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SDS-PAGE analysis of protein profile in mackerel tuna (*Euthynnus affinis*) preserved with bilimbi (*Averrhoa bilimbi)* extract

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Abstract: Mackerel tuna (*Euthynnus affinis*) is a valuable source of animal protein but is highly perishable due to its high water content. To extend its shelf life, natural preservatives offer a promising alternative to synthetic additives. Bilimbi (*Averrhoa bilimbi*) has potential as a natural preservative owing to its bioactive compounds, such as saponins, flavonoids, and polyphenols, which possess antioxidant and antimicrobial properties. This study aimed to evaluate the effect of bilimbi extract on the protein profile of mackerel tuna using SDS-PAGE electrophoresis to determine the most effective concentration for preservation. An experimental approach was used, with samples consisting of tuna flesh subjected to 24-hour storage, both untreated and treated with bilimbi extract at concentrations of 50%, 75%, and 100%. SDS-PAGE analysis was employed to assess protein degradation, supported by organoleptic evaluation to observe changes in appearance, texture, odor, and overall acceptability. The results demonstrated that treatment with 50% bilimbi extract preserved the protein integrity and sensory quality of the fish most effectively, showing characteristics closest to those of fresh tuna. These findings suggest that bilimbi extract at 50% concentration is optimal as a natural preservative for mackerel tuna, maintaining both protein structure and organoleptic quality during storage.

Keywords: Bilimbi; Mackerel Tuna; Protein Profile

INTRODUCTION

Mackerel tuna (*Euthynnus affinis*) is a widely consumed marine fish known for its high protein content. However, its high moisture level makes it highly susceptible to rapid spoilage. Common forms of fish deterioration include biological damage due to microbial activity, physical damage such as bruising and breakage, and chemical degradation involving lipid oxidation and protein denaturation[1]. Among these, microbial spoilage primarily caused by bacteria and fungi is the leading factor contributing to the decline in fish quality during storage[1].

To maintain quality and safety, appropriate preservation techniques are essential. While synthetic preservatives are commonly used, there is increasing concern regarding their potential health risks. Consequently, there is a growing interest in natural preservatives that are safe for consumption and capable of inhibiting microbial growth[2]. One promising natural preservative is bilimbi (*Averrhoa bilimbi*), a tropical fruit known for its antimicrobial and antioxidant properties.

Previous studies have demonstrated the antimicrobial activity of bilimbi extract against various pathogenic bacteria, including *Staphylococcus aureus*, *Propionibacterium acnes*, and *Escherichia coli*[3–8]. These studies highlight bilimbi's

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ability to suppress microbial growth, but they primarily focus on its bacteriostatic properties rather than its impact on biochemical aspects of fish preservation, such as protein stability. This presents a critical research gap, as protein degradation is a key indicator of fish spoilage and nutritional loss.

Bilimbi contains several bioactive compounds such as saponins, flavonoids, and polyphenols[9]. Among them, flavonoids are known to disrupt bacterial cell membranes, leading to leakage of cellular contents and enzyme inactivation, ultimately resulting in microbial death[10]. These mechanisms support bilimbi's role in preserving fish, but its direct effect on the structural integrity of fish proteins remains underexplored.

Limited research has investigated the molecular effects of bilimbi extract on fish protein stability using analytical techniques such as SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis). SDS-PAGE is a reliable method for profiling protein patterns based on molecular weight, enabling the detection of protein degradation during storage[11]. Assessing protein profiles through this method allows a deeper understanding of how natural preservatives influence fish quality at the molecular level. Therefore, this study aims to evaluate the effectiveness of bilimbi extract as a natural preservative by analysing the protein profile of mackerel tuna using SDS-PAGE. Addressing this gap is crucial for validating bilimbi extract's role not only as an antimicrobial agent but also as a stabilizer of protein integrity during fish storage offering a safe, accessible, and effective alternative to chemical preservatives, particularly in regions with limited access to refrigeration and modern preservation technologies.

