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THE EFFECT OF CHITOSAN AND ANTIOXIDANT COATING ON THE QUALITY OF FRESH GOLDEN REDFISH (*SEBASTES NORVEGICUS*) FILLETS DURING CHILLED STORAGE

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ABSTRACT

This study aimed to evaluate the effectiveness of chitosan coating and *Garcinia cambogia* solution in improving the shelf life of golden redfish (*Sebastes norvegicus*). During this trial four groups of golden redfish fillets were tested. Four groups of golden redfish fillets were tested and treated with different solutions: water (control), 1% acetic acid–1.5% chitosan (chitosan), 10% *Garcinia* (*Garcinia*), and 2% chitosan combined with 10% *Garcinia* (chitosan – *Garcinia*). The fillets were stored at 4 °C for 14 days to assess physical, chemical, microbiological, and sensory changes during preservation. The results revealed that the chitosan–Garcinia-based coating significantly delayed and decreased lipid hydrolysis in redfish fillets during cold storage ($p < 0.05$). It also slowed down the increase in TVC and *Pseudomonas spp*. count during cold storage. Additionally, the chitosan-*Garcinia* coating had an impact on reducing the TVB-N value of the fillets. Sensory evaluation indicated that redfish fillets treated with *Garcinia* had a longer shelf life (14 days) than the control group (12 days). Both *Garcinia* and chitosan individually exhibited effects on shelf life and quality; however, their combination demonstrated superior efficacy.

Key words: *Garcinia cambogia*, fish quality, fish preservation, chitosan coating, redfish shelf life, Sri Lanka

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1 INTRODUCTION

1.1 Background

1.1.1 Fish and shelf life.

Fish is widely recognised as a highly nutritious food source rich in protein, healthy polyunsaturated fatty acids, minerals, and vitamins, which are crucial for supporting the body's metabolic functions. However, its inherent perishable nature poses a challenge in maintaining its nutritional value over an extended period. Spoilage begins soon after harvest and is influenced by chemical, microbiological, and physical factors. The shelf life of fish (the duration during which it remains suitable for consumption) is compromised as spoilage alters its texture, odour, and flavour. Protein degradation, lipid oxidation, and the accumulation of nitrogenous compounds contribute to these changes. Additionally, the growth of pathogenic microorganisms poses a risk of foodborne diseases associated with fish consumption. At room temperature, the shelf life of fish is remarkably short, typically six to twelve hours after death, resulting in a rapid decline in quality (Frazier, 1998).

1.1.2 Sri Lankan fisheries of Yellowfin tuna

In Sri Lanka, fish account for over 50% of animal protein intake, which is approximately three times higher than the global average. More than 0.9 million individuals receive full- or part-time direct or indirect work from coastal and marine fisheries, supporting the livelihoods of approximately 3.6 million Sri Lankans. The fisheries industry plays a pivotal role in Sri Lanka's economy, contributing 1.1% of the country’s annual GDP in 2021, of which 0.9% comes from the marine sector (Ministry of Fisheries, 2022).

Notably, yellowfin tuna (YFT) is a major contributor to the country's foreign exchange earnings in the fisheries sector. According to data from 2020 and 2021, the annual commercial catch for YFT in Sri Lanka reached 37,013 and 31,318 metric tons, respectively (FAO, 2023). The primary exports of YFT were 3,176 and 2,836 metric tons in 2020 and 2021, including fresh and frozen tuna, with a special emphasis on sashimi-grade tuna (FAO, 2023). The industry commands high prices in the fiercely competitive and discerning sashimi markets, particularly in Japan (Jinadasa et al., 2015). YFT is not only exported but is also a popular choice among the Sri Lankan population, often enjoyed as a regular part of their meals.

1.1.3 Yellowfin tuna challenges

In Sri Lanka, chilled YFT products have problems with freshness owing to chemical spoilage (Jinadasa et al., 2015). In addition to chemical spoilage, YFT spoilage occurs because of the presence of microorganisms, such as *Pseudomonas, Shewanella, Morganella, and Acinetobacter* (Wang et al., 2021). Chemical spoilage increases the biogenic amines in fish, leading to rancidity and colour changes (Hansamali et al., 2020; Jinadasa et al., 2015; Wang et al., 2021). Therefore, finding ways to overcome these problems and extend the shelf life and quality of fresh YFT would be of interest to the Sri Lankan economy.

The original aim of this study was to examine the impact on yellowfin tuna; however, owing to the unavailability of tuna in Iceland, it was redirected to investigate golden redfish, which share a similar nutritional value. This study now centres on evaluating the efficacy of incorporating *Garcinia cambogia* or *Tamarindus indica* into chitosan coatings to extend the shelf life of golden redfish.

This test will be conducted later for yellowfin tuna. The aim is to determine whether coating tuna with chitosan and extract would increase shelf life. If this is the case, it could lead to the

large-scale commercial production of both *Garcinia cambogia* or *Tamarindus indica* extract and chitosan in Sri Lanka.

1.2 Objective

The primary objective of this study is to investigate the efficacy of a coating made of chitosan and/or extracts of *Garcinia cambogia* or *Tamarindus indica*, to extend the shelf life of chilled golden redfish stored (at 4ºC).

This study aims to achieve the following objectives:

1. Evaluate the efficiency of chitosan coating in extending the shelf life of golden redfish.
2. Evaluate the efficiency of spice or fruit extract in extending the shelf life of golden redfish.

2 LITERATURE REVIEW

2.1 Yellowfin tuna

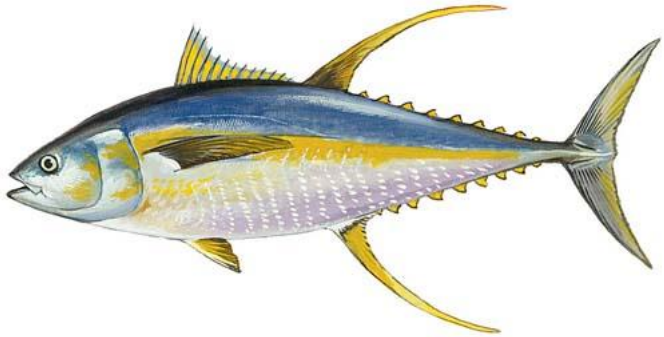

Figure 1: Yellow fin tuna (Source: https://norrik.com/saltwater-fish/yellowfin-tuna)

Yellowfin tuna (YFT; *Thunnus albacares*) is a large pelagic fish belonging to the Scombridae family of marine fish (Figure 1). It is one of the most significant species traded for commerce worldwide (Hansamali et al., 2020). YFT is a large pelagic predator with a silver belly, dark blue backs, and yellow sides. Their large, vivid yellow dorsal fin and yellow stripe along the side distinguish them from other tuna species. They are predominant in tropical and subtropical water. They prefer surface waters and are often associated with schools of other tuna species. They are carnivorous predators that primarily feed on smaller fish, squid, and crustaceans, see Fishbase, 2023 (Froese and Pauly, 2023). YFT can grow to impressive sizes. Adults typically range from 4 to 7 feet in length and can weigh between 100 and 400 pounds. Some individuals may even exceed these averages.

2.2 Golden redfish (*Sebastes norvegicus*)

Figure 2: Golden redfish (Source: https://www.britannica.com/animal/redfish#ref74968)

Golden redfish, scientifically known as *Sebastes norvegicus*, (Figure 2) is a marine species with a rich nutritional profile, characterised by high protein and moderate fat content. Golden redfish and YFT exhibit distinct nutritional profiles. In 100 grams of raw fish, golden redfish contains 19 grams of protein and 4 grams of total fat, with higher levels of polyunsaturated fats (Iceland Responsible Fisheries, 2023). Comparatively, yellowfin tuna offers 23.7 grams of protein and approximately 1 gram of total fat per 100 grams, along with elevated vitamin B12 levels. While both species are rich sources of high-quality protein, golden redfish tends to have a marginally higher fat content, especially in polyunsaturated fats, which makes it an ideal fish to conduct the experiment. In fish spoilage, various aspects can affect nutritional quality beyond fat content. Protein degradation, loss of vitamins, formation of harmful compounds, reduction in omega-3 fatty acids, and changes in mineral content are all potential consequences of spoilage that can impact the nutritional value of fish.

The fact that golden redfish is found in cold waters, whereas YFT is found in warm waters, could influence the experiment, as the quality and spoilage of fish can indeed differ based on the water environment in which the fish resides. Factors such as temperature, salinity, microbial flora, oxygen levels, pollutants, and pH levels play crucial roles in influencing fish spoilage patterns. Warmer waters may accelerate the growth of spoilage-causing microorganisms and enzymatic activities, leading to quicker deterioration.

2.3 Problems related to fish spoilage and its effects on YFT and golden redfish.

Fish muscles are composed of two types: light and dark. Dark muscle is a strip of dark tissue that runs the length of the body and is located beneath the skin. Dark muscle can also be found close to the vertebrae in tuna (Sánchez-Zapata et al., 2011). As a migratory species, it requires higher levels of fat, glycogen, and myoglobin to fuel its lengthy migrations. Therefore, it has been identified that the darker muscle has higher lipid contents, less stable proteins, higher sarcoplasmic protein concentrations, and lower final pH values, which may be factors in the challenges it faces in the storage of raw materials.

2.4 Shelf life and spoilage

To address this limitation, it is imperative to either consume the fish promptly after harvesting or employ preservation techniques, such as chilling, freezing, canning, smoking, and drying. These methods mitigate fish deterioration, extend its shelf life, and ensure its availability for consumption over a more prolonged period.

YTF is highly susceptible to spoilage during fresh storage and transportation as it contains high protein and moisture content, which provide favourable conditions for rapid microbial growth and chemical degradation (Wang et al., 2021). Regarding refrigerated storage, myoglobin and lipid oxidation are the primary causes of tuna spoilage (Kaewprachu et al., 2017). Lipid oxidation and myoglobin oxidation lead to rancidity and browning of tuna flesh (Kaewprachu et al., 2017). Hansamali et al. (2020) stated that the shelf life of yellow fin tuna that was vacuum packed and stored in ice at 0ºC was 15 days. Moreover, freshly harvested fish degrade because of naturally occurring microbial spoilage. According to the study by Wang et al. (2021), among the microbial community, *Pseudomonas* are the dominant spoilage bacteria of tuna. Studies have identified *Pseudomonas* as a generator of biogenic amines, such as putrescine, cadaverine, and histamine (Economou et al., 2017). Studies have also highlighted that ice used to refrigerate fish can act as a vector for bacterial pathogens and histamine-producing bacteria in fish (Economou et al., 2017).

Similar to many seafood products, golden redfish is susceptible to spoilage during chilled storage. Spoilage is primarily attributed to microbial activity and enzymatic reactions accelerated at higher temperatures, resulting in a decline in quality (Martin, 2012). Effective control of storage conditions, including temperature management and proper handling practices, is essential to mitigate spoilage and extend the shelf life of golden redfish, ensuring that consumers receive a high-quality and nutritious product.

