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Combination of Chitosan Nanoparticles and Cellulose Derivatives in the Preparation of Edible Coatings: Effects on Bacterial Activity and Organoleptic Properties of Tenggiri Fish Pempek.

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ABSTRACT

Pempek is a very popular traditional Indonesian food because it has a delicious taste. However, the shelf life of pempek is only about 16 hours at room temperature. This study aims to develop and evaluate edible coatings based on cellulose and chitosan nanoparticles (CSNPs) to extend the shelf life of pempek from Tenggiri fish (Scomberomorus commerson). This study is experimental research divided into two stages. The first stage involves characterizing the edible coating using a Completely Randomized Design (CRD), with tests including antimicrobial activity, water absorption, and viscosity, conducted with 5 treatments and 4 repetitions. The second stage involves applying the coating to pempek and evaluating its shelf life, focusing on TPC and organoleptic qualities using a two-factor CRD with 3×6 treatments and 3 repetitions. The results showed a significant effect ($P<0.05$) of various formulations on the characteristics of edible coatings. The results showed that the variation of cellulose derivatives and CSNP in the preparation of edible coating significantly affected the coating characteristics ($P<0.05$). The combined formulation of CMC and CSNP (P5) was selected as the pempek coating, with antibacterial activity of 9.31 mm, water absorption of 1.23%, and solution viscosity of 33.50 cPa.s. In the second stage, the treatment of pempek coating with CMC and CSNP (C2) effectively maintained the quality of pempek until day 3 at room temperature and day 12 at refrigerator temperature.

Keyword: Pempek; Coating; Chitosan Nanoparticles; Innovation

INTRODUCTION

Pempek is a popular processed fish in Indonesia and originated from Sumatra Island. Currently, pempek contributes to protein intake, especially in Indonesia because of its high protein content of around 6.47% and its relatively cheap price (Afriani *et al.,* 2015). However, pempek products have a limited shelf life and distribution, which only lasts about 16 hours at room temperature, making this product vulnerable to microorganisms that cause spoilage (Pratama *et al.,* 2016). Currently, pempek is marketed outside the region by traditional sellers, who only coat their products with tapioca flour or cooking oil, and some cheaters will even add preservatives such as formalin and borax to extend the shelf life of the product (Falahudin *et al.,* 2016). On an industrial scale, synthetic food preservatives can cause allergic reactions and long-term health problems (Bialangi *et al.,* 2023). The demand for healthy and safe food with minimal use of synthetic preservatives continues to increase, encouraging the development of edible coating technology. Edible coatings are thin edible layers that protect food and control the transfer of water vapor, oxygen, and fat (Kamal *et al.,* 2017).

The constituent components of edible coatings

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can be hydrocolloids, fats, and composites (Wang *et al.,* 2023a). The use of cellulose-based materials that are part of the hydrocolloid group as raw materials for edible coatings/films has been widely researched and applied. Cellulose commonly used as coating/films are cellulose derivatives including hydroxypropyl cellulose (HPC), hydroxypropyl methylcellulose (HPMC), methylcellulose (MC), and carboxymethylcellulose (CMC). Films/coatings made from cellulose and its derivatives have the potential to be used as food coatings because they have characteristics that are transparent, flexible, odorless and tasteless, resistant to oil and fat, and have moderate resistance to water vapor and oxygen diffusion (Suyadri *et al.,* 2020).

Although its resistance to water vapor and gas diffusion is better than starch-based materials, these characteristics still need to be optimized. Low coating/film stability can shorten shelf life because water vapor and microbes entering through the film will damage food ingredients (Menezes and Athmaselvi, 2018). To improve the physical and functional characteristics of cellulose-based coatings, the addition of biopolymers or other materials such as hydrophobic materials and/or those with antimicrobial properties is required.

Improving the characteristics of cellulose-based coatings can be achieved by adding chitosan nanoparticles (CSNP) to form nanocomposites. Nanocomposites are materials containing a polymer matrix and nanofillers (≤100 nm) that can improve the functional properties, morphology, and stability of the polymer matrix as a film (Moulia, 2018). Chitosan converted into nanoparticles can increase its specific surface area which allows better distribution and penetration into the polymer matrix thus more effectively inhibiting the passage of permeates such as O2, CO2, and water vapor (Algarni *et al.,* 2022).

In addition, chitosan nanoparticles exhibit a higher antibacterial effect than chitosan solution due to the higher density of positively charged amino groups against the negatively charged bacterial surface so the interaction causes bacterial cell death (Perinelli *et al.* 2018). Chitosan nanoparticles are safe to use if the concentration is less than 180 mg/ml and can interact with various molecules through electrostatic interactions (Hartawan *et al.,* 2023). Chitosan nanoparticles are also able to increase the stability and effectiveness of coatings and extend shelf life (Chandrasekaran *et al.,* 2020).

The addition of chitosan nanoparticles so that it becomes a nanocomposite is not only able to improve the mechanical properties of the coating but also its function as an antibacterial agent that can protect food products from microbial contamination during storage (Perinelli *et al.* 2018). Therefore, this study aims to determine the formulation of a combination of chitosan nanoparticles and cellulose derivatives on the characteristics of edible coatings and to determine the effect of the best edible coating formulation on bacterial contamination and organoleptic quality of Tenggiri fish pempek during storage at room temperature and cold temperature.

