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Effect of garlic extract as a natural preservative based on protein profile of red snapper

Indonesian title: Pengaruh ekstrak bawang putih sebagai pengawet alami terhadap profil protein kakap merah

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ABSTRAK

Ikan kakap merah (Lutjanus bitaeniatus) merupakan salah satu sumber protein hewani yang memiliki kadar air cukup tinggi sehingga mudah mengalami pembusukan. Pembusukan dapat diatasi dengan menggunakan pengawetan. Bawang putih dapat digunakan sebagai pengawet alami ikan karena mengandung zat antibakteri antara lain allicin yang berperan dalam menghambat dan membunuh bakteri pembusuk, serta terdapat senyawa antimikroba lainnya seperti alkaloid, flavonoid, saponin, dan tanin. Penelitian ini bertujuan untuk mengetahui pengaruh ekstrak bawang putih sebagai pengawet alami terhadap profil protein ikan kakap merah. Rancangan penelitian dibagi menjadi lima bagian yaitu ikan kakap merah segar, disimpan 24 jam tanpa perendaman, dan tiga bagian direndam dengan ekstrak bawang putih masing-masing konsentrasi 5%, 10%, dan 20%. Tipe penelitian ini adalah eksperimental. Metode penelitian ini yaitu pembuatan ekstrak bawang putih dengan konsentrasi 5%, 10%, dan 20%. Perhitungan konsentrasi protein dengan metode Bradford, kemudian dilakukan SDS-PAGE untuk memisahkan protein berdasarkan berat molekulnya. Analisis hasil SDS-PAGE menggunakan aplikasi GelAnalyzer 19.1 untuk menghitung berat molekul protein dan profil protein dari perlakuan dibandingkan dengan ikan segar. Hasil penelitian menunjukkan konsentrasi protein tertinggi yaitu perlakuan ekstrak bawang putih konsentrasi 5% dibandingkan konsentrasi 10%, dan 15%. Profil protein ikan kakap segar dan perlakuan ekstrak bawang putih 5% tidak jauh berbeda dengan ikan kakap segar dengan jumlah pita protein 13 (9 pita mayor dan 4 pita minor), sedangkan ikan kakap segar 16 pita protein (13 pita mayor dan 3 pita minor). Kesimpulan dari penelitian ini adalah perlakuan perendaman ekstrak bawang putih dapat digunakan sebagai pengawet alami dengan konsentrasi terbaik adalah ekstrak bawang putih 5%.

Keywords: *bawang putih; kakap merah; profil protein.*

ABSTRACT

Red snapper (Lutjanus bitaeniatus) is a source of animal protein with a high-water content, making it prone to spoilage. Spoilage can be prevented through preservation. Garlic can be used as a natural preservative for fish because it contains antibacterial substances, including allicin, which plays a role in inhibiting and killing spoilage bacteria, as well as other antimicrobial compounds such as alkaloids, flavonoids, saponins, and tannins. This study aims to determine the effect of garlic extract as a natural preservative on the protein profile of red snapper. The research design was divided into five parts: fresh red snapper stored for 24 hours without soaking, and three parts soaked in garlic extract at concentrations of 5%, 10%, and 20%, respectively. This type of research was

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experimental. The research method was the preparation of garlic extract at concentrations of 5%, 10%, and 20%. Protein concentration was calculated using the Bradford method, followed by SDS-PAGE to separate proteins based on their molecular weight. The SDS-PAGE results were analyzed using the GelAnalyzer 19.1 application to calculate the molecular weight of the proteins and the protein profiles of the treatments compared to fresh fish. The results showed that the highest protein concentration was found in the 5% garlic extract treatment compared to the 10% and 15% concentrations. The protein profiles of fresh snapper and the 5% garlic extract treatment were not significantly different from fresh red snapper, with 13 protein bands (9 major bands and four minor bands). In contrast, fresh red snapper had 16 protein bands (13 major and three minor bands). This study concludes that garlic extract immersion treatment can be used as a natural preservative, with the optimal concentration being 5% garlic extract.

