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Note

Preservation of yellowfin tuna (*Thunnus albacares*) chunks using edible wraps prepared from under-utilised *Averrhoa bilimbi* fruit juice, infused with chitosan and fish gelatin

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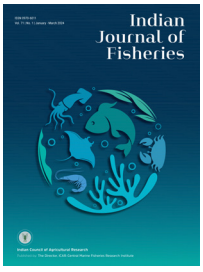

Abstract

The study aimed to investigate the bioactive properties of *Averrhoa bilimbi* fruit juice, focusing on its antioxidant and antimicrobial activities, and to evaluate its use as edible wraps infused with chitosan and fish gelatin for preserving tuna chunks under chilled conditions (4 °C). The wrap comprised *A. bilimbi* fruit juice (BJW 50% and BJW 100%) blended with fish gelatin (5%) and chitosan (0.5%). Antioxidant activity increased in a concentration-dependent manner and was positively correlated with the phenolic content of bilimbi juice. In the disc diffusion antimicrobial assay, 100% BJW showed the largest zone of inhibition against *Lysteria monocytogenes* (24.70±0.01 mm). During refrigerated storage, the psychrophilic bacterial counts in the control, GCW (gelatin-chitosan wrap), and BJW 50% wraps exceeded the acceptable limit of 7 log CFU g^{-1} by the 12th day of storage. In contrast, the samples wrapped with the BJW 100% remained within the acceptable limits until the 15th day, which corresponded well with the results of the antimicrobial assay, demonstrating the preservative potential of BJW. Similar trends were observed for lipid oxidation and spoilage indicators, Thiobarbituric acid reactive substances (TBARS) and total volatile base nitrogen (TVB-N) values, of the control, GCW, and BJW 50% samples exceeded permissible limits on the 6th day of storage, while BJW 100% treated samples exceeded the limits only after the 15th day. Overall, *A. bilimbi* juice-based edible wraps effectively maintained the microbiological and biochemical quality of tuna chunks, demonstrating their potential as a safe and effective biopreservative for seafood applications.

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Tuna fisheries in India are expanding, with an estimated resource potential of 213,000 t within the exclusive economic zone (INCOIS, 2013), contributing nearly 47.8% of large pelagic landings (CMFRI, 2023). Among these, yellowfin tuna dominates with 54% of the catch, followed by skipjack tuna (40%) and big-eye tuna (6%) (Hari Kumar, 2013). The tuna market is growing both nationally and internationally due to its high nutritional value, especially its protein and PUFA content, which increases consumer demand. This trend has prompted Indian processors to focus on producing sushi- and sashimi-grade tuna, as well as ready-to-eat and canned products, enhancing their global market appeal (Servusova *et al*., 2021). Tuna products are commonly marketed as frozen chunks, fillets, or canned goods. However, they face significant challenges in maintaining quality because of their high perishability. Processed tuna is highly susceptible to spoilage from lipid oxidation and microbial activity during processing, storage, and transportation, resulting in substantial economic losses for the industry (Bahram *et al*., 2020). While chemical preservatives have been used to prevent spoilage, concerns over their safety have led industries to seek natural preservatives with antioxidant and antimicrobial properties. These alternatives

aim to ensure product safety, protect consumer health, and promote environmental sustainability (Sathish *et al*., 2023a).

Bioactive edible packaging systems have recently emerged as innovative solutions to improve the nutritional content, quality, and safety of food products (Sathish *et al*., 2023b). Edible wrapping, a notable example, offers benefits such as being edible, biodegradable, bioactive, and having effective barrier properties (Sabir *et al*., 2021). It enhances the nutritional value and sensory qualities of products while incorporating active antimicrobial and antioxidant agents for controlled release, thereby prolonging product quality (Merino *et al*., 2019; Liu *et al*., 2020). These wrappings are typically composed of polysaccharides, proteins, lipids, or their combinations. In general, polysaccharide- and protein-based composite films provide improved mechanical and barrier properties (Bourtoom *et al*., 2009; Mohamed *et al*., 2020). Common materials such as chitosan and fish gelatin are often used to prepare composite films or wraps, demonstrating desirable characteristics like mechanical strength, transparency, and edibility (Wang *et al*., 2022). However, they exhibit lower antioxidant and antimicrobial activities than synthetic preservatives. To overcome this, bioactive compounds from natural extracts can be incorporated into wraps, allowing controlled release onto food surfaces to enhance preservation and maintain quality.