MATERIALS AND METHODS

This research was conducted at the Education Laboratory of the Faculty of Fisheries and Marine Sciences, Universitas Airlangga from March to August 2024. The materials used in this study include Tenggiri fish (*Scomberomorus commerson*) with a size of 50-55 cm and a weight of 1-1.1 kg with an organoleptic value of 9 (SNI 2729: 2021), salt, tapioca flour (SNI 3451: 2011), flavoring, eggs, chitosan, acetic acid solution, sodium tripolyphosphate (STPP), Methyl cellulose (MC), Carboxymethyl cellulose (CMC), distilled water, sorbitol, commercial edible coating brand 'Chitasil', strains of gram-

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negative *Escherichia coli* bacteria provided by LIHTR laboratory of Universitas Airlangga, Nutrient Broth (NB) media, plate count agar (PCA), Muller Hinton Agar (MHA) media, and phosphate buffer. The equipment used in this study includes a pan, spatula, stove, food processor (Mitochiba), mixer (Han River), basin, knife, sieve, magnetic stirrer, hotplate (Thermo Scientific Cimarec), viscometer (Brookfield), pan, analytical scales (OHAUS), autoclave, ose, petri dish, erlenmeyer, incubator, blank disc, measuring flask, tray/cooling rack, plastic wrap, refrigerator, vortex, test tube, micropipette, organoleptic test form, stationery, tissue, oven, and particle size analyzer/PSA (model Biobase BK-802N).

Research methods

This research uses a laboratory experimental method which is divided into two stages. Stage 1. Preparation and characterization of edible coatings from cellulose derivatives and chitosan nanoparticles. The research at this stage used a completely randomized design (CRD) with five treatments and each treatment was repeated four times. The treatment of edible coating composition is presented in Table 1.

Table 1. Edible coating formulation composition

No	Material	Total composition				
		P1	P2	P3	P4	P5
1	CNSP solution (ml)	-	-	99	-	99
2	MC (g)	-	1	1	-	-
3	CMC (g)	-	-	-	1	1
4	Sorbitol (ml)	-	0,5	0,5	0,5	0,5
5	Aquadest (ml)	-	100	-	100	-
6	Coating commercial (ml)	100				

Research Implementation

Preparation and Measurement of Chitosan Nanoparticles.

Chitosan nanoparticles were prepared by ionic gelation method using tripolyphosphate anion based on the method of Wang *et al.* (2023b) with slight modification. Chitosan as much as 210 mg was dissolved in 70 ml of 4.5% acetic acid solution, then sodium tripolyphosphate (STPP) with a concentration of 1.2 mg/ml was added to 29 ml slowly at a rate of 1 ml/min while stirring at 1000 rpm. The duration of stirring was carried out according to different treatments, including 60, 120, and 180 minutes at 1500 rpm. After that, the nano chitosan particle suspension was measured using a Particle Size Analyzer/PSA (model Biobase BK-802N), and the average was calculated based on the results of three repetitions per sample.

Stage 1. Preparation and Characterisation of Edible Coating

Preparation of Edible Coating Composite

The preparation of the coating solution was based on Wang *et al.* (2023b) with slight modifications. First, 1 g of methylcellulose (MC) or carboxymethyl cellulose (CMC) powder was mixed with 100 ml of distilled water and stirred with a magnetic stirrer at 850 rpm at 50°C for 90 minutes until fully dissolved. After that, sorbitol was added as a plasticizer and chitosan nanoparticles.

Stage 2. Edible coating application of pempek storage

This study used two-factor CRD, where Factor I was coating treatment (C) and Factor II was storage duration (H), each treatment was repeated three times. Factor I coating treatment; C0: No coating (control), C1: Commercial coating (Chitasil), C2: Best formulation coating from Stage 1. Factor 2 storage duration treatment; Room temperature (25-27 °C): Day 0, 3, and 6 Cold temperature (5-10 °C): Day 6, 12, and 181). The edible coating solution was then stirred at the same speed at 50°C for 30 minutes (Wang *et al.,* 2023b).

Viscosity Analysis of Composite Solution

The viscosity of the coating solution was measured using a viscometer with spindle 2 at 50 rpm. Dial readings were recorded 5 minutes after the start to allow for equilibration (Moulia, 2018).

Water Absorbency Analysis

The water absorption test was carried out based on Moulia (2018) by converting the coating into a film first with an area of 2×2 cm. The conversion into a film was done by drying at 38-42°C for 48 hours. Then the test was carried out by leaving the film at room temperature (25-27°C) and weighing it until the weight was constant for 4 days. Calculation of water absorption was done based on the following equation.

$$\textit{Water absorption} = \frac{w2-w1}{w1} \times 100\%$$

Where:
W1 = Initial weight (grams)
W2 = Final weight (grams)

Antibacterial Activity Analysis

The antimicrobial test procedure used the disc diffusion method (Jenkins and Maddocks, 2019). First of all, gram-negative test bacteria *Escherichia coli* was cultured with NB media incubated for 24 hours at 37°C. Then as much as 100 µl of culture that has reached Optical Density 0.1 is applied to a Petri dish containing MHA media. Antimicrobial samples (code P1, P2, P3, P4, P5) as much as 30 µl were dripped on a sterile blank disc, and each petri dish used for one sample concentration was repeated four times. After that, the Petri dish was incubated for 24 hours at 37°C and then the diameter of the inhibition zone around the disc was measured using a caliper.

Stage 2. Application of Edible Coatings in the Preparation of Tenggiri Fish Pempek

Pempek preparation follows Pitayati (2021) with slight modifications. First, fresh Tenggiri fish is separated from the bones, offal, and skin, and the meat is ground using a food processor. Next, 1 kg of ground Tenggiri meat is mixed with 50 g of salt and flavoring, 120 ml of egg, and 480 ml of water using a mixer until the mixture becomes fluffy and not liquid. Once the fish and seasoning mixture has risen, 600 g of tapioca flour is gradually added until the mixture is homogeneous. The dough is then shaped by hand into a lenjer form, approximately 7 cm in length and 3 cm in diameter, and boiled for 15 minutes. After cooking, the pempek is drained and allowed to cool. The cooled pempek can then proceed to the coating stage.

