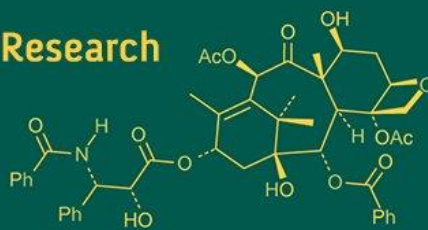


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Effects of whey protein isolate enriched with thyme essential oil on the extension of shelf life of skipjack tuna (*Katsuwonus pelamis*) steak under refrigerated condition

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Abstract

The seafood industry increasingly considers seafood shelf life as a critical problem. Some essential oils contain antibacterial and antioxidant characteristics that helps to keep meat safe. Edible coatings have beneficial effect on quality of fish and act as barrier against moisture transfer and uptake of oxygen. Edible coating made up of biodegradable materials is helpful to control the quality deterioration and enhance the shelf life. The present study evaluated the effect of whey protein isolate (WPI) coating enriched with thyme essential oil (TEO; 4, 6 and 8% v/v) on skipjack tuna (*Katsuwonus pelamis*) steak during storage at 4°C. Samples were analyzed at 0, 5, 10, 15, 20, 25 and 30 days for biochemical analyses (pH, Total volatile base nitrogen-TVB-N, pH, Trimethylamine nitrogen TMA-N, Peroxide value-PV), Microbiological (Total plate count, psychrotrophic bacteria, *E. coli*, *Staphylococcus aureus*), color characteristics (a* value, b* value and L* value) and sensory quality (color, odor, texture, appearance and overall acceptability). 5%WPI+8%TEO (T₄) coated samples had significantly lower variation in all these parameters over the time of storage compared to other coated samples (5%WPI (T₁), 5%WPI+4%TEO (T₂) and 5%WPI+6%TEO(T₃)), while these parameters increased greatly in control T₀(C) samples. As a result of this study, the addition of thyme essential oil to WPI coatings enhanced quality significantly better than that of the control and WPI coating without thyme essential oil during refrigerated storage and this effect increased with increase in the thyme EO concentration in WPI coating. Additionally, the coatings successfully provided antioxidant activity that could help to increase fish steak quality and favour their preservation. In conclusion, coating fish with whey protein isolate: Thyme essential oil (5:8) enhances the shelf life and quality of fish steak.

Keywords: Whey protein isolate, Thyme essential oil, bioactive coatings, Skipjack tuna (*Katsuwonus pelamis*), improved quality, Refrigerated storage, Shelf life

Introduction

Tuna fishes are considered the largest and most specialized commercially important group of species among all fish. One of the most popular raw meals is fresh tuna meat, which is frequently eaten as sushi or sashimi. Tunas are of great importance because of their high catches, Market demand for tuna remains high, higher economic value and extensive international trade. Among major tuna fisheries, skipjack tuna (*Katsuwonus pelamis*) is the largest fishery in all the oceans around the world and the largest marine fishery in India as well. Skipjack tuna is a major food both regionally and internationally (Mahaliyana *et al.*, 2015) ^[1].

Additionally, the muscle of marine fish such as tuna has long-chain fatty acids particularly omega-3 and omega-6 fatty acids, as well as important amino acids that are useful in the treatment and prevention of inflammatory disorders, tumours, hypertension and cardiovascular illnesses (Shi *et al.*, 2016) ^[2]. One of the most popular food commodities traded globally is still fish and fishery products.

In addition, because of deterioration from lipid oxidation and microbial contamination, it loses its freshness more quickly than any other muscle food. Refrigeration and freezing have the least impact on the nutritional attributes of fish (FAO, 2020) ^[3]. The activity of bacteria and enzymes embedded in fish causes fish deterioration (Arannilewa *et al.*, 2005) ^[4]. Due to these actions occurring in fish, ammonia and tri-methylamine (TMA) are produced, which

results in the hardness and spoilage of seafood. Refrigeration and freezing have the least impact on the nutritional attributes of fish (FAO, 2020)^[3].

In addition to refrigeration or other packaging systems, edible film coatings have proven to be a successful preservation technique for extending the shelf-life of food and preserving the quality of several fresh chilled food products. Edible coatings could also function as a vehicle for antibacterial substances (Quintavalla & Vicini, 2002; Zhou *et al.*, 2010)^[5, 6].

Since they can extend the shelf life of the product, coatings made from polysaccharides, lipids and protein components are employed in food preservation. The thin layer of food that forms on the surface of foods, which may be consumed with the food and arises from natural sources, is known as an edible film or coating. They increase food's ability to be preserved for an extended period and improve its organoleptic qualities, nutritional profile and quality when made from food and natural sources (Ayana & Turhan, 2010)^[7]. Additionally, the use of coatings on food surfaces can develop a physical barrier against gases and moisture, which helps to inhibit the development of microorganisms on the surface of coated foods (Khodaei *et al.*, 2021)^[8].

The production of coating and film from various sources of proteins and carbohydrates allows for the safe application of these materials to increase seafood quality and extend shelf life. Edible coatings and films are thin layers of edible ingredients that are immersed, brushed, sprayed or wrapped around foods or even placed inside of them (Gómez-Estaca *et al.*, 2009)^[9].

The main purpose of edible films and coatings is to improve the quality, extend the shelf life and ensure the safety of the food by preventing the transfer of moisture, gases (including O₂ and CO₂), lipids, smells and oils into or out of food (Lacroix & Vu, 2014)^[10]. Proteins have a higher potential to produce films with stronger mechanical and barrier properties than polysaccharides (Cuq & Guilbert, 1998)^[11]. Whey protein isolated (WPI) which is edible, biodegradable and readily available can be used to create active packaging as an oxygen barrier (Ramos *et al.*, 2012)^[12].

The number of microorganisms on the outer coating of food can be reduced by adding additives like preservatives and antioxidants to edible films and coatings (Ayana & Turhan, 2010)^[7].

An edible film combined with an antibacterial or antioxidant substance is of great significance for filling this gap in fish preservation. Since ancient times, plants, herbs and their essential oils have been utilized in the food industry. Natural substances with antimicrobial and/or antioxidant properties, such as essential oils and herb extracts have recently been thoroughly studied as potential substitutes for synthetic preservatives in packaging films and materials. These substances give an additional barrier against potentially harmful contaminating microorganisms and a substitute for synthetic antioxidants in reducing rancidity (Yasin & Abou-Taleb, 2007; Ojagh *et al.*, 2010; Song *et al.*, 2011)^[13, 14, 15].

Essential oil (EO) application is an effective preservation technique that enhances the shelf life of fresh foods (Anyanwu *et al.*, 2017; Quital *et al.*, 2009)^[16, 17]. Carotenoids, retinoids, tocopherols, ascorbic acid, phenolic acids, flavonoids and polyphenols derived from plant extracts are known for their antibacterial action. EOs are liquids with strong aromas that are derived from plant matter. TEO has also reportedly been shown to have

antioxidant properties similar to those of -tocopherol and BHT (Miguel *et al.*, 2004)^[18]. *Thymus vulgaris*, which is utilized as a flavoring ingredient, has significant antibacterial potential due to the presence of thymol in it (Burt, 2004)^[19]. The inhibitory effects of various essential oils on various microorganisms asserted that gram-positive bacteria are a bit more susceptible than gram-negative organisms (Burt, 2004; Hammer *et al.*, 1999)^[19, 20]. Gram-negative bacteria's hydrophilic lipopolysaccharide cell wall prevented hydrophobic essential oils from penetrating the cell membrane. On the other hand, Gram-positive bacteria that lack such cell wall features are more susceptible to essential oil accumulation (Bajpai *et al.*, 2008)^[21].

Among the saltwater commodities with great nutritional and economic value are tuna and fish species related to tuna that belong to the Scombridae family. The biggest and most commercially significant species of fish are considered to be tuna fish. canned tuna is the most widely consumed efficient protein seafood item that is simple to ship and store with little to no refrigeration expense (Chakma *et al.*, 2022)^[22]. Tuna, especially skipjack tuna (*K. pelamis*), is commercially significant in international trade, where the European Union represents its main market and holds a dominant position in the global seafood market. Tuna is an extremely nutritious and economically exportable marine fishery product to developed countries (Lewis *et al.*, 2011)^[23].

The objective of the present investigation was

1. To study the effect of whey protein isolate enriched with thyme essential oil on the quality of refrigerated stored tuna steak (*Katsuwonus Pelamis*).
2. To find out the effect of whey protein isolate enriched with thyme essential oil on the shelf life extension of tuna steak (*Katsuwonus Pelamis*) stored at refrigerated condition under air pack.

Materials and Methods

Materials

Fresh skipjack tuna (*Katsuwonus pelamis*) with an average weight of 2.15 ± 0.2 kg and a total length of 43.26 ± 4.87 cm was purchased from the Veraval fish landing center and transported to the Laboratory of Fish Processing Technology in an iced Styrofoam box with a temperature that ranged from 0 °C to 4 °C. Whey protein isolate was purchased from local shop of Veraval city of (Gujarat). Thyme essential oil was supplied from a local herbal store. All the chemicals and reagents were procured from M/S Fisher Scientific, India Pvt. Ltd. and SRL (Sisco Research laboratories) Pvt. Ltd, India.