2.5 Edible films for the preservation of fresh fish

Edible films have emerged as a novel and effective approach for preserving fresh fish. These coatings can be composed of proteins, polysaccharides, resins, and lipids. When applied as coatings, these films create a protective layer on the fish surface, thereby hindering the growth of spoilage-and pathogenic bacteria. Moreover, coatings reduce lipid oxidation and minimise moisture loss, thereby slowing down deterioration (Zaman et al., 2018). These sustainable and eco-friendly practices are making promising avenues in the quest for innovative and responsible seafood preservation techniques. In addition, the incorporation of herbs and spices into these bio-coatings further enhances their preservation properties for fresh fish (Qiu et al., 2014; Shahidi et al., 1999; Zaman et al., 2018).

2.6 Chitosan as a coating

Chitosan is a natural polymer characterised by low acetyl substitution, derived from chitin. It primarily consists of glucosamine and 2-amino-2-deoxy-b-d-glucose, as shown in Figure 3 (Shahidi et al., 1999).

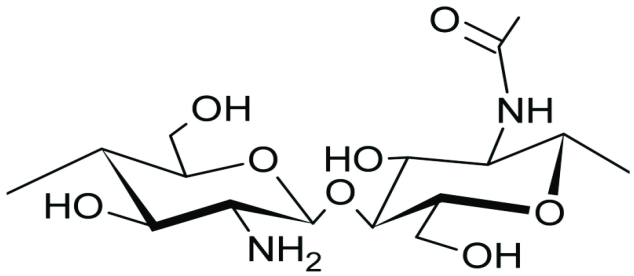

Figure 3: Chemical structure of Chitosan. (Source: https://www.researchgate.net/figure/Chemical-structure-of-chitosan_fig4_355787682/)

This polysaccharide nature provides it with film-forming properties, allowing it to create thin, flexible films when processed. Chitosan contains amino groups (NH_2) along its polymer chain.

These amino groups make chitosan positively charged under acidic conditions, enhancing its interaction with negatively charged surfaces, such as those of food products (Dutta et al., 2004; Shahidi et al., 1999).

Rising consumer demand for chemical-free foods has spurred interest in natural antimicrobials. Chitosan exhibits better antimicrobial activity because of the positive charge on C-2 of the glucosamine monomer at pH < 6. Chitosan is an excellent barrier to the permeation of oxygen, while exhibiting relatively low vapour barrier characteristics. By incorporating fatty materials, hydrophobicity can be increased, thereby producing composite films resistant to water transmission (Dutta et al., 2004; Shahidi et al., 1999; Zaman et al., 2018).

In addition, the antioxidant ability of chitosan is attributed to its ability to act as an electron donor, and the primary amino groups of chitosan can form stable complexes with volatiles (Qiu et al., 2014; Sathivel, 2005).

The addition of various herbs and spices, such as thyme, oregano, and green tea, as antioxidants to edible films improved their efficacy (Qiu et al., 2014; Zaman et al., 2018). Studies on Japanese sea bass (*Lateolabrax japonicas*) fillets stored at 4 °C with chitosan coatings incorporated with citric acid or liquorice extract showed that citric acid or liquorice extract significantly enhanced the preserving function of chitosan by retarding lipid oxidation and inhibiting microbial growth (Qiu et al., 2014).

2.7 Use of *Tamarindus indica* and *Garcinia cambogia* as a fish preservative

Tamarind (*Tamarindus indica*) and *Garcinia cambogia* are used in Sri Lanka as spices and souring agents to provide the desired acidity in various food recipes. Studies on these two spices have reported that they have antimicrobial and antioxidant effects, which can inhibit the growth of spoilage bacteria in fish and reduce lipid oxidation (Apang et al., 2020; Jain et al., 2011).

2.7.1 Tamarindus indica

Tamarindus indica, commonly known as tamarind, is a tropical evergreen tree native to Africa but widely cultivated in tropical and subtropical regions globally. Tamarind trees are distributed across different regions of Sri Lanka, including both lowland and upland areas. They thrive in a variety of environments, from coastal areas to inland regions. The tree, which can reach up to 25 meters in height, produces distinctive pod-like fruits (Figure 4) with a sweet and sour pulp. Widely used in culinary applications, tamarind adds a tangy flavour to an array of dishes, sauces, and beverages in South Asian, Middle Eastern, and Latin American cuisines. Beyond its culinary uses, tamarind boasts nutritional value, containing vitamin C, potassium, magnesium, and antioxidants. Traditional medicine recognises its potential health benefits, including anti-inflammatory and laxative properties. Tamarind seed extracts are used in the textile and pharmaceutical industries. With adaptability to various soil types and arid conditions, tamarind trees are not only culturally significant but also environmentally adaptable, often serving as shade trees in agroforestry systems. The global cultivation of *Tamarindus indica* highlights its versatility and importance in diverse aspects of human life (Jain et al., 2011).

The analysis of bioactive phytochemical constituents of tamarind fruit pulp revealed the presence of tannins, terpenoids, and citric acid, which are related to the antimicrobial properties of the fruit. Adeniyi et al., (2017) study on the antibacterial activity demonstrated by different tamarind extracts shows tamarind pulp and leaf extracts exhibited antibacterial activities against *Pseudomonas* and *E. coli* compared to its seed extract. The presence of flavonoids and phenolic compounds in tamarind fruit pulp suggests a positive impact on human health, particularly as natural antioxidants. Importantly, these extracts maintain their antioxidant activity even after heat treatment, which is commonly employed in food processing (Tril et al., 2014).

Figure 4: *Tamarindus indica* fruit (Source: https://www.researchgate.net/figure/Tamarindus-indica-fruits-seeds_fig2_334605506)

2.7.2 Garcinia cambogia

Garcinia cambogia is a small to medium-sized tree that is native to Southeast Asia and India. In Sri Lanka, G. cambogia is primarily distributed in the wet and intermediate zones, where the climate is suitable for its growth. The tree belongs to the family Clusiaceae and produces a pumpkin-shaped fruit with a greenish-yellow colour known as the Malabar tamarind (Figure 5). *Garcinia cambogia* has been utilised in traditional culinary practices in parts of Southeast Asia for its sour taste.

Figure 5: *Garcinia cambogia* fruit is shown in the bottom and dried rind in the upper. (Source: https://www.aboticaorganica.com.br/garcinia-cambogia-verdadeira-fruta-importado-sri-lanka-100gr/)

The Garcinia cambogia fruit rind contains hydroxycitric acid, tartaric acid, citric acid, and malic acid (Apang et al., 2020). Studies on the shelf life of Indian mackerel (*Rastrelliger kanagurta*) in chilled storage with *Garcinia incorporated* ice medium showed that it extended the shelf life of fish by 8 days. It also demonstrated the antioxidant ability of the *Garcinia rind* extract, which significantly inhibited the generation of peroxides. The presence of phenolics in the ice and *Garcinia* medium prevented the development of hydroperoxide by scavenging free radicals that are reactive to fatty acids, thereby interrupting the chain reaction that leads to lipid oxidation (Apang et al., 2020).

The antimicrobial activity of *Garcinia cambogia*, which has been extensively studied, is attributed to its lower pH values. These values effectively inhibit microbial growth and enzymatic processes, thereby contributing to their efficacy (Apang et al., 2020; Bhuvana et al., 2020).

3 METHODOLOGY

3.1 Materials

3.1.1 Golden redfish fillet

Sixty-two golden redfish fillets used in this study were provided by the Brim Company. The fillets were used for both the main experiments and the pre-trials.

3.1.2 Chitosan

Chitosan (ChitoClear® fg95) with DDA ≥ 95% was supplied by Primex Company.

3.1.3 Garcinia cambogia and Tamarindus indica

Dried *Garcinia cambogia* fruit rinds and dried *Tamarindus indica* fruit (without the shell) were purchased from a local market in Matale, Sri Lanka. They were stored in airtight polyethylene packages until their use in the experiment.

3.2 Pre study

Before the main experiment, pre-trials were conducted to identify the best antioxidant extract for the experiment and to prepare a chitosan solution with the appropriate viscosity for application as a coating.

3.2.1 Pre-trial 1: Extraction of Garcinia and tamarind

In accordance with the methodology outlined by Apang et al. (2020), the extraction process for the experiment involved soaking the dried fruit rind of *Garcinia and* the ground pulp of tamarind fruit in distilled water at a ratio of 1:10 (w/v) overnight. This extraction was conducted in two variations: with the fruit samples ground and unground, to determine the most effective extraction method. Subsequently, the mixture was stirred and boiled for 15 minutes. After cooling to room temperature, the mixture was filtered through cheesecloth (45 µm). The resulting extracts were stored in plastic cups and refrigerated (4–6 °C) for later analysis of their antioxidant properties.

3.2.2 Pre-trial 2: Antioxidant analysis of Fruits extracts

Colorimetric analysis of the total phenolic content (TPC) was performed using the Folin–Ciocalteu method, as described by Singleton and Rossi. Here the preparation steps include the preparation of 7.5% sodium carbonate, 0.2N Folin-Ciocalteu solution, 0.1mg/mL Gallic acid standard and 0.1mg/mL Phloroglucinol standard. Then the standards were diluted to create a curve.

In a transparent microplate, 20 µL of standards or samples, 100 µL of Folin-Ciocalteu 0.2N, and 80 µL of sodium carbonate solution were added as per the microplate layout. The microplate was then incubated for 5 minutes at room temperature, closed with a plastic cap to prevent evaporation, microwaved for 10 seconds at 500 W, and shaken at room temperature for 30 minutes. After removing the plastic cap, absorbance readings were taken at 720 nm.

Reducing power (RP) assay: According to the methodology, a phosphate buffer of 0.2M (pH 6.6) and a 1% potassium ferricyanide, 0.1% ferric chloride (FeCl3), 1 mg/mL L-ascorbic acid standards were created. The L-ascorbic acid standard was then diluted according to the reference table to obtain a curve.

A total of 63 µL of phosphate buffer (pH 6.6), 63 µL of potassium ferricyanide solution, and 13 µL of standards or samples were added to a transparent microplate. The microplate was then incubated for 30 minutes. After incubation, the plastic film was removed, and 63 µL of

trichloroacetic acid (TCA) and 40 µL of $FeCl_3$ were added to the microplate. Absorbance readings were obtained at 720 nm.

The extract exhibiting the highest antioxidant properties was selected for subsequent experiments.

3.2.3 Pre-trial 3: Preparation of Chitosan solution for the control group

To prepare the chitosan extract alone, the methodology outlined by Huynh (2019) was employed. The dissolution of 1.5% chitosan was facilitated using a 1% acetic acid solution (acetic acid with 97.5–100% purity), and the entire process was carried out at room temperature. This approach eliminated the need for heating and aimed to achieve effective chitosan dissolution while preserving the sensory attributes of the coated fillets.

Some tests were conducted by dissolving chitosan in the fruit extract produced in the pre-trial, which had shown the best antioxidant activity (pre-trial 2). Additional tests of chitosan dissolution at different concentrations were performed to determine whether it dissolved properly. The drying time after the fillets were dipped into the solution was also determined during the pre-trial.

