Formulation of Carrageenan and Sodium Chloride in Edible Coatings for Enhanced Tilapia Fillet Preservation

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ABSTRACT

While carrageenan coatings offer promising preservation for fish, limitations like water absorption and insufficient antimicrobial strength necessitate more investigation on combining them with natural substances. This study aimed to extend the shelf life of tilapia fillets using an edible coating made from carrageenan and sodium chloride (NaCl). Response Surface Methodology (RSM) was employed to optimize the coating formulation, considering film properties such as thickness, swelling index, opacity, and water vapor transfer rate. A Central Composite Design (CCD) identified the optimal composition as 1.4 grams of carrageenan and 0.6 grams of NaCl, minimizing undesirable characteristics. The optimized coating was applied to fresh tilapia fillets and compared to uncoated controls during five days of refrigerated storage (4.5 °C). Quality parameters, including bacterial count, total volatile basic nitrogen (TVB-N), pH, weight loss, and color, were monitored. The carrageenan-NaCl coating significantly reduced bacterial growth, with values increasing from log 6.31 CFU/ml on day 0 to log 6.34 CFU/ml on day 5, compared to uncoated fillets exhibiting TNTC (too numerous to count) bacteria by the end of storage. Additionally, the coated fillets displayed a lower increase in TVB-N (22.50%) compared to uncoated samples (31.38%), maintaining a stable pH and experiencing less weight loss (27.54%) compared to uncoated controls (32.96%). The coating also effectively preserved color, indicating its potential to maintain sensory attributes. These findings suggest that the optimized carrageenan-NaCl edible coating offers a promising strategy for extending the shelf life and maintaining the quality of fresh tilapia fillets, potentially reducing losses within the seafood supply chain.

Keywords: Carrageenan, Edible coating, NaCl, Response Surface Methodology, Tilapia fillets

Introduction

Fish has been well known as a highly nutritious food source, providing a wealth of essential nutrients, including high-quality protein and unsaturated fatty acids. However, its inherent perishability poses a significant challenge as it is susceptible to deterioration due to various factors, including autolysis, bacterial spoilage, and oxidative stress. These intrinsic factors are further exacerbated by external conditions such as temperature, light, and oxygen exposure, accelerating the spoilage process and decreasing the quality and safety of fish products.

To address the problems and extend the shelf life of fish, specifically for fresh fish products, various preservation methods have been widely explored, including the addition of preservatives, canning or plastic packaging, vacuum packaging techniques, modified atmosphere packaging, and others (de Rezende *et al*., 2022). Innovative approaches like the use of edible coatings have come to prominence as particularly promising among such techniques. A thin layer of edible materials called edible coating was placed to the surface of fish products and has several advantages, such as barrier qualities, antioxidant and antibacterial activity, and sensory preservation (Kumar *et al*., 2022; Umaraw *et al*., 2020).

The effectiveness of edible coatings depends on the selection of coating materials and their properties. Alginate, agar, and carrageenan, extracted from seaweed, are commonly used biopolymers for edible coatings due to their film-forming ability and biocompatibility. These coatings had demonstrated effectiveness in extending the shelf life of various food products, including fruits, vegetables, dairy, and meat (Jayakody *et al*., 2022). Specifically, carrageenan had gained attention for its unique properties, including its ability to form strong gels, its biodegradability, and its compatibility with various additives (Fathiraja *et al*., 2022). Moreover, carrageenan offers advantages over other polysaccharides, such as superior gel strength, high transparency, and excellent stability under a wide range of pH and temperature conditions, making it particularly suitable for food preservation applications (Blakemore, 2016; Udo *et al*., 2023).

Studies have shown that carrageenan-based coatings can effectively preserve fish quality by reducing microbial growth and lipid oxidation. Khojah, (2020), employed biobased coatings made from a combination of fish gelatin, carrageenan, and pomegranate peel extracts. Volpe *et al*. (2015), achieved promising results by coating fresh trout fillets with carrageenan and lemon essential oil, observing notable antibacterial activity and reduced lipid oxidation during storage at 4°C for 15 days. He *et al*. (2020) applied a two-layered, unidirectionally permeable film with an inner hydrophilic layer composed of ê-carrageenan, gelatin, and curcumin, and an outer hydrophobic layer of zein. This novel coating significantly suppressed microbiological development, extending the shelf life of grass carp fillets.