MATERIAL AND METHOD

Research Design

This laboratory-based experimental study aimed to investigate the effects of bilimbi (*Averrhoa bilimbi*) extract on the protein profile and organoleptic properties of mackerel tuna (*Euthynnus affinis*). The research was conducted from April to May 2022 at the Molecular Biology Laboratory and Chemistry Laboratory, University of Muhammadiyah Semarang.

Tools and Materials

The instruments used included a food processor (blender), centrifuge (Gemmy PLC-03), mortar, cuvettes, visible spectrophotometer, conical tubes, SDS-PAGE electrophoresis apparatus (Atto), micropipettes (Bio-Rad), pipette tips, savelock microtubes, droppers, vortex mixer (VM-300), power supply unit (JY-ZY5), beakers, 5 mL Erlenmeyer flasks, analytical balance, and heat block. The materials used included Bovine Serum Albumin (BSA), Bio-Rad Protein Assay (BPA), bilimbi fruit, mackerel tuna, 1× PBS solution (pH 7.4), sterile distilled water (dH_2O), sample buffer, electrode buffer, Tris/HCl buffers (pH 8.8 and 6.8), 30% acrylamide solution, Coomassie Brilliant Blue R-250 (CBB) staining solution, destaining solution, 10% acetic acid, 10% SDS, 10% ammonium persulfate (APS), and TEMED.

Bilimbi Extract Preparation

Fresh bilimbi fruit was washed, chopped into small pieces, and blended. The extract was filtered and diluted to prepare three concentrations: 50% v/v, 75% v/v, and 100% v/v, each prepared in a volume of 50 mL.

Sample Treatment

Each treatment used 10 g of mackerel tuna, with three replicates per concentration (50%, 75%, and 100%). Samples were immersed in bilimbi extract for 15 minutes, then removed and drained. Treated fish samples were stored at room temperature for 24 hours. Control groups included fresh fish (untreated) and a sample stored for 24 hours without bilimbi treatment.

Organoleptic Assessment

Organoleptic evaluation was conducted by trained panelists using a descriptive method. Assessment criteria included appearance, texture, and odor, recorded on a standardized evaluation sheet. The results were analyzed descriptively to determine quality differences among treatments[12].

Protein Concentration Determination

Protein concentration was determined using the Bradford method. Tuna samples were cut into small pieces, and 3 g were homogenized with 1× PBS to achieve a paste-like consistency. The homogenate was transferred to a conical tube, vortexed, and centrifuged at 3,000 rpm for 15 minutes. A 2,000 µL aliquot of the supernatant was transferred to microtubes. For each reading sample, 798 µL of dH_2O, 2 µL of sample, and 200 µL of BPA reagent were mixed in a microtube. For the blank, 800 µL of dH_2O and 200 µL of BPA were combined. All tubes were vortexed and incubated at room temperature for 10 minutes. Absorbance was measured using a visible spectrophotometer at 595 nm. A standard curve using BSA was used to calculate protein concentration.

SDS-PAGE Protein Electrophoresis

Sample preparation involved mixing protein extract, sample buffer, and 1× PBS (pH 7.4) according to the required volume, followed by heat denaturation in a dry bath at 100°C for 2 minutes. Tubes were then cooled on ice. SDS-PAGE was performed to separate proteins based on molecular weight. Electrophoresis was run at 100 V for 90 minutes. Gels were stained with Coomassie Brilliant Blue R-250 for 1 hour on a shaker, then destained using destaining solution until background clarity was achieved, leaving only protein bands stained. Gels were fixed with 10% acetic acid, dried using a plastic press for approximately two days, and stored in a dark place until analysis.

Data Analysis

Protein bands from SDS-PAGE were analyzed using GelAnalyzer 19.1 software to determine molecular weight and assess protein degradation profiles.

RESULTS AND DISCUSSION

Organoleptic Test

The organoleptic evaluation revealed noticeable changes in mackerel tuna (*Euthynnus affinis*) after 24 hours of storage. As shown in Table 1, untreated fish samples exhibited faded coloration, less compact and elastic texture, and the emergence of slightly rancid odors. Similar characteristics were observed in fish treated with 75% and 100% bilimbi (*Averrhoa bilimbi*) extract concentrations. In contrast, samples treated with 50% bilimbi extract retained more favourable sensory attributes, closely resembling fresh fish in texture and odor.