Methods

Edible Coating Preparation

The whey protein isolates and thyme coating was prepared according to the method described by Seydim & Sarikus (2006)^[24]. Whey protein isolate was dissolved in distilled water and glycerol was added. The pH was adjusted to 8.0 with 2N NaOH. Then, solutions were heated to 90 ± 2 °C while being stirred continuously. Candelilla wax was added during heating. The coating solutions were filtered through a layer of cheesecloth. For the preparation of thyme oil, it was mixed with tween 80 as a surfactant. Thyme oil in 4%, 6% and 8% ratios (v/v) were added to the coating solutions. After homogenizing for 2 min, solutions were cooled to room temperature for 1.5 h.

Treatment and Storage of Fish Samples

The prepared skipjack tuna steak was divided into five treatments. WPI coatings were then applied to the fish fillets by the dipping method for 2 min at room temperature. In the first treatment control (T_0) skipjack tuna steak was packed in air in LDEP pouches without any treatment and stored at refrigerated temperature (0-4 °C). The control fish steaks were dipped into distilled water using the same manner as the treated sample. In T_1 treatment skipjack tuna steak was dip-treated with whey protein isolate (5%). In T_2 treatment skipjack tuna steak was dip-treated with thyme oil (4%) and whey protein isolate (5%). In the T_3 treatment skipjack tuna steak was dip-treated with thyme oil (6%) and whey protein isolate (5%). In the T_4 treatment skipjack tuna steak was dip-treated with thyme oil (8%) and whey protein isolate (5%). After being treated for 2 minutes, the steak was removed from the solution, skipjack tuna steak was drained and packed individually in pouches followed by packing and stored at refrigerated temperature (0°C±4 °C). During refrigerated storage, the samples (5 bags each) were randomly taken and subjected to analyses every 5 days up to 30 days.

Proximate Composition of Fish

AOAC 2006 ^[25] official methods used to determine moisture, ash content, crude fat contents and crude protein.

Biochemical quality evaluation

Determination of Trimethylamine Nitrogen (TMA-N)

TMA was determined as trimethylamine nitrogen (TMA-N) by the micro diffusion method described by Beatty and Gibbons (1937) ^[26].

Determination of Total Volatile Base Nitrogen (TVB-N)

Determination of the total volatile base in the sample was determined as total volatile base nitrogen (TVB-N) by the micro diffusion method described by Beatty and Gibbons (1937) ^[26].

Determination of pH (pH meter method)

Determination of pH in the sample was determined by the pH meter method described by AOAC (2006) ^[25]. A 10 g of skipjack tuna steak meat was homogenized in 20 ml of distilled water and the mixture was filtered. The pH of the filtrate was measured using a digital pH meter (U TECH INSTRUMENTS - 733060) at ambient temperature.

Determination of Peroxide Value (PV)

The peroxide value (PV) of skipjack tuna steak determined from the lipid extract iodometrically method according to Jacobs (1958) ^[27].

Physical quality evaluation

Instrumental color analysis

The colour of the fish sample was measured using a portable colorimeter. Food colour is typically measured using the international standard colour parameter 'L*' -value, 'a*' -value and 'b*' -Value (Subhashree *et al.*, 2020) ^[28]. The sensor was standardized with white and black tiles for the analysis. 'L*', 'a*' and 'b*' -values were recorded. The initial values of 'L*' 'a*' and 'b*' in the fish sample were 46.17, -5.04 and 5.33, respectively.

The 'L*' -value represents lightness and ranges from zero (the darkest black) to 100 (the brightest white). The 'a*' -

value represents "redness" or "greenness" and ranges from +120 for absolute red to -120 for absolute green, while the 'b*' -value represents "yellowness" or "blueness" and ranges from +120 for absolute yellow to -120 for absolute blue.

Sensory Characteristics

Sensory assessments conducted by a panel of multiple members were recognized as the most often utilized methods for assessing meat products' organoleptic quality and spoilage judgments (Luong *et al.*, 2020) ^[29]. Sensory evaluation is based on a 9point hedonic scoring method described by (Peryam & Pigram, 1957). Where stored samples were assessed by 10 panelists a score of 9 indicates the better quality, 1 for very poor quality with all signs of decomposition and 5 was acceptable level. Lipid formation and myoglobin oxidation in fresh meat is often associated with changes in acceptable odour, taste, colour and rancid flavor (Pires *et al.*, 2018; Luong *et al.*, 2020) ^[29]. Tuna was considered unsuitable for human consumption when a final score < 5. The appearance, colour, odour, texture and overall acceptability of the samples were examined.

Microbiological Analysis

Preparation of sample 10g of skipjack tuna steak sample was taken in a sterile sample dish and transferred to a sterile plastic bag. The sample was homogenized with 90 ml of sterile phosphate buffer or normal saline solution. Then the homogenized sample was transferred in a stomacher blender and diluted 10 times i.e., 10^{-1} . For further dilution, 1 ml from 10^{-1} dilution was mixed with 9 ml of the appropriate for plating i.e., 10^{-2} , 10^{-3} , and 10^{-4} .

Total Plate Count

The microbiological characteristic of fish sample was carried out according to the standard method recommended by AOAC (2006) ^[25]. Plate Count Agar (PCA) was used for the enumeration of total plate count. 1ml each from the required dilutions was transferred in separated serial petri dishes in duplicates. 18-20 ml of sterile Plate Count Agar (PCA) was spread on petri dishes. Mixed well and allowed to solidify. Incubation of petri dishes was done at 37 ± 1 °C for 48 hours. After 48 hours the colonies were counted.

TPC (CFU/g) = Average No. of colonies x Dilution factor.

Total Psychrotrophic Count The microbiological characteristic of fish sample was carried out according to the standard method recommended by AOAC (2006) ^[25]. Plate Count Agar (PCA) was used for the enumeration of total plate count. 1ml each from the required dilutions was transferred in separated serial petri dishes in duplicates. 18-20 ml of sterile Plate Count Agar (PCA) was spread on petri dishes. Mixed well and allowed to solidify. Incubation of petri dishes was done at 7 ± 1 °C for 5 days. After 5 days the colonies were counted.

TPC (CFU/g) = Average No. of colonies x Dilution factor.

Escherichia coli

Determination of E. coli in fish sample was carried out according to the standard method described by AOAC (2006) ^[25]. 10 g of fish sample was taken aseptically and homogenized with 90 ml of physiological saline, which give 10^{-1} dilution. 1 ml of inoculums from the dilution was spread on 0-15 ml of sterile Tergitol-7 (T-7) agar plats. The plates were incubated at 37 ± 1 °C for 18-24 hours. E. coli

colonies on T-7 agar plates appeared as circular, non-mucoid, flat, and yellow colour with a pinkish tinge. The addition of Triphenyl tetrazolium chloride (TTC) permits the confirmation of *E. coli*. Positive colonies were counted and the result was expressed as the number of colony forming units per gram of sample.

Staphylococcus aureus

Determination of *S. aureus* of fish sample was carried out according to the standard method recommended by AOAC (2006) [25]. 1 ml of inoculums from the dilution was spread on 0-15 ml triplicate plate of Baird parker agar (BPA) medium and equitably distributed 1 ml inoculum over the triplicate plate. The inoculum was spread over the agar surface using sterile bent glass streaking rods (hockey sticks). The plates were incubated in an upright position in the 35-37 °C incubator for about 1 hour. Then the plate was inverted and incubated at 35-37 °C for 45-48 hours. The colonies were counted and calculated and the result was expressed as the number of *S. aureus* per gram of sample.

Statistical Analysis

Statistical analysis was based on triplicate analysis for each sample at each specific storage time. ANOVA (Analysis of variance) statistical technique was used to find out the significant difference in samples between the treatments as per the standard statistical methods (Snedecor and Cochran, 2014). The analysis of variance (ANOVA) was carried out based on the experimental data using IBM BASIC Windows release 1.13 to know the significant difference between different treatment combinations and to find the best treatment combination. Duncan's multiple range tests and variance analysis were used to evaluate the significance level for statistical differences.

Results and Discussion

Biochemical Changes

TVB-N

TVB-N is a freshness indicator and its level increases due to the formation of volatile compounds such as trimethyl amine nitrogen (TMA-N) and ammonia in fish by bacterial action (Fraser & Sumar, 1998) [30]. Depending on the species, handling and processing methods the TVB-N values in fish can vary. Its increase during storage is related to the activity of spoilage bacteria and endogenous enzymes. The limit of acceptability of TVB-N value is generally considered as 30-35 mg/100. According to the quality classification, the TVB-N value for "very good" products should be up to 25 mg/10 g, for "good" products up to 30 mg/100 g, for "marketable" products up to 35 mg/100g and for "spoilage" food greater than 35 mg/100 g was examined (Varlik *et al.*, 1993). The TVB-N values of skipjack tuna steak were 10.50, 10.42, 10.30, 10.12 and 10.04 (at initial storage day) and 51.32, 47.14, 44.05, 42.12 and 40.36 (at the end of storage day) for control (T_0), T_1 , T_2 , T_3 , and T_4 , respectively.