However, carrageenan-based coatings also exhibit limitations, such as their hydrophilic nature, which can lead to increased moisture transfer and reduced water resistance (Sedayu *et al*., 2019). Additionally, the antimicrobial properties of carrageenan alone may not be sufficient to fully protect fish products from spoilage (Roy *et al*., 2019).

Presently, with increasing consumer awareness regarding synthetic chemical additives, the food industry is exploring natural antimicrobial compounds. Plant essential oils, volatile compounds from plants, have a long history of being used in food preservation. Their antimicrobial and antioxidant properties, as validated by numerous studies, render them appealing for the preservation of fishery products. However, their application was constrained by extraction costs and their potential to adversely impact food taste (Panahi & Mohsenzadeh, 2022).

To overcome these issues, researchers have explored incorporating alternative substances such as sodium chloride (NaCl) into polymeric coating materials. The main reason for adding NaCl is its ability to enhance ionic interactions within the polymer matrix, thereby improving film structure, cohesion, and mechanical stability (Cui *et al*., 2023; Shi *et al*., 2018). Panahi & Mohsenzadeh (2022) demonstrated that a sodium alginate coating containing essential oil, nisin, and NaCl could successfully preserved and increased the shelf life of chicken breasts during refrigerated storage. Fabra *et al*. (2012) noted that adding NaCl to carrageenan film enhances the film's uniformity and surface properties, improving barrier properties and reducing moisture loss. Compared to the standard requirements of edible films (such as low water vapor transmission rate, adequate tensile strength, and minimal moisture loss) NaCl incorporation helps coatings approach these desirable qualities. However, it is important to consider that high concentrations of NaCl could create osmotic pressure, potentially decreasing membrane stability (Yuan *et al*., 2022). Despite the potential benefits of incorporating NaCl into carrageenan coatings, there is a lack of comprehensive investigation on the optimal concentration of NaCl included in carrageenan edible coating, and its performance in preserving fish quality.

The effectiveness and stability of edible coatings are determined by their composition and properties. In this present study, NaCl was used to modify the barrier and mechanical properties of the carrageenan coating film, while to achieve the optimum formulation of the edible film, the RSM was used (Fathiraja *et al*., 2022; Tessema *et al*., 2023). This study aimed to determine the optimal formulation of carrageenan and NaCl concentrations to produce edible film coatings, particularly for preserving the quality of fresh Nile tilapia fillets during refrigeration.

Material and Methods

Coating materials

The materials used to produce the coating film include k-carrageenan purchased from CV Karagen Indonesia, NaCl obtained from PT UniChemCandi Indonesia, and glycerol (ROFA Laboratorium Centre, Indonesia).

Preparation of coating solutions

The coating preparation followed a modified Hanani & Husna (2018) method. Carrageenan was dissolved

in 100 ml of distilled water and heated to 80°C. NaCl was then added according to Table 1. Glycerol was added at 1% concentration and stirred vigorously at 80°C for 30 minutes. After eliminating air bubbles at 65°C, the solution was used as a coating for tilapia fillets. The coating's properties were assessed by transforming the solution into films, drying at 50°C for 20 hours, and analyzing physical, mechanical, and barrier characteristics. The films were peeled off from trays for further analysis.

Coating preparation

Fresh Nile tilapia (*Oreochromis niloticus*) sourced from a local fish farm in Bantul Regency, Yogyakarta, Indonesia, were promptly processed after harvesting. After being killed, gutted, and washed, the fish were chilled with ice in a cool box for transportation to the laboratory. Following filleting, the fillets were cold stored for approximately 4 hours before preparation for coating.

For coating, the fillets were cut into 3 × 3 × 1 cm^3 squares and divided into two groups: the control group (uncoated) and the coated group. The coated group's pieces were immersed in a coating solution for one minute using plastic basket trays, drained, and allowed to dry. Both groups were then stored at 4.5 ^oC and 32% RH, and quality observations were made over a 5-day period.

Experimental design

RSM was used to find the best formula for the edible coating, with Carrageenan and NaCl as independent variables. Glycerol was added at a constant 1% rate. A Central Composite Design (CCD) model assessed 13 formulations, considering thickness, swelling index, opacity, and water vapor transmission rate (WVTR). Mathematical models analysed the effects of Carrageenan, NaCl, and plasticizer. Minitab 21 software was used for experimental design, regression model, and statistical analysis. Contour plots showed results. The optimal Carrageenan-NaCl combination was chosen based on factors like minimum thickness and WVTR. Response data were validated against projected response using absolute percentage error. Mean with standard deviation was provided, with $p < 0.05$ indicating significance. All experimental runs are listed in Table 1.