Table 1. Assessment of Mackerel Tuna Organoleptic Test

Concentration (%v/v)	Organoleptic		
	Appearance	Texture	Smell
0 (fresh)	Whole, red color	Compact, elastic	Fresh
0 (24 hours incubation)	Whole, faded red color	Sufficiently elastic, rather compact	Less fresh, slightly rancid
50	Whole, faded red color	Sufficiently elastic, rather compact	Less fresh, slightly rancid
75	Whole, faded red color	Less elastic, soft	Starting to rot, slightly rancid
100	Whole, faded red color	Less elastic, soft	Starting to rot, slightly rancid

The faded colour observed in treated samples may result from protein denaturation triggered by the acidic nature of bilimbi extract, which disrupts protein structures and leads to coagulation[13]. Acidic conditions also affect water-holding capacity, initially producing a firmer texture; however, over time, this capacity decreases, resulting in softening of the fish tissue[14].

Protein Concentration

Table 2 illustrates that mackerel tuna soaked in 50% bilimbi extract maintained protein concentration similar to fresh samples. However, at 75% and 100% extract concentrations, protein levels declined significantly. This decline suggests that excessive acidity from higher extract concentrations accelerates protein denaturation and degradation.

Table 2. Sample protein concentration

Concentration of bilimbi Extract (v/v%)	Concentration (kDa)
0 (Fresh)	33,7575
0 (24-hour incubation)	32,2915
50	33,588
75	19,39
100	19,715

The reduction in protein concentration after 24 hours of storage without treatment is attributed to bacterial proteolytic activity. In samples treated with bilimbi extract, protein loss was primarily due to acid-induced denaturation. Acidic conditions destabilize protein structures and reduce essential amino acid availability, lowering the nutritional value of the fish[15].

Protein Profile Analysis (SDS-PAGE)

Protein profile analysis using SDS-PAGE revealed distinct differences in the number and intensity of protein bands between fresh and treated samples, as shown in Figure 1 and Table 3.

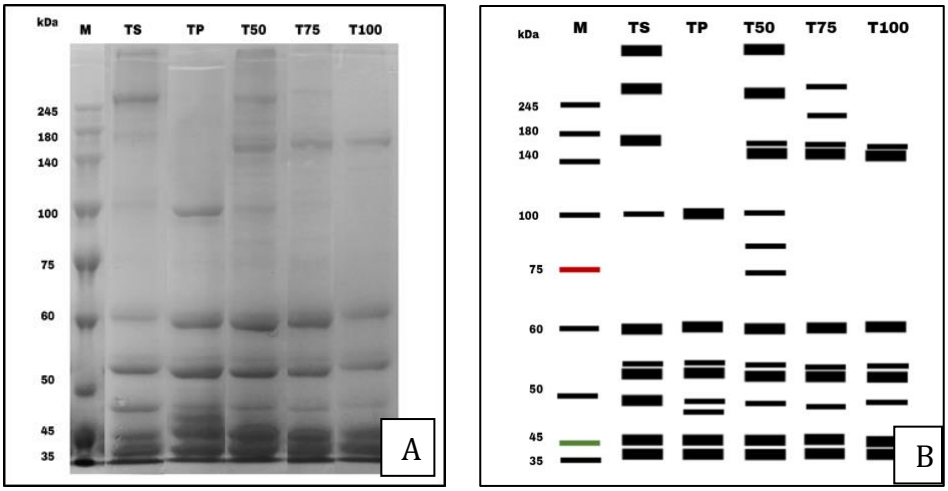

Figure 1. Results of SDS-PAGE electrophoresis of the sample (A) and visualization the result of SDS-PAGE

Caption:

M : Marker Protein
TS : Fresh mackerel tuna
T : Mackerel tuna with 24 hours storage without soaking in bilimbi extract
T50 : Mackerel tuna with 24 hours storage by soaking bilimbi extract at a concentration of 50% for 15 minutes
T75 : Mackerel tuna with for 24 hours storage by soaking bilimbi extract at a concentration of 75% for 15 minutes
T100 : Mackerel tuna with for 24 hours storage by soaking bilimbi extract at a concentration of 100% for 15 minutes