3.3 Experimental design

A flowchart of the experimental design is presented in Figure 6. Here, the experiment was carried out as four treatments.

1. T1: Control - dipped in water.
2. T2: Chitosan coating only (1.5% chitosan)
3. T3: *Garcinia cambogia* or tamarind extract only. (10% extract in water w/v)
4. T4: Chitosan coating and *Garcinia cambogia* or tamarind extract (2% chitosan in 10% extract)

The fillets were dipped in the above treatments for 15 seconds. Then, all coated fish fillets were removed, drained, and dried at 1-2 °C for 1.5 hours (Figure 1 & 2). All samples were placed on styrofoam trays wrapped with LLDPE and stored at 4 °C to evaluate the parameters (Figure 3).

Sensory evaluation was conducted on days 0, 4, 7, 10, 12, and 14.

The sensory panel decision was used as a threshold (indicating whether the product was still suitable for consumption), and the highlighted parameters in Figure 6 (TVB-N and microbiological analysis) were measured.

1. At the initial point, 0 days
2. After the rejection of the sensory panel

All other parameters were analysed on six sampling days (0, 4, 7, 10, 12, and 14).

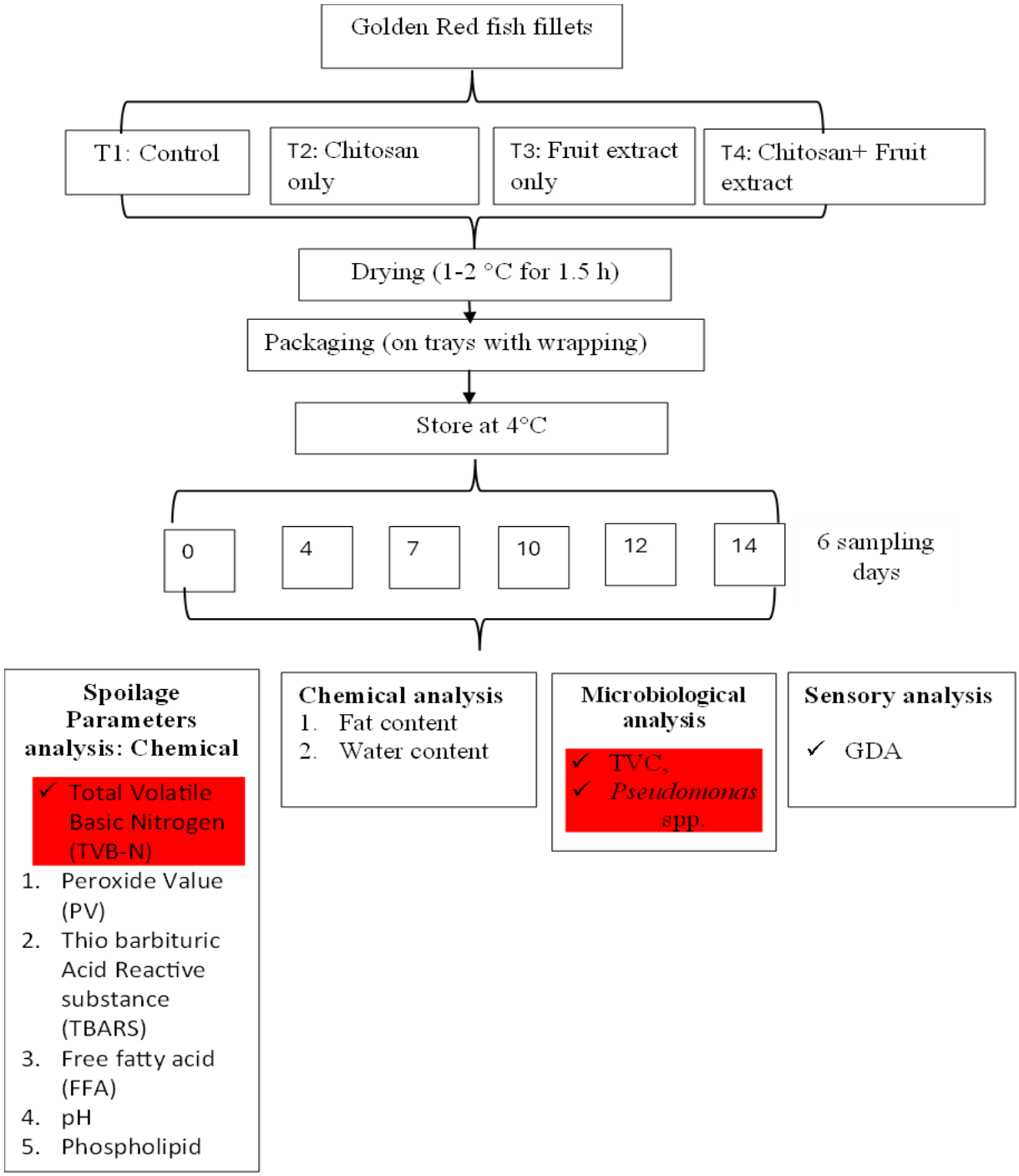

Figure 6: Flowchart of the experiments for preservation of golden redfish fillet by chitosan and chitosan-fruit extract coating.

3.4 Water content

The water content was measured according to ISO 6496:1999. Approximately 5.0 g of the homogenised sample was weighed and placed in a small porcelain bowl. The porcelain bowl containing the sample was left to dry in an oven at 103 ± 2 °C for 4 hours. The bowl was removed from the oven and allowed to cool to ambient temperature in a desiccator for approximately 30 min. The water content was calculated using the following formula:

$$W = \frac{(m2 - m3)}{(m2 - m1)} \times * \ 100\ (\%)$$

Where: m1 is the mass of the bowl (g),

m2 is the mass of the bowl with the test portion (g)

m3 is the mass of the dish with the dried test portion (g).

3.5 Lipid content

Initially, 25 g of the grounded sample was weighed and transferred into 250- or 500-mL centrifuge bottles. A correction table was used to adjust the sample weight and water content based on the moisture content of the samples when it was lower than 80%.

Subsequently, a mixture comprising 25 mL of chloroform and 50 mL of methanol was added to the sample, followed by homogenisation for 2 min to ensure thorough mixing. An additional 25 mL of chloroform was added to the mixture, further enhancing fat solubilisation, and the mixture was subsequently mixed for another minute.

After the addition of 25 mL of 0.88% potassium chloride (KCl), centrifugation was conducted at 2500 rpm for 20 min at 4°C. The lower chloroform phase, containing the extracted fat, was carefully extracted using pipettes. This phase was subjected to filtration under suction to eliminate solid impurities. The filtered chloroform phase was then transferred into a 50 mL volumetric flask, ensuring the removal of any residual aqueous phase. Finally, the volumetric flask was filled with chloroform to the mark for precise volumetric measurement, thereby enabling an accurate analysis of the extracted fat content. Through this systematic process, the fat content was effectively extracted from the sample and prepared for further analytical procedures.

After extraction, 3 mL of the chloroform phase of the Bligh and Dyer extraction was evaporated at 55°C under a nitrogen jet. The weight of the tube was recorded before adding the sample (m1) and after evaporation (m2). The weight of the sample used to conduct the Bligh and Dyer extraction was recorded and denoted as w. The fat content was calculated using the following formula:

$$\text{Fat}\ (\%) = \frac{\frac{(m2 - m1) \times 50}{3}}{w} \times 100$$

3.6 Spoilage parameters analysis: chemical

3.6.1 Determination of total volatile basic nitrogen (TVB-N)

The experimental procedure by Malle and Poumeyrol (1989) was used here. First, 100 mL of a 7.5% aqueous trichloroacetic acid solution was added to 50 g of fish muscle and homogenised in a Waring blender. The resulting mixture will then be filtered through Whatman no. 3 filter paper. Subsequently, steam distillation was performed using a Kjeldahl-type distillator (Struer TVN).

For the distillation process, 25 mL of the filtrate was transferred into a distillation flask, followed by the addition of 6 mL of 10% NaOH. A beaker containing 10 mL of 4% boric acid, along with 0.04 mL of methyl red and bromocresol green indicators, was positioned under the condenser for the subsequent titration of ammonia. Distillation was initiated, and steam distillation was sustained until a final volume of 50 mL was achieved in the beaker, resulting in 40 mL of distillate.

The boric acid solution will exhibit a green colour upon alkalinization with distilled TVB-N, which is then titrated with an aqueous 0.0444N sulfuric acid solution using a 0.05 mL graduated burette. Complete neutralisation was confirmed when the colour changed to pink after the addition of an extra drop of sulfuric acid. The concentration of TVB-N was determined using the following formula:

$$(\mathbf{mgN/100g}) = \frac{\mathbf{14mg/mol \times a \times b \times 300}}{\mathbf{25}\boldsymbol{ml}}$$

Where: a is the volume of sulphuric acid (mL)

b is the normality of sulphuric acid

This analysis was performed by the chemical analysis laboratory at MATIS.

3.6.2 Phospholipid content

Lipid extracts were used to measure phospholipid content (PL) using the colorimetric method developed by Stewart in 1980. This method is based on the formation of a complex between PL and ammonium ferrothiocyanate.

In the procedure, 25 µL of extract (Bligh and Dyer, 1959) were added to 5 µL of chloroform followed by the addition of 1 mL of thiocyanate reagent. This mixture was then vortexed for 1 min to ensure thorough mixing. Subsequently, the solution was centrifuged at a low speed (approximately 2000 rpm) for 5 min. Following centrifugation, the lower layer of the solution, which appeared red in colour, was carefully removed using a plastic 3 mL transfer pipette. The absorbance of the solution was then measured at 488 nm using a spectrophotometer.

To determine the concentration of the phospholipid, the absorbance reading was compared with known amounts of a standard phospholipid solution. A standard curve was prepared using phosphatidylcholine in chloroform (5–150 µg/mL) by evaluating the absorbance at 488 nm using a UV1800 spectrophotometer (Shimadzu, Kyoto, Japan). The results were expressed as a percentage of the total lipid content.

3.6.3 Free Fatty Acids (FFA)

FFAs were determined according to the method developed by Lowry and Tinsley in 1976, with a modification made by Bernárdez, Pastoriza, Sampedro, Herrera, and Cabo in 2005. The lower phase (3 mL), resulting from lipid extraction (Bligh & Dyer, 1959), was added to a screw-cap culture tube. Any solvent present was removed at 55°C using a nitrogen jet. After cooling, 3 mL of cyclohexane was added, followed by 1 mL of cupric acetate–pyridine reagent, and the mixture was vortexed for approximately 40 seconds. After centrifugation at 2000 rpm for 10 min at 4°C, the upper layer was measured at 710 nm in a spectrophotometer. The FFA concentration in the sample was calculated as µmol oleic acid based on a standard curve spanning a 0–22 µmol range. The results were expressed as micrograms of oleic acid per 100g lipid of the sample.