Averrhoa bilimbi, commonly known as bilimbi, is a tropical fruit native to South-east Asia and other tropical regions. It is considered an underutilised fruit with significant potential (Kumar *et al*., 2013; Seebaluck *et al*., 2019). The fruit is rich in ascorbic acid, oxalic acid, and various phytochemicals, which contribute to its antioxidant, antimicrobial, and pharmacological properties (Ferreira *et al*., 2021). Bilimbi extract has demonstrated significant antioxidant and antimicrobial properties (Norhana *et al*., 2009; Alhassan and Ahmed, 2016), making it an ideal candidate for food preservation. Incorporating bilimbi extract into chitosan-fish gelatin-based composite films enhances these properties and enables controlled release of active compounds, thereby preserving food quality for longer periods (Fitria *et al*., 2023). Therefore, the current research aims to explore the bioactive properties of bilimbi juice and to develop edible wraps infused with chitosan and fish gelatin to preserve tuna chunks under chilled conditions.

Fresh *A. bilimbi* fruits were collected from Thrissur District in Kerala, India, for the study. Chitosan, with a deacetylation degree of 90% and a viscosity of 150–500 cP, was purchased from SRL, Mumbai, India. Commercial fish gelatin was obtained from Sidi Vinayaka Industries in Gujarat, India. All other reagents and chemicals used in the study were of analytical grade and procured from Merck, Mumbai, India. Muller-Hinton Agar (MHA) and Brain Heart Infusion Broth (BHI broth) (Sigma-Aldrich) were used for antimicrobial evaluation. Bacterial cultures, *Listeria monocytogenes* (ATCC 19115), *Vibrio cholerae* (ATCC 14035), *Salmonella typhi* (ATCC 6539), *Pseudomonas fluorescens* (ATCC 1352), and *Escherichia coli* (ATCC 25922) were procured from Hi-Media Laboratories. The juice of *A. bilimbi* fruits was extracted using a commercial fruit juice extractor.

The total phenolic content of bilimbi fruit juice was estimated using Folin-Ciocalteu's method (Singleton, 1999). DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) assays were conducted to evaluate the antioxidant activity of bilimbi fruit juice. DPPH assay followed the method of Wangcharoen and Morasuk (2007). Briefly, a mixture consisting of 500 µl of bilimbi fruit juice, 500 µl of 99.5% ethanol, and 125 µl of 0.02% DPPH in absolute ethanol was prepared in amber test tubes. For edible wrap samples, 50 mg of wrap material was dissolved in distilled water, and an aliquot was used with slight modifications. The solutions were vortexed and incubated in the dark for 60 min, and their absorbance was measured at 517 nm using a UV-spectrophotometer. A control solution, containing only ethanol and DPPH, was used for comparison. The results were expressed as:

DPPH (% of inhibition) = (Control - Sample)/Control x 100

The FRAP assay was performed according to Benzie *et al*. (1999) with minor modifications. The FRAP reagent was freshly prepared by mixing 10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3 \cdot 6H_2O$ in distilled water, and 300 mM acetate buffer at pH 3.6. In a 96-well microtiter plate, 30 µl of extract at various concentrations was mixed with 270 µl of FRAP reagent. Ethanol served as a positive control. A calibration curve was created by plotting absorbance at 593 nm against $FeSO_4$ concentrations, which were then correlated with Trolox concentrations. FRAP values were expressed as mM Trolox equivalents (TE) per 100 mg l^{-1} of extract by comparing absorbance changes in the test mixture with those observed at increasing Fe^{3+} concentrations.