The higher TVB-N content was also in agreement with the higher pH value (Fig.1). Generally, the development of TVB-N in meat is correlated well with microbial growth (Kilincceker *et al.*, 2009) [32]. Coating fish with the WPI alone may indirectly result in microbial inhibition by limiting oxygen concentration inside the fish steak for aerobic microbes. The lower TVB-N content in samples coated with thyme essential oil may be attributed to the

antibacterial properties of essential oil and more specifically to carvacrol content. Yasin and Abou-Taleb (2007) [13] found that edible coatings with 5% thyme and marjoram were more effective in delaying the rate of TVB-N during the subsequent cold storage of semi fried mullet fish fillets. Yildiz (2016) [33] also observed that the effect of thyme and rosemary essential oils on marinated rainbow trout caused a reduction in TVB-N values during refrigerated storage. Goulas and Kontominas (2007) [13] found that TVB-N of treatment oregano oil is lower than that non treatment sample.

pH

The pH in fresh fish flesh is almost neutral. The increase in pH indicates the loss of quality (Can, 2011). The pH value is crucial in determining the quality of meat. The increased TVB-N content was in agreement with the higher pH value. The pH of the fish flesh rises during the post-mortem period as a result of the decomposition of nitrogenous substances. The alteration in metabolic, bacterial and enzymatic activities that take place in fish muscles after capture is the cause of a change in hydrogen ion concentrations in fish muscles. The pH values of skipjack tuna steak were 5.90, 5.88, 5.85, 5.83 and 5.80 (at initial storage day) and 6.75, 6.60, 6.45, 6.38 and 6.325 (at the end of storage day) for control (T_0), T_1 , T_2 , T_3 and T_4 , respectively (Table 1 and Fig. 1).

The growth of spoilage bacteria is accelerated by a higher pH (Hazards & Panel, 2016). The samples treated with essential oil T_2 , T_3 and T_4 showed lower pH values as compared with control samples and coating with WPI. Similar findings were reported by other researchers. The lower pH values of these samples might have suppressed microbial growth and inhibited the activity of the endogenous proteases at high concentrations of thyme oil (Song *et al.*, 2011) [15]. This is most likely due to antioxidant and antibacterial components of EOs preventing chemical, microbiological and physical lipid/protein damage hence preventing nitrogen and high alkaline volatile base generation such as ammonia creation and minimizing pH increase.

Socaciu *et al.* (2021) observed that whey protein isolates with tarragon essential oil coating lower the pH rate in brook trout fish during refrigerated storage. Erbay *et al.* (2017) also found a similar increasing trend with the value rising from 5.80 to 7.00 during this entire study on rainbow trout fillets coated with whey protein isolate containing electrospun poly nanofibers with *Urtica dioica* L. extract.

PV

Aldehydes, ketones and alcohols, which are volatile and contribute to off-flavor in products are produced during the breakdown of peroxides an unstable molecule. Fatty acid hydroperoxide measured as peroxide value (PV) is the main byproduct of lipid oxidation. The initial PV of fresh skipjack tuna steak was 1.30 (Table 1). The PV of all samples increased with storage time. In comparison to other treatments, the control (T_0) sample had comparatively higher PV contents (Table. 1 and Fig. 1).

The control (T_0) had the highest value of peroxide measured at 16.76 ± 0.16 meq/kg, whereas the sample treated with 5% WPI+8%TEO (T_4) had the lowest value at 10.76 ± 0.08 meq/kg. The oxidation of fat might have resulted in increased PV. The use of thyme oil to protect muscle foods

against oxidation has been reported by (Oğuzhan & Yangilar, 2017). (Erkan and Bilen, 2010; Mariutti *et al.*, 2008) found that garlic oil and thyme oil was effective means of controlling lipid oxidation in chicken and fish meat. Sabzipour-Hafshejani *et al.* (2022) announced a similar increasing trend during the storage of whey protein edible coating containing fish gelatin hydrolysates chicken breast fillets. Oğuzhan and Yangilar (2017) also reported similar changes during the study of whey protein isolate-based coating enriched with *Zingiber officinale* and *Matricaria recutita* on refrigerated rainbow trout. An increase in PV of chicken breast fillet samples during the shelf-life study was also reported by (Bazargani-Gilani *et al.*, 2015; Kamkar *et al.*, 2021).

TMA-N

The most widely used chemical technique for determining fish quality is TMA, which is generally present in marine fish. In the control (T_0), T_1 , T_2 , T_3 and T_4 samples, the changes in TMA-N content showed steadily increasing patterns as the refrigerated storage time progressed (Table 4.5 and Fig. 4.4). At the end of storage period TMA-N value were found to be 25.4 ± 0.18 , 21.47 ± 0.21 , 19.21 ± 0.24 , 18.16 ± 0.22 and 16.62 ± 0.21 in control (T_0), 5%WPI (T_1), 5%WPI+4%TEO (T_2), 5%WPI+6%TEO (T_3) and 5%WPI+8%TEO (T_4) respectively (mean + SD).

Zeng *et al.*, 2005 showed that the initial TMA value of the shrimp (*P. borealis*) was 5 mg N kg⁻¹ at the beginning of storage. TMA-N values in each tested sample, especially in the control (T_0) increased rapidly, while a fish steak coated with the 5%WPI+8%TEO (T_4) showed the slowest increase in the TMA values throughout the storage period ($p < 0.05$). This was probably due to the greater antimicrobial activity of carvacrol content in thyme oil, resulting in lowering the metabolic microbial-producing compounds, particularly TMA as well as TVB-N. Probably thymol could prevent enzymatic activities.

The TMA-N value of the skipjack tuna steaks during refrigerated storage increased progressively; Due to a bacterial enzyme that combines with trimethylamine oxide to form trimethyl amine, TMA was predominantly produced by certain microorganisms, e.g., *Shewanella putrefaciens* and *Aeromonas spp.* TMA is predominantly related to the fishy odour in fish which is produced by bacterial degradation of trimethylamine oxide.

Concerning the critical limit of acceptability control (T_0) was found fresh up to 10 days, while 5%WPI (T_1) was found to be fresh up to 15 days and 5%WPI+4%TEO (T_2), 5%WPI+6%TEO (T_3) was found to be fresh up to 20 days. Sample 5%WPI+8%TEO (T_4) was found to be fresh even after 25-day storage. (Chaijan *et al.*, 2020) also reported the reduced TMA content in all the WPI coating containing extract from ginger, lemongrass, or green tea samples compare to control of chilled stored Asian sea bass steak. Similar results were obtained by (Günlü and Koyun, 2013) for the seabass treated with chitosan coating.

Microbiological Characteristics

Total Plate Count (TPC) (Log CFU/g)

TPC of all samples increased gradually as the storage period increased ($p < 0.05$). The TPC values of skipjack tuna steak was 3.83, 3.78, 3.73, 3.68 and 3.58 (at initial storage day) and 6.11, 5.76, 5.51, 5.43 and 5.38 (at end of storage day) for control (T_0), T_1 , T_2 , T_3 and T_4 , respectively (Table 4.7,

Fig 4.6). The maximum permissible microbiological load in fresh fish, as per the FSSAI, is 5 log CFU/g. (FSSAI, 2017). All of the coated treatments led to a significant reduction in TPC in skipjack tuna steak compared to the UC (uncoated) fish steak. From the results, the fish steak coated with WPI-containing 5% WPI+8%TEO (T_4) had the highest microbial inhibition at 25 days.

The inhibitory properties of whey protein isolate and concentrate coatings have been reported by (Oğuzhan Yıldız and Yangilar, 2017; Seifzadeh, 2014) [33]. Carvacrol a major component of the EOs of oregano and thyme, is thought to be the major active compound to inhibit many microorganisms such as *Clostridium perfringens*, *Bacillus cereus*, *L. monocytogenes*, *E. coli* and *Staphylococcus aureus* through breakdown of the bacterial membrane, leading to cell death (Burt, 2004; Di Pasqua *et al.*, 2006) [19]. Shakeri *et al.* (2011) found that the inhibitory effects of Thyme EO on microbial load have been found to increase as the concentration of EO increased. Tokur *et al.* (2016) stated that WPI coating enriched with thyme essential oils in the preservation of rainbow trout fillets retarded the growth of aerobic microorganisms during refrigeration storage. Tokur *et al.* (2016) also found that initial microbial load was inhibited by using whey protein isolate coatings.

Total Psychrotrophic count (log CFU/g)

The spoilage of aerobically stored fish is mainly due to the Gram-negative psychrotrophic non-fermenting rods. These bacteria are capable of growth at 0°C but with optimum around 25°C. The initial psychrotrophic count (log CFU/g) was found to be 3.51 ± 0.46 in control, 3.01 ± 0.45 in 5%WPI (T_1), 2.81 ± 0.55 in 5%WPI+4%TEO (T_2), 2.76 ± 0.29 in 5%WPI+6%TEO (T_3) and 2.5 ± 0.06 in 5%WPI+8%TEO (T_4). At the end of the storage period TPC in control (T_0), 5%WPI (T_1), 5%WPI+4%TEO (T_2), 5%WPI+6%TEO (T_3), 5%WPI+8%TEO (T_4) increased to 9.14 ± 0.30 , 8.76 ± 0.27 , 8.23 ± 0.24 , 8.12 ± 0.17 and 7.74 ± 0.09 log₁₀ CFU/g, respectively.

The highest average psychrotrophs bacteria counts were determined for the control sample (T_0) with 9.14 ± 0.30 log CFU/g. While the lowest counts were for the test sample based on 5% WPI+8%TEO (T_4) with 7.74 ± 0.09 log CFU/g. Psychrotrophs bacteria growth increased during the storage in all test samples (T_0 to T_4) but the in-control sample (T_0) increased rapidly during storage. Tokur *et al.* (2016) reported the WPI edible films with thyme oil were better than those with WPI alone in delaying total microbial and psychrotrophic growth. WPI-containing ginger extract had the highest microbial inhibition on the steak during preservation (Chaijan *et al.*, 2020).

