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EFFECTIVENESS OF ASCORBIC ACID AS A NATURAL SUBSTITUTE FOR CARBON MONOXIDE IN MAINTAINING THE QUALITY OF GOLDBANDED JOBFISH FILLETS

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ABSTRACT

The use of carbon monoxide (CO) in the fishing industry to improve the bright red color of fish fillets has been controversial. Despite its effectiveness and buyer demand, CO results in food safety risks and there are strict regulations in some countries. The objective of this study was to examine the effectiveness of ascorbic acid as a substitute for CO to enable products to penetrate the European Union market. The research method employed was a combination of organoleptic and microbiological testing of Goldbanded Jobfish fillets treated with CO, whereas treatment with ascorbic acid was only evaluated through organoleptic testing. The results showed that the use of CO in Goldbanded jobfish fillets resulted in bright red color and a soft texture. However, European countries prohibit the injection of CO in meat products, so the fisheries industry cannot export Goldbanded jobfish (*Pristipomoides multidens*) fish fillets to the European Union. Therefore, innovation in natural ingredients is needed to maintain the red color of fish fillets. The addition of 0.03% ascorbic acid, can maintain the bright red color of fish fillets and produce a firm and dense texture. To our knowledge, this research is the latest alternative solution to replace CO gas adjuvant in improving the texture of frozen Goldbanded jobfish products.

Keywords: Goldbanded Jobfish; *Pristipomoides multidens*; CO Gas; Ascorbic Acid; Microbiology

INTRODUCTION

Indonesia is the second-largest producer of fish and seafood in the world, after China. In Indonesia, the fisheries and marine sector plays an important role in the country's economy, as fisheries production in 2023 reached 24.74 million tons. According to data from the Ministry of Marine Affairs and Fisheries in 2023, Indonesia's per capita fish consumption reached 58.48 kg. Fish contains good sources of nutrients, including marine long-chain omega-3 polyunsaturated fatty acids (n-3 LCPUFA), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and many micronutrients (such as vitamin A, vitamin B12, vitamin D, zinc, selenium, and iodine) (Reksten *et al.*, 2020; Triyastuti *et al.,* 2023).

Goldbanded jobfish (*Pristipomoides multidens*) is one of the most popular marine fish species, especially among seafood lovers. Its dense and nutrient-rich meat texture and delicious flavor make it a top choice for various classy dishes and are often called the jewel of the sea (Younis *et al*., 2021). Goldbanded jobfish have nutrients as a source of protein and fat that are good and beneficial for health. The distribution area of Goldbanded jobfish (*Pristipomoides multidens*) covers the Indo-Pacific region, an area with enormous marine biodiversity (Ovenden *et al*., 2002). The chemical content of Goldbanded jobfish muscle includes moisture content of 77.90 ± 0.01^{b}, protein content of 16.74 ± 0.51^{c}, fat content of 2.53 ± 0.40^{b}, and ash content of 5.07 ± 0.49^{b}. High water, protein, and fat content make myoglobin pigment susceptible to oxidation and color degradation (Younis *et al*., 2021; Howes *et al*., 2019).

The process of fish quality deterioration after death generally occurs through the pre-rigor, rigor mortis, and post-rigor phases. However, in terms of fillet color quality, the most decisive phase is post-mortem degradation during storage. (Li *et al*., 2023). Several factors, including enzyme activity, bacterial activity, chemical processes, fish sex, fish size, environmental conditions, physical treatments, and the number of microorganisms, can cause deterioration in fish quality. The decline in fish quality can be observed physically, including odor, texture, color, mucus, and an unattractive appearance (Wang, 2018). The process most often applied to large amounts of raw fish is freezing. The application of GMP (Good Manufacturing Practices) in processing is a preventive measure in quality assurance to improve fish handling during freezing to minimize or prevent food hazards (Zhang *et al*., 2022).