Edible wraps were prepared using two concentrations of bilimbi juice extract, namely 50% and 100% (v/v). Initially, 5 g of gelatin powder was added to the juice extracts, followed by 25 ml of 0.5% chitosan and 1 g of glycerol as a plasticiser. Similarly, a control wrap solution was prepared without bilimbi juice. The solutions were then stirred continuously at 45°C using a magnetic stirrer for 3 h. Subsequently, the wrap solutions were poured into uniform square silicone dishes and dried in a hot air oven at 45°C overnight. After drying, the wraps were carefully peeled from the drying dishes. The tensile strength of the edible wraps thus prepared was evaluated as per the method of Fitria *et al*. (2023).

Antimicrobial activity of the bilimbi fruit juice was evaluated by the well diffusion and disc diffusion methods. For the well diffusion assay, 10 mm wells were created in MHA plates, and 0.1 ml of inoculum was carefully applied to each well. Subsequently, 100 µl of bilimbi fruit juice (BJC 50% and BJC 100%) was introduced into the wells. For disc diffusion, 10 mm discs of bilimbi juice wraps (BJW 50% and BJW 100%) and a gelatin-chitosan wrap (GCW) as a control were placed on the same plate adjacent to two wells. The plates were incubated at 37°C for 24 h. Finally, the zone of inhibition was measured by determining the diameter with a Haloes caliper.

The edible wraps prepared were then used to evaluate their potential preservative effects on tuna chunks. For the study, yellowfin tuna fish (*Thunnus albacares*) was collected from a local fish market in Kochi, Kerala, India. The fish was transported to the research laboratory on ice and cut into approximately 50 g chunks. The chunks were wrapped with 50% and 100% BJW or GCW (control wraps) as the primary packaging method. Unwrapped fish chunks were kept as positive controls. The wrapped chunks were placed in sterile pouches and stored at 4°C, and samples were collected at 3-day intervals to evaluate their biochemical, microbial, and sensory qualities.

The oxidative stability of fish samples was analysed using the Thiobarbituric acid reactive substances (TBARS) value, following the method of Tarladgis *et al*. (1960), with some modifications. Briefly, 10 g of the macerated fish sample was treated with 100 ml of 2% HCl and then placed in the distillation unit. Fifty millilitre of distillate was collected from the distillation unit. Five millilitre each of the distillate and TBA reagent were combined in a test tube, whereas 5 ml of distilled water served as the blank. Finally, the test tubes were placed in boiling water for 30 min, and the absorbance was measured at 510 nm. The results were expressed as milligrams of malonaldehyde per kilogram of the sample (mg MDA kg^{-1}).

Changes in trimethylamine (TMA) and total volatile base nitrogen (TVB-N) values of tuna chunks were determined using the Conway diffusion method of Ali *et al*. (2008), with slight modifications. Briefly, 4 g of the sample was macerated with 16 ml of trichloroacetic acid (TCA) solution using a mortar and pestle. About 1% boric acid solution with bromocresol green and methyl red indicators was added to the inner chamber of the Conway unit. Then, 1 ml each of the sample and saturated potassium carbonate was added to the inner and outer rings, respectively. Then, 1 ml of 10% formaldehyde was added to the outer ring of the TMA unit, immediately followed by clockwise and anticlockwise rotations to ensure proper mixing. The unit was then incubated at 37°C for one hour. Finally, titration was performed in the inner ring using 0.02 N HCl and the total TVB-N content was expressed as mg of Nitrogen per 100 g of sample.

The pH of tuna chunk samples during chilled storage was measured using a digital pH meter (Kumar *et al*., 2023). Mesophilic and psychrophilic plate counts were performed according to the standard AOAC method (2023). The sensory scores of the tuna chunk samples during chilled storage were assessed following the methods of Meilgaard *et al*. (1999).

The phenolic content of the BJW extract increased with increasing concentrations from 20% to 100%. The total phenolic content of BJW ranged from 8.00±0.01 to 38.00±0.00 mg ml^{-1} gallic acid equivalent (Table 1). Hasanuzzaman *et al.* (2013) reported a slightly higher total phenolic content (50.23±0.56 mg of GAE per g of extract) in the aqueous extract of *A. bilimbi* fruits. Flavonoids and tannins are significant phenolic compounds that function as primary antioxidants or free radical scavengers (Danlami *et al*., 2013).