Table 1. Matrix of experimental design for the optimization of carrageenan-NaCl edible coatings

Run	Carrageenan (g)	NaCl (g)
T1	2.0	1.5
T2	3.0	1.5
T3	3.0	0.0
T4	3.0	3.0
T5	1.0	0.0
T6	2.0	3.0
T7	2.0	1.5
T8	1.0	3.0
T9	2.0	0.0
T10	2.0	1.5
T11	2.0	1.5
T12	2.0	1.5
T13	1.5	1.5

Film Characteristics Analysis

Thickness

The measurement of film layer thickness was conducted using a digital micrometer at five randomly selected film spots according to Shojaee-Aliabadi *et al*. (2014). Subsequently, the average was calculated.

Swelling Index

The swelling index was measured as a percentage of the weight difference between the initial and final weights of the samples after immersion in water following the procedure outlined by Susmitha *et al*. (2021) with slight modification. The film samples were cut into square sized 1.5 cm x 1.5 cm^2, dried in an

oven at 105°C for 24 hours, and weighed (W0). The samples were then soaked in a 30 ml of distilled water for 5 minutes at room temperature (± 25°C), dried on a paper towel, and reweighed (W1). W1 represents the weight of the wet sample, and W0 represents the weight of the dry sample. The following equation was used to calculate the absorption of water:

$$\text{Swelling Index } (\%) = [(W1 - W0)/W0] \text{ x } 100$$

Opacity

Film opacity was measured using the method described by Hanani & Husna, (2018). A rectangular film sample (5 cm x 0.8 cm) was placed in cuvette and mounted into the test cell of UV-Vis spectrophotometer (Agilent Cary 60 serial MY 19459217, USA). The opacity of each film sample was measured at a wavelength of 600 nm, with an empty cuvette served as the reference. The opacity was calculated using the following equation.

$$\text{Op} = \frac{\text{Abs}_{600}}{\text{X}}$$

The value of Abs_{600} represents the absorbance at a wavelength of 600 nm, whereas x denotes the thickness of the film. Each sample was measured three times. Lower opacity values are indicative of high transparency.

Water vapor transmission rate (WVTR)

WVTR was measured following the method described by Shojaee-Aliabadi *et al*. (2014), with some modifications based on the ASTM E96 standard. Circular film samples (dia. 0.6 cm) were used to seal 2.0 ml glass vials containing silica gel. The vials were then placed in a desiccator filled with distilled water. The initial weight of each vial was recorded under controlled ambient conditions. WVTR measurements were taken every 24 hours for 7 days.

The WVTR may be calculated using the following equation:

$$\text{WVTR} = \frac{(\text{slope})}{(\text{film area})} = \frac{\Delta \text{m}}{\Delta \text{t}}$$

In this context, the variable (m) represents the mass of water, (A) is the area of the film, and (t) signifies the time interval.

Fish Quality

Total Plate Count (TPC)

The TPC assessment in fillet samples followed the protocol outlined in SNI 2897:2008 (BSN, 2008). Fillet samples weighing up to 10 g were placed in a sterile container with 90 ml of 0.1% Butterfield's Phosphate Buffered (BPW) solution. After 120 seconds of homogenization, resulting in a dilution ratio of 10^{-1}, a series of dilutions (10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) were prepared by transferring 1 mL of each dilution into 9 mL of 0.1% BPW solution. Each dilution was vigorously shaken at least 25 times. Then, 1 mL of suspension from each dilution was pipetted onto sterile petri dishes in duplicate. Plate Count Agar (PCA) was added (15-20 mL) to each dish, and the dishes were rotated to ensure thorough mixing. The dishes were then inverted and incubated at 35 ± 1°C for 24-48 hours.