Keywords: *Garlic; Red snapper; Protein profile.*

INTRODUCTION

Indonesia is a maritime nation, predominantly oceanic. Its abundant natural resources offer the potential to meet its population's food and nutritional needs. Human resource development can be supported by a key dietary factor: adequate animal protein intake (Nastiti, 2013). Fishery products are a source of animal protein containing vitamins, micro-minerals, and long-chain unsaturated fatty acids (especially omega-3 fatty acids), and are relatively inexpensive compared to other protein sources, such as eggs, milk, and beef (Azhar, 2016; Kaiang et al., 2016). One such fishery product is red snapper (*Lutjanus bitaeniatus*) (Wahyuningsih et al., 2013) Lamongan-Jawa Timur. Penelitian ini bertujuan untuk mengetahui beberapa aspek parameter populasi (Lc, Lm, L , t 0, K, Z, E, M, F.

Red snapper (*Lutjanus bitaeniatus*) is one of the products exported abroad, especially from Sulawesi (Fitriani et al., 2023). Fresh red snapper has a water content of 79.31%, ash 1.92%, protein 16.30%, fat 0.05%, and carbohydrate 0.23% (Jacoeb et al., 2015). A frequent problem in the fisheries sector is maintaining quality because fish is a highly perishable food ingredient, requiring special handling to maintain its freshness, shelf life, and quality (Arifin & Nugroho, 2016). The fish spoilage process is caused by a reasonably high water content (80%), a near-neutral pH of the fish body, and fish meat that is easily digested by autolysis enzymes, causing the meat to be very soft, thus becoming a growth medium for spoilage bacteria (Adawiyah, 2014; Fitria et al., 2025; Fitria et al., 2024).

Fish damage or decay often occurs, including biological damage, chemical damage due to chemical reactions such as rancidity caused by fat oxidation, protein denaturation, and physical damage (Meliana et al., 2024; Fitria et al., 2025). Proper fish handling is necessary to maintain good condition when consumed by preserving fish. Preservation aims to prevent spoilage bacteria from entering the fish (Fitria et al., 2025). Natural preservatives are needed to ensure the safety of food products and can inhibit microbial growth in fish, thus reducing the risk of food spoilage due to microbial activity (Anggraini & Yuniningsih, 2016). Preservatives used include two types: chemical and natural preservatives. Chemical preservatives such as borax and formalin have harmful effects if ingested and continuously consumed, so awareness is needed from various parties to use natural ingredients that are inexpensive, readily available, safe, and have antimicrobial potential. One natural ingredient that can be used as a preservative is garlic (*Allium sativum*) (Tamal & Aryanto, 2018)the actual number of HF hospitalizations remains >1 million annually. More than 80% of patients who are hospitalized are initially seen in the emergency department (ED.

Garlic (*Allium sativum*) can be used as a natural preservative because it contains antioxidants and antimicrobials such as allicin, alkaloids, flavonoids, saponins, and tannins to inhibit the growth and development of microbes (Borlinghaus et al., 2014)allicin is a reactive sulfur species (RSS. Allicin acts as an antimicrobial substance that is more bacteriostatic than bactericidal, effective in inhibiting bacterial growth by damaging cell walls and inhibiting protein synthesis, as well as

preventing the degradation of proteins into simple molecules (Moulia et al., 2018; Syifa et al., 2013). The phenol group in antioxidants in garlic (*Allium sativum*) functions to donate hydrogen atoms for oxidation reactions, thereby maintaining the protein content in meat (Mayasari, 2015).

Pure garlic (*Allium sativum*) juice exhibits antibacterial potential against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Bacillus subtilis* (Gosal et al., 2021; Farizal, 2018; Aini & Shovitri, 2018; Ramadhani et al., 2021). Soaking in garlic extract provides better organoleptic values than the control, thereby maintaining fish meat's appearance, odor, and texture (Al Hakim et al., 2016). At optimal doses, garlic (*Allium sativum*) can reduce bacterial populations and maintain protein content (Sari et al., 2016). The protein content in treated fish can be determined by looking at the characteristics of its protein profile.

The characteristics of the protein profile can be determined using the SDS-PAGE electrophoresis method (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis). This polyacrylamide-based electrophoresis technique analyzes proteins by separating protein sub-units based on their molecular weight (Saputra, 2014). Previous research on the analysis of the protein profile of red snapper (*Lutjanus* sp.) based on SDS-PAGE with variations in marination time and acetic acid concentration showed that vinegar soaking affected red snapper protein; namely, the higher the concentration and duration of vinegar soaking in red snapper, the protein contained in the fish would experience denaturation (Jufri, 2017).