3.6.4 Peroxide Value

Lipid hydroperoxides (PV) were determined using a modified version of the ferric thiocyanate method. Samples weighing 5 g were recorded and transferred into 50 mL tubes with red screw caps. To each tube, 10 mL of ice-cold solvent, including a chloroform: methanol (1:1) solution with 500 ppm BHT previously stored in the freezer, was added. The mixture was homogenised at 6000 rpm for 10 seconds. Following homogenisation, 5 mL of sodium chloride solution was added, and the mixture was thoroughly mixed using a homogeniser. Subsequently, the tubes were centrifuged at 5100 rpm (2350 g) for 5 minutes at 4 °C. After centrifugation, the bottom layer (~3 mL) was collected using a transfer pipette and transferred into 15 mL tubes for

freezing for later analysis. According to the study, the sample (bottom layer): solvent ratio was determined. It was then added to Eppendorf tubes followed by 5 µL of ammonium thiocyanate and ferrous chloride solution (1:1). The mixture was vortexed and allowed to stand for 10 minutes at room temperature. Finally, 100 µL of the solution was placed in a PP microplate and read at 500 nm using a spectrophotometer.

3.6.5 Thio barbituric acid reactive substance (secondary oxidation product)

In the experiment, a modified method based on Lemon's approach from 1975 was employed to measure thiobarbituric acid reactive substances (TBARS). The procedure involved homogenising 5 g of the sample with a 10 mL trichloroacetic acid (TCA) extraction solution comprising 7.5% TCA, 0.1% propyl gallate, and 0.1% ethylenediaminetetraacetic acid (EDTA) in ultrapure water using a homogeniser at maximum speed for 10 seconds (Ultra-Turrax T-25 basic, IKA, Germany). Subsequently, the homogenised samples were centrifuged at 5100 rpm for 20 min using a TJ-25 Centrifuge (Beckmann Coulter, USA). A 0.1 mL supernatant was collected and combined with 0.9 mL thiobarbituric acid (0.02 M). The mixture was heated in a water bath at 95 °C for 40 minutes. Following heating, the samples were cooled on ice and promptly loaded into 96-well microplates (NUNC A/S Thermo Fisher Scientific, Roskilde, Denmark) for measurement at 530 nm using a Tecan Sunrise spectrophotometer (Tecan, Austria). To quantify the results, a standard curve was prepared using 1, 1, 3, 3-tetraethoxypropane (TEP). The results were expressed as µmol of malonaldehyde diethyl acetal per kg of the sample.

3.7 Microbiological Analysis

3.7.1 Total Viable Count

Microbiological analysis was performed by mixing a 20 g sample with 180 g of dilution buffer (0.85% NaCl + 0.1% peptone), blending the mixture in the Stomacher for 1 min, and diluting the solution extracted from the mixture to the desired decimal dilutions. Subsequently, 1 mL of the decimal dilution was pipetted and poured into a Petri plate. Approximately 15 mL of melted iron agar (IA) medium was then poured and mixed with the dilution in culture plates. Once the medium solidified, the plates were covered with a thin layer of melted iron agar medium and incubated at 22°C for 48 hours.

Total viable bacteria, including both white and black colonies, were counted on the plates using a colony counter, and the results were expressed as colony forming units per gram (CFU/g). This measurement was performed in a microbiology analysis laboratory.

3.7.2 Pseudomonas spp.

Pseudomonas aeruginosa was enumerated using modified Cephaloridine Fucidin Cetrimide (mCFC) agar, as described by Stanbridge and Board in 1994. Plates were spread-plated and incubated at 22°C for 3 days.

Pseudomonas Agar Base from Oxoid, UK, was used as the base for the agar medium, supplemented with CFC selective Agar Supplement from Oxoid.

These measurements were conducted by the microbiology analysis laboratory team.

3.8 Sensory evaluation

Sensory evaluation was conducted using the Generic Descriptive Analysis (GDA) method. GDA was used only with short descriptors, focusing on freshness and spoilage attributes (Appendix 1. Table 1). Only odour and flavour were analysed (texture and overall appearance were not evaluated). Five trained panellists evaluated duplicates of all sample groups. The

sensory evaluation was conducted for six sampling dates (0, 4, 7, 10, 12, and 14). The sensory evaluation was conducted by Aðalheiður Ólafsdóttir, the sensory panel leader.

3.9 Statistical analysis

Microsoft Excel for Office 365 was used to compute means, standard deviations, and construct graphs. Since the data exhibited a non-normal distribution, the Friedman test was employed to analyse differences between treatment groups across sampling days. Additionally, a two-sample t-test was conducted to compare data between two different sampling days. Significance of differences was determined at $P < 0.05$. The analysis was conducted using the Minitab statistical software, 2024.

4 RESULTS

4.1 Pre-trial 1: Extraction of *Garcinia and* Tamarind

When extracted ungrounded and grounded *Garcinia* and Tamarind coloured the water to different concentrations (Figure 7).

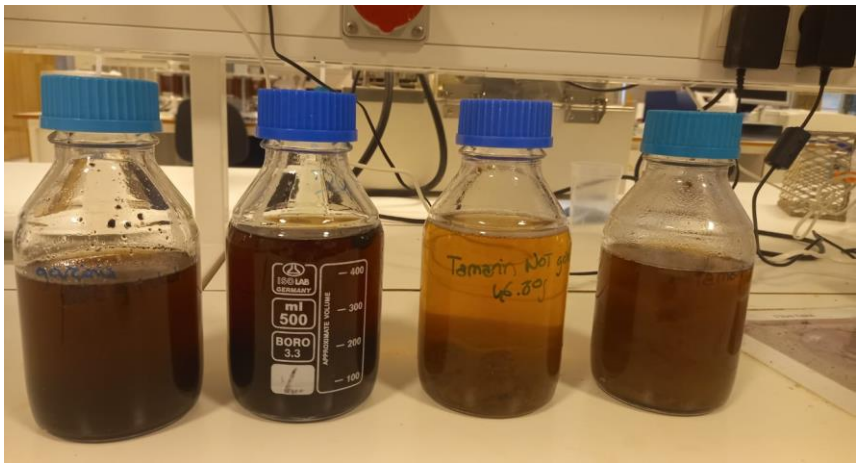

Figure 7: Fruit extract prior to boiling, from left to right; *Garcinia* unground, *Garcinia* ground, Tamarind unground & Tamarind ground respectively.

Following extraction, the pH values of the fruit extracts were analysed, and the values are shown in Table 1.

Table 1:pH value of the extracts

Extracts	**pH value**
Garcinia unground	2.10
Garcinia ground	2.10
Tamarind unground	2.79
Tamarind ground	2.81

The lowest pH values were observed in *Garcinia* solutions compared to tamarind solutions, even if both were acidic.

4.2 Pre-trial 2: Antioxidant analysis of fruit extracts

The results from TPC were derived from a calibration curve ($y = 6.0236x + 0.0427$ $R^2 = 0.9999$) of gallic acid (0–0.1mg/mL) and expressed in milligrams of gallic acid equivalents per gram dry extract weight in Table 2.

The results indicate that *Garcinia cambogia* had the greatest phenolic content in both the unground and ground versions, approximately twice that of tamarind. Surprisingly, the unground extracts had a stronger phenolic content than the ground versions, indicating that extraction was more successful when the spices were unground.

Table 2: TPC results for *Garcinia* and Tamarind fruit extracts.

	Unground *Garcinia* (mg GAE /g)	Ground *Garcinia* (mg GAE/g)	Unground Tamarind (mg GAE/g)	Unground Tamarind (mg GAE/g)
Standard Gallic acid (mg/L)	52.92±0.72	45.78±0.83	20.73±0.25	11.59±0.96

Reducing Power Assay results derived from a calibration curve y = 0.0049x + 0.0752 R^2 = 0.9999 of L- ascorbic acid (0– 0.2 mg/mL) and expressed in L- ascorbic acid equivalents (AAE) in Table 3. According to the results, *Garcinia cambogia* showed a three times higher reducing power value than tamarind.

Table 3: RP assay results for fruit extracts

	Unground *Garcinia* (AAE/g)	Ground *Garcinia* (AAE/g)	Unground Tamarind (AAE/g)	Unground Tamarind (AAE/g)
L- Ascorbic acid standard (mg/L)	618.69±9.06	488.02±7.41	230.92±16.70	336.93±5.47

Based on the overall results from the antioxidant analysis, *Garcinia cambogia* was selected for the main experiment. Additionally, it was confirmed that the unground version yields the best extraction for *Garcinia rinds*.

4.3 Pre-trial 3: Preparation of Chitosan extracts

4.3.1 Solubility of chitosan in Garcinia solutions

The pH value of the *Garcinia solutions* after dissolution of chitosan increased with increasing chitosan concentration (Table 4). The thickness of the solution also increased, indicating that a higher chitosan concentration is required to obtain a good dipping solution (Table 5). The findings revealed that chitosan readily dissolved in *Garcinia solutions* at concentrations of 1%, 1.5%, and 2%. However, only the combination of 2% chitosan and *Garcinia solution* exhibited the desired viscous and sticky properties ideal for coating purposes.

Table 4: pH values of Ch-*Garcinia solutions*.

Chitosan solution	pH value of Ch-*Garcinia solutions*
1%	2.20
1.5%	2.34
2%	2.46

Table 5:Attributes of chitosan -*Garcinia solution*.

Chitosan solution	**Dissolution**	**Description about the solution**
1%	Fully dissolved	Too liquid, almost non-sticky liquid.
1.5%	Fully dissolved	Too liquid, almost non-sticky liquid.
2%	Fully dissolved	Viscous and sticky liquid. Easy for dipping fish fillets, a layer of sticky liquid maintains on the fillet surface after coating.

The results of the three pre-trials indicated that *Garcinia cambogia* extract exhibited superior antioxidant activity compared to tamarind extract, indicating its potential for the main experiment. The extraction of *Garcinia and* tamarind fruits revealed that the extraction efficiency was higher when used in the unground form. Additionally, a 2% chitosan solution in Garcinia imparted the ideal viscosity for coating the fillets. It was also observed that the *Garcinia*-incorporated coating imparted a strong smoky odour, which could potentially influence the sensory attributes of the fillets. Additionally, the coating exhibited a light brown appearance compared to the fillets treated with chitosan or water alone.

4.4 Water content

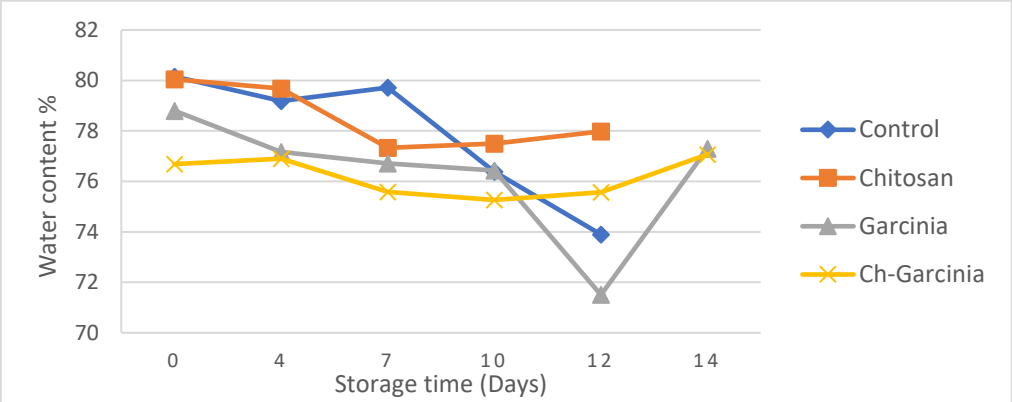

Figure 8: Variation of water content with storage time.