Total volatile base Nitrogen (TVBN)

The TVBN concentration of fish sample was measured by the Conway's dish method with triplicate determinations. Five grams of the fillet sample was mixed with 10 ml of 7% trichloroacetic acid (Merck) in a glass beaker and then homogenized and filtered using filter paper for analysis. TVBN was released with the addition of saturated K_2CO_3 and was absorbed by a boric acid solution and then titrated with 0.02 N HCl after incubating the samples for 20-24 hours at ambient temperature. The TVBN concentration was expressed in milligrams of N/100 g of fish (Arulkumar *et al*., 2017).

pH

The fillet samples are fragmented into smaller fragments, each weighing up to 5 g, and after that, homogenized using a mortar, using 45 ml of distilled for 60 seconds. Subsequently, the liquid should be transferred into a beaker glass with a capacity of 10 ml, followed by determining its pH using a pH meter as described by Albertos *et al*. (2015) with slight modification.

Weight Loss

The fillet samples for each treatment were subjected to weighing at the commencement of the

storage period (day 0) and the conclusion of the storage period (day 5). The concept of weight reduction may be quantified using an equation by Chan *et al*. (2020):

$$W_{loss} = (W_0 - W_1)x\frac{100}{W_0}$$

W_{loss} represents the percentage weight loss (%), W0 represents the initial weight at the beginning of the storage (g), and W1 represents the weight 5 days into the storage.

Color

The measurements of color fillet samples including lightness (L*), redness/greenness (a*), and yellowness/blueness (b*) were performed with a chromameter manufactured by Konica, Minolta, Japan. utilizing the techniques of Xiong *et al*. (2021).

Data Analysis

All treatments were conducted in triplicate. The obtained values of the parameter analysis are subjected to examination using a one-way analysis of variance (ANOVA). The statistical significance of the difference between the mean values, as determined by a significance level of Pd≤0.05, was assessed using the Fisher least significant difference (LSD) method for the film characteristics and the T-test for fish sample quality. The selection of optimal film conditions considered the minimum values, which included thickness, opacity, swelling index, and water vapor transmission rate of all the specimens.

Results and Discussions

Optimization of the film-forming solutions

The results of the analysis for the 13 formulation samples conducted using CCD are presented in Table 2. The objective was to identify the optimal film formulation by considering several parameters. The foremost requirement was transparency in the film to enhance the visual appeal of the coated fillet, aligning with consumer preferences. Consequently, a film formulation with minimal opacity was selected. Following this, efforts were made to minimize the permeability of the film, as indicated by the WVTR value. This was essential to restrict the diffusion of water vapor, either from the material to the environment or vice versa (Bertolo *et al*., 2022). This serves to impede the deterioration of fish quality due to oxidation processes. Additionally, the selection criteria included a minimum swelling index, indicating the film's efficiency in absorbing water from the fillet sample. A low swelling index signifies the film's ability to preserve its integrity upon contact with water.

Table 2. Results of the analysis of thickness, opacity, WVTR, and swelling index of the 13 film formulation from CCD.

Run	NaCl (g)	Carrageenan (g)	Thickness (mm)	Opacity (mm^{-1})	WVTR (g /mm^2 day)	Swelling index (%)
T1	1,5	2,0	0,11 ± 0,012 ^{ef}	5,64 ± 1,34 ^{de}	0,048 ± 0,002 ^{a}	24,55 ± 3,63 ^{h}
T2	1,5	3,0	0,13 ± 0,010 ^{c}	4,79 ± 0,66 ^{e}	0,047 ± 0,001 ^{a}	33,63 ± 4,63 ^{ef}
T3	0,0	3,0	0,08 ± 0,009 ^{g}	0,42±0,08 ^{g}	0,046 ± 0,001 ^{ab}	43,51 ± 1,84 ^{ab}
T4	3,0	3,0	0,16 ± 0,034 ^{b}	5,62±0,54 ^{de}	0,048 ± 0,001 ^{a}	32,03 ± 1,21 ^{fg}
T5	0,0	1,0	0,05 ± 0,023 ^{h}	2,13±0,41 ^{f}	0,047 ± 0,002 ^{a}	41,13 ± 1,45 ^{bc}
T6	3,0	2,0	0,17 ± 0,029 ^{b}	7,86±0,57 ^{bc}	0,042 ± 0,009 ^{bc}	27,53 ± 0,77 ^{gh}
T7	1,5	2,0	0,09 ± 0,016 ^{fg}	6,50 ± 0,78 ^{cd}	0,044 ± 0,005 ^{abc}	39,73 ± 0,02 ^{bc}
T8	3,0	1,0	0,22 ± 0,045 ^{a}	6,15±0,39 ^{de}	0,045 ± 0,001 ^{abc}	12,92 ± 3,49 ^{i}
T9	0,0	2,0	0,06 ± 0,010 ^{h}	0,70 ± 0,09 ^{fg}	0,042 ± 0,001 ^{bc}	47,41 ± 0,63 ^{a}
T10	1,5	2,0	0,12 ± 0,017 ^{cde}	10,56±1,08 ^{a}	0,037 ± 0,003 ^{d}	38,05±0,15 ^{cde}
T11	1,5	2,0	0,11 ± 0,019 ^{de}	8,12 ± 3,89 ^{b}	0,042 ± 0,004 ^{c}	39,20±0,06 ^{bcd}
T12	1,5	2,0	0,13 ± 0,018 ^{cd}	6,18 ± 0,64 ^{de}	0,040 ± 0,002 ^{cd}	34,07±0,03 ^{def}
T13	1,5	1,5	0,11 ± 0,020 ^{ef}	7,18 ± 0,51 ^{bcd}	0,044 ± 0,001 ^{abc}	42,55±4,57 ^{abc}