***Application of Composite Edible Coating on* Pempek**

Coating of fish pempek refers to Wang *et al.* (2023b) which is done by dipping method for 60 seconds in the best formulation coating solution from stage 1 and commercial coating solution, then drained with a cooling tray.

TPC Analysis

Total Plate Count (TPC) testing in this study was carried out with the pour plate technique and calculated using the TPC calculation formula as follows (BSN, 2015a).

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

Description:
N = Number of sample colonies (col/ml)
$\sum C$ = Number of colonies counted on all cups
$n1$ = Number of cups in the first dilution counted
$n2$ = Number of cups in the second dilution counted d = First dilution counted

Organoleptic Analysis

Organoleptic analysis was conducted by 6 trained panelists using a rating scale of 1-9 according to the sensory assessment scoresheet on fishery products SNI 2346: 2015 (BSN, 2015b).

Data Analysis

Data on nanoparticle size, viscosity, water absorption, and antibacterial activity were analyzed using one-way ANOVA, while the TPC test employed two-way ANOVA. If significant differences were found, Duncan's post-hoc test was conducted at a significance level of α = 5%. Organoleptic data were analyzed using the Kruskal-Wallis test. All analyses were performed using IBM SPSS Statistics version 26.

RESULTS AND DISCUSSION

Preparation and Measurement of Chitosan Nanoparticles.

The nanoparticle chitosan (CSNP) particles prepared with different stirring times such as 60, 120, and 180 minutes, had mean nano chitosan particle sizes of 18.73, 15.71, and 11.69 nm, respectively (Table 2).

Table 2. Particle size of CSNPs Synthesized with Different Stirring Duration

Treatment	Stirring Time (minutes)	Particle Size (nm)
CSNP1	60	18.73 ± 0.18^{c}
CSNP2	120	15.71 ± 0.28^{b}
CSNP3	180	11.69 ± 0.25^{a}

Note: the data is the mean of three repetitions ± standard deviation. Different superscript indicates significantly different results ($p<0.05$).

The results of the statistical analysis using one-way ANOVA indicated that different stirring times had a significant effect ($p < 0.05$) on the size of the chitosan nanoparticles produced (Table 2). The treatment with a three-hour stirring time (CSNP3) resulted in the smallest nanoparticle size of 11.69 nm. Particle size influences material properties such as reactivity and mechanical strength. The particle size of nano chitosan prepared by the ionic gelation method is affected by the duration of the stirring process, as longer stirring times lead to an increased number of cross-links between chitosan and STPP, thereby enhancing the mechanical strength of the chitosan matrix (Ardean *et al.,* 2021). Smaller particle sizes can enhance performance in terms of interaction with the polymer matrix, improving the functional and barrier properties of edible coating nanocomposites against gas and water vapor (Wang *et al.,* 2023a).

Characterization of Edible Coating

The edible coatings prepared in this study had an average antibacterial activity value of 0-9.31 mm, a viscosity of 30.18 - 40.23 cPa.s, and a water absorption of 1.23- 4.83% (Table 3).

Table 3. Edible coating characterization analysis result

Treatment	Test Result± SD		
	Antibacterial Activity (mm)	Viscosity (cPa.s)	Water Absorbency
P1 (Control)	8.26 ± 0.17^{b}	40.23 ± 0.22^{a}	1.69 ± 0.36^{a}
P2 (MC)	0.00 ± 0.00^{c}	32.00 ± 2.10^{cd}	4.82 ± 0.80^{c}
P3 (MC+CSNP)	8.71 ± 0.52^{b}	30.18 ± 0.17^{d}	1.64 ± 0.08^{a}
P4 (CMC)	0.00 ± 0.00^{c}	35.35 ± 0.45^{b}	3.58 ± 0.39^{b}
P5 (CMC+CSNP)	9.31 ± 0.62^{a}	33.50 ± 2.38^{bc}	1.23 ± 0.08^{a}

Note: the data is the mean of four repetitions ± standard deviation. Different superscript indicates significantly different results ($p<0.05$).

The results of statistical analysis by ANOVA on the treatment of formulations P1, P2, P3, P4, and P5 showed a significant difference ($P<0.05$) in antibacterial activity, viscosity, and water absorption of edible coatings (Table 3). The results of Duncan's further test indicated that the highest antibacterial activity of edible coating was found in edible coating made with formulation P5 (CMC-CSNP) of 9.31 mm. The lowest antibacterial activity was found in edible coating formulations P2 and P4 at 0.00 mm or had no antibacterial activity at all. Antibacterial activity was measured based on the diameter of the edible coating inhibition zone in suppressing the growth rate of gram-negative bacteria *E. coli*.

The results of this study showed that antibacterial activity varied in various edible coating formulation treatments (Table 3). The treatment that has antibacterial activity is an edible coating formulation that contains chitosan in it. Chitosan has the potential to be used as an antibacterial material because it has aminopolysaccharide groups that can inhibit bacterial growth and has the efficiency of chitosan inhibition against bacteria (Wulandari *et al.,* 2015). Chitosan solution is bacteriostatic, which works by inhibiting bacterial cell metabolism so that bacterial growth is inhibited (Ardean *et al.,* 2021). The bacteria used for the test in this study is *E. coli* which is a gram-negative bacterium. The membrane of Gram-negative bacteria is a thin two- dimensional layer containing peptidoglycan thus creating a hydrophilic surface.