The initial water contents of the redfish fillets were 80.15%, 80.04%, 78.8%, and 76.7% for the control, chitosan, *Garcinia,* and Ch-*Garcinia* groups, respectively (Figure 8). These values decreased to 73.9%, 77.98%, 77.29%, and 77.07% for the respective groups by the end of the storage period. However, as only one sample was measured for each group at each given time, these results are only indicative and not statistically different. The differences between the days could be attributed to individual fish.

4.5 Lipid content

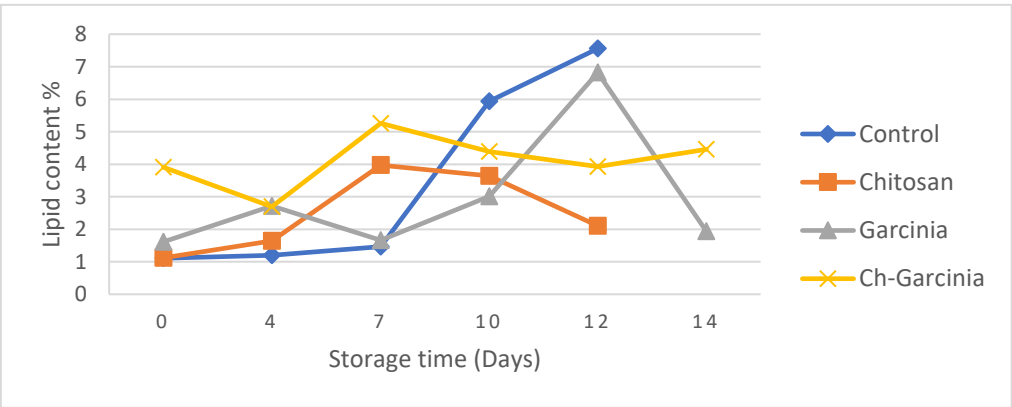

Figure 9: Variation of Lipid content with the storage time.

The lipid content in the redfish fillets was initially between 1 and 4% and evolved between 1 and 8% at the end of the trial (Figure 9). As visible in the graphs, water and fat contents were inversely correlated. When the fat content increases in a fish, the water content decreases. Here

again, as only one sample was measured per sampling point and group, these results are only indicative of the composition of the fillets.

4.6 Spoilage parameters analysis: Chemical

4.6.1 TVB-N content

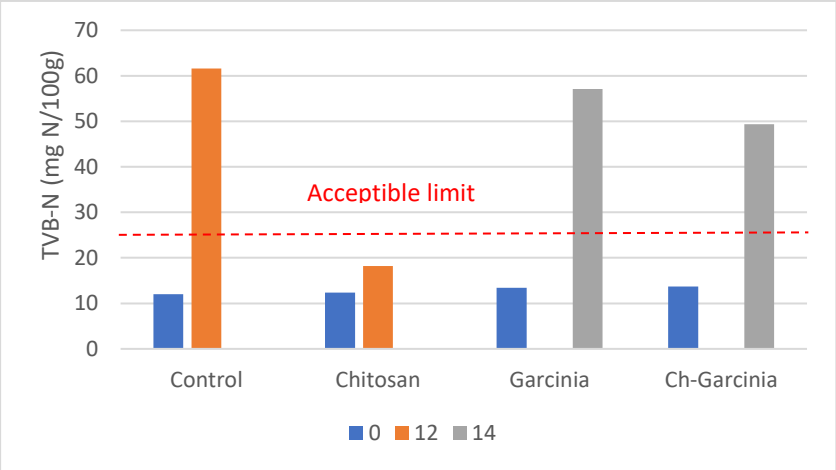

Figure 10: Variation of TVB-N content with storage time.

The TVB-N value increased over time (Figure 10). At day 0, the control group exhibited a TVB-N value of 12 mgN/100 g, whereas the chitosan, *garcinia,* and chitosan–garcinia *groups* had values of 12.4, 13.4, and 13.7 mgN/100 g, respectively. By day 12, the control and chitosan groups recorded values of 61.6 mgN/100 g and 18.2 mgN/100 g, respectively. Subsequently, by day 14, TVB-N values were recorded for the *garcinia* and chitosan–garcinia *groups*, with readings of 57.1 and 49.4 mgN/100 g, respectively. Notably, all three groups exceeded the acceptable limit (25 mgN/100 g) for consumption on days 12 and 14 after storage.

At day 12, the chitosan group showed notably lower values, indicating chitosan effects on TVB-N content. Moreover, the group treated with *Garcinia* exhibited a lower TVB-N value than the control group two days later, indicating a positive effect. The combination of chitosan and *Garcinia* demonstrated a more effective reduction in TVB-N levels after 14 days, suggesting an enhanced impact (TVBN value lower at day 14 than TVB-N value of control group at day 12).

However, it is important to note that these findings are based on the analysis of only one sample, which limits the generalisability of the results. Therefore, further studies with larger sample sizes are needed to confirm these preliminary findings and to establish the effectiveness of chitosan, *Garcinia,* and their combination in reducing TVB-N levels.

4.6.2 *Lipid hydrolysis: Phospholipid content*

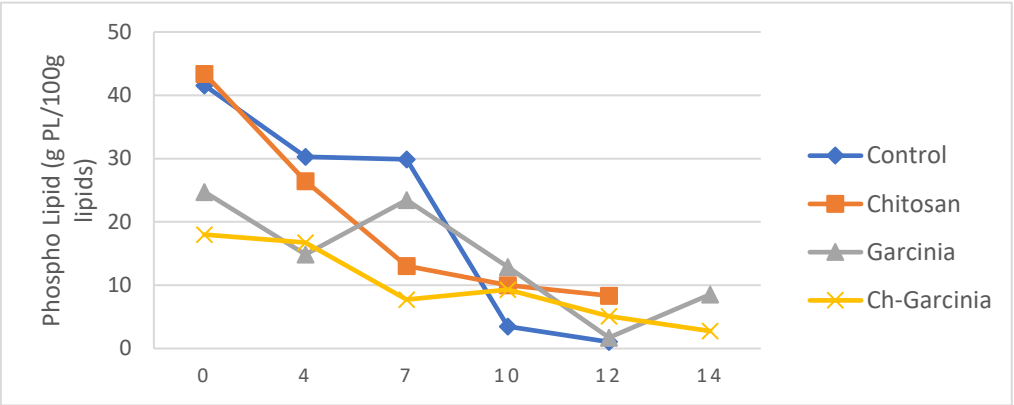

Figure 11: Variation of Phospholipid content with the storage time.

PL decreased over time (Figure 11); in the groups without *Garcinia,* it decreased faster than in the groups with *Garcinia.* In fact, the groups treated with *Garcinia* only decreased by 20 g of PL/100 g of lipid, whereas the groups not treated with *Garcinia* decreased by 40 g of PL/100 g of lipid and 35 g of PL/100 g lipid (when chitosan was added). This means *that Garcinia* impacted lipid hydrolysis reduction, as well as chitosan when used alone, even if it was less effective.

4.6.3 *Lipid hydrolysis: Free Fatty Acid (FFA) Content*

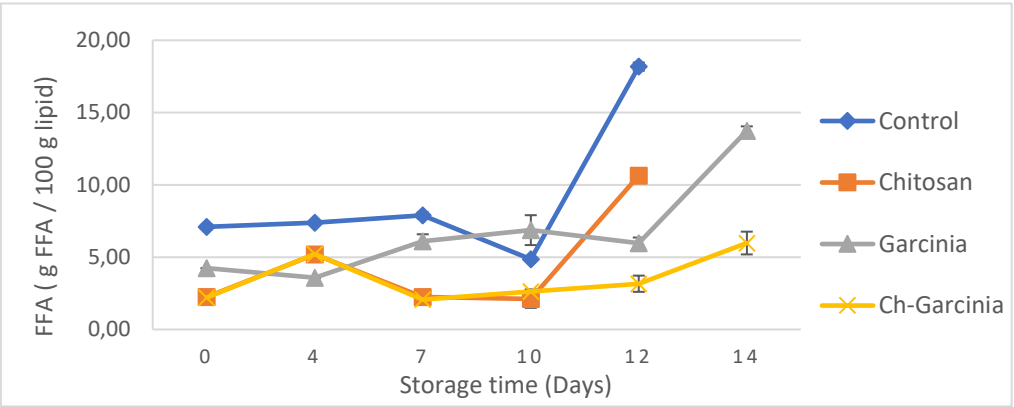

Figure 12:Variation of the FFA with storage time.

FFA increased in all groups through storage time (Figure 12). In both groups without *Garcinia,* FFA levels increased significantly after day 10, whereas the groups treated with *Garcinia showed a* sharp increase only after day 12. When compared at day 12, the groups without *garcinia (control and chitosan only)* had different levels of FFA. According to the two-sample t-test, chitosan had significantly lower FFA levels than the control ($P = 0.001$, $P < 0.05$). This means that even if it did not delay lipid hydrolysis, it decreased it.

The use of *Garcinia* delayed lipid hydrolysis (increasing only between days 12 and 14). When combined with chitosan, both their actions were effective (delay and decrease); as the group treated with both was significantly lower than the group treated with only *Garcinia* on day 14 ($P = 0.049$, $P < 0.05$) and significantly lower than the two (2) groups without *Garcinia* on day 1 ($P = 0.019$, $P < 0.05$ and chitosan ($P = 0.038$, $P < 0.05$), as determined by the two-sample t-test.

4.6.4 Lipid oxidation: Peroxide Value (PV) and Thiobarbituric acid reaction substances (TBARS) value.

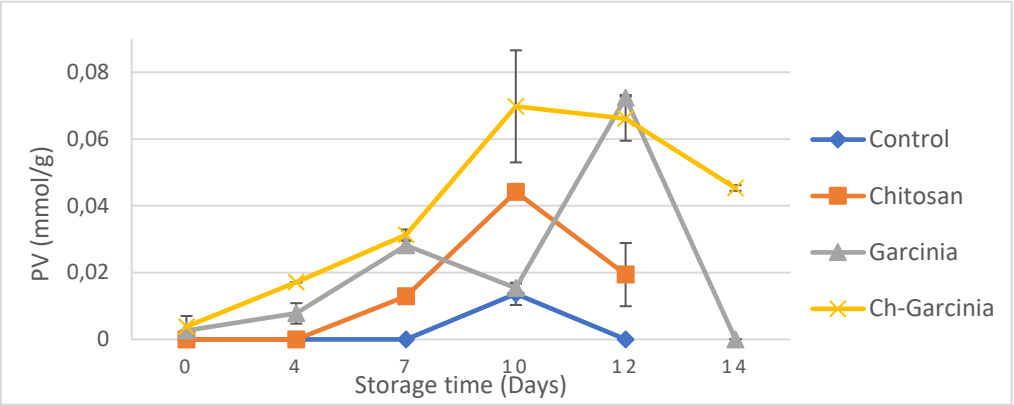

Figure 13:Variation of PV value with the storage time.