In the same column, values with different superscript letters indicate statistically significant differences by ANOVA and Tukey (p d ≤ 0.05).

The experimental data in Table 1 was subsequently utilized to find the optimal point through the RSM. The resulting output was in the form of a response surface plot in Figure 1 below.

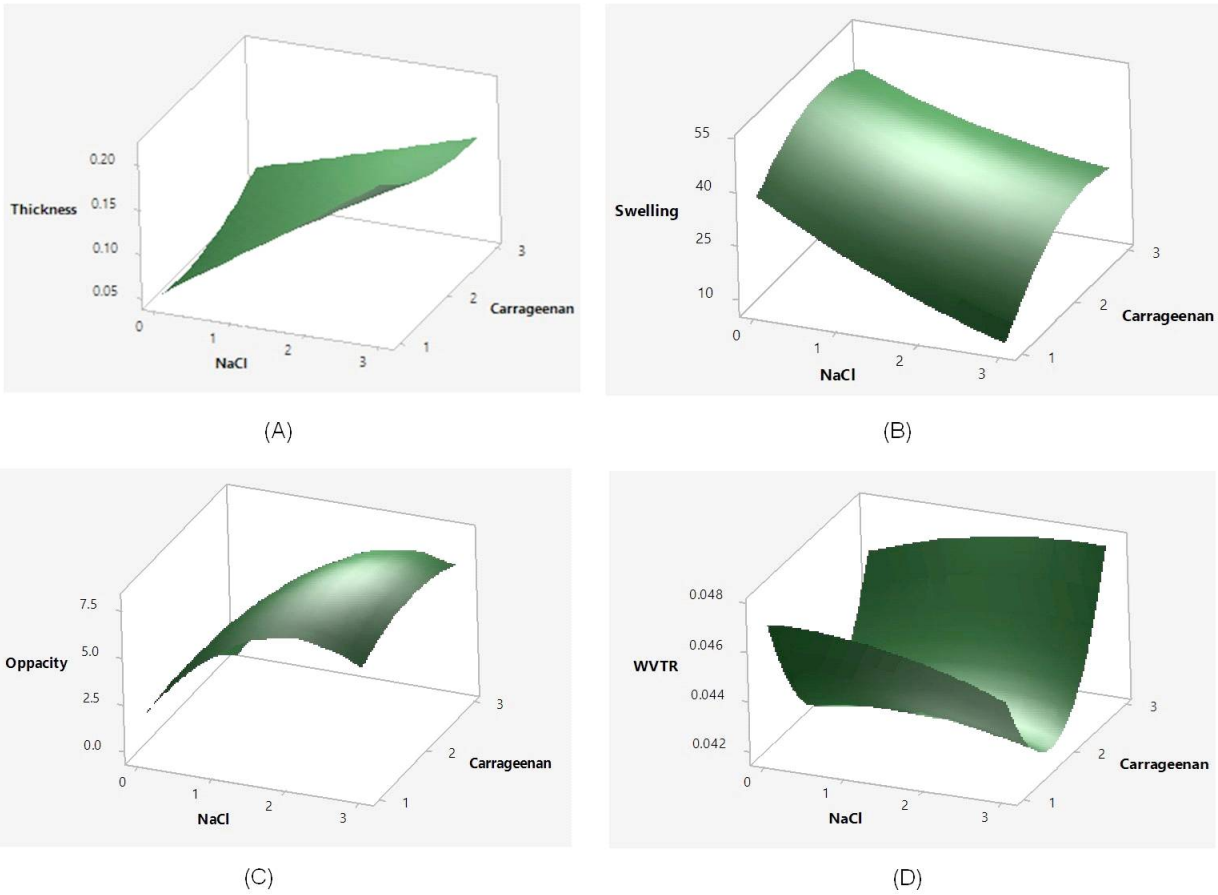

Figure 1. Response surface plots of the interaction between NaCl and carrageenan for CCD film-forming solutions in terms of film thickness (A), swelling (B), opacity (C), and WVTR (D).

Figure 1A illustrates the correlation between film formulations and the resulting film thickness. The data indicates that the lowest thickness was observed in the 0.0 g NaCl/1.4 g carrageenan formulation with a 0.048 mm, while the highest thickness was observed in the 3.0 g NaCl/1.0 g carrageenan formulation, with a thickness of 0.216 mm. Furthermore, the results of statistical analysis indicate that the quantities of both components, NaCl and carrageenan, influence the resulting film thickness. A similar finding was also observed by (Razavi *et al*., 2015; Tessema *et al*., 2023), where they noted that the ingredient composition of the coating film affects its final thickness. However, it is noteworthy that the addition of NaCl in the film formulation demonstrates a more significant impact on the increased film thickness compared to the addition of carrageenan. NaCl can provide more nucleation sites for the film formation, which can increase the crystallization rate and the â-phase content of the film. This resulted in a higher film thickness compared to the addition of carrageenan, which is a polysaccharide that might not have had the same effect on the film structure (French *et al*., 2020; Wang *et al*., 2021).

Different results were obtained from the observations of the swelling index of the samples. The contour plot graph above (Fig. 1B) illustrates that an increase in carrageenan content is associated with an elevated swelling index in the resulting film, while in contrast, an increase in NaCl content within the formulation corresponds to a decrease in its swelling index. The minimum swelling index (7.80%) was observed in the formulation with 3.0 