Colour Characteristics

'L*' -value

'L*' -value of all skipjack tuna steak samples showed a decreasing trend during the storage. Initially the 'L*' -value ranged from 46.17 ± 36.78 , 46.47 ± 39.18 , 46.74 ± 41.49 , 46.79 ± 41.58 , 47.19 ± 42.045 in control (T_0), 5% WPI (T_1), 5%WPI+4%TEO (T_2), 5%WPI+6%TEO (T_3), 5%WPI+8%TEO (T_4). The value decreased to 36.78 ± 0.65 , 39.18 ± 0.20 , 41.49 ± 0.61 , 41.58 ± 0.53 and 42.045 ± 0.70 . Alotaibi and Tahergorabi (2018) observed a similar trend during the analysis of sweet potato starch-based coated fresh meat of shrimp.

'a*' -Value

The changes in 'a*' -value in all skipjack tuna steak samples showed decreasing trends toward redness with the increasing storage periods, where Whey Protein Isolate with Thyme Essential Oil treated sample (T₂, T₃, and T₄) showed minor changes in redness (Table 3 and Fig. 3). The lowest value was noted in the control (T₀) sample after 30 days of storage. 'a*' -value was highest in treatment 5%WPI+8%TEO (T₄) 4.06±0.06 followed by 5%WPI+6%TEO (T₃), 5%WPI+4%TEO (T₂), 5%WPI (T₁) and control (T₀) at the end of storage period. A similar result was observed by Kalkan and Erginkayan, 2019 during the study of whey protein isolate coating with different microbial agents.

'b*' -value

The highest value for 'b*' denotes yellowness. Consequently, a rise in the 'b*' -value represents an increase in yellowness. The 'b*' significantly increased with storage time, probably due to the oxidation of lipids. From an initial value of 5.33 to 12.08 in the control (T₀), 5.14 to 11.22 in 5%WPI (T₁), 4.86 to 9.22 in 5%WPI+4%TEO (T₂), 4.86 to 9.14 in 5%WPI+6%TEO (T₃) and 4.73 to 9.04 in 5%WPI+8%TEO (T₄) (Table No. 3 & Fig. 3). This could be attributed to the pigmentation of TEO. The values of 'b*' were generally lower in 5%WPI+8%TEO (T₄) samples than those in (T₀), (T₁), (T₂) and (T₃) samples. The highest 'b*' variation was noted in control (T₀). least the degree of change seen in T₄. Similarly, (Ozturk *et al.*, 2015) observed an increase in the 'b*' -value of sucuk treated with TEO compared with control treatment. A similar result was reported by (Socaciu *et al.*, 2021) during a whey protein isolate-based film incorporated with tarragon essential oil on refrigerated brook trout.

Sensory Characteristics

Appearance

Table No. 4 and Fig. 4 display the variances in the general appearance of refrigerated tuna steaks. The early period of all samples had good general appearance characteristics, although it reduced throughout the time of storage. During refrigerated storage, the highest score (4.70±0.67) was recorded in the T₄ treatment followed by T₃ (4.20±1.03), T₂ (3.80±0.79), T₁ (2.80±0.42) and T₀ (1.70±0.95) at the end of 30 days of refrigerated storage. After 30 days of storage coated samples showed a good appearance than uncoated samples (control).

Colour

Throughout the storage period, the colour of the skipjack tuna showed a decreasing trend. The samples' initial score displayed their brightest colour but as the days passed by their brightness decreased, indicating a colour change. The T₄ treatment had the greatest score (4.50±0.71) during refrigerated storage followed by T₃ (4.10±0.99), T₂ (3.80±0.92), T₁ (2.60±0.52) and T₀ (1.60±0.52) at the end of refrigerated storage of 30 days. The colour of T₂, T₃ and T₄ change less than the control (T₀). The colour change in fish steaks is generally attributed to light absorption and scattering by the surface due to protein denaturation, protein/lipid degradation and pigment oxidation. The primary cause of meat colour deterioration during storage is the oxidation of red myoglobin to brown metmyoglobin, which results in a decrease in the intensity of redness in the

flesh. Suman and Joseph (2013) pointed out that aldehydic lipid oxidation products could stimulate myoglobin oxidation.

Odor

The levels of FFA are related to the quality of fat and oils in food products, with high levels of FFA leading to the development of off-odour products (Thiansilakul *et al.*, 2010). During storage or processing steps, microorganisms change the protein structure and provoke unpleasant odours. At the beginning of storage, the result displayed a typical natural odour but it decreased during storage. Throughout the storage time, T₂ odour changed the least out of all the treatments. The fish steaks coated with the 5%WPI+4%thyme essential oil (T₂) showed a higher odour likeness score than those coated with the WPI alone T₁, T₃, T₄ and T₀ (control). A similar trend of odour score evaluation was observed by (Ruelas-Chacon *et al.*, 2020) in tilapia fish fillets coated with guar gum with thyme oil. Kykkidou *et al.* (2009) observed that the use of thyme essential oil at higher concentration would result in increase of shelf life of fish, but higher concentration probably imparts thymy odour on the fish.

Texture

For the consumer acceptability of any fishery product, texture is an important factor. Protein oxidation has an adverse effect on the organoleptic properties, particularly texture of fish muscle during low-temperature storage. The outcome showed that the texture was good enough in the initial period of storage but it softened during the end of the storage.

The Texture value significantly decreases with storage time from 8.60 to 1.50 in the control (T₀), from 8.70 to 2.30 in the 5% WPI (T₁), from 8.70 to 3.30 in the 5%WPI+4%TEO (T₂), from 8.80 to 3.80 in the 5%WPI+6%TEO (T₃), from 8.90 to 4.10 in the 5%WPI+8%TEO (T₄). control (T₀) scored the lowest (1.50±0.53) texture value at the end of the refrigerated storage of 30 days. The texture of the T₄ (5%WPI+8%TEO) sample scored highest (8.90±0.32). Whey protein isolate and thyme essential oil combined treatment related to the maintain texture in fish sample.

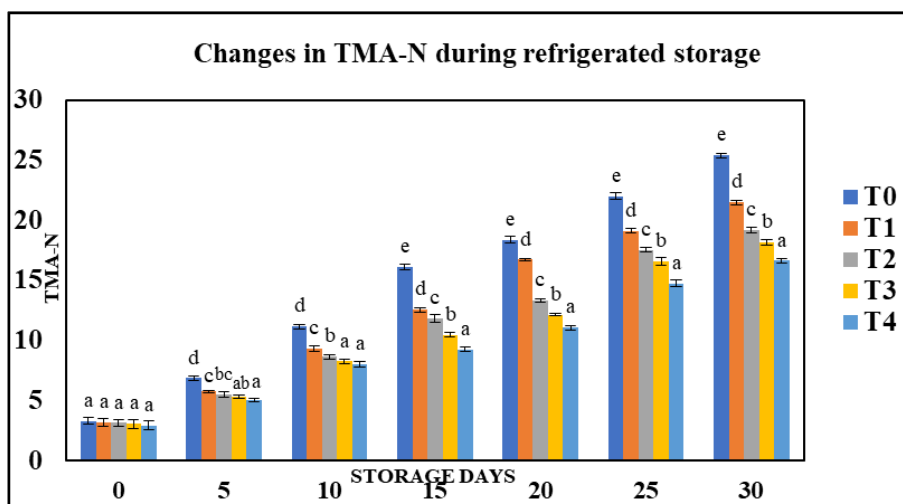
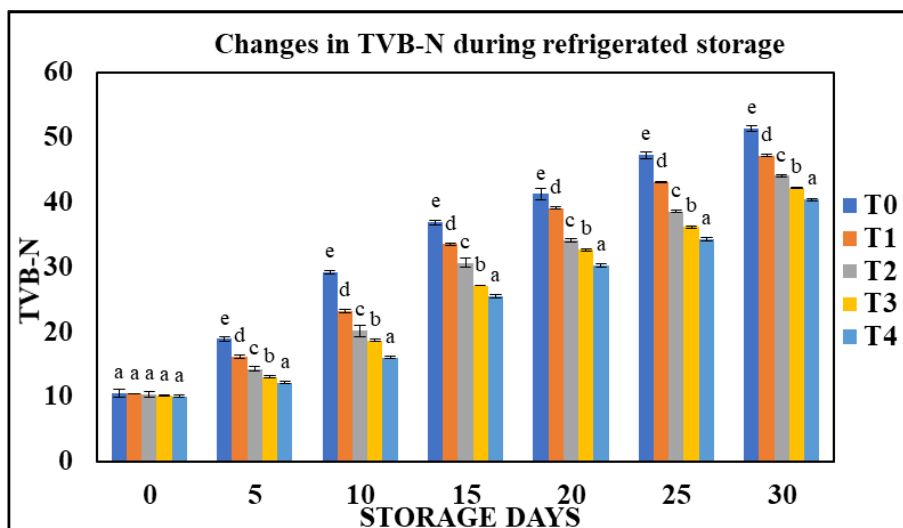
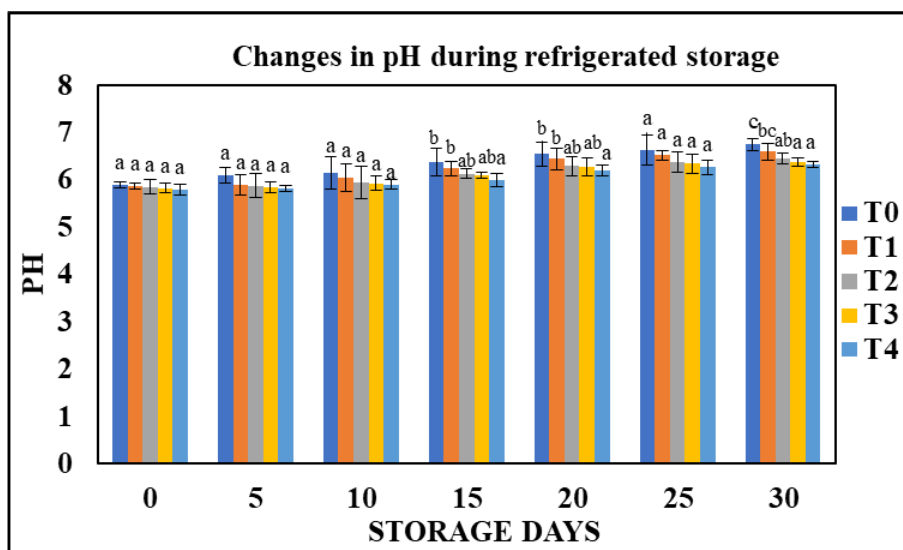
Overall acceptability