The physical parameter commonly used by consumers to determine the quality of fish fillets is the change in reddish color. Bright red fish fillets are of higher quality and cost more than brown fillets. The bright red color of fish meat indicates the product's freshness and wholesomeness (Anne *et al*., 2017). To maintain the reddish color of fish fillets, the fisheries processing industry uses carbon monoxide (CO). The use of CO in Goldbanded jobfish fillets is at the buyer's request. However, the use of CO in fish is controversial because it relates to fraud detection (disguising decay) and consumer safety. In the United States, the Food and Drug Administration (FDA) has recognized 0.4% CO gas as safe (FDA, 2004). However, regulations in EU countries and Japan state that the use of CO gas is an economic fraud offense (disguising the deterioration of fish meat) (Ariyani

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et al., 2020). The controversy over the use of CO arose because of its ability to improve the color and texture of fish meat, making it appear bright red. Still, several countries banned it because of the potential economic risks posed by fraud and the residual CO2 gas contained in treated samples.

This is due to concerns that the use of CO can disguise microbial spoilage in fish meat, thereby misleading consumers about product freshness and affecting consumer safety (Anne *et al.*, 2017). According to Commission Regulation (EU) 2022/1923, ascorbic acid and its derivatives are permitted food additives in the European Union and function as antioxidants to maintain food product quality. In the context of fishery products, ascorbic acid can be used to inhibit the oxidation of pigments and lipids, thereby helping to preserve the natural color and sensory quality of fish meat during storage. Although effective, CO poses food safety risks and is subject to strict regulations in some countries. Thus, a solution is needed to develop fish fillet products without CO gas. According to Gu *et al*. (2020), ascorbic acid can effectively improve texture and color; however, its effectiveness as a substitute for CO has not been directly compared in industrial simulations. Ascorbic acid, as a natural alternative, has shown the potential to improve the quality of fish products. Therefore, the purpose of this study is to evaluate the effectiveness of ascorbic acid in maintaining the organoleptic and microbiological quality of Goldbanded jobfish (*Pristipomoides multidens*) fillets, as an alternative to CO use to make them safer, higher quality, and more sustainable.

RESEARCH METHODS

Time and Location

This research was conducted from January to December 2025 at the Bitung Marine and Fisheries Polytechnic and the Fisheries Product Processing Engineering Laboratory.

This study employed experimental methods in the fish processing industry. The sample of Goldbanded jobfish used was fish fillets. Observations were made on the flow of the Goldbanded jobfish processing process using CO gas as a reference to improve sensory quality of product, which aligns with the standards of the quality assurance system and the safety of fishery products. The results provide alternative solutions to replace the use of CO auxiliary materials to improve the product's sensory and safety. The analysis includes fish quality, as assessed through sensory analysis of fish samples in the fisheries processing industry. CO injection is performed at 6 kg/cm² for 1-3 minutes (Volume of CO = 55 mL). Given that the weight of the Goldbanded jobfish fillet is 500 grams, the volume of CO = 55 mL = 0,055 L, the purity of CO = 100%, the molecular mass of CO = 28,01 g/mol, and the molar volume of gas at STP = 22,4 L/mol. The following is the formula for the percentage of CO:

$$\text{Percentage of CO to fillet weight} = \frac{\text{mCO}}{\text{mikan}} \text{X } 100 \text{ ..... (1)}$$

Ascorbic Acid Treatment

Ascorbic acid treatment in this study was conducted as an alternative to the use of CO gas to maintain and improve the sensory quality of Goldbanded jobfish fillets. The fish fillets were treated by immersion in a 0.03% ascorbic acid solution so that the solution could evenly contact the entire surface of the fillets. The immersion process lasted 10 minutes at 0–4°C, after which the fillets were removed. This method was used to evaluate ascorbic acid's ability to maintain sensory characteristics, particularly color and texture, of fillets as an alternative that complies with food safety regulations. The research experiment design is shown in Figure 1.