Table 1. Polyphenol content in *A. bilimbi* fruit juice and its antioxidant activities. Results are expressed as Mean±SD (n=3)

Concentrations	Polyphenols (mg ml^{-1} gallic acid equivalent)	FRAP (µg ml^{-1} ascorbic acid equivalent)	DPPH (% of inhibition)
20%	8.00±0.01	68.90±0.00	40.40±0.01
40%	14.00±0.00	77.60±0.00	56.40±0.01
60%	25.00±0.00	82.70±0.01	64.50±0.05
80%	34.00±0.00	86.90±0.03	76.60±0.05
100%	38.00±0.00	96.40±0.01	90.40±0.01

Table 2. clearly shows increase in DPPH scavenging activity of BJW with rising concentrations. The maximum percentage of inhibition was observed at 100% BJW (90.40±0.01%). The edible wrap solution with 100% BJW (combined with chitosan and gelatin) demonstrated 98.00±0.10% inhibition (Table 2). The FRAP assay yielded similar results, with higher concentrations showing increased activity (Table 2). The results of this study demonstrated a strong, positive correlation between total phenolic content and antioxidant assays.

Tensile strength measures stretchability, whereas elongation at break assesses the flexibility of the edible film. The elongation of the edible food wrap increased significantly relative to the control wrap when BJW was combined with chitosan and gelatin, resulting in a more flexible wrap. In contrast, the control wraps, which contained only bilimbi juice, which is rich in pectin, exhibited poor elongation properties (Table 2). However, the incorporation of chitosan and gelatin reduced the tensile strength of the edible wraps, while their combined use enhanced the plasticising effect. A similar trend was noted by Hosseini *et al*. (2013) during the preparation of fish gelatin and chitosan-blended edible films.

Bilimbi juice at 50% (BJC50%) concentration inhibited only *E. coli* (11.10±0.00 mm), indicating that this concentration was insufficient to effectively retard or minimise bacterial growth. Whereas bilimbi juice at 100% concentration (BJC100%) effectively suppressed the growth of all tested bacteria, with the maximum zone of inhibition against *V. cholerae* (18.40±0.01 mm), followed by *P. fluorescens* (16.10±0.01 mm) and *L. monocytogenes* (16.10±0.01 mm) (Table 3). Similar antimicrobial activity of *A. bilimbi* against both Gram-positive and Gram-negative bacteria has been reported previously (Zakaria *et al*., 2007; Prastiyanto *et al*., 2020; Phat *et al*., 2021), which has been attributed mainly to its flavonoid content. Among edible wraps, BJW 100% showed the maximum zone of inhibition against *L. monocytogenes* (24.70±0.01 mm), followed by *P. fluorescens* (23.50±0.01 mm) and *V. cholerae* (21.00±0.00 mm). On the whole, the incorporation of chitosan and gelatin into bilimbi juice-based wraps (BJW) significantly enhanced the zones of inhibition against all tested bacteria compared with BJW alone, demonstrating a synergistic antimicrobial effect.

TBARS value are widely used as indicators of lipid oxidation and fish spoilage (Connel, 1995). In this study, the initial TBARS value of wrapped samples was 0.34±0.03 mg MDA kg^{-1}, reflecting the freshness of the tuna chunks. During chilled storage, TBARS values

Table 2. Antioxidant properties and mechanical properties of edible food wraps. Results are expressed as Mean±SD (n=3)

Antioxidant properties			Mechanical properties	
Samples	DPPH (% of inhibition)	FRAP (µg ml^{-1} Trolox equivalent)	Elongation (%)	Tensile strength (kgf cm^{-2})
GW (Control)	21.30±0.94	54.00±0.60	4.78±0.01	33.00±3.20
BJW50%	71.30±2.30	125.00±0.20	129.00±0.80	26.00±2.40
BJW100%	98.00±0.10	136.60±0.57	141.00±0.03	13.00±0.60

Table 3. Antimicrobial activity of edible food wraps evaluated by disc and well diffusion assays (mm). Results are expressed as Mean±SD (n=3)