Colour, odour, texture and appearance were considered in the scoring of total acceptance. TVB-N compounds are toxic and change the quality (colour, textural and taste) of food products, which will directly affect the acceptability of these food products by consumers. The total acceptance decreased during the storage time for all the samples. The Overall acceptability significantly decreases with storage time from 8.50 to 1.60 in the control (T₀), from 8.50 to 2.50 in the 5% WPI (T₁), from 8.60 to 3.70 in the 5%WPI+4%TEO (T₂), from 8.60 to 3.90 in the 5%WPI+6%TEO (T₃), from 8.70 to 4.10 in the 5%WPI+8%TEO (T₄). The highest overall acceptable score at the end of the analysis was observed in the fish steak coated with the 5%WPI+8%TEO (T₂) (p < 0.05) and 5%WPI+4%TEO (T₄) which indicates that this coating significantly helped to retain the sensorial characteristics of the skipjack tuna steak.

The study reported that the 5%WPI+4%TEO (T₂) and 5%WPI+8%TEO (T₄) treated skipjack tuna steak had a shelf life of 25 days without any significant loss in sensory acceptability compared to just 10 days for the control

samples. A similar trend of the sensory evaluation was observed by (Farsanipour *et al.*, 2020) in *Scomberoides commersonianus* fillets. Erginkaya, Z. and Kalkan, S. (2019) reported WPI coatings containing thyme essential oil were the most sensory coating types. Alotaibi, S. and

Tahergorabi, R. (2018) found that the TEO incorporation resulted in numerically better characteristic of sensory properties. This can be related to the intrinsic sensory characteristics of thyme oil, which improve the acceptability of the product for the consumer.



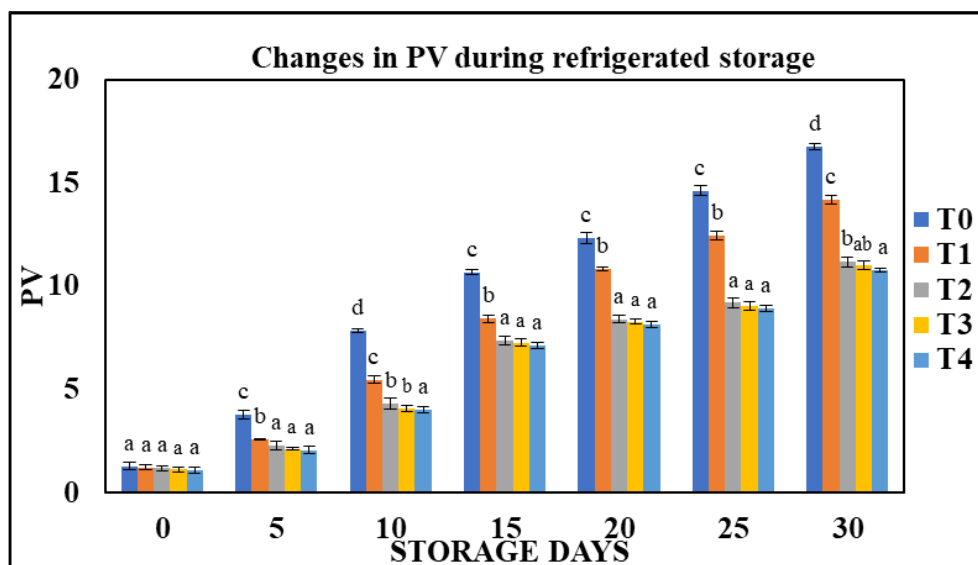


Fig 1: Changes in Biochemical Characteristics (pH, TVB-N, TMA-N & PV) of skipjack tuna fish (*Katsuwonus pelamis*) during refrigerated storage

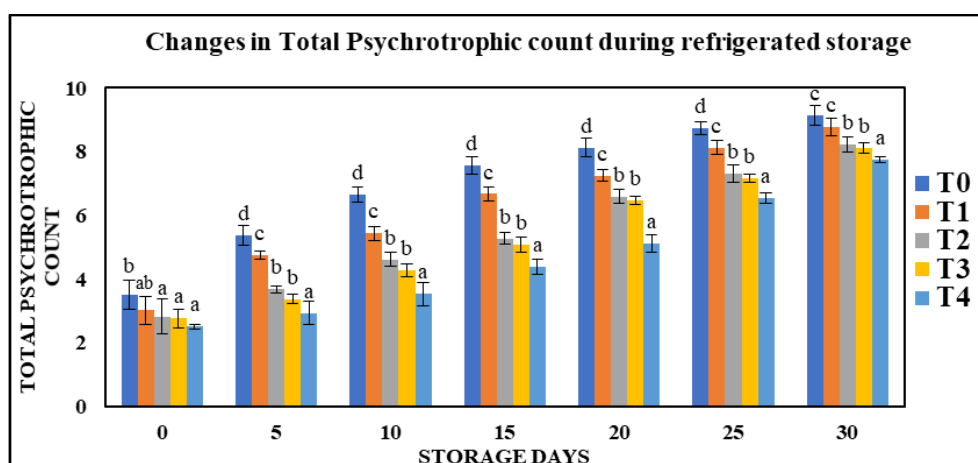
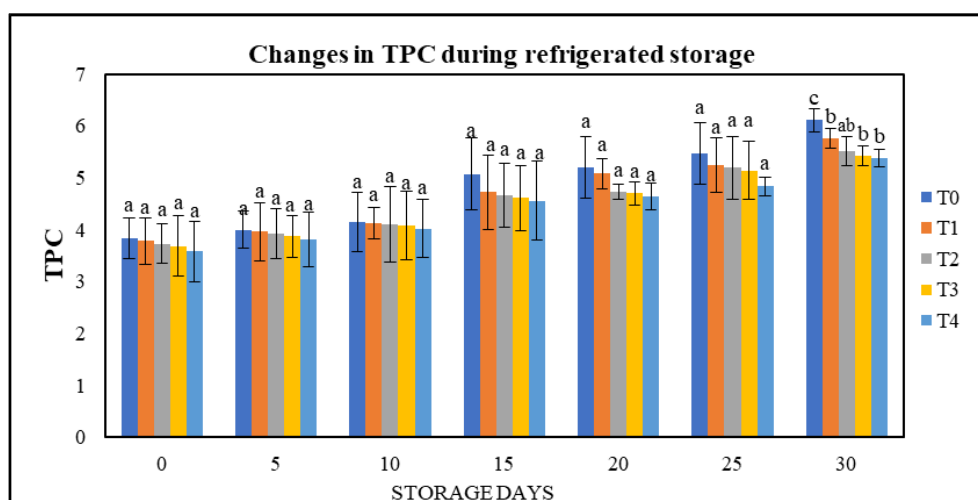


Fig 2: Changes in Microbiological Characteristics (TPC & Total Psychrotrophic count) of skipjack tuna fish (*Katsuwonus pelamis*) during refrigerated storage