According to the description, there have been no reports on the protein profile of red snapper fish treated with garlic extract as a natural preservative using SDS-PAGE. This research evaluates the impact of garlic extract (*Allium sativum*) as a natural preservative, analyzed through the protein profile of red snapper fish (*Lutjanus bitaeniatus*) using SDS-PAGE.

Method

The study was conducted in December 2022 at the Molecular Biology Laboratory of Muhammadiyah University Semarang. The study design was experimental, detecting the effects of garlic extract (*Allium sativum*) on the organoleptic characteristics and protein profile of red snapper (*Lutjanus bitaeniatus*).

The materials used were red snapper from Tambak Lorok Market in Semarang, garlic (*Allium sativum*), Bovine Serum Albumin (BSA HIMEDIA MB083-25G), Biorad Protein Assay (BIO-RAD), dH_2O, loading buffer, electrode buffer, stacking gel, separating gel, Comassie Brilliant Blue (CBB) R-250 staining solution, destaining solution, and protein marker (SMOBIO PM5100) (Triwahyuni et al., 2018).

The equipment used was SDS-PAGE (ATTO WSE-1150 PageRunAce), a Thermo Scientific vis spectrophotometer (GENESYS 20), a centrifuge (HERMLE Z 326 K), a micropipette (Bio-Rad), a vortex (VM-300), a rotator (Daihan Scientific SHO-ID), an analytical balance (Mettler Toledo), and a digital dry bath.

Garlic Extract Production

Garlic extract (*Allium sativum*) is derived from juice. The garlic is cleaned well and diced into tiny pieces, then put in a blender and blended. After that, the garlic is strained to acquire the extract. Garlic extract is prepared in three concentrations: 5%, 10%, and 15%, using dH_2O as the solvent. Each concentration is prepared in 50 mL batches.

Sample Treatment

There were five distinct treatments for the samples: fresh control (fresh fish - KS), negative control (fish kept for 24 hours - KTP), fish treated with 5% garlic extract (KBP 5%), 10% (KBP 10%), and 15% (KBP 15%). The red snapper weighed approximately 200g and was soaked in pineapple extract at a ratio of 20% (w/v) between the sample and the extract. The fish were soaked in the extract for 15 minutes, taken out, and allowed to drain. Subsequently, they were kept in a container

for 24 hours. Table 1 displays the design of the treatment stage.

Table 1.
Treatment level plan

Sample Code	Group	Garlic extract soaking treatment	Storage Time
KS	Fresh	Without soaking	No storage
KTP	Control	Without soaking	24 hours
KBP 5%	5%	Soak for 15 minutes	24 hours
KBP 10%	10%	Soak for 15 minutes	24 hours

Source: Author (2022)

Organoleptic test

The tests were conducted by panelists using descriptive methods. Two panelists are laboratory assistants at the Biochemistry Laboratory. The assessment of the tested samples, which included the appearance, texture, and odor of each sample, was described on an assessment sheet. The results were analyzed based on specifications.

Preparation of Protein Standard Curve

The 12 microtubes were prepared, then BSA (Bovine Serum Albumin), distilled water, and BPA (Biorad Protein Assay) were pipetted according to Table 2 into a microtube. The concentration of BSA is one µg/µL, and BPA is 100% (ready to use). The mixture is then homogenized using a vortex and then incubated at room temperature for 10 minutes. The levels of each sample were read using a visible spectrophotometer at a wavelength of 595 nm. The results are recorded and entered into Microsoft Excel to obtain the equation of the line. The standard curve of protein was obtained by entering the absorbance value on the y-axis and the protein content of each standard solution on the x-axis. The equation formula is obtained after creating a standard curve (Meliana et al., 2024).

Table 2.
Preparation of BSA for Standard Curve

BSA (µL)	dH_2O (µL)	BPA (µL)
0 (blank)	800	200
0,5	799,5	200
1	799	200
2	798	200
3	797	200
4	796	200
5	795	200
6	794	200
7	793	200
8	792	200
9	791	200
10	790	200

Source: Meliana et al. (2024)

Determination of protein concentration

Fresh red snapper meat without treatment and red snapper with various garlic extract soaking treatment concentrations are prepared. Each piece of red snapper meat is cut into small pieces, weighed as much as 3 grams, ground with a mortar, added PBS 1X until the texture is like baby porridge, and homogenized.