Microorganisms	BJC 50%	BJC 100%	BJW50%	BJW100%	GW (control)
Listeria monocytogenes	-	16.10±0.01	17.00±0.00	24.70±0.01	-
Vibrio cholerae	-	18.40±0.01	24.00±0.00	21.00±0.00	-
Salmonella typhi	-	11.30±0.01	-	17.40±0.01	-
Pseudomonas fluorescens	-	16.10±0.01	18.40±0.01	23.50±0.01	-
Escherichia coli	11.10±0.00	14.40±0.05	12.10±0.01	18.00±0.00	-

increased progressively with duration of storage (Fig. 1). The BJW 50% exceeded the acceptance limit of 2 mg MDA kg^{-1} (Connel and Howgate, 1996) on the 9th day, reaching 3.5±0.10 mg MDA kg^{-1}, while BJW 100% surpassed the limit only on the 12th day with a value of 3.54±0.30 mg MDA kg^{-1}. In contrast, gelatin-chitosan-wrapped (GCW) samples (2.1±0.02 MDA kg^{-1}) and control samples (2.63±0.01 MDA kg^{-1}) exceeded the limit by the 6 day of storage. The results clearly indicated that bilimbi juice effectively retarded lipid oxidation in tuna chunks during chilled storage. The phenols and polyphenolic compounds, including flavonoids, present in bilimbi juice provide significant antioxidant activity that plays a vital role in controlling oxidation during storage (Adeyemi and Olorunsanya, 2012; Adeyemi *et al*., 2013).

TVB-N values of samples wrapped with 50 and 100% BJW increased gradually from 4.5±0.00 to 35.75±0.20 mg 100 g^{-1} and 28.47±0.10 mg 100 g^{-1}, respectively, after 15 days of storage (Fig. 2). Generally, a TVB-N level of 30-35 mg 100 g^{-1} of muscle is recommended for iced fish (Connell and Howgate, 1986). In this study, samples wrapped in 50% BJW exceeded the acceptable limit on the 15th day of storage (35.75±0.20 mg 100 g^{-1}), whereas 100% BJW samples did not exceed the limit by that time. However, the control sample surpassed the limit (39.67±0.20 mg 100 g^{-1}) on the 12th day. TMA values of tuna chunks wrapped with BJW and stored under chilled conditions are shown in Fig. 3. TMA values of samples wrapped with GCW, 50% BJW, and 100% BJW increased from 1.49±0.00 to 18.38±0.00 mg 100 g^{-1}, 16.69±0.10 mg 100 g^{-1}, and 15±0.00 mg 100 g^{-1}, respectively, by the 12th day of storage. Generally, a TMA content of 10-15 mg 100 g^{-1} is considered acceptable for fish (Connell and Howgate, 1996). In this study, there was no significant difference ($p<0.05$) among tuna chunks wrapped with BJW, GCW, and the control.

The pH values of the control, GCW, 50%, and 100% BJW-wrapped samples decreased from the initial value of 6.0±0.00 to 5.83±0.00, 5.76±0.00, 5.90±0.00 and 6.00±0.005, respectively, on the third

