of chitosan into nanoparticle form can increase the antibacterial activity of chitosan (Hu *et al*., 2020), through increasing surface area, so it will increase the number of chitosan interactions with negative charges on the surface of bacterial cells. Chitosan nanoparticles have a good dispersion rate in solution, making it easier to penetrate the bacterial cell wall. This can disrupt bacterial cell permeability which can lead to bacterial cell death (Melo *et al*., 2018). In addition, the incorporation of WPI and CSNP results in a network structure that fills empty spaces in the polymer matrix. Edible coatings from these blends also form structures that restrict the movement of polymer chains, thus making the network more stable and reducing oxygen transfer (Muley *et al*., 2020). The lack of oxygen availability in food products coated with edible coatings causes bacterial growth to be inhibited (Farsanipour *et al*., 2020).

Sensory Analysis Score of Otak-Otak Ikan Tenggiri Products

The sensory test of this study used 6 trained panelists who gave scores through a test form with a scale of 5 to 9 according to the specifications contained in the sensor assessment sheet SNI (2022). The parameters studied in this study include the appearance, odor, taste, and texture of otak-otak ikan tenggiri products.

Appearance

Based on the Kruskal Wallis analysis, it shows that the application of different coatings has a significant effect on the appearance value with different temperatures and lengths of storage time (P<0.05) (Table 4).

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Table 4. Sensory analysis value of the appearance of otak-otak ikan tenggiri products

Treatment	Appearance							Standard (BSN, 2022)
	Room Temperature (27°C)			Cold Temperature (5°C)				
	H0	H3	H6	H0	H6	H12	H18	
C0	8.0^{Ba} ± 1.10	6.7^{Bb} ± 0.82	5.0^{Bd} ± 0.00	8.0^{Ba} ± 1.10	7.3^{Bb} ± 0.82	7.0^{Bb} ± 0.00	6.0^{Bc} ± 1.10	
C1	8.7^{Aa} ± 0.82	7.3^{Ab} ± 0.82	5.3^{Ad} ± 0.82	8.7^{Aa} ± 0.82	7.7^{Ab} ± 1.03	7.3^{Ab} ± 0.82	6.7^{Ac} ± 0.82	7
C2	8.7^{Aa} ± 0.82	7.7^{Ab} ± 1.03	5.3^{Ad} ± 0.82	8.7^{Aa} ± 0.82	8.3^{Ab} ± 1.10	7.7^{Ab} ± 1.03	7.0^{Ac} ± 0.00	

Note: Capital letter difference (A-C) in the same column indicates a significant difference ($p<0.05$) in coating treatment. Lowercase difference (a-d) in the same row indicates significant differences ($p<0.05$) in storage duration. C = treatment coating; C0 = No coating (kontrol), C1 = Commercial coating, C2 = Best EC coating stage 1 (EC modified tapioca-WPI and CSNP), dan H = Days; H0 = Day 0, H3 = Day 3, H6 = Day 6, H12 = Day 12, H18 = Day 18.

Mann-Whitney further test showed that the highest average appearance value at the end of cold temperature storage time (5 ºC) was found in otak-otak ikan tenggiri products with modified tapioca-WPI and CSNP coatings (C2). The lowest appearance value was found in otak-otak ikan tenggiri products without coating (C0) stored at room temperature (27 °C) on the sixth day with an appearance value of 6.68 and those stored at cold temperature (5 °C) on the 18th day with an appearance value of 6.0. Otak-otak ikan tenggiri products with coating treatments Cl and C2 showed the highest appearance value on the first day of storage (Table 4). This happens because the edible coating makes the product look shiny, thus increasing the acceptability of the product. The difference in appearance value between treatments of otak-otak ikan tenggiri products along with the length of storage time is due to color changes that tend to darken in products that are not coated with an edible coating (C0). Discoloration is related to the process of fat oxidation due to the interaction between oxygen and the product during storage (Venkatachalam & Lekjing, 2020). In addition, the process of protein decomposition that occurs during storage can cause the brightness of the product to decrease rapidly in products without edible coating (Yani *et al*., 2022). The treatment of samples coated with edible coatings from modified tapioca-WPI and CSNP had a slow decrease in appearance value during storage. This is due to the ability of edible coatings made from CSNP to cover the surface of the product to the pores, thereby reducing the rate of oxygen and carbon dioxide respiration optimally (Safitri *et al*., 2021). This reduction in gas respiration helps slow down product discoloration during storage (Alves *et al*., 2018).

Odor

Based on Kruskal Wallis analysis, it shows that different coatings dont have a significant effect on the value of odor stored with different temperatures and lengths of storage time ($P>0.05$) (Table 5).