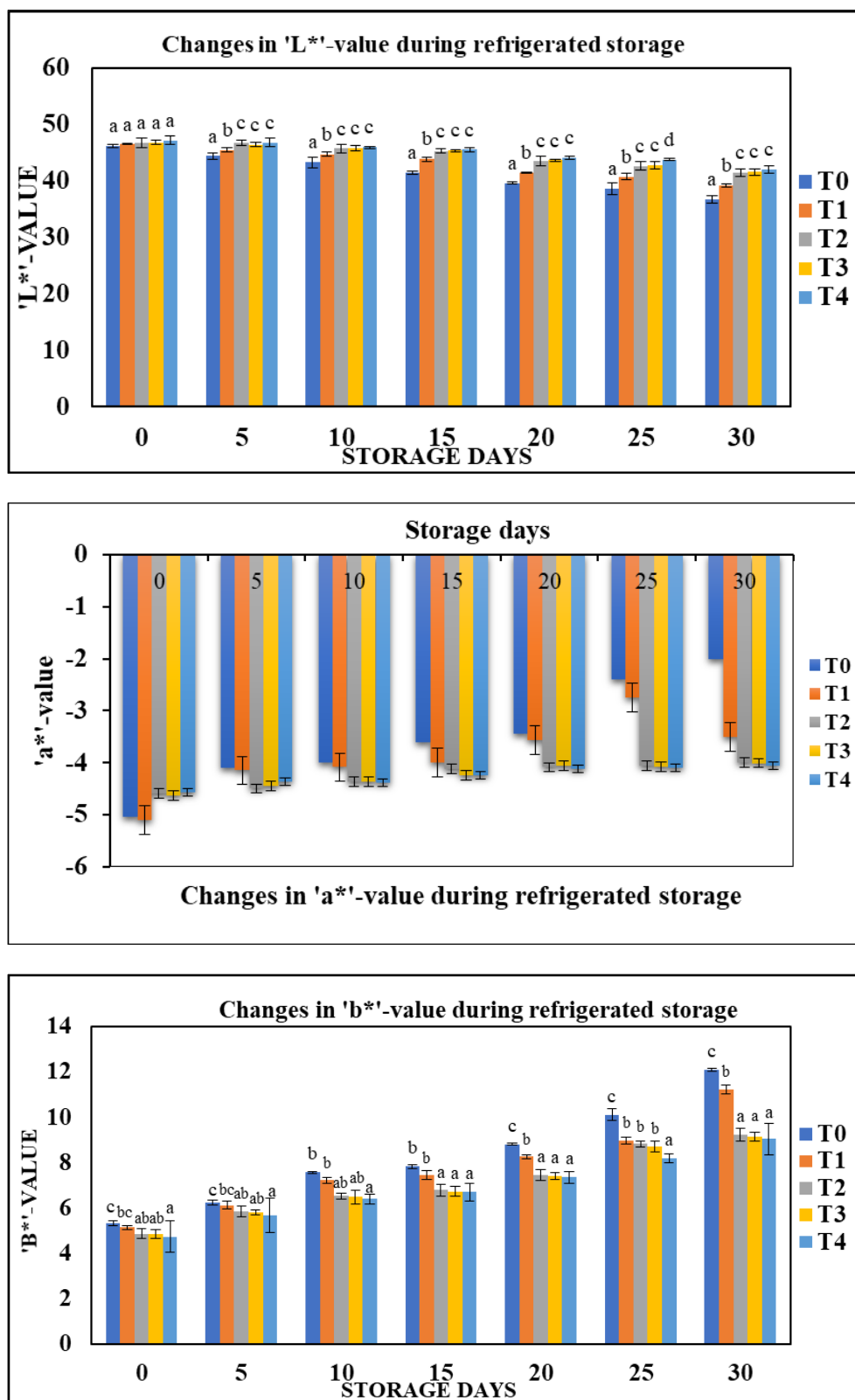
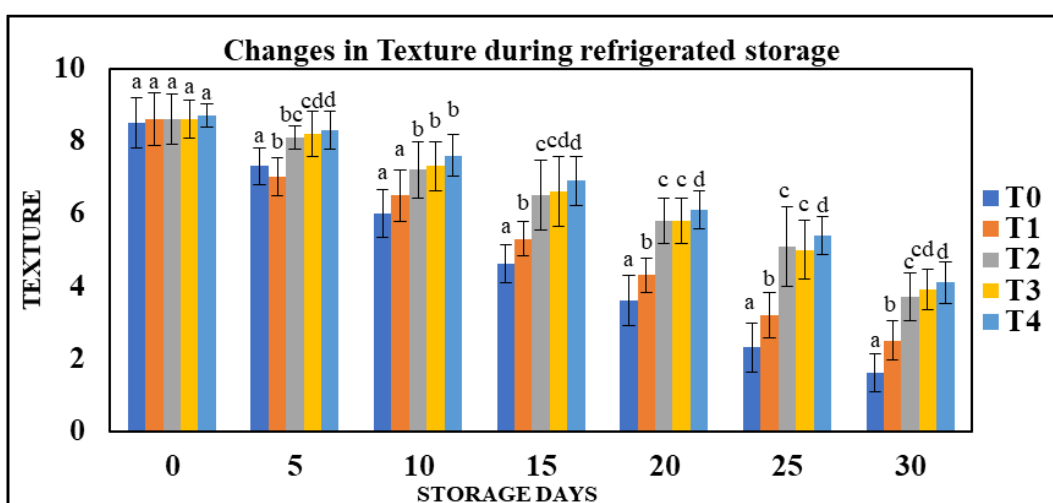
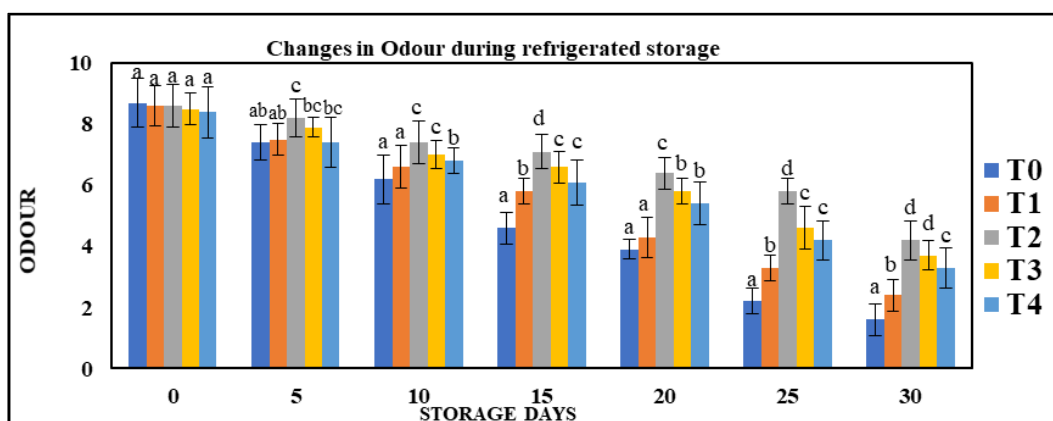
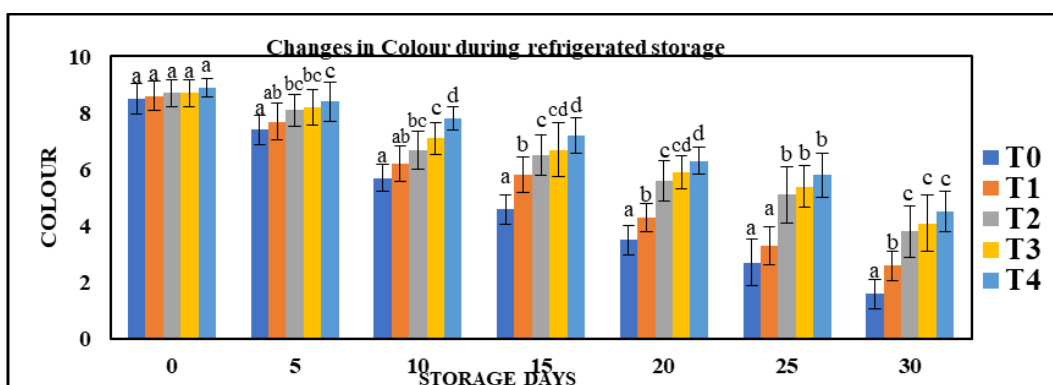
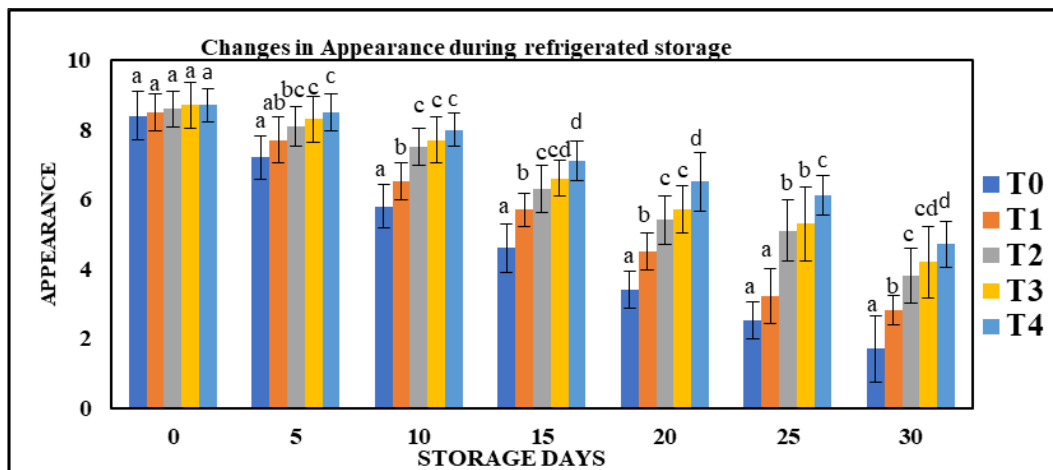


Fig 3: Changes in Colour Characteristics (L*-value, a*-value, & b*-value) of skipjack tuna fish (*Katsuwonus pelamis*) during refrigerated storage



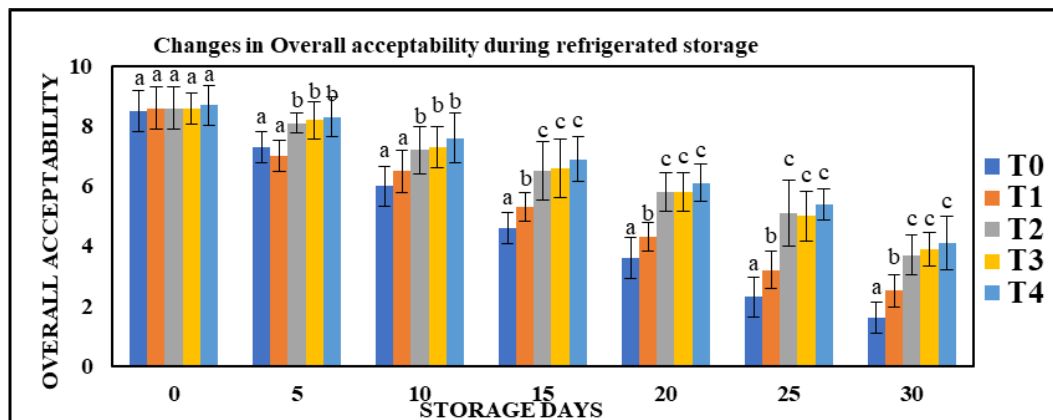


Fig 4: Changes in Sensory Characteristics (Appearance, Colour, Odour, Texture & Overall acceptability) of skipjack tuna fish (*Katsuwonus pelamis*) during refrigerated storage