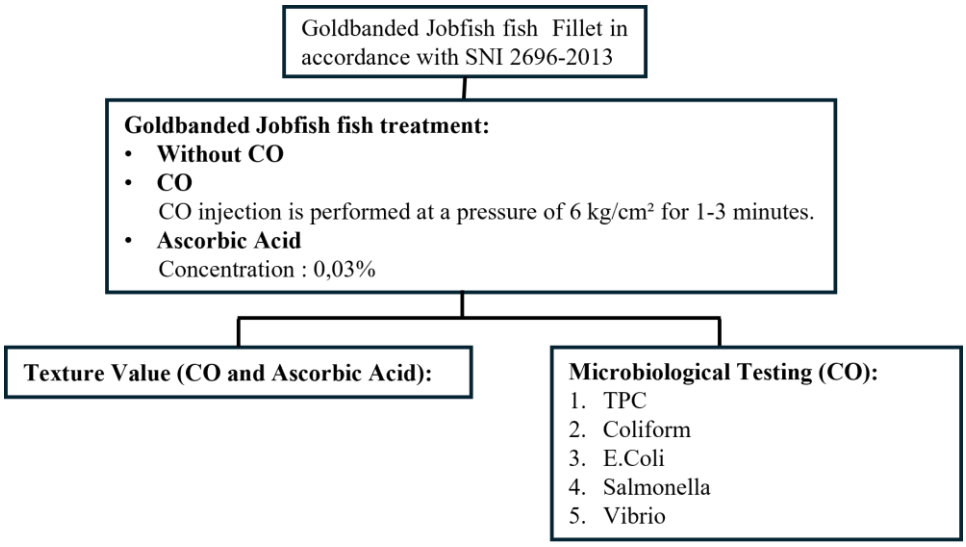

Figure 1. Research Experiment Design

Sampling

Microbiological testing for Coliform and E. coli based on SNI 2332.1:2015/2021, Salmonella based on SNI 01-2332.2-2006, and Vibrio SP based on SNI 2332.4:2009. Microbiological testing of fish involves sampling 1-3 times a week with a minimum of 2 fish per day. TPC analysis is performed 3 times a week, while testing for Coliform, E. coli, Salmonella, and Vibrio SP parameters is performed once a week.

Total Plate Count (TPC)

TPC testing includes sample preparation, inoculation, and incubation. Sample preparation involves weighing 25 g of sample, placing it in a blender container, and adding 225 mL of

Butterfield's phosphate-buffered (BFP) solution. Homogenize for 2 minutes. Take 1 mL of the homogenate solution (10^{-1} dilution) and add it to 9 mL of BFP solution for a 10^{-2} dilution. Add 1 mL of the 10^{-2} dilution to 9 mL of BFP solution to obtain a 10^{-4} dilution.

Perform the inoculation process by taking 1 mL of the 10^{-2} to 10^{-3} to 10^{-4} dilutions in duplicate. Add 12-15 mL of standard plate count agar at ±45°C to the sample, then spread it evenly.

Incubation process: After the PCA agar has completely hardened, the petri dishes are incubated upside down at 35°C ± 0.5°C for 48 hours.

Coliform

The Coliform testing process begins with sample preparation, followed by a preliminary test and a confirmatory test (also called a follow-up test). Sample preparation involves diluting the sample with BFP solution according to the estimated microbial density. Dilution is performed in triplicate (three repetitions) using 90 mL of sterile distilled water.

The preliminary test is performed by taking 1 mL from each dilution level and placing it into 3 or 5 tubes containing LSB media equipped with Durham tubes. Homogenize all tubes using a vortex mixer. Incubate the tubes at 35°C for 48 hours. After the incubation period, observe gas formation in the Durham tubes. Tubes showing gas formation are considered positive and are separated for further confirmation testing, while negative tubes are discarded.

Confirmation Test (Further Test): From the LTB tubes showing positive results, inoculate using a sterile needle into tubes containing BGLB medium equipped with Durham tubes. Re-homogenize all tubes with a vortex mixer. Incubate at 35°C for 48 hours. After incubation, observe gas formation in the Durham tube. Tubes that produce gas are declared positive as confirmation test results.

Escherichia Coli

The stages of E. coli testing include sample preparation, presumptive testing, confirmatory testing, and further presumptive testing. Sample preparation involves diluting the sample with BFP solution to the estimated level of microbial contamination.

Presumptive testing is performed by taking 1 mL from each dilution level and placing it into 3 tubes containing LSB media with Durham tubes. Homogenize all tubes with a vortex mixer. Incubate at 35°C for 48 hours. After incubation, observe gas formation in the Durham tubes. Tubes that produce gas are declared positive and separated for further confirmatory testing.