The cytoplasmic membrane consists of lipoproteins, lipopolysaccharides, and phospholipids. When the protonated amino groups of chitosan interact with negatively charged bacterial surfaces (such as carboxylate or phosphate residues), electrostatic binding can occur, especially in low molecular weight chitosan. This results in a decrease in cell permeability and osmotic stability of the bacterial wall so that chitosan enters the cell and disrupts physiological activity or causes leakage of bacterial nucleotides and enzymes (Ardean *et al.,* 2021). Edible coatings with the highest inhibition zone are formulations that use a mixture of chitosan nanoparticles (P3 and P5). Chitosan nanoparticles have better adsorption power than chitosan due to its specific surface and smaller size (Sivakami *et al.* 2013). Tovar *et al.* (2022) also explained that small chitosan nanoparticles can easily enter bacterial cells. The treatment without the addition of chitosan (P2 and P4) does not have antibacterial activity due to hydrogels consisting only of cellulose without additional antibacterial agents can't kill or inhibit bacterial growth (Bao *et al.,* 2022).

The Duncan test results on viscosity showed that the commercial edible coating (P1) had the highest viscosity value of 40.23 cPa.s, while the lowest viscosity value was found in the edible coating formulation of the combination of MC and CSNP (P3), which was 30.18 cPa.s. Viscosity is the flowability of the edible coating. Viscosity is the flowability of a solution. A higher viscosity value indicates that the solution is more concentrated, which indicates that the liquid resistance is higher (Naiu and Yusuf, 2024). The viscosity of edible coating added with chitosan nanoparticles (P3 and P5) is lower than the viscosity of chitosan solution (P1).

The addition of chitosan nanoparticles can reduce the total hydrodynamic volume of the solution (Tovar *et al.* 2022). Abdou *et al.* (2012) also explained that the viscosity of chitosan nanoparticles is lower than that of ordinary chitosan solution because chitosan is converted into chitosan nanoparticles through ionic gelation process, causing cross-linking between chitosan and tripolyphosphate (TPP) to form denser particles resulting in smaller hydrodynamic volume than pure chitosan chains. If the cross-linked chitosan molecules are more than the free chitosan chains, the total hydrodynamic volume of chitosan in solution will decrease, resulting in decreased viscosity. Viscosity measurement is necessary because it affects the quality of the coating produced. Edible coating solutions that are too dilute can produce poor coatings, while solutions that are too thick can produce coatings that are too thick (Chotimah *et al.,* 2022). The results of Duncan's further test on the ability of water absorption showed that the edible coating formulation of the combination of CMC and CSNP (P5) had the lowest water absorption value of 1.23% and the highest water absorption value was found in the edible coating formulation of MC (P2) which was 4.82% (Table 3.) The addition of chitosan can reduce the value of water absorption because chitosan is hydrophobic so that it is resistant to water absorption.

Stage 2. Edible Coating Application on *Tenggiri* Fish Pempek During Storage Total Plate Count (TPC) Analysis

The observation of the TPC value of *Tenggiri* fish pempek with the best coating treatment from Stage 1, namely CMC and CSNP combination formulation (C2), commercial coating (C1), and without coating (C0) stored at room temperature and cold in this study was 1.55 - 5.70 log CFU/g (Figure 1).

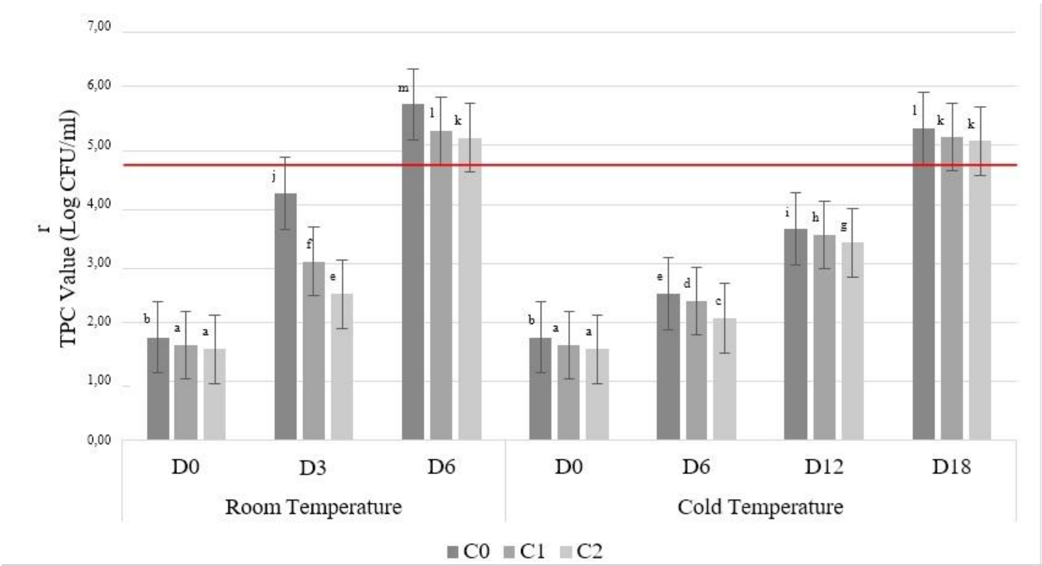

Figure 1. Diagram of TPC value of Tenggiri fish pempek

Notes: Different superscript indicates significantly different results in Duncan's further test ($p<0.05$). The red line on the horizontal axis shows the minimum limit of TPC value in pempek that has been required by SNI, which is at least 5 x 104 CFU/g or 4.7 Log CFU/g. Treatment: C = Coating treatment; C0 = non-coating (control), C1 = Commercial coating, C2 = Best coating from Stage 1 (CMC+CSNP), and H = Storage Day; H0 = Day 0, H3 Room = Day 3 at room temperature, H6 Room = Day 6 at room temperature, H6 Cold = Day 6 at cold temperature, H12 Cold = Day 12 at cold temperature, H18 Cold = Day 18 at cold temperature.