g NaCl /1.0 g carrageenan, while the maximum (49.71 %) was identified in the absence of NaCl. This can be explained by the inherent water-absorbing properties of carrageenan, while the addition of NaCl in the film matrix may impede the water uptake. According to Santamaría Vanegas *et al*., (2019) NaCl increased the ionic strength of the solution, which reduced the electrostatic repulsion between the negatively charged sulphate groups on the carrageenan chains. This led to a more compact and rigid film structure, which resisted water penetration.

Figure 1C showcases this relationship of effect NaCl and carrageenan on opacity of the film. The lowest opacity (-0.19 mm^{-1}) occurs at 3.0 g carrageenan and 0.0 g NaCl, while the highest (7.93 mm^{-1}) is observed at 1.9 g carrageenan and 2.3 g NaCl. Carrageenan addition decreased the opacity parameter, as its colourless properties facilitate the production of transparent film sheets (Putri *et al*., 2023). Conversely, increasing NaCl concentration raised opacity by thickening the film, aligning with findings that transparency values decreased with film thickness (Zhao *et al*., 2022).

The water vapor transmission rate (WVTR) measures a film's resistance to the passage of water vapor. Ahmed & Ikram, (2016) emphasize the importance of maintaining low WVTR values to create effective barriers against moisture migration. Figure 1D illustrates the response model, showing a decrease in WVTR as concentrations of carrageenan and NaCl increase. The effect was more pronounced with NaCl, although carrageenan also contributed to a slight reduction. The minimum WVTR (0.042 g / mm^2 day) occurs at 3.0 g NaCl and 1.9 g carrageenan, while the maximum (0.048 g /mm^2 day) is observed at 2.7 g NaCl and 3.0 g carrageenan. Interestingly, the decrease in WVTR appears to be proportional to the square of the NaCl concentration. Santamaría Vanegas *et al*. (2019) and Lei *et al*. (2022) highlighted that addition of NaCl can increase the intermolecular interactions between carrageenan chains, resulting in a more compact and dense film or matrix. This dense structure can hinder the movement of water vapor molecules through the material, acting as a barrier to their transmission.