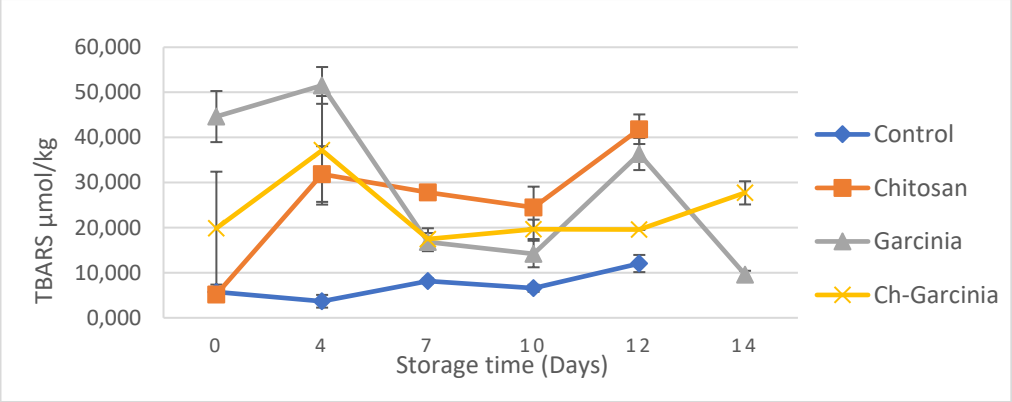

Figure 14: Variation of TBARS value with the storage time.

PV (Figure 13) and TBARS values (Figure 14) fluctuated during storage. No specific trend or interpretation could be made from these measurements. It appears that chitosan or Garcinia neither reduced nor delayed lipid oxidation.

4.7 Microbiological Analysis

4.7.1 Total Viable Count

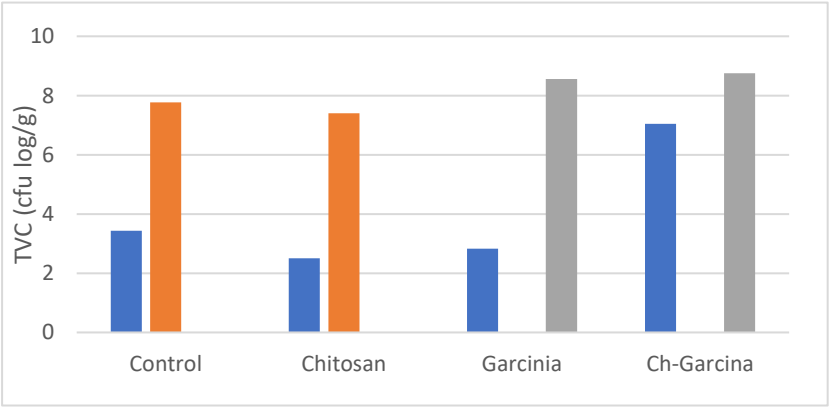

Figure 15: Variation of TVC with storage time.

The TVC increased with storage time (Figure 15). The initial TVC of the control, chitosan, and *Garcinia* fillets at the beginning of the experiments was 3.34 log CFU/g, 2.50 log CFU/g, and

2.82 log CFU/g, respectively. Meanwhile, the Ch-*Garcinia* group showed 7.04 log CFU/g, over the maximum limit of 5 log CFU/g is considered as freshness limit for fresh fish (Huynh, 2019).

The TVC increased during the storage period and reached 7.77 log CFU/g and 7.39 log CFU/g and 8.56 log CFU/g and 8.74 log CFU/g on days 12 and 14 for the control, chitosan, *Garcinia,* and Ch-*Garcinia* groups, respectively. The increment of the TVC value in the Ch-*Garcinia* group was low (1.7 log CFU/g) compared to other groups (more than 4 log CFU/g), indicating improvement in the inhibition of microbes. However, only one sample was measured in each group; therefore, these differences could be due to individual differences between the fillets.

4.7.2 Pseudomonas spp.

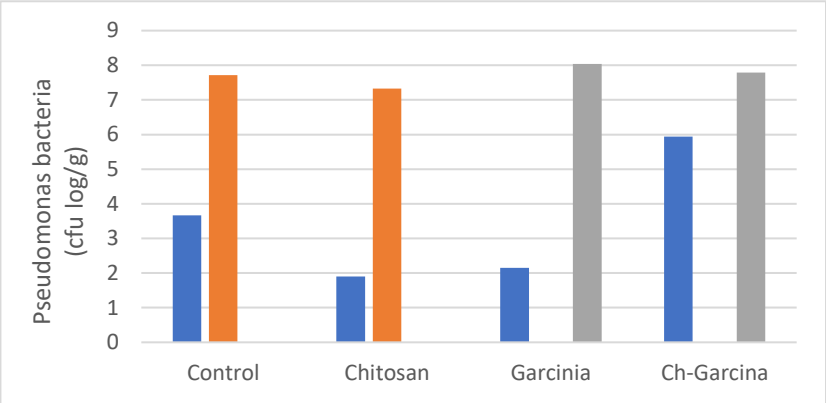

Figure 16: Variation of Pseudomonas spp. with storage time.

Pseudomonas spp. increased over time during storage of the fillets (Figure 16). At the beginning of the experiments, the fish fillets had Pseudomonas spp. values of 3.66 log CFU/g, 1.90 log CFU/g, 2.16 log CFU/g, and 5.94 log CFU/g for the control, chitosan, Garcinia, and Ch-Garcinia treatments, respectively. Increment of *Pseudomonas spp*. count during the storage period reached approximately 7.71 log CFU/g, 7.32 log CFU/g and 8.04 log CFU/g, 7.79 log CFU/g respectively on day 12 and 14. After 12 days of storage, chitosan treatment showed a lower value, indicating the impact of chitosan. After 14 days of storage, the Chitosan and *Garcinia* treatments worked to delay/decrease the development of those bacteria.

4.8 Sensory Evaluation

4.8.1 Freshness odours

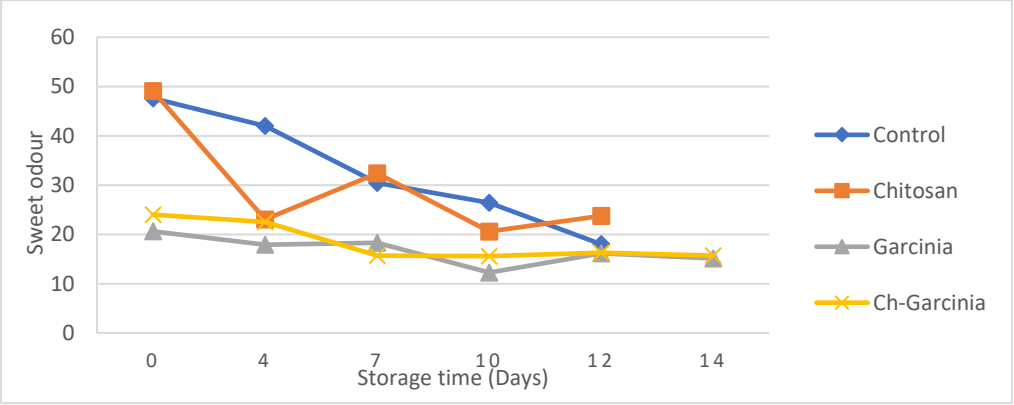

Figure 17: Variation of sweet odour with storage time.

The sweet odour decreased over storage time (Figure 17). At the start of the trial on day 0, all groups displayed notably high sweetness scores, with the control and chitosan groups scoring 48 and 49, respectively, followed by *garcinia* (21) and ch-garcinia (24). This initial assessment revealed a significant difference in sweetness perception between the control (a) and chitosan (a) groups and the *garcinia* (b) and ch-garcinia (b) groups ($P = 0.001$, $P < 0.05$). This could be explained by the strong smoky smell detected initially with *garcinia*, which can mask the sweetness. The decrease in sweet odour occurred more quickly in the groups without *garcinia*, which could also be related to the masking smell of the spice (smoky).

4.8.2 Spoilage odours

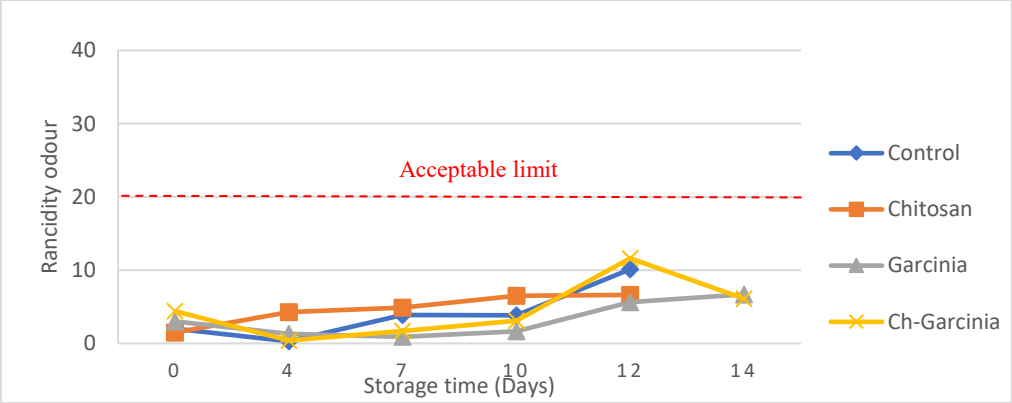

Figure 18:Variation of Rancid odour sensory score for storage time.

For rancid odour (Figure 18) at day 0, no significant differences were observed among the Control, Chitosan, *Garcinia*, and Ch-*Garcinia* groups ($P= 0.597$. $P > 0.05$). Similarly, at subsequent time points (days 4, 7, 10, 12, and 14), there were no statistically significant differences in rancid odour perception scores among the groups (P-values ranging from 0.137 to 0.855; $P > 0.05$). Despite no difference between the groups in sensory analysis, all groups increased in rancidity, indicating that sample degradation was detected by the panellists.