The results of TPC analysis on Tenggiri fish pempek with Two Way ANOVA showed a significant difference ($p<0.05$) in the amount of TPC of pempek based on the length of storage, both at room temperature and cold temperature. The TPC value of Tenggiri fish pempek increased as the storage time increased. The lowest TPC value was C2 treatment on day 0 of storage, which was 1.52 log CFU/g. Meanwhile, the highest TPC value was found in C0 on day 6 of storage at room temperature, which was 5.70 log CFU/g, and C0 on day 18 at cold temperature, which was 5.29 log CFU/g.c. The TPC value illustrates the amount of microbial contamination in food products. Microbial growth in food is affected by oxygen gas transfer during storage. Edible coatings can reduce the transfer of oxygen from the environment to the product so that the growth of aerobic bacteria can be suppressed. Utami's research (2017) reported that edible coatings were able to inhibit the entry of O_2 and water vapor in kurisi fish sausage so that microbial growth was inhibited. The maximum limit of TPC count required by SNI 7661.1:2019 is 5 x 104 CFU/g (BSN, 2019). The use of nanocomposite coating on *Tenggiri* fish pempek (C2) proved to be more effective in suppressing microbial contamination compared to no coating (C0) and better than commercial coating (C1) (Figure 1). TPC values are influenced by external factors such as storage conditions and product handling. In Tenggiri fish pempek, starch from tapioca flour is one of the main components of a potential sugar source for microbes to grow (Hanifah & Kanetra, 2018). Microbial activity is influenced by storage conditions, where the longer the storage, the more the number of bacteria. Rahmi *et al.* (2018) also explained that the increase in the number of bacterial colonies was related to temperature during storage. Room temperature of 25-30°C favors microbial growth, as evidenced by pempek stored at this temperature becoming unfit for consumption on day 6, while pempek stored at cold temperatures can survive until day 18. However, the coated pempek found in this study proved effective in reducing microbial contamination at both room and cold temperatures due to chitosan nanoparticles that have antibacterial activity. Chitosan is bacteriostatic by inhibiting bacterial cell metabolism, thus inhibiting bacterial growth (Ardean *et al.,* 2021).

Organoleptic Analysis

Organoleptic or sensory testing has an important meaning in food products because involves 6 trained panelists. Sensory testing was carried out using a scoresheet that refers to SNI 2346:2015 concerning sensory assessment of fishery products which includes the parameters of appearance, odour, taste, and texture of pempek.

Appearance

The observation results of the appearance value of Tenggiri fish pempek with coating treatment of CMC and CSNP combination formulation (C2), commercial coating (C1), and without coating (C0) stored at room and cold temperature in this study. The results of the Kruskall-Wallis analysis showed that the appearance of pempek was significantly different ($P<0.05$) between storage days but not significantly different ($P>0.05$) in the coating treatment. The Mann- Whitney further test showed that the highest appearance value was found in the treatment without coating (C0) and with the treatment of CMC+CSNP combination formulation (C2) on the 0th day of storage, which was 8.33. The lowest appearance value was found in the treatment without coating (C0) on the 6th day of storage at room temperature, which was 6.33. Appearance is a criterion that determines the initial preference of consumers. Pempek coated with edible coating looked shiny on the surface, resulting in a clean surface impression.

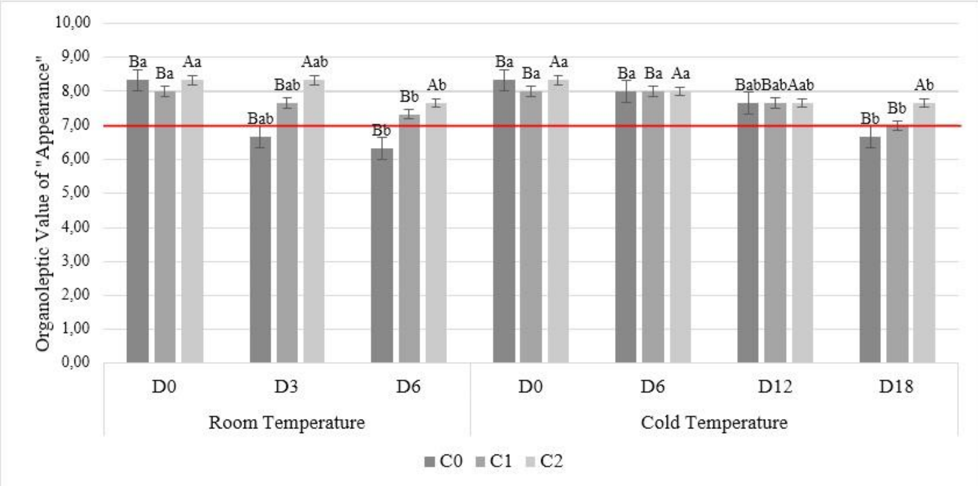

Figure 2. Diagram of the appearance value of *Tenggiri* fish pempek

Notes: Capital letter difference (A-B) in the same column indicates a significant difference ($p<0.05$) of coating treatment. Lowercase differences (a-b) in the same row indicate significant differences ($p<0.05$) in storage duration. The red line on the horizontal axis shows the minimum organoleptic value limit on pempek that has been required by SNI, which is at least 7. Treatment: C = Coating treatment; C0 = non-coating (control), C1 = Commercial coating, C2 = Best coating from Stage 1 (CMC+CSNP), and D= Storage Days; D0 = Day 0, D3 Room = Day 3 at room temperature, D6 Room = Day 6 at room temperature, D6 Cold = Day 6 at cold temperature, D12 Cold = Day 12 at cold temperature, D18 Cold = Day 18 at cold temperature.