Table 5. Sensory analysis value of the odor of otak-otak ikan tenggiri products

Treatment	Odor							Standard (BSN, 2022)
	Room Temperature (27°C)			Cold Temperature (5°C)				
	H0	H3	H6	H0	H6	H12	H18	
C0	$8.7^{Ba} \pm 0.82$	$6.0^{Bc} \pm 1.10$	$5.0^{Bd} \pm 0.00$	$8.7^{Ba} \pm 0.82$	$7.0^{Bb} \pm 0.00$	$6.3^{Bc} \pm 1.03$	$6.0^{Bc} \pm 1.10$	7
C1	$8.3^{ABa} \pm 1.03$	$7.0^{ABc} \pm 0.00$	$5.0^{ABd} \pm 0.00$	$8.3^{ABa} \pm 1.03$	$7.7^{ABb} \pm 1.03$	$6.7^{ABc} \pm 0.82$	$6.3^{ABc} \pm 1.03$	
C2	$8.0^{Aa} \pm 1.10$	$7.3^{Ac} \pm 0.82$	$5.7^{Ad} \pm 1.03$	$8.0^{Aa} \pm 1.10$	$7.7^{Ab} \pm 1.03$	$7.0^{Ac} \pm 0.00$	$6.7^{Ac} \pm 0.82$	

Note: Capital letter difference (A-C) in the same column indicates a significant difference ($p<0.05$) in coating treatment. Lowercase difference (a-d) in the same row indicates significant differences ($p<0.05$) in storage duration. C = treatment coating; C0 = No coating (kontrol), C1 = Commercial coating, C2 = Best EC coating stage 1 (EC modified tapioca-WPI and CSNP), dan H = Days; H0 = Day 0, H3 = Day 3, H6 = Day 6, H12 = Day 12, H18 = Day 18.

Mann-Whitney further test showed that there was an effect of storage time on the odor value of otak-otak ikan tenggiri products. The C2 otak-otak ikan tenggiri products on the first day had the lowest average odor value of 8.0. This occurred due to the use of acetic acid to dissolve modified tapioca-WPI and CSNP in the formation of edible coatings so that the otak-otak ikan tenggiri products had a slightly sour odor. The long storage time of otak-otak ikan tenggiri products with C2 coating has a lower odor value. This is because the C2 treatment otak-otak ikan tenggiri products have low bacterial activity so the process of protein and fat decomposition in otak-otak ikan tenggiri products has decreased. The process of protein decomposition produces simple compounds such as amines, ammonia peroxides, and free fatty acids known to produce unpleasant odors in products (Abdel-Naeem *et al*, 2021). The fat oxidation process produces volatile compounds (aldehydes and ketones) and causes product rancidity during storage (Yani *et al.*, 2022). The modified tapioca-WPI and CSNP edible coatings have a compact structure with improved mechanical resistance and low gas permeability properties that prevent the absorption of odors from the environment (Kurek *et al.*, 2014).

Taste

Based on Kruskal Wallis analysis it was shown that different coatings had significant effect on the taste value with different temperature and storage time ($P<0.05$) (Table 6).

Table 6. Sensory analysis value of the taste of otak-otak ikan tenggiri products

Treatment	Taste							Standard (BSN, 2022)
	Room Temperature (27°C)			Cold Temperature (5°C)				
	H0	H3	H6	H0	H6	H12	H18	
C0	$8.3^{Ba} \pm 1.03$	$6.0^{Bb} \pm 1.10$	$5.0^{Bc} \pm 0.00$	$8.3^{Ba} \pm 1.03$	$7.7^{Ba} \pm 1.03$	$7.0^{Bb} \pm 0.00$	$5.0^{Bc} \pm 0.00$	7
C1	$8.3^{Aa} \pm 1.03$	$7.0^{Ab} \pm 0.00$	$5.7^{Ac} \pm 1.03$	$8.3^{Aa} \pm 1.03$	$8.0^{Aa} \pm 1.10$	$7.0^{Ab} \pm 0.00$	$5.7^{Ac} \pm 1.03$	

Continued on the next page

Treatment	Taste							Standard (BSN, 2022)
	Room Temperature (27°C)			Cold Temperature (5°C)				
C2	8.3^{Aa} ± 1.03	7.3^{Ab} ± 0.82	5.7^{Ac} ± 1.03	8.3^{Aa} ± 1.03	8.0^{Aa} ± 1.10	7.7^{Ab} ± 1.03	7.0^{Ac} ± 0.00	

Note: Capital letter difference (A-C) in the same column indicates a significant difference (p<0.05) in coating treatment. Lowercase difference (a-d) in the same row indicates significant differences (p<0.05) in storage duration. C = treatment coating; C0 = No coating (kontrol), C1 = Commercial coating, C2 = Best EC coating stage 1 (EC modified tapioca-WPI and CSNP), dan H = Days; H0 = Day 0, H3 = Day 3, H6 = Day 6, H12 = Day 12, H18 = Day 18.