Based on the film characteristics obtained by varying concentrations of carrageenan and NaCl, the most optimal features were identified using the RSM method. Specifically, an optimal film formulation was determined, consisting of 1.4 grams of carrageenan and 0.6 grams of NaCl. This mixture was later used as an edible covering to preserve tilapia fillets.

Effects of selected coatings on refrigerated tilapia fillet quality

The performance of the selected edible coating formulation for preserving fresh tilapia fillets through the RSM method is presented in Table 3. Key quality parameters, including total volatile basic nitrogen (TVB), total plate count (TPC), pH, weight loss, and lightness, were observed. A comparison between uncoated and coated tilapia fillets was made at the initial stage and after 5 days of storage, with the corresponding difference values presented.

Table 3. Result of evaluation tilapia fillet quality

Parameters	Day 0		Day 5		Change (%) Increase (+) Decrease (-)	
	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated
TVB (mg/100 g)	13.45 ± 1.83	14.22 ± 0.95	20.67 ± 0.47	17.00 ± 0.86	(+) 31.38 ± 5.03 [a]	(+) 22.50 ± 3.54 [b]
TPC (log cfu/ml)	5.56 ± 0.26	6.31 ± 0.05	TNTC	6.34 ± 0.05	TNCT	(+) 1.13 ± 0.65
pH value	6.62 ± 0.06	6.69 ± 0.04	6.50 ± 0.08	6.69 ± 0.03	(-) 0.13 ± 0.04	0.00 ± 0.03
Weight loss (g)	10.91 ± 2.44	16.22 ± 4.64	6.99 ± 2.24	11.81 ± 3.66	(-) 32.96 ± 2.73 [a]	(-) 27.54 ± 1.97 [b]
Lightness (L value)	53.37 ± 0.25	42.63 ± 0.12	37.23 ± 0.45	41.27 ± 1.07	(-) 30.23 ± 0.52 [a]	(-) 3.20 ± 2.71 [b]
Redness (a value)	-2,70 ± 0,10	-0,97 ± 0,06	2,60 ± 1,10	1,23 ± 0,12	(+) 5,30 ± 1,15 [a]	2,20 ± 0,10 [b]
Yellowness (b value)	0,90 ± 0,30	2,53 ± 3,06	6,83 ± 0,61	7,70 ± 0,30	(+) 5,93 ± 0,84 [a]	5,17 ± 2,80 [a]

TNTC : too numerous to count; In the same row, values with different superscript letters indicate statistically significant differences by ANOVA and Tukey (p d≤0.05).

TVB

Total volatile base, a common indicator of fish freshness, is prompted by the decomposition of nitrogen-based compounds by bacteria and enzymes. Table 3 demonstrates that the coated fillets had significantly lower TVB values than the uncoated fillets after 5 days preservation in refrigerated storage. While the uncoated fillets experienced a significant increase in TVB (31.38%), the coated ones showed a much smaller increase (22.50%), remaining within the acceptable range at the end of preservation (maximum 35 mg N/100 g) (Volpe *et al*., 2015). These results suggest that carrageenan coating effectively slows down the chemical and enzymatic deterioration of the fillets, likely due to its ability to prevent water penetration and hinder the growth of spoilage bacteria (He *et al*., 2020). These results were also consistent with the total bacteria counts of the tilapia fillets during storage.

The edible coating, comprising carrageenan and NaCl, functions as a barrier, minimizing the water diffusion rate to delay decomposition, as evidenced by a low WVTR value. The presence of NaCl indicated a moderately rigid conformation of ê-carrageenan macromolecules. According to Bercea & Wolf, (2019), The presence of NaCl suggests a relatively stiff structure of ê-carrageenan macromolecules. The addition of NaCl reduced electrostatic repulsion between helices, enabling their interaction for K^+ induced gelation. As a result, as the concentration of NaCl increases, so do the stiffness and rate of gelation (Nguyen *et al*., 2014).

TPC

The results of microbial growth observation on the coated fish fillet samples showed promising outcomes. The edible coating demonstrated effectiveness in slowing down the growth of spoilage bacteria during storage. The bacterial growth rate on the coated tilapia fish fillet could be suppressed, as indicated by the TPC values. The TPC value was Log 6.31 CFU/ml on day 0 and only increased to Log 6.34 CFU/ml on day 5. On the other hand, uncoated fillets exhibited TNTC bacteria by the end of the storage period. Generally, a TPC of 7 Log CFU/g is the maximum acceptable limit for fresh, high-quality fish products, according to research by Xiong *et al*. (2021). The coated tilapia fillet sample reached a final TPC of 6.34 Log CFU/ml on day 5, suggesting its quality was moderate.