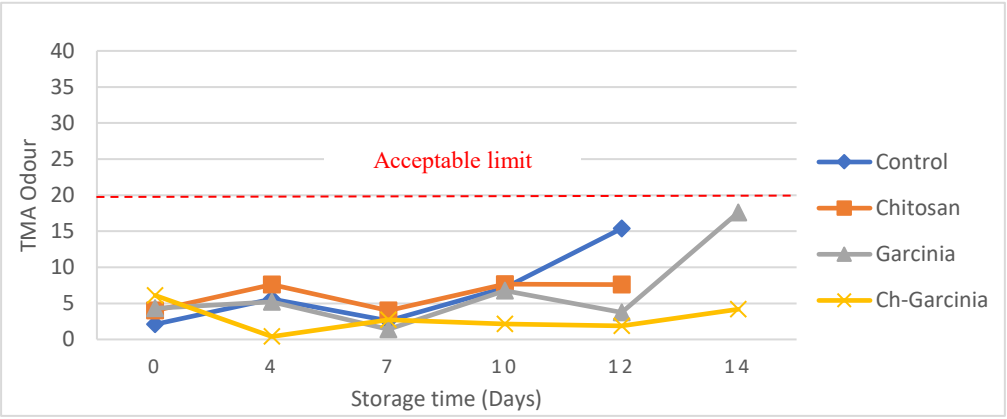

Figure 19:Variation of TMA odour sensory score for storage time.

On day 0, there were no notable differences in TMA odour Figure 19perception among the control, chitosan, *garcinia*, and chitosan–garcinia groups (Figure 18). This trend persisted throughout subsequent time points (days 4, 7, 10, 12, and 14), with no statistically significant variations in TMA odour perception scores across the groups (p-values ranging from 0.085 to 0.679, $P > 0.05$). Despite the lack of significant differences between the groups, higher values

were observed in the control and chitosan groups on day 12, whereas the chitosan–garcinia group exhibited notably low values. By day 14, the *garcinia* group displayed values four times higher than those of the chitosan–garcinia group.

Similar phenomena were also observed with cloth odour, particularly on days 10 and 14. On day 10, the control (a) and chitosan (a) groups had values of 12 and 11, respectively, followed by *garcinia* (4) and chitosan–garcinia (1) (b) groups, respectively, and the differences were significant ($P = 0.031$, $P < 0.05$). On day 14, *the garcinia* group value (16) was twice that of the chitosan–*garcinia* group (8).

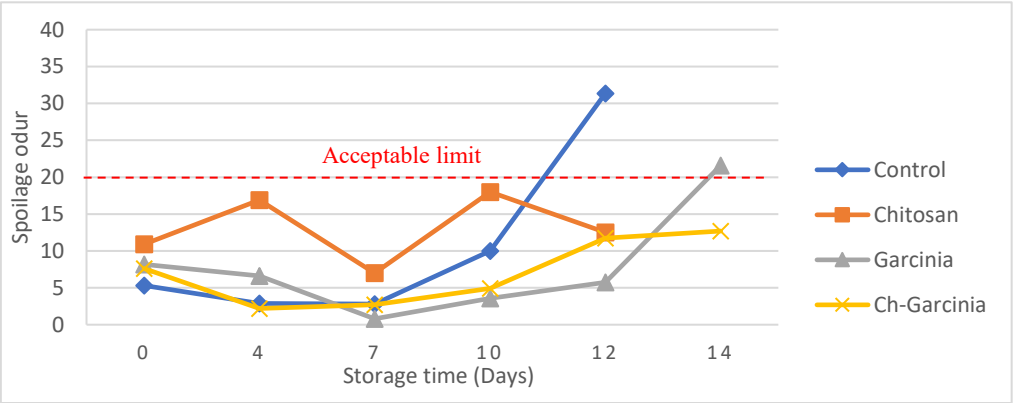

Figure 20:Variation of Spoilage odour with storage time.

The sensory evaluation scores for spoilage odour (Figure 20) showed that on day 0, there were no significant differences among the control, chitosan, *garcinia*, and ch-garcinia groups ($P = 0.493$, $P > 0.05$). By day 4, although not statistically significant, there was a noticeable increase in spoilage perception scores in the chitosan group compared to the other groups ($P = 0.062$). As the storage period progressed, the differences in spoilage perception became more apparent. By day 10, the control group had the highest spoilage perception score of 10, while the chitosan group had a higher score of 18, indicating a stronger perception of spoilage in this group ($P= 0.053$, $P > 0.05$). However, on day 12, the control group (a) had the highest score, and the chitosan group (b) followed by the ch-garcinia (b) and *garcinia* (b) groups had significantly lower scores ($P= 0.023$, $P < 0.05$). By day 14, spoilage perception scores were higher in the *garcinia* group than in the ch-garcinia group; however, the difference was not statistically significant ($P= 0.429$, $P > 0.05$), and they were still showing lower values than the control group on day 12, indicating that *garcinia* was effective in decreasing spoilage odour.

4.8.3 Freshness flavour

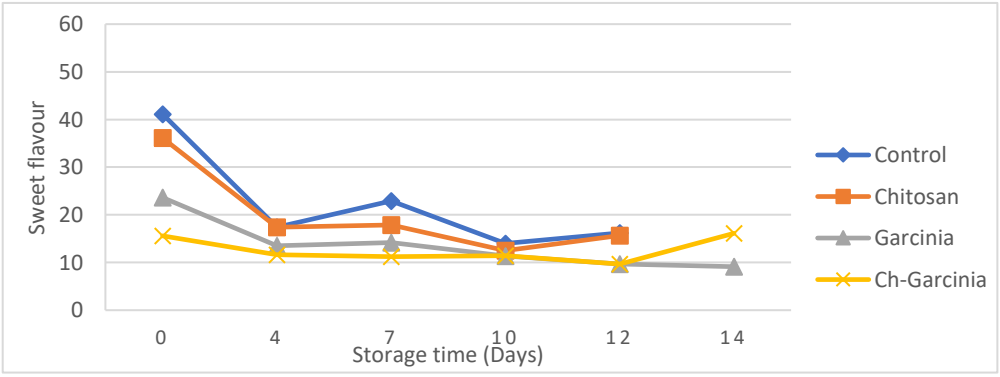

Figure 21:Variation of Sweet Flavour with storage time.

The sensory evaluation scores for sweet flavour (Figure 21) in different treatment groups decreased over the course of the storage trial. At day 0, significant differences in sweet taste perception scores were observed among the Control (a), Chitosan (a), *Garcinia* (b), and Ch-*Garcinia* (b) (P= 0.002, P < 0.05). Throughout the storage trial, sweetness flavour values were found to be stable for the *garcinia-treated* groups and decreasing for the groups without *garcinia*. This can be related to the high smoky flavour, which masked the sweet flavour.

4.8.4 Spoilage flavours

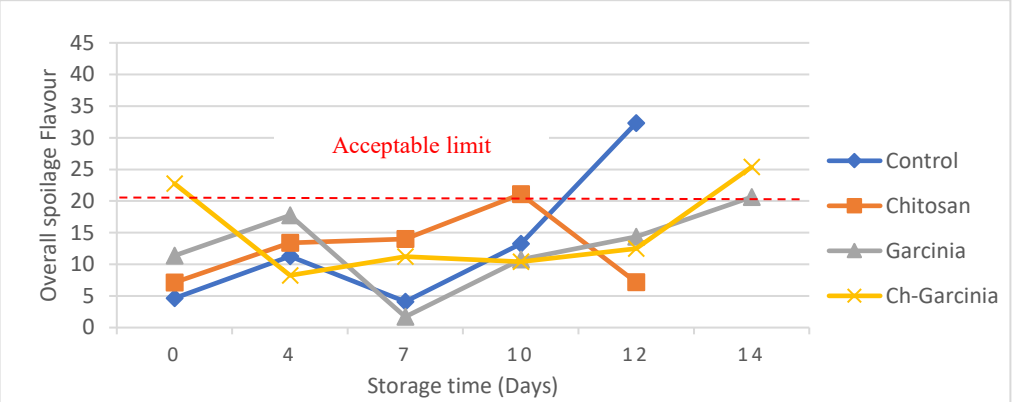

Figure 22:Variation of overall spoilage flavour with storage time.

Spoilage flavour changed throughout storage time (Figure 22Figure 22). On day 0, significant differences in spoilage flavour scores were evident between the control (b), chitosan (b), *and garcinia* with chitosan (a) (P = 0.033, P < 0.05). Notably, the chitosan–garcinia group recorded the highest score, indicating heightened perception of spoilage compared to the other groups. However, as the storage trial progressed, these differences diminished. It is worth noting that this analysis was based on individual fillets, which may have contributed to the variability in the results.

By day 4, no statistically significant variations in spoilage perception scores were observed among the groups (P = 0.741, P > 0.05); this trend persisted through days 7, 10, and 14 (P-values ranging from 0.320 to 0.428, P > 0.05). However, on day 12, significant differences resurfaced, with the control (a) group exhibiting the highest score, followed by the garcinia (b) group, while the chitosan (b) and ch-garcinia groups recorded lower scores (P = 0.041, P< 0.05).

The sensory panel rejected the control and chitosan groups on day 12, whereas the two *garcinia* groups lasted until day 14. This was due to diminished freshness, with reduced sweetness in odour and flavour, and notably higher levels of spoilage-related odours, such as TMA, rancidity, and cloth, in the former groups. The overall results from the sensory evaluation are presented in Appendix 1. Table 2 and Appendix 1. Table 3.

5 DISCUSSION

5.1 Pre-trial

Garcinia cambogia is an excellent candidate for the main experiment because of its high levels of phenolic compounds and strong reducing power values, particularly when compared with *Tamarindus indica*. In fact, its TPC surpasses that of well-known antioxidants like *Rosmarinus officinalis* and *Citrus sinensis* (L.) Osbeck, as reported by Tawaha et al., (2007). However, when compared with oregano and thyme, the TPC values of *Garcinia cambogia* were found to be lower (Mutlu-Ingok et al, 2021).

When preparation of chitosan for chitosan group only, 1.5 % was used instead of 2 % chitosan in 1% of acetic acid because the solution was too dense and thick made it difficult to dip fish fillets in. According to studies of Huynh (2019) pH value of the 1.5 % chitosan in 1% acetic acid was founded to be 4.1. This may be the reason for the dense and thick solution compared to 2% chitosan dipped in *Garcinia* solution (pH= 2.46)

5.2 Main experiment

5.2.1 Garcinia and its Effect on shelf life

This study used *Garcinia cambogia* to utilise its antioxidant properties to extend the shelf life of golden redfish fillets and reduce lipid degradation. Analysis revealed that *Garcinia* positively contributed to prolonging shelf life by mitigating lipid hydrolysis, which can cause off-flavours and odours and accelerate spoilage. The observed delay and reduction in FFAs, particularly after 14 days of storage, indicate that *Garcinia* effectively slowed down lipid hydrolysis, thereby preserving fillet quality for a longer duration.

Additionally, studies by Apang et al. (2020) have highlighted the antimicrobial properties of *Garcinia cambogia* extract when incorporated into icing mediums to enhance the shelf life of Indian mackerel. Similarly, Bhuvana et al. (2020) investigated the antimicrobial efficacy of *Garcinia cambogia* fruit extract against foodborne bacterial pathogens in chicken meat, demonstrating inhibition of *Pseudomonas spp*. The results indicated a significant impact on microbial growth with the addition of *Garcinia* extract, likely attributed to its lower pH values due to its higher polyphenolic content, particularly hydro citric acid.