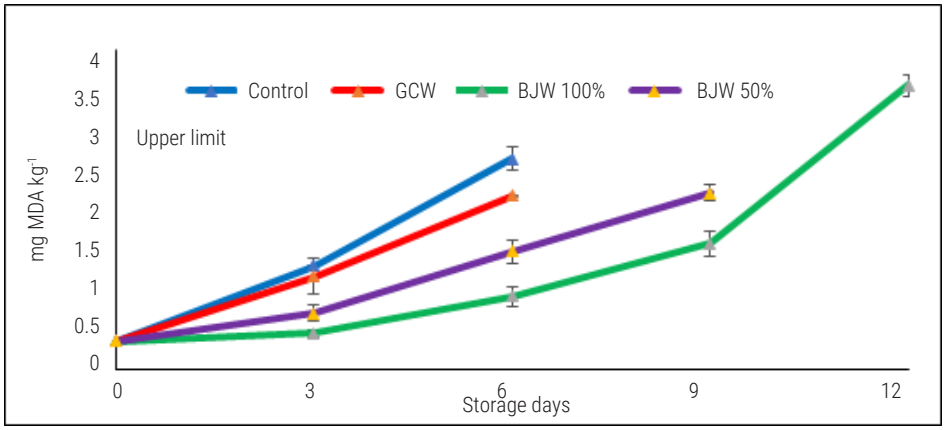

Fig. 1. TBARS value of tuna chunks during chilled storage at 4°C

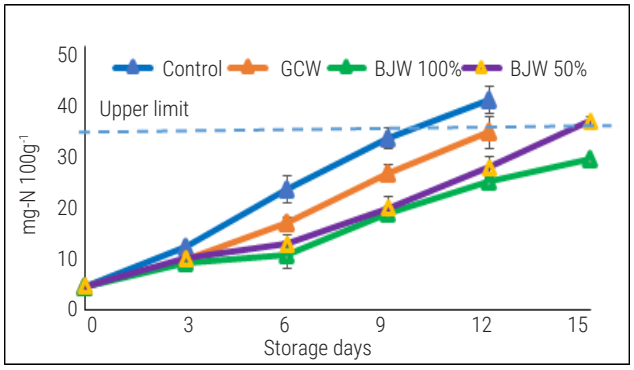

Fig. 2. TVB-N value of tuna chunks during chilled storage at 4°C

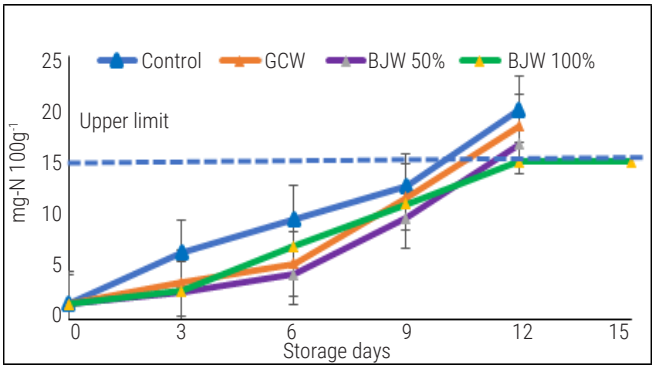

Fig. 3. TMA value of tuna chunks during chilled storage at 4°C

day of storage (Fig. 4). Subsequently, they declined further with increasing storage days. Overall, a significant reduction in pH was observed in all samples over 12 days of chilled storage, likely due to lactic acid production through anaerobic glycolysis and the release of inorganic phosphate, a product of ATP degradation (Sathish *et al*., 2023).

The initial mesophilic count of the sample was 4.86±0.02 log CFU g^{-1}, indicating the freshness of the tuna chunks (Fig. 5) . Throughout storage, mesophilic counts increased gradually in all samples. On the third day, in samples with BJW wraps, the mesophilic count decreased, likely due to the antimicrobial activity, of the wraps, which reduced bacterial growth. After three days, the bacterial count rose significantly. The control, GCW, and 50% BJW samples exceeded the acceptable limit of 7 log CFU g^{-1} on the 12th day of storage (8.71±0.00, 8.70±0.00, and 7.2±0.00 log CFU g^{-1}, respectively), while samples with 100% BJW surpassed this limit on the 15th day (7.27±0.02 log CFU g^{-1}). This highlights the effectiveness of the BJW wrap in controlling bacterial growth during chilled storage.

The psychrotrophic bacterial count, a key indicator of fish spoilage under chilled conditions, started at 4.04 log CFU g^{-1} and increased in all samples during storage (Fig. 6) . The psychrotrophic bacterial counts of the control and GCW samples surpassed the upper limit of 7 log CFU g^{-1} on the sixth day, reaching 8.12±0.02 and 7.24±0.02 log CFU g^{-1}, respectively. In contrast, samples wrapped with 50 and 100% BJW recorded counts of 7.35±0.00 and 7.16±0.00 log CFU g^{-1} on the 9th and 12th days, respectively. Overall, BJW demonstrated a superior antibacterial effect against both mesophilic and psychrotrophic bacteria, emphasising the potential of bilimbi juice as a natural antimicrobial agent.