Further confirmatory testing is carried out by inoculating the LSB tubes that show positive results with a sterile needle into EC broth media containing Durham tubes. Homogenize all tubes using a vortex mixer. Incubate in a water bath at 44°C for 48 hours. After incubation, record the number of Durham tubes showing gas formation as positive results, then separate them for the confirmatory test stage.

Confirmatory test: take one culture loop from the EC broth tube that shows a positive result, then streak it on the surface of the LEMB agar medium. Incubate the dish at 35°C in an incubator for 48 hours. After incubation, observe the growth of colonies suspected to be E. coli, namely those with a black center and, if present, a metallic green sheen. Colonies suspected to be E. coli are then transferred using an inoculating needle to slanted PCA media using the streak method, then incubated at 35°C for 24 hours.

Salmonella

The stages of Salmonella testing include sample preparation (pre-enrichment), enrichment, and isolation. Prepare the sample by weighing 25 g aseptically, then place it in a sterile blender. Add 225 mL of lactose broth solution, then homogenize for ±2 minutes at low speed. Incubate the suspension at 35°C for 24 hours. The resulting homogenate is a 1:10 dilution.

Enrichment is carried out by gently stirring the pre-enriched culture, then taking 1 mL each and transferring it to 10 mL of TTB and RV media. Incubate the tubes at 43°C for 24 hours.

Isolate Salmonella by taking one loop from each enrichment medium, then streaking it onto the surface of the selective media HE, XLD, and BSA. Incubate all media at 35°C for 24 hours. Select colonies that show the characteristic features of Salmonella from the three media (HE, XLD, and BSA) using a loop. Next, inoculate the TSI medium by streaking the slanted agar surface and piercing the upright agar. Without taking new colonies, use the same needle to inoculate the LIA medium by piercing the upright agar first, then streaking the slanted part. Inoculate TSI and LIA media at 35°C for 24 hours with the tube caps slightly loosened to prevent pressure from excessive gas formation.

Vibrio Cholerae

The steps for testing Vibrio cholerae include sample preparation, enrichment, and isolation. Prepare a sample by aseptically weighing 25 g, then place it in a sterile blender. Add 225 mL of APW (Alkaline Peptone Water) solution, then homogenize for 2–3 minutes. The resulting suspension is a 1:10 dilution solution.

Enrichment is carried out by preparing a dilution series up to 10^{-2} by diluting 1 mL of the suspension in APW. Perform a further dilution based on the estimated microbial population density in the sample. At each dilution stage, shake at least 25 times to ensure homogeneity. Incubate the enrichment tubes at 35°C for 6–8 hours. After the initial incubation, streak the enriched culture from APW onto the surface of TCBS agar. Re-incubate the streaked media at 35°C for 16–24 hours.

Isolate Vibrio cholerae by taking one loopful from the tube showing turbidity (positive) at each dilution level, approximately 1 cm below the liquid surface, without shaking the tube. Restreak on TCBS agar, then incubate at 35°C for 18–24 hours. Observe the growth of colonies suspected to be Vibrio cholerae on TCBS medium. Colonies are generally large, smooth-surfaced, somewhat flat, with a cloudy center, lighter edges, and yellow in color, indicating a positive reaction to sucrose fermentation.

Organoleptic Analysis

Organoleptic analysis of fish acceptance is evaluated using the SNI 2729: 2013 reference on organoleptic assessment of fresh fish, with the lowest value score of 1 indicating low quality and the highest value of 9 indicating the best fish quality. Organoleptic testing of Goldbanded jobfish raw materials consists of 4 aspects of assessment, namely appearance (eyes, gills, body surface lender), meat, odor, and texture. Quality Control determines whether fish from suppliers are accepted.

Fish fillets have various quality requirements that are important for maintaining product quality. A good fish fillet is characterized by white, specific, brilliant, clean meat, a specific fresh odor, and an elastic, dense, and compact texture. Texture is an important parameter that can determine the physical quality of a food or food product. Texture measurements were performed using organoleptic methods by 25 trained panelists with assessment criteria of elasticity (score 1-9), density (score 1-9), and compactness (score 1-9). Fish freshness is one of the factors that affect fillet texture, with deteriorated fish having a softer texture (Lastri & Putra, 2020).