Table 1: Changes in Biochemical Characteristics of skipjack tuna fish (*Katsuwonus pelamis*) during refrigerated storage

Treatments	Storage Time (Days)				
	T _{0(c)}	T ₁ (5WPI%)	T ₂ (5WPI% + 4% TEO)	T ₃ (5WPI% + 6% TEO)	T ₄ (5WPI% + 8% TEO)
pH					
0	5.90±0.06 ^a	5.88±0.06 ^a	5.85±0.15 ^a	5.83±0.10 ^a	5.80±0.12 ^a
5	6.10±0.17 ^a	5.90±0.21 ^a	5.88±0.25 ^a	5.85±0.12 ^a	5.825±0.06 ^a
10	6.15±0.35 ^a	6.05±0.30 ^a	5.95±0.35 ^a	5.93±0.15 ^a	5.90±0.10 ^a
15	6.38±0.30 ^b	6.25±0.15 ^a	6.13±0.10 ^{ab}	6.10±0.06 ^{ab}	6.00±0.15 ^a
20	6.55±0.25 ^b	6.45±0.23 ^a	6.30±0.20 ^{ab}	6.28±0.20 ^{ab}	6.20±0.12 ^a
25	6.63±0.32 ^a	6.53±0.10 ^a	6.38±0.21 ^a	6.35±0.20 ^a	6.275±0.15 ^a
30	6.75±0.12 ^c	6.60±0.17 ^{bc}	6.45±0.12 ^{ab}	6.38±0.10 ^a	6.325±0.06 ^a
TVB-N					
0	10.50±0.55 ^a	10.42±0.40 ^a	10.30±0.43 ^a	10.12±0.42 ^a	10.04±0.22 ^a
5	18.87±0.41 ^e	16.14±0.39 ^d	14.25±0.32 ^c	13.05±0.27 ^b	12.15±0.20 ^a
10	29.17±0.32 ^e	23.14±0.44 ^d	20.15±0.85 ^c	18.64±0.21 ^b	16.02±0.20 ^a
15	36.85±0.34 ^e	33.48±0.81 ^d	30.60±0.68 ^c	27.12±0.28 ^b	25.44±0.24 ^a
20	41.23±0.93 ^e	39.10±0.26 ^d	34.01±0.26 ^c	32.64±0.22 ^b	30.25±0.31 ^a
25	47.37±0.54 ^e	43.12±0.52 ^d	38.52±0.18 ^c	36.16±0.13 ^b	34.21±0.27 ^a
30	51.32±0.40 ^e	47.14±0.34 ^d	44.05±0.21 ^c	42.12±0.80 ^b	40.36±0.12 ^a
TMA-N					
0	3.30±0.27 ^a	3.21±0.33 ^a	3.14±0.27 ^a	3.05±0.40 ^a	2.94±0.34 ^a
5	6.86±0.21 ^d	5.75±0.12 ^c	5.51±0.20 ^{bc}	5.35±0.13 ^{ab}	5.06±0.12 ^a
10	11.15±0.16 ^d	9.35±0.23 ^c	8.63±0.21 ^b	8.25±0.21 ^a	8.01±0.27 ^a
15	16.15±0.23 ^e	12.55±0.18 ^d	11.80±0.31 ^c	10.45±0.17 ^b	9.25±0.18 ^a
20	18.36±0.26 ^e	16.72±0.13 ^d	13.35±0.14 ^c	12.15±0.13 ^b	11.05±0.16 ^a
25	22.01±0.28 ^e	19.12±0.19 ^d	17.52±0.20 ^c	16.57±0.34 ^b	14.75±0.31 ^a
30	25.40±0.18 ^e	21.47±0.21 ^d	19.21±0.24 ^c	18.16±0.22 ^b	16.62±0.21 ^a
PV					
0	1.30±0.19 ^a	1.24±0.11 ^a	1.20±0.11 ^a	1.14±0.11 ^a	1.09±0.13 ^a
5	3.76±0.21 ^c	2.61±0.03 ^b	2.30±0.20 ^a	2.14±0.08 ^a	2.07±0.18 ^a
10	7.84±0.10 ^d	5.47±0.17 ^c	4.34±0.26 ^b	4.11±0.15 ^{ab}	4.02±0.14 ^a
15	10.67±0.12 ^c	8.43±0.17 ^b	7.37±0.21 ^a	7.27±0.19 ^a	7.12±0.16 ^a
20	12.32±0.27 ^c	10.84±0.08 ^b	8.41±0.18 ^a	8.29±0.13 ^a	8.14±0.16 ^a
25	14.62±0.25 ^c	12.46±0.20 ^b	9.19±0.25 ^a	9.04±0.22 ^a	8.92±0.13 ^a
30	16.76±0.16 ^d	14.17±0.20 ^c	11.17±0.23 ^b	11.01±0.21 ^{ab}	10.76±0.08 ^a

Table 2: Changes in Microbiological Characteristics of skipjack tuna fish (*Katsuwonus pelamis*) during refrigerated storage

Treatments	Storage Time (Days)				
	T _{0(c)}	T ₁ (5WPI%)	T ₂ (5WPI% + 4% TEO)	T ₃ (5WPI% + 6% TEO)	T ₄ (5WPI% + 6% TEO)
TPC (Total Plate Count)					
0	3.83±0.39 ^a	3.78±0.45 ^a	3.73±0.38 ^a	3.68±0.58 ^a	3.58±0.58 ^a
5	4.00±0.35 ^a	3.96±0.56 ^a	3.92±0.48 ^a	3.87±0.41 ^a	3.81±0.52 ^a
10	4.14±0.57 ^a	4.13±0.30 ^a	4.10±0.72 ^a	4.07±0.66 ^a	4.02±0.56 ^a
15	5.07±0.69 ^a	4.72±0.71 ^a	4.66±0.61 ^a	4.61±0.62 ^a	4.56±0.77 ^a
20	5.20±0.59 ^a	5.08±0.29 ^a	4.73±0.15 ^a	4.70±0.22 ^a	4.64±0.25 ^a
25	5.47±0.60 ^a	5.24±0.52 ^a	5.19±0.61 ^a	5.14±0.56 ^a	4.83±0.18 ^a

30	6.11±0.22 ^c	5.76±0.19 ^b	5.51±0.28 ^{ab}	5.43±0.19 ^b	5.38±0.17 ^b
Total Psychrotrophic count					
0	3.51±0.46 ^b	3.01±0.45 ^{ab}	2.81±0.55 ^a	2.76±0.29 ^a	2.50±0.06 ^a
5	5.37±0.31 ^d	4.74±0.12 ^c	3.68±0.11 ^b	3.37±0.14 ^b	2.93±0.36 ^a
10	6.64±0.24 ^d	5.43±0.21 ^c	4.61±0.22 ^b	4.27±0.19 ^b	3.53±0.36 ^a
15	7.57±0.28 ^d	6.67±0.23 ^c	5.27±0.19 ^b	5.08±0.23 ^b	4.38±0.23 ^a
20	8.13±0.30 ^d	7.25±0.17 ^c	6.58±0.22 ^b	6.47±0.14 ^b	5.11±0.29 ^a
25	8.73±0.21 ^d	8.13±0.22 ^c	7.31±0.26 ^b	7.16±0.13 ^b	6.53±0.16 ^a
30	9.14±0.30 ^c	8.76±0.27 ^c	8.23±0.24 ^b	8.12±0.17 ^{ab}	7.74±0.09 ^a

Table 3: Changes in Colour Characteristics of skipjack tuna fish (*Katsuwonus pelamis*) during refrigerated storage