This is per the research of Utami *et al.* (2017) that semi-refined carrageenan edible coating gives a shiny appearance to kurisi fish sausage so that it gives the impression of a good appearance that will affect the assessment of other organoleptic parameters such as smell, texture, and taste. Pempek with nanocomposite coating (C2) has a higher appearance value than pempek with commercial coating (C1) due to the content of cellulose derivatives as hydrocolloids that are better at forming gels than chitosan alone (Goff and Guo, 2019). This makes C2 pempek more attractive and shiny comparing without coating (C0) and with commercial coating (C1). Statistical analysis showed significant differences between days of storage in both room temperature and cold temperature. Pempek without coating (C0) stored at room temperature experienced the fastest deterioration in appearance, presumably due to high microbial contamination that triggers various chemical processes that can cause discoloration and mucus formation (Shabrina, 2023). However, pempek with coating showed better appearance due to the ability of chitosan to control the transfer of oxygen gas thus reducing the occurrence of chemical processes in the product (Cahyani *et al.,* 2022). Pitayati's research (2021) showed that chitosan-soaked pempek did not change color up to the 4th day of storage, presumably because chitosan is able to control the transfer of soluble solids which maintains the natural colour of the product (Cahyani *et al.,* 2022).

Odour

The observation results of the odour value of Tenggiri fish pempek with coating treatment of CMC and CSNP combination formulation (C2), commercial coating (C1), and without coating (C0) stored at room and cold temperature in this study were 5.33-8.67 (Figure3).

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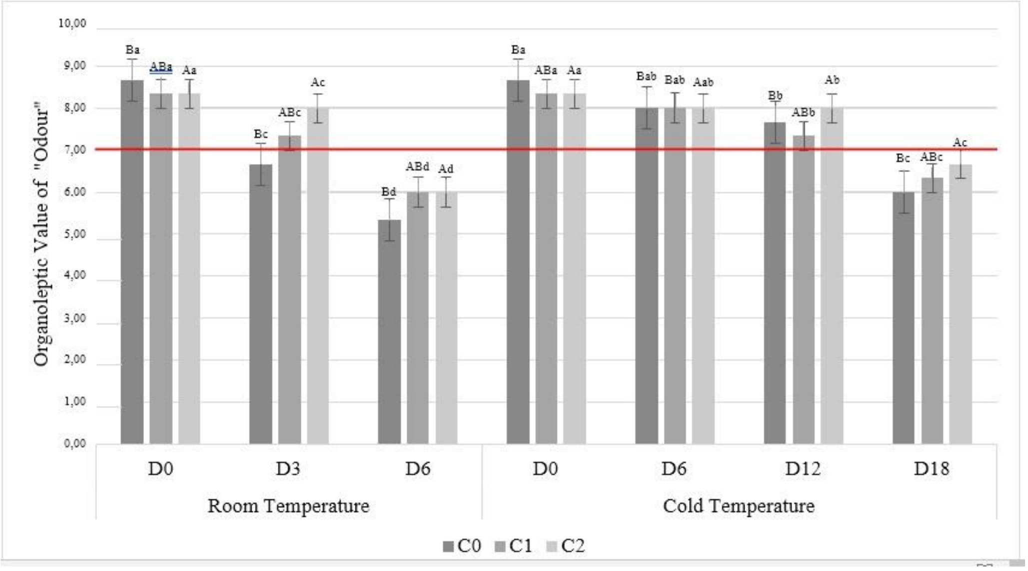

Figure 3. Diagram of the odour value of Tenggiri fish pempek

Notes: Capital letter difference (A-B) in the same column indicates significant difference ($p<0.05$) of coating treatment. Lowercase differences (a-b) in the same row indicate significant differences ($p<0.05$) in storage duration. The red line on the horizontal axis shows the minimum organoleptic value limit on pempek that has been required by SNI, which is at least 7. Treatment: C = Coating treatment; C0 = non-coating (control), C1 = Commercial coating, C2 = Best coating from Stage 1 (CMC+CSNP), and D= Storage Days; D0 = Day 0, D3 Room = Day 3 at room temperature, D6 Room = Day 6 at room temperature, D6 Cold = Day 6 at cold temperature, D12 Cold = Day 12 at cold temperature, D18 Cold = Day 18 at cold temperature.

The results of the Kruskall-Wallis statistical analysis showed that the odour of pempek was significantly different ($P<0.05$) between storage days but not significantly different ($P>0.05$) in the coating treatment. The Mann-Whitney further test showed that the highest odour value was found in the treatment without coating (C0) on day 0 of storage at 8.67 as well as the lowest odour value which was also found in the C0 treatment but on day 6 at room temperature at 5.33. Pempek from Tenggiri fish has a distinctive aroma that decreases with the length of storage, triggering the onset of rancidity. This is caused by fat breakdown that produces peroxide breakdown compounds or free fatty acids (Azizah *et al.,* 2019).