Mann-Whitney further test showed that the highest average taste value at the end of the cold temperature storage time was with a range of taste values from 8.3 to 5.7. Otak-otak ikan tenggiri products that are not coated with edible coatings (C0) have a taste value that does not meet SNI 2022 standards on day 3 of storage at room temperature (27 °C) of 6.0 and on day 18 at cold temperature (5 °C) of 5. Otak-otak ikan tenggiri products with modified tapioca-WPI and CSNP coating (C2) can maintain the taste of the product according to the standard until day 3 at room temperature storage of 7.3 and day 18 at cold temperature storage of 7. The taste value that is maintained until the end of storage is a savory taste at cold temperature storage, while the others have a change in taste to acid. This occurred because C2 products coated with edible coating modified tapioca-WPI and CSNP were able to inhibit fat oxidation and prevent the formation of volatile compounds (monoglycerides, glycerol, and free fatty acids) that can cause an unfavorable taste in the product (Namaskara *et al.*, 2018). In addition, CSNP has high antibacterial activity that inhibits the growth of bacteria that produce acidic compounds (free amino acids and lactic acid) from the protein degradation process that affect the taste of the product (Werdiyaningsih dan Kanetro, 2018).

Texture

Based on Kruskal Wallis analysis, it shows that different coatings do not have a significant effect on the value of texture stored with different temperatures and lengths of storage time (P>0.05) (Table 7).

Table 7. Sensory analysis value of the texture of otak-otak ikan tenggiri products

Treatment	Texture							Standard (BSN, 2022)
	Room Temperature (27°C)			Cold Temperature (5°C)				
	H0	H3	H6	H0	H6	H12	H18	
C0	8.7^{Ba} ± 0.82	6.7^{Bcd} ± 0.82	5.0^{Be} ± 0.00	8.7^{Ba} ± 0.82	7.7^{Bb} ± 1.03	7.0^{Bc} ± 0.00	6.0^{Bd} ± 1.10	
C1	8.7^{Aa} ± 0.82	6.7^{Acd} ± 0.82	6.3^{Ae} ± 1.03	8.7^{Aa} ± 0.82	8.0^{Ab} ± 1.10	7.3^{Ac} ± 0.82	6.7^{Ad} ± 0.82	7
C2	8.7^{Aa} ± 0.82	7.0^{Acd} ± 0.00	6.3^{Ae} ± 1.03	8.7^{Aa} ± 0.82	8.3^{Ab} ± 1.03	7.3^{Ac} ± 0.82	7.0^{Ad} ± 0.00	

Note: Capital letter difference (A-C) in the same column indicates a significant difference (p<0.05) in coating treatment. Lowercase difference (a-d) in the same row indicates significant differences (p<0.05) in storage duration. C = treatment coating; C0 = No coating (kontrol), C1 = Commercial coating, C2 = Best EC coating stage 1 (EC modified tapioca-WPI and CSNP), dan H = Days; H0 = Day 0, H3 = Day 3, H6 = Day 6, H12 = Day 12, H18 = Day 18.

Mann-Whitney further test showed that there was an effect of storage time on the texture value of otak-otak ikan tenggiri products. Changes in texture value (Table 7) show that otak-otak ikan tenggiri products with modified tapioca-WPI and CSNP coating (C2) can maintain product texture according to SNI standards (2022) until 3 days at room temperature storage (27 °C) and 18 days at cold temperature storage (5 °C). Otak-otak ikan tenggiri products with no coating (C0) showed a significant decrease in texture value on 3 days of storage at room temperature and 18 days of storage at cold temperature, with values below the standard SNI texture value (2022).

Otak-otak ikan tenggiri products that have been stored for three days in room temperature storage and 18 days in cold storage, are not liked by panelists because the texture is less compact, hard, and slimy because otak-otak ikan tenggiri products without edible coating has a rapid growth of microorganisms and protein degradation process (Kandasamy *et al*., 2021). The hardened texture of otak-otak ikan tenggiri products occurs due to the product losing water (Lasimpala *et al*., 2014). The process of protein denaturation by microorganisms causes changes in the secondary and tertiary structures of proteins that can reduce the ability of proteins to hold water so that the texture of the product becomes harder (Ramadhani & Murtini, 2017).

CONCLUSION

Based on the results of the study, it can be concluded that the combination of different edible coating formulations has a different effect on antibacterial activity, water absorption and viscosity of edible coatings with the best formulation using modified tapioca-WPI formulation treatment with CSNP (P4) producing antibacterial activity with an inhibition zone of 9.16 mm, water absorption value of 3.46%% and viscosity 15.33 cP. Edible coating combination of modified tapioca-WPI with CSNP gave a significantly different effect on microbial growth and organoleptic of otak-otak ikan tenggiri products. Edible coating formulation of modified tapioca-WPI with CSNP (C2) can maintain the total of microbes and organoleptic of otak-otak ikan tenggiri products that are still suitable for consumption until 3 days storage at room temperature and 12 days storage at cold temperature.

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AUTHOR'S CONTRIBUTION

The contributions of each author are as follows FRH: collected data drafted the manuscript and designed tables and diagrams. ASM and GM: designed the main conceptual ideas and made critical revisions to the article. All researchers discussed the results and contributed to the final manuscript

CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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