Table 3. Molecular weight of the sample

Sample	Molecular Weight (kDa)	
	Mayor	Minor
TS	474, 274,186, 55,48, 45, 44, 43	100, 49
TP	94, 54, 47, 44, 43	49, 45, 45
T50	445, 262, 161, 53, 48, 44, 43	178, 95, 79, 68, 49, 45
T75	169, 54, 48, 44, 43	283, 220, 185, 49, 45
T100	165, 55, 48, 44, 43	188, 49, 45

The fresh sample (TS) displayed eight major bands and two minor bands, indicating intact protein subunits. In contrast, the control sample stored for 24 hours (TP) exhibited a reduced number of bands, reflecting protein breakdown during storage. Samples treated with 75% and 100% bilimbi extract also showed a decrease in the number and variety of protein subunits, consistent with protein hydrolysis under acidic conditions.

Interestingly, the 50% extract-treated sample (T50) preserved a broader range of major and minor bands, suggesting reduced denaturation. The acidic pH of bilimbi extract likely caused protein coagulation via isoelectric shifts, disrupting peptide bonds and promoting hydrolysis[16,17]. This aligns with prior findings where vinegar and lime juice caused similar protein breakdown in fish[15].

The 50% bilimbi extract concentration appears optimal, maintaining protein integrity and limiting denaturation. These results demonstrate the extract's potential as a natural preservative with both antimicrobial and protein-stabilizing properties. Previous research has shown bilimbi's efficacy in inhibiting *Aeromonas salmonicida* and *Propionibacterium acnes* at relatively low concentrations[20,21], supporting its broad-spectrum preservative action.

The application of 50% bilimbi extract for 15 minutes followed by 24-hour storage at room temperature effectively preserved the organoleptic and molecular quality of mackerel tuna. These findings bridge the knowledge gap between bilimbi's antimicrobial potential and its molecular effects on protein preservation, highlighting its promise as a natural, food-safe preservative in fish products.

CONCLUSION

This study demonstrated that a bilimbi (*Averrhoa bilimbi*) extract concentration of 50% is optimal for preserving the quality of mackerel tuna (*Euthynnus affinis*). The 50% treatment effectively maintained protein integrity and organoleptic properties comparable to those of fresh fish, as evidenced by SDS-PAGE protein profiling and sensory evaluation. In contrast, higher concentrations of bilimbi extract (75% and 100%) led to excessive protein denaturation, likely due to increased acidity, which altered protein subunits and reduced nutritional value. These findings highlight the potential of bilimbi extract as a natural, eco-friendly, and sustainable alternative to synthetic chemical preservatives in fish preservation. Further research is recommended to expand the understanding of bilimbi's preservative capabilities by exploring the use of other plant parts such as the leaves or flowers. Future studies should also investigate variations in extract concentration, soaking duration, and storage time to optimize preservation efficacy. Additionally, incorporating microbiological analysis would provide valuable insights into the extract's effectiveness in inhibiting specific spoilage microorganisms and pathogens, thereby strengthening its application as a natural food preservative.

AUTHORS' CONTRIBUTIONS

MSF carried out the SDS-PAGE, determination of molecule weight, and compiling publication manuscript, SS performed extraction of bilimbi and protein isolation, and AIK carried out the sample treatment and measure the protein concentration.

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DATA AVAILABILITY STATEMENT

The utilized data to contribute to this investigation are available from the corresponding author on reasonable request.

DISCLOSURE STATEMENT

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors. The data is the result of the author's research and has never been published in other journals.