The decrease in odour value in pempek without coating (C0) is faster than those using coating (C1 and C2) because the coating can withstand the oxidation rate due to bacterial activity. On day 0, the highest odor value was obtained by pempek in the treatment without any dressing (C0) followed by pempek with commercial dressing (C1) and then pempek with CMC and CSNP combination dressing (C2). Pempek with both commercial (C1) and combined CMC and CSNP (C2) coatings had a similar aroma and tended to smell of acetic acid (CH_3COOH) because both coatings used acetic acid to dissolve the chitosan inside.

However, in subsequent storage, pempek without coating (C0) experienced a decrease in odor value with the largest range because it is related to the high value of microorganism pollution whose activities can trigger an accelerated oxidation rate, while pempek with coating is better at maintaining its aroma. Edible coatings help inhibit the activity of spoilage bacteria, as stated in the research of Ridwan *et al.* (2015), which showed that chitosan is able to suppress the activity of acid odor-producing bacteria (ammonia). During storage, the acetic acid odor in the coating evaporates, so it no longer disturbs the distinctive aroma of pempek. The odor in question is not a foul odor (ammonia) as in pempek without coating (C0). Pitayati's (2021) research supports this finding, where pempek with chitosan solution produces an unobtrusive acetic acid odor, in contrast to the foul odor of pempek without coating.

The treatment that is most able to maintain the smell value of pempek is the one coated with an edible coating combining CMC and chitosan nanoparticles (C2) with the best storage time is up to the 3rd day at room temperature and the 12th day at cold temperature. The standard for assessing the sensory odor of pempek fish has been regulated in SNI 2346:2015, which is a minimum of 7 with product-specific neutral odor specifications. During storage, the smell of acetic acid in the coating evaporates, so it no longer disturbs the typical aroma of pempek. The smell in question is not foul (ammonia) like in uncoated pempek (C0). Pitayati's research (2021) supports this finding, where pempek with chitosan solution produces an unobtrusive acetic acid aroma, in contrast to the foul smell of uncoated pempek.

Taste

The observation results of the taste value of Tenggiri fish pempek with coating treatment of CMC and CSNP combination formulation (C2), commercial coating (C1), and without coating (C0) stored at room and cold temperature in this study were 5-9 (Figure 4).

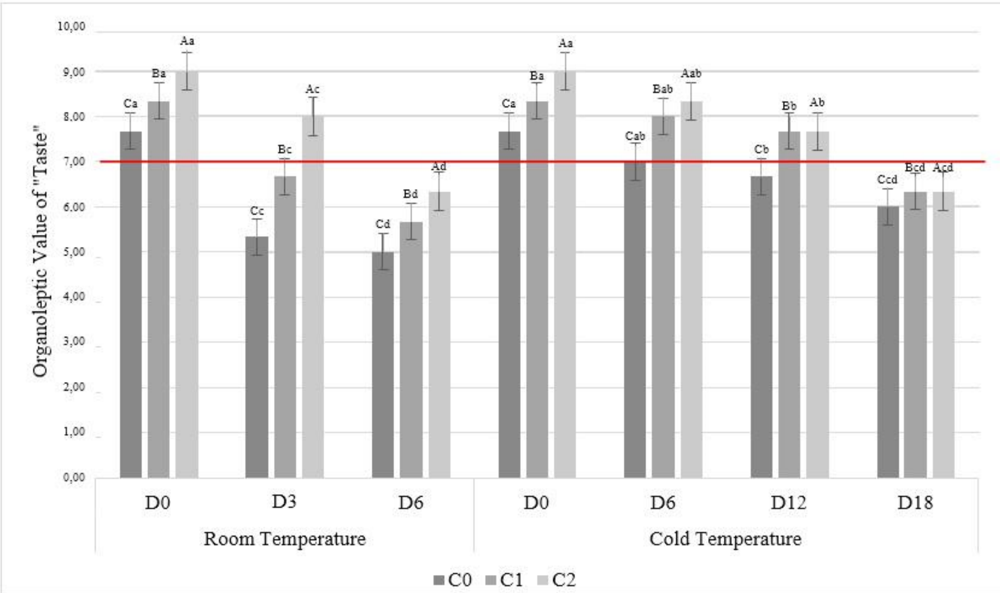

Figure 4. Diagram of the taste value of *Tenggiri* fish pempek

Notes: Capital letter difference (A-B) in the same column indicates a significant difference ($p<0.05$) in coating treatment. Lowercase differences (a-b) in the same row indicate significant differences ($p<0.05$) in storage duration. The red line on the horizontal axis shows the minimum organoleptic value limit on pempek that has been required by SNI, which is at least 7. Treatment: C = Coating treatment; C0 = non-coating (control), C1 = Commercial coating, C2 = Best coating from Stage 1 (CMC+CSNP), and D= Storage Days; D0 = Day 0, D3 Room = Day 3 at room temperature, D6 Room = Day 6 at room temperature, D6 Cold = Day 6 at cold temperature, D12 Cold = Day 12 at cold temperature, D18 Cold = Day 18 at cold temperature.

The results of Kruskall-Wallis statistical analysis showed that the taste of pempek was significantly different ($P<0.05$) between treatments and days of storage. The Mann-Whitney further test showed that the highest flavour value was found in the CMC+CSNP combination formulation (C2) on day 0 of storage at 9, while the lowest flavor value was found in the treatment without coating (C0) on day 6 of storage at room temperature at 5. The taste is influenced by the appearance and smell of pempek, where the rancid smell is often followed by an unpleasant taste. The rancid smell is caused by the activity of several microbes that produce enzymes that can break down fats into free fatty acids and other compounds that smell bad. Free fatty acids, even a small amount, can cause a bad taste (Kusuma *et al.,* 2016). The taste of food is influenced by basic ingredients, processing processes, additives, chemical compounds, temperature, flavor interactions, and ingredient concentrations (Novianti *et al.,* 2019). The standard taste of pempek fish that has been regulated in SNI 2346:2015 is a minimum of 7 with specific neutral taste specifications. In this study, the taste value of pempek without *coating* (C0) decreased below 7 on the 6th day of storage at cold temperatures. In *coating* treatments (C1 and C2), taste values reached 7 on day 3 at room temperature and day 12 at cold temperature, with *commercial coating* treatment (C1) showing a drop below 7 on day 3 at room temperature.