The sample was placed into a conical tube, and PBS 1X was added up to ± 7 mL and vortexed, then centrifuged at a speed of 3000 rpm for 15 minutes. After centrifugation, the supernatant was removed as much as 2 mL and separated into another microtube. Then, a microtube was prepared for sample and blank readings. For sample reading microtubes, dH_2O was pipetted 798 µl, then 2 µl of the sample, and 200 µl BPA. While the microtube is for blank, dH_2O pipette 800 µl, then added with 200 µl BPA. Each microtube for sample and blank readings was homogenized by vertexing and incubated at room temperature for 10 minutes. After incubation, the samples and blanks were poured into a cuvette and then read on a visible spectrophotometer with a wavelength of 595 nm (Bradford, 1976). Protein concentration is calculated using the equation from the standard curve.

SDS-PAGE Electrophoresis

Sample preparation is prepared by pipetting the protein sample, sample buffer, and PBS 1X pH 7.4 according to calculations and putting into savelock microtubes. The calculation is in Table 3, and the concentration is made the same, which is 20 µg/µL. The total volume of each protein sample was 20 µL. The save lock microtubes are heated in a dry bath for 2 minutes at 100°C to break down the protein chains in the sample. Then, lifted and placed in a bowl containing water and ice cubes.

Table 3.
The calculation of the sample preparation

Sample	Protein Sample (µL)	PBS (µL)	Sample Buffer (µL)
KS	1.4	14.6	4
KTP	1.4	14.6	4
KBP 5%	2.2	13.8	4
KBP 10%	1.9	14.1	4
KBP 15%	1.7	14.3	4

Source: Authors (2022)

SDS-PAGE was conducted to distinguish proteins according to their molecular weight. Electrophoresis was conducted at 100 V for 90 minutes. Gels were colored with Coomassie Brilliant Blue R-250 for 1 hour on a shaker, followed by destaining using a destaining solution until the background was clear, retaining only the protein stripes marked. Gels were stabilized with 10% acetic acid and dried with a plastic press for roughly two days and kept in a dark location until examination (Laemmli, 1970).

Data Analysis

Data was analyzed by comparing red snapper's organoleptic characteristics and protein bands. The molecular weight of each protein band was analyzed using GelAnalyzer 19.1 software. The data obtained is then presented in the form of a descriptive narrative.

RESULTS AND DISCUSSION

Organoleptic Test

A panel of experts conducted the organoleptic testing. The results of the average organoleptic assessment are presented in Table 3. The results show a clear difference between fresh fish and fish with soaking treatment. In this assessment, the fish soaked in 5% garlic extract was almost the same as fresh red snapper, with the appearance of intact meat cuts, a dense and compact texture, and a neutral or non-rotten odor.

Table 4.
Organoleptic Test Assessment of Red Snapper

Garlic Extract Concentration	Organoleptic		
	Appearance	Texture	Odor
0 (fresh)	Whole, clean, bright milky white meat cut	Solid, compact, elastic	Very fresh, specific type of sea fish
0 (left for 24 hours)	Whole, clean, brownish cut of meat	Solid, compact, somewhat elastic	Not fresh, starting to rot
5	Whole, clean cut, milky white color less bright	Solid, compact, somewhat elastic	Neutral, onion smell is quite clear
10	Whole, clean, bright milky white cut of meat	Dense, less compact, starting to become soft	Not fresh, the smell of onions is clear
15	Whole, clean, bright milky white cut of meat	Not compact and soft	Not fresh, the smell of onions is clear

Source: Author's Analysis (2022)

The results of organoleptic tests on red snapper meat with treatment soaking in garlic extract produce fish with a distinctive appearance, which is cleaner and brighter, and

the special smell of onions produced is not too much stinging. Soaking garlic extract can increase the organoleptic value of fish (Al Hakim et al., 2017). The administration of garlic affects the elasticity and compactness of meat. Meat protein is closely related to water-holding capacity. The pH of garlic ranges from 5.93 to 5.97, which is acidic (Nurwantoro et al., 2012).