Treatments	Storage Time (Days)				
	T _{0(c)}	T ₁ (5WPI%)	T ₂ (5WPI% + 4% TEO)	T ₃ (5WPI% + 6% TEO)	T ₄ (5WPI% + 6% TEO)
L*-Value					
0	46.17±0.25 ^a	46.47±0.1 ^a	46.74±0.79 ^a	46.79±0.35 ^a	47.19±0.70 ^a
5	44.39±0.59 ^a	45.51±0.36 ^b	46.25±0.5b ^c	46.41±0.4b ^c	46.76±0.75 ^c
10	43.27±0.91 ^a	44.75±0.32 ^b	45.72±0.78 ^c	45.84±0.49 ^c	45.93±0.20 ^c
15	41.49±0.26 ^a	43.84±0.36 ^b	45.28±0.37 ^c	45.36±0.22 ^c	45.53±0.38 ^c
20	39.58±0.2 ^a	41.47±0.1 ^b	43.53±0.8 ^c	43.63±0.22 ^c	44.15±0.26 ^c
25	38.63±0.96 ^a	40.79±0.51 ^b	42.67±0.8 ^c	42.73±0.62 ^c	43.81±0.20 ^d
30	36.78±0.65 ^a	39.18±0.2 ^b	41.49±0.61 ^c	41.58±0.53 ^c	42.04±0.70 ^c
a*-Value					
0	5.04±0.18 ^b	5.10±0.10 ^b	4.60±0.10 ^a	4.63±0.14 ^a	4.58±0.20 ^a
5	4.10±0.22 ^a	4.15±0.15 ^a	4.50±0.21 ^a	4.45±0.15 ^a	4.37±0.06 ^a
10	4.00±0.10 ^a	4.09±0.11 ^a	4.36±0.15 ^b	4.37±0.10 ^b	4.38±0.24 ^b
15	3.60±0.06 ^a	4.00±0.21 ^b	4.12±0.16 ^{bc}	4.24±0.08 ^c	4.25±0.12 ^c
20	3.45±0.15 ^a	3.57±0.01 ^a	4.09±0.18 ^b	4.06±0.02 ^b	4.13±0.19 ^b
25	2.40±0.10 ^a	2.75±0.12 ^b	4.06±0.08 ^c	4.08±0.10 ^c	4.10±0.20 ^c
30	2.01±0.02 ^a	3.50±0.21 ^b	4.00±0.06 ^b	4.01±0.10 ^b	4.06±0.06 ^b
b*-Value					
0	5.33±0.12 ^c	5.14±0.09 ^{bc}	4.86±0.21 ^{ab}	4.86±0.20 ^{ab}	4.73±0.35 ^a
5	6.24±0.10 ^c	6.12±0.16 ^{bc}	5.85±0.25 ^{ab}	5.81±0.10 ^{ab}	5.67±0.30 ^a
10	7.56±0.06 ^b	7.21±0.15 ^b	6.52±0.14 ^{ab}	6.48±0.31 ^{ab}	6.39±0.06 ^a
15	7.82±0.10 ^b	7.44±0.19 ^b	6.78±0.25 ^a	6.73±0.21 ^a	6.70±0.12 ^a
20	8.81±0.06 ^c	8.25±0.10 ^b	7.44±0.23 ^a	7.41±0.16 ^a	7.35±0.21 ^a
25	10.11±0.28 ^c	8.98±0.15 ^b	8.82±0.12 ^b	8.71±0.25 ^b	8.17±0.17 ^a
30	12.08±0.05 ^c	11.22±0.20 ^b	9.22±0.27 ^a	9.14±0.21 ^a	9.04±0.31 ^a

Table 4: Changes in Sensory Characteristics (Appearance, Colour, Odour, Texture & Overall acceptability) of skipjack tuna fish (*Katsuwonus pelamis*) during refrigerated storage

Treatments	Storage Time (Days)				
	T _{0(c)}	T ₁ (5WPI%)	T ₂ (5WPI% + 4% TEO)	T ₃ (5WPI% + 6% TEO)	T ₄ (5WPI% + 6% TEO)
Appearance					
0	8.40±0.70 ^a	8.50±0.53 ^a	8.60±0.52 ^a	8.70±0.67 ^a	8.70±0.48 ^a
5	7.20±0.63 ^a	7.70±0.67 ^{ab}	8.10±0.57 ^{bc}	8.30±0.67 ^c	8.50±0.53 ^c
10	5.80±0.63 ^a	6.50±0.53 ^b	7.50±0.53 ^c	7.70±0.67 ^c	8.00±0.47 ^c
15	4.60±0.70 ^a	5.70±0.48 ^b	6.30±0.67 ^c	6.60±0.52 ^{cd}	7.10±0.57 ^d
20	3.40±0.52 ^a	4.50±0.53 ^b	5.40±0.70 ^c	5.70±0.67 ^c	6.50±0.85 ^d
25	2.50±0.53 ^a	3.20±0.79 ^a	5.10±0.88 ^b	5.30±1.06 ^b	6.10±0.57 ^c
30	1.70±0.95 ^a	2.80±0.42 ^b	3.80±0.79 ^c	4.20±1.03 ^{cd}	4.70±0.67 ^d
Colour					
0	8.50±0.53 ^a	8.60±0.52 ^a	8.70±0.48 ^a	8.70±0.48 ^a	8.90±0.32 ^a
5	7.40±0.52 ^a	7.70±0.67 ^{ab}	8.10±0.57 ^{bc}	8.20±0.63 ^{bc}	8.40±0.70 ^c
10	5.70±0.48 ^a	6.20±0.63 ^{ab}	6.70±0.67 ^{bc}	7.10±0.57 ^c	7.80±0.42 ^d
15	4.60±0.52 ^a	5.80±0.63 ^b	6.50±0.71 ^c	6.70±0.95 ^{cd}	7.20±0.63 ^d
20	3.50±0.53 ^a	4.30±0.48 ^b	5.60±0.70 ^c	5.90±0.57 ^{cd}	6.30±0.48 ^d
25	2.70±0.82 ^a	3.30±0.67 ^a	5.10±0.99 ^b	5.40±0.74 ^b	5.80±0.79 ^b
30	1.60±0.52 ^a	2.60±0.52 ^b	3.80±0.92 ^c	4.10±0.99 ^c	4.50±0.71 ^c
Odour					
0	8.70±0.79 ^a	8.60±0.67 ^a	8.60±0.70 ^a	8.50±0.53 ^a	8.40±0.84 ^a
5	7.40±0.57 ^{ab}	7.50±0.53 ^{ab}	8.20±0.63 ^c	7.90±0.32 ^{bc}	7.70±0.82 ^{bc}
10	6.20±0.79 ^a	6.60±0.70 ^a	7.40±0.70 ^c	7.00±0.47 ^c	6.80±0.42 ^b
15	4.60±0.52 ^a	5.80±0.42 ^b	7.10±0.57 ^d	6.60±0.52 ^c	6.10±0.74 ^c
20	3.90±0.32 ^a	4.30±0.67 ^a	6.40±0.52 ^c	5.80±0.42 ^b	5.40±0.70 ^b
25	2.20±0.42 ^a	3.30±0.42 ^b	5.80±0.42 ^d	4.60±0.70 ^c	4.20±0.63 ^c

30	1.60±0.52 ^a	2.40±0.52 ^b	4.20±0.63 ^d	3.70±0.48 ^d	3.30±0.67 ^c
Texture					
0	8.60±0.70 ^a	8.70±0.48 ^a	8.70±0.48 ^a	8.80±0.42 ^a	8.90±0.32 ^a
5	7.20±0.79 ^a	7.80±0.42 ^b	8.00±0.47 ^{bc}	8.40±0.70 ^{cd}	8.60±0.52 ^d
10	6.40±0.52 ^a	6.70±0.67 ^a	7.40±0.70 ^b	7.70±0.67 ^b	7.90±0.57 ^b
15	4.70±0.48 ^a	5.50±0.71 ^b	6.30±0.67 ^c	6.80±1.40 ^{cd}	7.30±0.67 ^d
20	3.60±0.52 ^a	4.20±0.42 ^b	5.80±0.42 ^c	6.00±0.47 ^c	6.50±0.53 ^d
25	2.10±0.74 ^a	3.10±0.57 ^b	4.30±0.67 ^c	4.70±0.48 ^c	5.60±0.52 ^d
30	1.50±0.53 ^a	2.30±0.82 ^b	3.30±0.67 ^c	3.80±0.63 ^{cd}	4.10±0.57 ^d
Overall acceptability					
0	8.50±0.70 ^a	8.60±0.71 ^a	8.60±0.70 ^a	8.60±0.52 ^a	8.70±0.67 ^a
5	7.30±0.52 ^a	7.60±0.52 ^a	8.10±0.32 ^b	8.20±0.63 ^b	8.30±0.67 ^b
10	6.00±0.67 ^a	6.50±0.71 ^a	7.20±0.79 ^b	7.30±0.67 ^b	7.60±0.84 ^b
15	4.60±0.52 ^a	5.30±0.48 ^b	6.50±0.97 ^c	6.60±0.97 ^c	6.90±0.74 ^c
20	3.60±0.70 ^a	4.30±0.48 ^b	5.80±0.63 ^c	5.80±0.63 ^c	6.10±0.63 ^c
25	2.30±0.67 ^a	3.20±0.63 ^b	5.10±1.10 ^c	5.00±0.82 ^c	5.40±0.52 ^c
30	1.60±0.52 ^a	2.50±0.53 ^b	3.70±0.67 ^c	3.90±0.57 ^c	4.10±0.88 ^c

Conclusion

Whey protein isolate coating enriched with thyme essential oil under refrigerated conditions has a beneficial effect on the quality of skipjack tuna fish. Further, the use of 5% WPI+8% TEO (T₄) coating was found to be more effective in minimizing deteriorative changes in chemically, microbiologically and sensory attributes along with further extension of shelf life up to 15 days at 4 °C. WPI coatings produced good antimicrobial effects during the initial stage of storage but did not do so later. The addition of thyme essential oil to WPI coatings significantly enhanced the quality of the product during refrigerated storage and this effect increased with increasing the thyme EO concentration in the WPI coating. The results obtained in this investigation show that the skipjack tuna steak shelf life was extended with the addition of 4% and 6% thyme EO in WPI solutions while maintaining the quality attributes. Nevertheless, 8% thyme EO in WPI coating solution was shown to be the most effective way to inhibit microbial load than 4% thyme EO & 6% thyme EO in WPI coating. Therefore, the present study demonstrated the preservative effect of whey protein isolate and thyme essential oil (TEO), which could be a potential source of antioxidants and antimicrobials that can be used as a natural preservative for shelf life extension of fish and fishery products.

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