RESULT AND DISCUSSION

Goldbanded Jobfish Fillets

Goldbanded jobfish fillets processed with CO injection are a processed fish product often produced in the fish processing industry. The use of CO gas aims to improve the color appearance and product attractiveness for consumers. However, it is important to understand that the implications of each stage of the process must comply with the applicable regulations regarding CO gas dosage and the residue left in the product.

Figure 2. Goldbanded Jobfish Fillets

The addition of CO gas to Goldbanded jobfish meat will react with myoglobin, making the meat appear fresher and more attractive. CO gas can help slow the oxidation process that causes meat to turn red. Goldbanded jobfish fillets added with CO gas can last longer on cold display shelves.

The use of CO gas in Goldbanded jobfish fillets, when used in the right amount and in accordance with regulations, is considered safe for consumption. However, excess CO can be harmful. Some consumers may prefer products without added chemicals. Strict regulations govern the use of CO in the food industry to ensure product safety and quality, so a substitute for CO gas that is safer for consumers is needed, one of which is ascorbic acid.

Ascorbic acid, also known as vitamin C, is widely used in the food processing industry, including in fish processing, to maintain a fresh red color in fish meat. Ascorbic acid is an effective natural antioxidant. Ascorbic acid inhibits the oxidation reaction that causes meat to turn reddish-brown. The red color is due to ascorbic acid's ability to stabilize myoglobin, prolonging the meat's red color. In addition, ascorbic acid can prevent bacterial growth because the acidic environment it creates inhibits the growth of certain bacteria that can damage meat quality.

Ascorbic acid works as an antioxidant in Goldbanded jobfish meat because it can donate electrons. These electrons then react with free oxygen, causing oxidation, thus protecting myoglobin from damage. Ascorbic acid can maintain pH by lowering the pH of fish meat, creating an acidic environment that is not conducive to the growth of bacteria that cause damage. The advantage of using ascorbic acid is that it can extend the shelf life. By maintaining a fresh red color, ascorbic acid indirectly extends the shelf life of fish products. Ascorbic acid will also increase attractiveness, as consumers tend to choose food products that look fresh.

Ascorbic acid is a food additive generally considered safe for consumption and is regulated by the BPOM and the rules of export destination countries, such as the European Union. Ascorbic acid concentration must be used correctly, as improper dosage can affect the meat's taste and texture. Ascorbic acid can be added to Goldbanded jobfish meat in various ways, such as soaking, spraying, or injecting.

Microbiological Analysis

One very important aspect of quality is the total number of microbes. Microbiological analysis was conducted on goldbanded jobfish fillet product samples and production sanitation water quality. Total microbes are a quality parameter to show the high and low levels of microbial contamination in a product. Microbes are the source of many public health problems, such as diarrhea caused by *Escherichia coli*, typhoid by *Salmonella*, and cholera by *Vibrio cholerae*. Fresh fish is an example of a product that has a high risk of microbial contamination. This is because fresh or raw fish that has just died undergoes a metabolic process, so catabolism occurs frequently, and the resulting products serve as growth substrates for microbes. Therefore, raw fish products spoil faster than processed fish products. In addition, pH, aw, temperature, RH, and cross-contamination greatly affect microbial contamination and growth (Palawe, 2016). In the fisheries processing industry, the microbiological testing methods used are TPC, *Coliform, E. coli, Vibrio Cholerae*, and *Salmonella* testing. The following are the results of microbiological testing and a table of reference standards for microbiological testing of goldbanded jobfish fillet product samples and production sanitation water quality.

A very important quality aspect of fishery products is the total microbial count. Microbiological analysis was conducted on golden-striped jobfish fillets and on production sanitation water quality using TPC (Total Plate Count), Coliform, E. coli, Vibrio, and Salmonella parameters, in accordance with fishery industry standards.

Microbiological test results on fish samples that had been injected with CO showed that the TPC value in golden-striped jobfish fillet samples ranged from 2.6 × 104 to 5.3x104CFU/g, which is still below the maximum limit standard of SNI 2696-2013. The success in maintaining Coliform, E. coli, Salmonella, and Vibrio parameters at negative levels across all samples indicates that implementing strict sanitation protocols and controlling storage temperature has effectively prevented cross-contamination and the growth of harmful pathogens.