Soaking in an acidic solution will cause the pH to decrease and the meat muscles to shrink, so that the meat's ability to digest is reduced, and the binding water will decrease. The soaking process with garlic can cause the meat to become soft and fall apart, which is indicated by damage or decomposition of the structure of fish meat tissue due to active bacteria and increased proteolytic enzymes (Rumondor et al., 2023). Flavonoids influence the work of enzymes, but the flavonoids contained in garlic are relatively low. Damage to the protein structure in meat is due to the strength of the acid decreasing until the water binding force decreases, which causes water to enter the fish meat so that the texture of the meat softens (Djafar et al., 2014).

Total Protein Analysis

Total protein analysis with a spectrophotometer was used as a tool, as shown in Table 4. The protein sample concentration was calculated using the equation from the standard curve as shown in Figure 1.

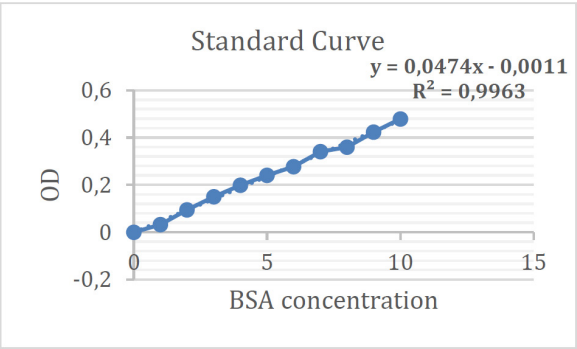

Figure 1.
Standard curve from protein standard (Bovine Serum Albumin)

Table 5.
Sample protein concentration

Sample	Concentration (µg/µl)
KS	14.589
KTP	14.263
KBP 5%	8.988
KBP 10%	10.465
KBP 15%	11.562

Source: Author's Analysis (2022)

The analysis of the protein concentration of fresh red snapper meat as a control showed the highest total protein compared to the others. The protein content of the treated fish was 14,589 µg/µl. Red snapper with 24 hours of storage without soaking has a protein concentration almost the same as fresh red snapper. There are differences in total protein based on the soaking treatment and storage. Red snapper with 5% garlic extracts soaking treatment has the lowest protein content, as shown in Table 4. The total protein content of red snapper meat treated with a concentration of 15% was 11.562 µg/µl, which gave a better value than the total protein content of red snapper meat treated with concentrations of 5% and 10% shown in Table 5.

This decrease in total protein concentration can be caused by protein denaturation. Dead fish will rapidly decompose due to the presence of microorganisms that break down the protein, resulting in protein damage (denaturation), which is characterized by the appearance of a foul odor because the sulfur-containing amino acids have changed into hydrogen sulfide (Wahyudi & Maharani, 2017). In addition, it is caused by the growth of microorganisms such as mesophilic bacteria, which continue to grow optimally at room temperature (25°C-30°C) (Karina, 2013). The number of bacteria in fish will increase with the length of storage due to the power supporting the increasing environment and protein content, which allows bacteria to grow optimally. In addition, the pH of the fish decreases so that it will activate autolysis enzymes, which will degrade proteins in fish (Lestari et al., 2020).

Results of Protein Profile Analysis Using the SDS-PAGE Method

The protein profile analysis of fresh red snapper meat after 24-hour storage without soaking and with soaking treatment shows garlic extract concentrations of 5%, 10%, and 15%, respectively shown in Figure 2.

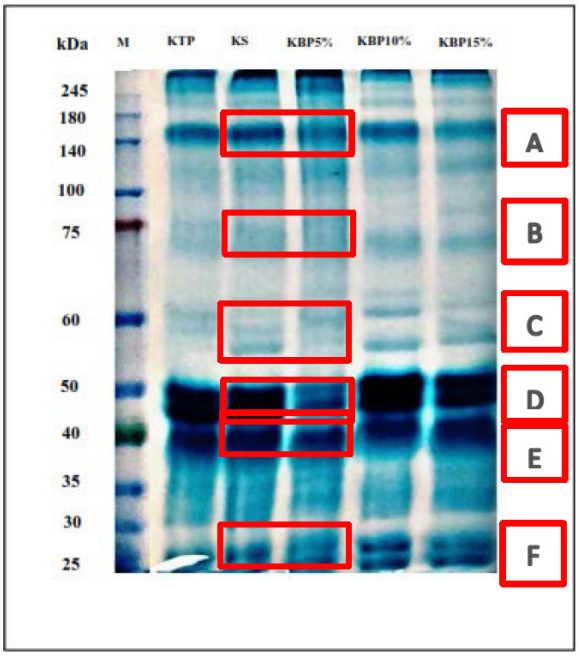

Figure 2.
SDS-PAGE electrophoresis results on sample (A) The red mark is a protein band that is common to both fresh fish and fish treated with 5% garlic extract
Source: Author's Analysis (2022)