REFERENCE

1. Mareta, D. T., & Awami, S. N. 2011. Pengawetan Ikan Bawal dengan Pengasapan dan Pemanggangan. *Jurnal Ilmu-Ilmu Pertanian*, *7*(2), 33–47. http://dx.doi.org/10.31942/mediagro.v7i2.532
2. Anggraini, S. P. A., & Yuniningsih, S. 2016. Optimalisasi Penggunaan Asap Cair dari Tempurung Kelapa sebagai Pengawet Alami pada Ikan Segar. *Jurnal Reka Buana*, 2(1), 11–18. https://doi.org/10.33366/rekabuana.v2i1.660
3. Rachmawaty, D. & Arisanty. 2021. Pemanfaatan Perasan Buah Belimbing Wuluh (*Averrhoa bilimbi*) Sebagai Pengawet Alami pada Daging Sapi Segar. *Media Farmasi*, XVII (1), 31-36. https://doi.org/10.32382/mf.v17i1.1971
4. Kambaya, P.P., Jumiati, & Masyhudi. 2021. Uji Efek Antibakteri Ekstrak Etanol Buah Belimbing Wuluh (*Averrhoa bilimbi* L.) Sebagai Kandidat Bahan Medikamen Saluran Akar Gigi Terhadap Pertumbuhan *Staphylococcus aureus* Secara In Vitro. *Mulawarman Dental Journal*, 1 (1), 1-9. https://e-journals.unmul.ac.id/index.php/MOLAR/article/view/5034
5. Pramiastuti, O., Rejeki, D.S., & Maghfiroh, I. 2020. Uji Antibakteri Kombinasi Ekstrak Daun Belimbing Wuluh (*Averrhoa bilimbi* L) dan Daun Kersen (*Muntingia calabura* L) Terhadap *Staphylococcus aureus*. Parapemikir: Jurnal Ilmiah Farmasi, 9 (2), 33-41. http://ejournal.poltektegal.ac.id/index.php/parapemikir/article/view/2026
6. Afifi, R., Erlin, E, & Rachmawati, J. 2018. Uji Antibakteri Ekstrak Daun Belimbing Wuluh (*Averrhoa bilimbi* L) Terhadap Zona Hambat Bakteri Jerawat *Propionibacterium acnes* Secara In Vitro. *Quagga: Jurnal Pendidikan dan Biologi*, 10 (1), 10-17. https://journal.uniku.ac.id/index.php/quagga/article/view/803/0
7. Abdullah, N & Munadirah. 2021. Efektivitas Ekstrak Buah Belimbing Wuluh (*Averrhoa bilimbi* L) Dalam Menghambat Bakteri *Staphylococcus aureus*. *Media Kesehatan Gigi*, 20 (2), 13-20. https://doi.org/10.32382/mkg.v20i2.2546
8. Agistia, A., Arifin, M.Z., & Setyorini, E. Uji Efektivitas Antimikroba Ekstrak Daun Belimbing Wuluh (*Averrhoa bilimbi* L) Terhadap Bakteri *Eschericia coli*. *Jurnal Insan Cendekia*, 8 (1), 29-38, https://digilib.itskesicme.ac.id/ojs/index.php/jic/article/view/639/560