The combination of carrageenan and NaCl certainly prevented bacterial contamination in tilapia fillets. This was accomplished by forming a barrier layer that decreased ambient contamination, while the presence of NaCl generated a hostile environment for the development of bacteria. Sedayu *et al*. (2019) highlighted the primary goal of using carrageenan in edible coatings for fresh and frozen fish: inhibiting spoilage and deterioration by microbial contamination. Panahi & Mohsenzadeh (2022) further emphasized this by demonstrating a synergistic antimicrobial effect among NaCl, sodium alginate, and nisin. This synergy stems from NaCl's ability to reduce water activity (a_w) in foods, creating an environment unfavorable for microbial growth.

pH value

The pH values of both coated and non-coated fish fillet samples are nearly the same during the preservation. The initial pH of uncoated and coated fillets was 6.62 and 6.69, respectively. After five days of storage, the pH of the uncoated fillet was declined slightly to 6.50, whereas the pH of the coated fillet was remained steady at 6.69. The slight decline in pH in uncoated fillets was associated with both the breakdown of ATP and the production of lactic acid, as reported by by Chen *et al*. (2022), that the initial accumulation of lactic acid resulting from metabolism of glycogen under anaerobic conditions contributed to the reduction in pH values in fish muscle. Similar findings were observed in grass carp fillets stored at 0 and 3 °C, as reported by He *et al*. (2020). While the constancy of pH in the coated fillets implies that the edible coating made of carrageenan NaCl inhibited microbial deterioration and enzyme activity to some extent.

Weight loss

After 5 days of storage, both groups experienced weight loss, however, the uncoated fillets lost considerably more weight (32.96%) compared to the coated samples (27.54%). The weight loss, in this case, was related to water evaporation of the samples. The loss differences can be due to fish muscle's high-water content of 75%, with only 10-15% bound water and the rest free water vulnerable to evaporation (Listrat *et al*., 2016). The edible coating, therefore, acted as a water vapor barrier (Santamaría Vanegas *et al*., 2019), effectively reducing evaporation, and preserving water content in coated fillets. (Li *et al*., 2022) studied the influence of coating treatments on fish under various storage temperatures and discovered a reduction in weight loss linked with the coating treatment.

Color

Over 5 days of storage, the coating treatment significantly preserved the color and appearance of tilapia fillets. Uncoated fillets exhibited a pronounced color shift, with the a* value changing from -2.70 to +2.60, indicating a transition from a greenish to a reddish hue, and the b* value rising sharply from 0.90 to 6.83, reflecting increased yellowness due to lipid oxidation. In contrast, coated fillets showed much smaller changes. The a* value increased slightly from -0.97 to 1.23, and the b* value grew from 2.53 to 7.70, demonstrating the coating's effectiveness in slowing oxidative color changes. The lightness (L* value) of uncoated fillets decreased notably due to dehydration, while coated fillets maintained higher L* values, consistent with findings by Li *et al*. (2022), who reported that coatings act as both water vapor and oxygen barriers, preventing dehydration and oxidation to preserve lightness and color. Similarly, Cardoso *et al*. (2019) found that coated meat remained color-stable after storage. The optimized carrageenan-NaCl formula, meeting edible film criteria of minimal thickness and opacity, was effective in preserving the tilapia fillet's appearance.

Conclusion

This research successfully obtained an optimal formulation of carrageenan-based edible coating incorporated with NaCl for preserving fresh tilapia fillets by using an RSM. The selected coating film formulation effectively enhanced the barrier properties of the films although with some compromise in transparency and swelling index. Throughout refrigerated storage, the selected coating formulation demonstrated promising ability to preserve the quality of tilapia fillet during refrigerated storage. Observations showed that the NaCl incorporated carrageenan edible coating notably reduced the bacterial growth and total volatile basic nitrogen rates, also maintained the pH and color of the samples during preservation. Those performances were primarily in accordance with the formidable barrier capability against moisture loss and bacterial growth, thereby extending the shelf life of tilapia fillets. Future research is needed to enhanced antioxidant properties of carrageenan coating by adding natural bioakctive compounds. Its performance should also be tested under extended storage and real conditions

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