The difference in TPC values between samples stored in a chilling room at a constant temperature of 1-3°C to keep the fish meat cold, and stored for at least 6 hours, so that the CO gas

added to the fish can react optimally. This indicates that temperature management is a critical factor in inhibiting microbial growth. This finding aligns with modern fisheries industry best practices that emphasize the importance of cold chain management to ensure food safety. Microbiological testing results of pristipomoides multidens fish fillets are presented in Table 2, and a diagram of goldbanded jobfish fillet sensory test results is presented in Figure 4.

Table 2. Microbiological Testing Results of *Pristipomoides multidens* Fish Fillets

Component	Sample			*Maximum Limit* (SNI 2696-2013, ICMSF (International Commission on Microbiological Specifications for Foods), EOSQC (Food Safety Objectives), AFNOR (Association Française de Normalisation))
	1	2	3	
TPC (CFU/g)	$2.6x10^4$	$5.2x10^4$	$5.3x10^4$	*5.0 x 10^5*
Coliform (MPN/g)	<3	<3	<3	<3
E.Coli (MPN/g)	<3	<3	<3	<3
Salmonella	Negative	Negative	Negative	Negative
Vibrio Cholerae	Negative	Negative	Negative	Negative

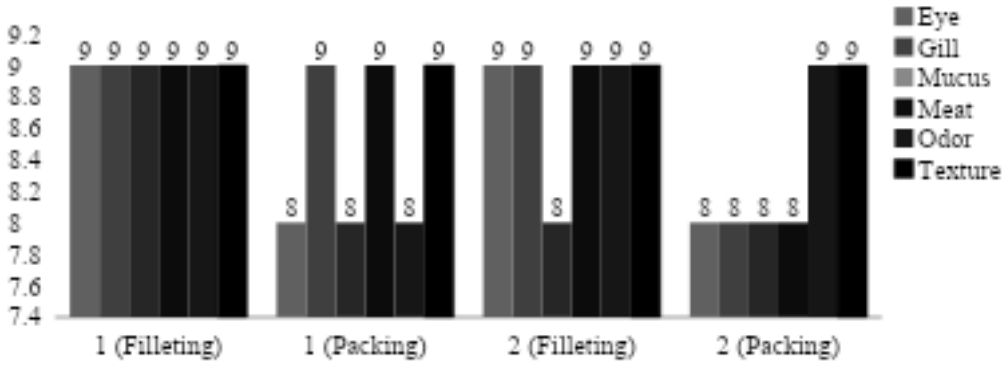

Figure 4. Diagram of Goldbanded Jobfish Fillet Sensory Test Results

Organoleptic Analysis

Organoleptic or sensory testing is a method that uses the five human senses as the primary tool to assess the quality of a product (Triyastuti *et al.,* 2023). Sensory testing is carried out by Quality Control on Goldbanded jobfish fillet raw material samples using a score sheet in accordance with SNI 2729:2013, which assesses 6 aspects: eye, gill, mucus, meat, odor, and texture.

In the process flow shown in Figure 1, the filleting process produces fillet products but has not been injected with CO. While the packing process sampling has been injected with CO. The sensory test results shown in Figure 4 indicate that Goldbanded jobfish fillets from the fish filleting process have a higher sensory value of 9 than those from the packing process (8). The sensory testing value in the filleting process is higher due to the use of high-quality raw materials, ensuring the fish meat remains fresh, compact, and red. However, the fish decreased in sensory value during the washing process due to increased moisture content, and because the tissues are exposed when in contact with water, making the fillets more mushy than before washing. So the company injects CO into fish fillets. The purpose of injecting CO is to give the fish fillets a bright red color. The addition of CO affects the color of tuna meat and panelist acceptance in the sensory test (Ariyani *et al*., 2020). According to Anne *et al.* (2017), the benefits of CO in fish in cold storage include increasing the value of fish meat packaging products by providing a red color effect, good color stability, improved taste, and improved fish meat quality, as it can reduce protein oxidation.