9. Sumiati, & Marjanah. 2020. Perbandingan Buah Belimbing Wuluh (*Averrhoa bilimbi*) dan Daun Kemangi (*Ocimum sanctum*) sebagai Bahan Pengawet Alami Ikan Kembung (*Rastrellinger sp*.). *Jurnal Jeumpa*, *7*(2), 422–432. https://doi.org/10.33059/jj.v7i2.3072
10. Rahmiati, A., Darmawati, S., & Mukaromah, A.H. 2017. Daya Hambat Ekstrak Etanol Buah Belimbing Wuluh (*Averrhoa bilimbi* L.) Terhadap Pertumbuhan *Staphylococcus aureus* dan *Staphylococcus epidermidis* secara In Vitro. *Prosiding Seminar Nasional Publikasi Hasil-Hasil Penelitian dan Pengabdian Masyarakat*. 669-774. https://jurnal.unimus.ac.id/index.php/psn12012010/article/view/2957
11. Saputra, F.R. 2014. Aplikasi Metode SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis) untuk Mengidentifikasi Sumber Gelatin pada Kapsul Keras. *Skripsi*. Uin Syarif Hidayatullah Jakarta. https://repository.uinjkt.ac.id/dspace/bitstream/123456789/26086/1/FAHRUR%20RAHMAN%20SAPUTRA-fkik.pdf
12. Darmawati, D., Natsir, H. & Dali, S. 2021. Analisis Total Volatile Base (TVB) dan Uji Organoleptik Nugget Ikan dengan Penambahan Kitosan 2,5%. *Indonesian Journal of Chemical Analysis*, 4 (1), 1-10. https://doi.org/10.20885/ijca.vol4.iss1.art1
13. Sitompul, Y. M. L., Sugitha, I. M., & Duniaji, A. S. 2020. Pengaruh Lama Perendaman dalam Air Perasan Buah Belimbing Wuluh (*Averrhoa Bilimbi Linn*) dan Lama Penyimpanan terhadap Karakteristik Ikan Tongkol (*Euthynnus Affinis*) pada Suhu Ruang. *Jurnal Ilmu Dan Teknologi Pangan (ITEPA)*, *9*(1), 71. https://doi.org/10.24843/itepa.2020.v09.i01.p09
14. Djafar, R., Harmain, R. M., & Dali, F. A. 2014. Efektivitas Belimbing Wuluh terhadap Parameter Mutu Organoleptik dan pH Ikan Layang Segar Selama Penyimpanan Ruang. *Jurnal Ilmiah Perikanan Dan Kelautan*, *II* (1), 23–28. https://docplayer.info/80817634-Efektivitas-belimbing-wuluh-terhadap-parameter-mutu-organoleptik-dan-ph-ikan-layang-segar-selama-penyimpanan-ruang.html
15. Asrullah, M. 2012. Denaturasi dan Daya Cerna Protein pada Proses Pengolahan Lawa Bale (Makanan Tradisional Sulawesi-Selatan) *Skripsi*. Fakultas Kesehatan Masyarakat. Universitas Hasanuddin Makassar.
16. Petalia, P., Julianti E., & Lubis L M. 2017. Pengaruh Berbagai Jenis Asam Jeruk terhadap Perubahan Mutu Ikan Mas Naniura Selama Waktu Display. *Jurnal Rekayasa Pangan dan Pertanian,* 5(1), 109-123. http://repositori.usu.ac.id/handle/123456789/21574
17. Nurjanna J, S., 2018. Profil Protein Ikan Bandeng *(Chanos-Chanos)* yang Direndam Jeruk Nipis *(Citrus Aurantifolia)* Berbasis SDS-PAGE. *Skripsi*. Universitas Muhammadiyah Semarang. http://repository.unimus.ac.id/3239/
18. Fadhila, R., & Darmawati, S. (2017). Profil Protein Daging Kambing, Kerbau dan Sapi yang Direndam Larutan Jahe Berbasis SDS-PAGE. *Prosiding Seminar Nasional & Internasional Semarang Indonesia*, 25-33. https://jurnal.unimus.ac.id/index.php/psn12012010/article/view/3109
19. Fitria, M.S., Sari, M.F., and Kartika, A.I. 2023. Effectiveness Of Kluwek Seeds (*Pangium edule*) As Natural Preservatives Material In Squid (*Loligo* Sp.) Viewed From Protein Profile Based On SDS-PAGE. Proceedings of the *International Conference of Community Health and Medical Sciences*, 1(1), 93-102. https://doi.org/10.17501/3021677X.2023.1111
20. Prayogo, Rahardja, B.S., dan Putri, R.W. 2011. Uji Potensi Sari Buah Belimbing Wuluh (*Averrhoa bilimbi* L.) dalam Menghambat Pertumbuhan Bakteri *Aeromonas salmonicida smithia* Secara *In Vitro*. *Jurnal Ilmiah Perikanan dan Kelautan*, 3(2), 165-168. https://doi.org/10.20473/jipk.v3i2.11602

21. Arisanty dan Dewi, R.P. 2018. Efektivitas Ekstrak Air Buah Belimbing Wuluh (*Averrhoa bilimbi*) Terhadap Pertumbuhan *Propionibacterium acnes*. *Media Farmasi*, 15(2), 66-71. https://doi.org/10.32382/mf.v14i2.601