A significant, gradual decline in sensory scores occurred across all samples during storage (p<0.05), consistent with findings by Sathish *et al*. (2023). The initial sensory score for tuna chunks was 8.5±0.00, with notable differences among samples from the third day of storage (Fig. 7). Control samples exceeded the acceptable limit on the sixth day (<6.00) due to odour development and colour changes. Conversely, samples wrapped with GCW, 50% BJW, and 100% BJW exceeded the acceptable limit on the 9th (5±0.00), 12th (5±0.00), and 15th (6±0.00) days of chilled storage, respectively. These changes were consistent with microbial and biochemical deterioration in the tuna chunks, suggesting that BJW can serve as a more effective primary edible wrap for extending shelf-life during chilled storage. Among the wraps, BJW 100% treated tuna chunks had a longer shelf-life, which directly correlated with antimicrobial activity.

The results of the study clearly demonstrate that the development of *A. bilimbi* fruit juice-infused composite edible wraps incorporating fish gelatin and chitosan represents a promising approach for fish preservation. Incorporation of this fruit juice into edible wraps along with other natural bioactive compounds can enhance the antioxidant and antimicrobial properties of the wraps, while reducing reliance on chemical preservatives and disposable packaging materials.

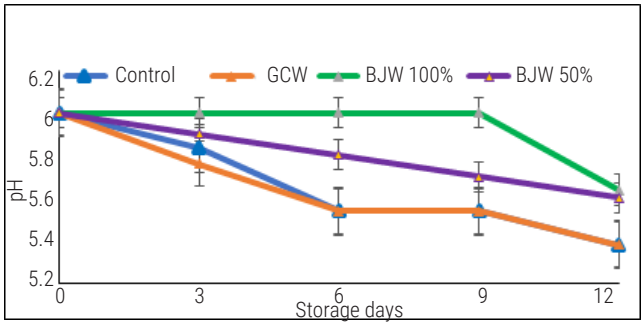

Fig. 4. pH value of tuna chunks during chilled storage at 4°C

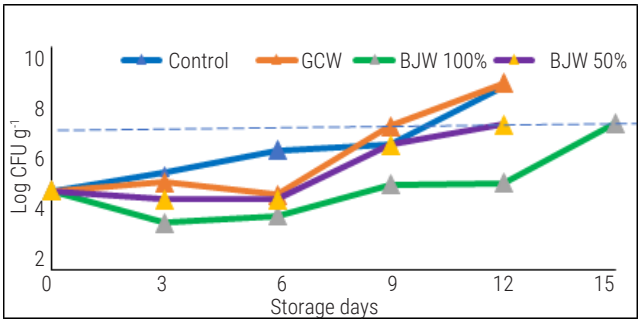

Fig. 5. Mesophilic bacterial count of tuna chunks during chilled storage at 4°C

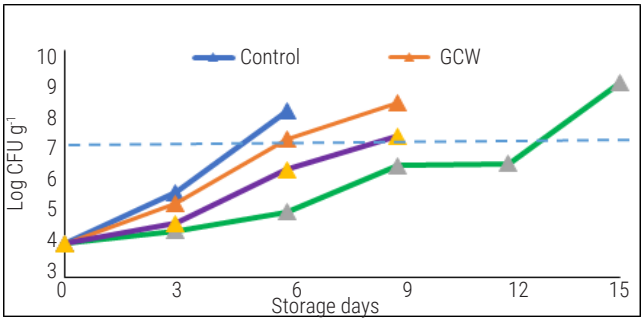

Fig. 6. Psychrotrophic bacterial count of tuna chunks during chilled storage at 4°C

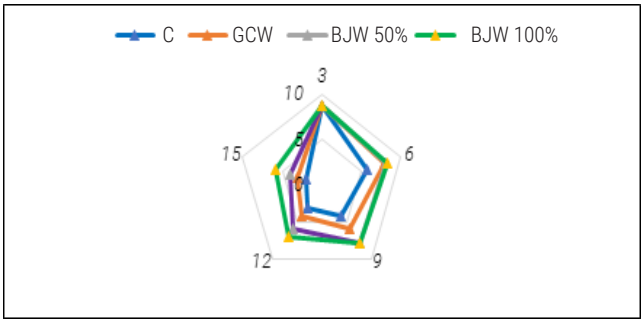

Fig. 7. Overall acceptability of tuna chunks during chilled storage at 4°C

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