Color is a key quality parameter that consumers can assess physically. CO is a colorless, tasteless, and odorless gas produced by the incomplete combustion of carbon-containing materials. However, the use of CO in fish meat to maintain a bright red color has been controversial due to its ability to hide signs of spoilage (changes in color and texture) and its potential impact on food safety. Therefore, product development innovations for bright red color additives in fish meat that are sustainable and safe are needed. Ascorbic acid is a natural additive that can enhance the bright red color of fish flesh, making it safe for consumption under European Union standards. The appearance of the addition of CO and ascorbic acid is shown in Figure 5.

Ascorbic acid is a natural additive and antioxidant permitted by European Union standards. In addition to helping maintain the red color of meat, AA works by reducing the oxidized form of myoglobin (metmyoglobin) back to a brighter colored form (deoxymyoglobin/oxymyoglobin, depending on conditions), thereby increasing color intensity without synthesizing or producing pigment gas complexes as CO does. CO injection can enhance a strong and stable red color. Ascorbic acid offers a safer and more functional alternative by enhancing red color. Therefore, the addition of ascorbic acid is recommended to enhance aesthetic freshness perception, and CO use should be considered only when combined with strict texture and microbiological quality control.

(a) (b) (c)

Figure 5. Fresh Goldbanded Jobfish Fillet (a), With Added CO (b), With Added Ascorbic Acid (c)

In the research of Kim *et al*. (2019), the addition of organic acids (ascorbic acid, malic acid) to processed meat with fermented spinach results in a higher redness value. Ascorbic acid is an organic acid that has been widely used in the meat industry as a preservative and acid regulator, extending the shelf life of fish products, and is generally recognized as safe by the Food and Drug Administration (FDA) (Gu *et al.,* 2020). Research has shown that adding ascorbic acid to tilapia meat products improves their color. This is based on research showing that frozen fish fillets soaked in an ascorbic acid solution showed a significant increase in brightness during storage compared to other samples. Ascorbic acid has a strong effect on the reddish color of meat, but using high concentrations of ascorbic acid decreases its reddish color (Botinestean *et al.*, 2020). Therefore, ascorbic acid with optimal concentration can maintain the bright red color of fish fillets significantly better than CO treatment.

Goldbanded jobfish fillets supplemented with ascorbic acid exhibited a denser texture than the control samples. This phenomenon is likely due to reduced water-holding capacity, which promotes water loss from muscle tissue. Consequently, muscle fiber shrinkage increases the compactness of the muscle structure, resulting in a firmer, denser fillet texture.

The difference in texture was found in samples from the fillet and the packing room. Texture profiles were assessed qualitatively by a trained sensory panel using the finger pressure method, evaluating attributes such as elasticity and muscle tension in accordance with SNI 2729:2013. The quality level is measured on a scale of 1-9. The samples taken from the fillet room have passed the cooling stage when stored in the chilling room which has a temperature of 1-3 ° C, causing the sample to have a texture when the fish is still fresh (before CO injection) because the texture of the meat is still soft unlike the samples taken in the packing room, where the samples have passed the CO injection and freezing stages in the Air Blast Freezer (ABF) with a temperature range of -35°C to -40°C. Therefore, samples taken from the packing room have a hard texture due to the frozen fish fillets. The samples taken from the packing room were then melted to examine their texture. In the melted-frozen state, the texture of the fish meat becomes very soft and is easily fragmented. This is because the exposed fish tissue will then bind to water. Meanwhile, the texture of unfrozen fish meat remains compact. The function of using CO in the packaging of frozen fish meat products is to produce a soft texture in an anaerobic packaging system (Anne *et al*., 2017; Klinmalai *et al.,* 2021).