Information:
M : Marker protein
KS : Fresh red snapper
KTP : Red snapper with 24-hour storage without soaking in garlic extract
KBP 5% : Red snapper with 24 hours storage with onion extract soaking white concentration 5%
KBP 10% : Red snapper with 24 hours storage with onion extract soaking white concentration 10%
KBP 15% : Red snapper with 24 hours storage with onion extract soaking white concentration 15%

Table 6.
Protein weight in the sample after calculation using the GelAnalyzer 19.1 application

Sample	Protein Bend (kDa)	
	Mayor	Minor
KS	193, 181, 145, 124, 76, 75, 69, 64, 60, 44, 40, 28, 25	121, 111, 85
KTP	173, 148, 67, 54, 49, 43, 28, 27	124, 93,76, 72, 61
KBP5%	185, 148, 124, 69, 54, 49, 41, 30, 28	104, 85, 64
KBP10%	181, 148, 116, 72, 61, 48, 41, 30, 28	100, 85, 76
KBP15%	181, 155, 69, 61, 54, 49, 41, 28, 27	133, 111, 95, 75

Source: Author's Analysis (2022)

Soaking treatment with garlic extracts also causes changes in protein subunits to occur, a decrease in the major band, and a change to the minor band, as shown in Figure 2, marked with letters B, C, and D. Table 6 shows the results of calculating the molecular weight of each sample using the GelAnalyzer 19.1 application. The protein profiles of fresh snapper and the 5% garlic extract treatment were not significantly different from fresh red snapper, with 13 protein bands (9 major bands and four minor bands). In contrast, fresh red snapper had 16 protein bands (13 major and three minor bands).

The study's results on visualizing the representation of red snapper protein bands from each soaking treatment caused changes in the protein subunits from major to minor bands, and some major protein bands slowly disappeared. The acid content found in garlic will speed up the work of enzymes in breaking down proteins into short-chain peptide groups or amino acids (Petalia et al., 2017). In addition, the contained tannins in garlic it will bind with protein to form H^+ ions, which can cause the pH to become acidic so that the protein will be denatured. Protein denaturation causes changes to the tertiary and qua-

ternary structures of proteins (Dewi et al., 2019).

Previous research has shown that garlic extract can help preserve fish food products. The compounds found in garlic are organosulfur compounds and organic compounds. Organosulfur compounds, namely allicin and ajoene, are unstable and not heat-resistant. Heat is the main bioactive sulfur component, which only forms when garlic is crushed or cut. Allicin is formed when the bulb part of the garlic is destroyed, so that it activates the allinase enzyme, hydrolyzes alliin, and produces intermediate compounds of sulfenic acid, pyruvate, and $NH3^{+}$. The working mechanism can suppress microbial growth so that meat decay activity can be inhibited, so that it can be used as a natural preservative (Al Hakim et al., 2016; Hendra, 2017; Mattulada et al., 2025; Borlinghaus et al., 2014allicin is a reactive sulfur species (RSS; Wu et al., 2015)as a template for investigations on the antibacterial activity of food ingredients. Staphylococcus epidermidis ATCC 12228 and the isogenic biofilm- forming strain ATCC 35984 were used to compare the activity of allicin against planktonic bacteria and bacterial biofilms. The minimal inhibitory concentration (MIC.

CONCLUSION

This study concludes that garlic extract can be used as a natural preservative for red snapper with an optimal concentration of 5% because the results of organoleptic tests and protein bands on SDS-PAGE are almost the same as fresh red snapper, even though the best protein concentration is red snapper without soaking treatment. The higher the concentration of garlic extract, the higher the level of protein denaturation, as indicated by the thinning and disappearance of protein bands. So, people can use garlic extract in small concentrations as a natural food preservative.

Research on the effects of garlic extract on red snapper preservation can be supplemented with bacterial count and identification to determine the number and types of bacteria that grow on red snapper. In addition, the protein profiles of preserved red snapper can be compared through immersion in natural extract, freezing, and cryopreservation.

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