Texture

The observation results of the texture value of *Tenggiri* fish pempek with coating treatment of CMC and CSNP combination formulation (C2), commercial coating (C1), and without coating (C0) stored at room and cold temperature in this study were 5.67 - 8 (Figure 5).

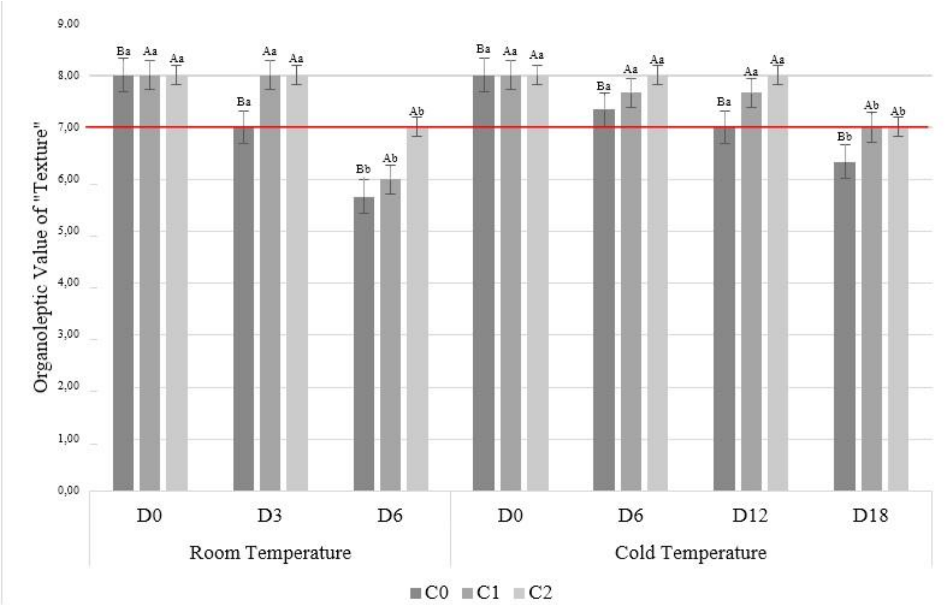

Figure 5. Diagram of the texture value of pempek *ikan tengiri*

Notes: Capital letter difference (A-B) in the same column indicates significant difference (p<0.05) of coating treatment. Lowercase differences (a-b) in the same row indicate significant differences (p<0.05) in storage duration. The red line on the horizontal axis shows the minimum organoleptic value limit on pempek that has been required by SNI, which is at least 7. Treatment: C = Coating treatment; C0 = non-coating (control), C1 = Commercial coating, C2 = Best coating from Stage 1 (CMC+CSNP), and D= Storage Days; D0 = Day 0, D3 Room = Day 3 at room temperature, D6 Room = Day 6 at room temperature, D6 Cold = Day 6 at cold temperature, D12 Cold = Day 12 at cold temperature, D18 Cold = Day 18 at cold temperature.

The results of the Kruskall-Wallis statistical analysis showed that the texture of pempek was significantly different (P<0.05) between treatments and days of storage. The Mann-Whitney further test showed that the highest texture value was found in all treatments on day 0 of storage which was 8, while the lowest texture value was found in the treatment without coating (C0) on day 6 of storage at room temperature which was 5.67. Texture decreased with increasing time and storage temperature. This change in texture is influenced by product moisture content, packaging type, and storage conditions. Microbial activity also plays a role in texture degradation, which increases with time and storage temperature (Shabrina, 2023). According to SNI 2346:2015, the standard for the texture value of pempek fish is at least 7, with a compact and chewy texture specification. In the study, pempek with *a* combination of CMC and CSNP (C2) coating treatment is the best in maintaining its texture, because *the coating* functions as a barrier against moisture from the environment (Kamal *et al.,* 2017). The texture is greatly affected by moisture content, where water supports the growth of putrefactive microbes that damage the texture of the product (Rosida *et al.,* 2018). Pitayati's research (2021) supports this finding where pempek without chitosan coating becomes softer with storage because there is no protective layer from moisture and environmental contaminants.

CONCLUSIONS

The results showed that different variations of edible coating formulations had different effects on their antibacterial activity, water absorption, and viscosity. The best formulation was found in the combination of CMC and CSNP (P5) which produced an antibacterial inhibition zone of 9.31

mm, a water absorption value of 1.23%, and a viscosity of 33.50 cPa.s. Pempek with edible coating combined with CMC and CSNP (C2) showed a significant influence on microbial contamination values and organoleptic properties of *Tenggiri* fish pempek. The edible coating formulation combining CMC and CSNP (C2) is effective in maintaining the microbial count and organoleptic quality of *Tenggiri* fish pempek better than the treatment without coating (C0) and with commercial coating (C1) so that the product remains suitable for consumption until the 3rd day at room temperature and the 12th day at cold temperature.

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AUTHOR'S CONTRIBUTION

The contributions of each author are as follows: FS was responsible for data collection, manuscript writing, and design of tables and diagrams. GM and ASM were responsible in designing the main concept and critically revising the article. All researchers participated in the discussion of results and contributed to the drafting of the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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