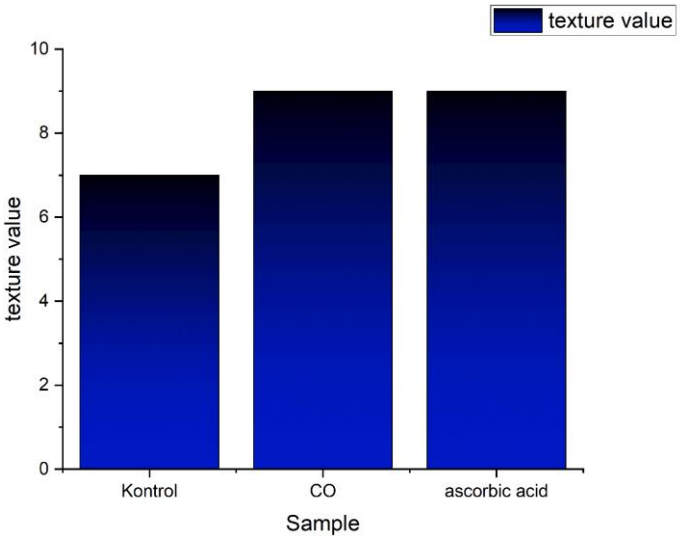

Figure 6. Texture Values on Goldbanded Jobfish Fillet Samples After Treatment

Ascorbic Acid Mechanism

Slow freezing forms large ice crystals that damage cell membranes and accelerate protein and lipid oxidation, resulting in reduced firmness and a brittle texture after thawing. Protein oxidation (increased carbonyls, decreased sulfhydryl groups, inactivation of Ca-ATPase) correlates with decreased firmness and gel properties of fish meat during frozen storage. Ascorbic acid is a water-soluble antioxidant that scavenges free radicals and helps maintain redox balance, including regenerating vitamin E, thereby reducing lipid and protein oxidation (Qi *et al*., 2022; T. Wang *et al*., 2017).

Fish fillet texture plays an important role in consumer acceptance of fish fillet products. Product development in improving texture quality by ensuring food safety and meeting nutritional needs. According to Botinestean *et al.* (2020), ascorbic acid is an alternative ingredient that can improve meat

tenderness and is acceptable to consumers. Ascorbic acid improves the chewiness of fish fillet meat (Klinmalai *et al.,* 2021). According to the research by Gu *et al.* (2020), the addition of ascorbic acid to tilapia meat products results in a chewy, compact texture. Thus, ascorbic acid is a natural ingredient, free of harmful chemicals, to improve the quality of tender, sustainable fish fillet products.

CONCLUSION

This study shows that ascorbic acid is a promising natural alternative to carbon monoxide (concentration 0.014%) in preserving the quality of Goldbanded jobfish fillets. A 0.03% ascorbic acid concentration was found to be optimal in this study, capable of maintaining the bright red color of the fish fillets while producing a firm, dense texture by inhibiting oxidation reactions in the fish meat. Although this study did not directly test the microbiological effects of the 0.03% ascorbic acid treatment, microbiological data from the use of CO shows that microbiological parameters can be maintained within safe limits with a Total Plate Count (TPC) ranging from 2.6×10^4 to 5.3×10^4 CFU/g, which is still well below the SNI 2696-2013 standard (5.0×10^5 CFU/g). More importantly, the test results confirmed the absence of harmful pathogens, with negative results for Salmonella and Vibrio cholerae, and Coliform and E. coli values below the detection limit (<3 MPN/g), in accordance with SNI, EU, and US standards. While the microbiological safety of CO-treated samples was confirmed to be within SNI and international standards, the 0.03% ascorbic acid treatment offers a promising alternative that aligns with EU safety regulations for natural additivesTherefore, further research is strongly recommended to conduct comprehensive microbiological testing on 0.03% ascorbic acid treatment to verify its ability to maintain microbiological safety parameters (Salmonella, Vibrio cholerae, Coliform, and E. coli) in accordance with SNI standards, (2) optimize the most effective duration of ascorbic acid immersion, (3) evaluate alternative concentrations for practical application in industry, and (4) test color stability and microbiological parameters during long-term storage. These practical recommendations will significantly contribute to the development of high-quality fish fillet products based on natural ingredients in the future, while meeting the increasingly stringent European market regulations on the use of synthetic additives. A limitation of the present study is that microbiological evaluations were performed only on CO-treated goldbanded jobfish fillets. As a result, a direct comparison of the effects of CO and ascorbic acid treatments on microbiological quality was not possible. Future research should incorporate microbiological evaluations of fillets treated with ascorbic acid to provide a more comprehensive assessment of the relative effectiveness of both treatments in maintaining fish fillet quality.

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