The inhibition of spoilage bacteria is linked to a decrease in ammonia production resulting from microbial activity, consequently leading to lower levels of TVB-N and protein degradation observed in the storage trial with *Garcinia* alone. These findings align with those of Apang et al. (2020) for Indian mackerel.

5.2.2 Chitosan and its Effect on Shelf Life.

Chitosan was selected because of its well-established antimicrobial properties. In our study, chitosan alone was effective in reducing TVC and suppressing the growth of *Pseudomonas bacteria* throughout the storage trial. As mentioned previously, a reduction in spoilage bacteria, including *Pseudomonas spp*., is correlated with a decrease in TVB-N levels.

Similar findings have been reported by Fan et al., (2009) and Bonilla et al., (2018), who reported that chitosan effectively reduced spoilage bacteria, resulting in decreased TVB-N levels in silver carp and catfish fillets, respectively. The presence of chitosan in fish samples hinders spoilage bacteria by interfering with the oxidative deamination process of nitrogen compounds. This interference occurs because of the positively charged amino groups of chitosan. These groups interact with negatively charged components on the bacterial cell membrane, disrupting its integrity and permeability (Dutta et al., 2004; Shahidi et al., 1999). Consequently, essential

cellular functions, such as nutrient uptake and waste expulsion, are compromised, ultimately inhibiting bacterial growth and proliferation. This mechanism primarily affects protein breakdown and lipid-related processes.

Chitosan alone decreased FFAs, lipid hydrolysis, and TVB-N; protein degradation was observed after 14 days of storage. Chitosan interferes with enzymatic processes involved in the oxidative deamination of both non-protein and protein nitrogen compounds by binding to enzymes or substrates, thereby impeding the breakdown of proteins and lipids and reducing the production of spoilage byproducts, such as FFAs and ammonia (Sathivel, 2005).

The analysis revealed that chitosan coatings alone exhibited some effectiveness in preserving fillet quality, as evidenced by acceptable sensory attributes for approximately 12 days of storage at 4°C. However, compared with the *Garcinia*-treated groups, the shelf life extension offered by chitosan alone was limited.

5.2.3 Combined effect of Garcinia and Chitosan

The aim of combining chitosan and *Garcinia* was to synergistically leverage their antimicrobial and antioxidant properties, thereby extending the shelf life of the product. The analysis suggests that using both chitosan and *Garcinia* in a dual-functional coating resulted in more significant improvements in shelf life than using either component alone.

This combined treatment showed enhanced effectiveness in reducing lipid hydrolysis, particularly after 14 days of storage. The synergistic effect indicates that chitosan's antimicrobial action complemented *Garcinia's* antioxidant properties, resulting in superior preservation of fillet quality. Chitosan's ability to reduce spoilage bacteria indirectly hindered lipid hydrolysis by limiting the availability of enzymes produced by these bacteria.

Similar results have been observed in previous studies, in which chitosan coatings incorporated with citric acid or liquorice extract improved the quality and shelf life of fish fillets (Qiu et al., 2014). Additionally, combined treatment led to a decrease in protein degradation, further supporting its effectiveness in extending shelf life by inhibiting spoilage microorganisms and oxidative processes.

Overall, the combination of chitosan and *Garcinia* shows promise in enhancing the shelf life of golden redfish fillets by addressing microbial spoilage and oxidative deterioration simultaneously.

6 CONCLUSION AND RECOMMENDATIONS

In conclusion, the investigation into the effects of *Garcinia cambogia* and chitosan on the shelf life of golden redfish fillets revealed promising strategies for extending freshness and quality. *Garcinia cambogia* demonstrated notable antioxidant properties, effectively mitigating lipid hydrolysis, and reducing TVB-N levels. This resulted in a delay in spoilage and preservation of sensory quality for at least 2 days. Similarly, chitosan, known for its antimicrobial properties, showed effectiveness in reducing spoilage bacteria and inhibiting oxidative processes. The combined treatment of chitosan and *Garcinia* exhibited a synergistic effect, surpassing the individual contributions of each component. This dual-functional coating demonstrated enhanced efficacy in mitigating lipid hydrolysis and reducing TVB-N levels, thereby significantly prolonging the freshness of the fillets.

These findings indicate that the application of Ch- *Garcinia* could potentially offer a solution to the challenges posed by chemical and microbiological spoilage in Sri Lanka's yellowfin tuna industry. Therefore, future studies should replicate this trial specifically for yellowfin tuna in Sri Lanka. Given the economic importance of yellowfin tuna to Sri Lanka's fishing industry, such studies could provide valuable insights into practical applications that may help improve product quality, reduce waste, and increase market competitiveness.

The increase in the production and selling prices of yellowfin tuna due to the application of a Ch–Garcinia coating is estimated to be approximately 7%–10%. Given the substantial benefits of the coating, including an extended shelf life for yellowfin tuna and potential marketing advantages, both fish processors and consumers would likely find the modest increase in production and selling prices acceptable.

However, further research with larger sample sizes and replication is necessary to validate these findings and optimise the formulation for practical implementation in the food industry. It is recommended to focus more on raw material-fresher fillet samples and monitoring microbiological and TVB-N contents throughout the storage period. Additionally, investigating the extraction process of *Garcinia* and optimising the properties of the coating would be beneficial for its application in fish preservation.

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APPENDICES

Appendix 1. Table 1: Sensory methodology, GDA scale for sensory evaluation of golden redfish fillets.

Sensory attribute	Short name	Definition
ODOUR		
sweet	O-sweet	Sweet odour of fresh redfish
smoke	O-smoke	Smoke odour, smoked salmon, smoked mackerel
cod liver	O-cod liver	Odour of boiled cod liver
shellfish, algae	O-shellfish	Characteristic, fresh odour of cooked redfish
kitchen cloth	O-cloth	Reminds of a damp kitchen cloth
TMA	O-TMA	TMA odour, reminds of dried salted fish, amine
rancid	O-rancid	Rancid odour
sour	O-sour	Spoilage characteristic, odour of sour milk, vinegar, butyric acid
spoilage	O-spoilage	Overall spoilage odour
FLAVOUR		
cod liver	F-cod liver	Flavour of boiled cod liver
metallic	F-metallic	Characteristic metallic flavour of fresh redfish
sweet	F-sweet	Characteristic sweet flavour of fresh redfish
smoke	F-smoke	Smoke flavour, smoked salmon, smoked mackerel
acidic	F-acidic	Fresh sour flavour, acidic
bitter	F-bitter	Bitter flavour
sour	F-sour	Spoilage characteristic, flavour of sour milk, vinegar, butyric acid
TMA	F-TMA	TMA flavour, reminds of dried salted fish, amine
rancid	F-rancid	Rancid flavour
spoilage	F-spoilage	Strength of spoilage flavour (all spoilage flavours)

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Appendix 1. Table 2: GDA results for odours of the fillets.

	odour								
Group	sweet	smoke	cod liver	shellfish	cloth	TMA	rancid	sour	spoilage
day 0									
CONTROL	48 **a**	0 **b**	4	10 **ab**	5	2	2	1 **b**	5
CHITOSAN	49 **a**	1 **b**	6	14 **a**	4	4	2	10 **a**	11
GARCINIA	21 **b**	52 **a**	3	5 **bc**	6	4	3	4 **b**	8
Ch-GARCINIA	24 **b**	52 **a**	6	6 **c**	7	6	4	3 **b**	8
p-value	0.001	0.000	0.454	0.004	0.765	0.586	0.597	0.010	0.493
day 4									
CONTROL	42 **a**	0 **c**	3	13 **a**	4 **b**	6	0	0	3
CHITOSAN	23 **b**	0 **c**	4	7 **b**	13 **a**	8	4	16 **a**	17
GARCINIA	18 **b**	41 **b**	3	4 **b**	5 **b**	5	1	2 **b**	7
Ch-GARCINIA	23 **b**	54 **a**	4	4 **b**	5 **b**	0	0	1	2
p-value	0.001	0.000	0.654	0.004	0.030	0.403	0.379	0.054	0.062
day 7									
CONTROL	30	1 **b**	8	11 **ac**	2	3	4	2	3
CHITOSAN	32	0 **b**	11 **a**	10 **a**	7	4	5	7	7
GARCINIA	18	51 **a**	4 **b**	6 **bc**	1	1	1	1	1
Ch-GARCINIA	16	52 **a**	2 **b**	3 **b**	1	3	2	2	3
p-value	0.029	0.000	0.012	0.003	0.140	0.679	0.471	0.480	0.303
day 10									
CONTROL	26	0 **b**	7	6	12 **a**	7	4	3 **b**	10
CHITOSAN	21	0 **b**	7	6	11 **a**	8	7	11 **a**	18 **a**
GARCINIA	12	38 **a**	3	2	4	7	2	2	4 **b**
Ch-GARCINIA	16	50 **a**	2	2	1 **b**	2	3	2 **b**	5 **b**
p-value	0.100	0.000	0.051	0.066	0.031	0.325	0.137	0.067	0.053
day 12									
CONTROL	18	0 **b**	11	3	11	15	10	9	31 **a**
CHITOSAN	24	0 **b**	11	7	1	8	7	3	13 **b**
GARCINIA	16	40 **a**	7	3	2	4	6	3	6 **b**
Ch-GARCINIA	16	48 **a**	3	1	0	2	12	2	12 **b**
p-value	0.530	0.001	0.439	0.207	0.244	0.085	0.855	0.413	0.023
day 14									
GARCINIA	15	11	4	5	16	18	7	6	22
Ch-GARCINIA	16	39	6	4	8	4	6	6	13
p-value	0.853	0.107	0.505	0.389	0.412	0.245	0.763	0.870	0.429

Appendix 1. Table 3: GDA results for flavours of fish fillets.

	flavour									
Group	cod liver	metallic	sweet	smoke	acidic	bitter	sour	TMA	rancid	spoilage
day 0										
CONTROL	6	17	41 **a**	0	4	3	2	3 **b**	1	5 **b**
CHITOSAN	9	23	36 **a**	0	5	4	6	3	2	7 **b**
GARCINIA	9	15	24 **b**	40 **a**	6	19	9	9	4	11
Ch-GARCINIA	11	20	16 **b**	23 **b**	8	16	11	13 **a**	14	23 **a**
p-value	0.874	0.326	0.002	0.001	0.686	0.038	0.345	0.050	0.316	0.033
day 4										
CONTROL	5	22	17	0 **b**	5	25	3	6	3	11
CHITOSAN	12	21	17	0 **b**	4	14	11	9	6	13
GARCINIA	9	25	14	19 **b**	4	29	13	8	4	18
Ch-GARCINIA	4	25	12	43 **a**	6	31	7	3	2	8
p-value	0.123	0.876	0.798	0.005	0.965	0.219	0.594	0.378	0.743	0.741
day 7										
CONTROL	13	27	23 **a**	1 **b**	4	8	1	3	4	4
CHITOSAN	18	27	18	0 **b**	1	7	11	7	12	14
GARCINIA	8	25	14	36 **a**	13	11	1	2	2	2
Ch-GARCINIA	12	23	11 **b**	42 **a**	7	11	11	10	9	11
p-value	0.327	0.677	0.037	0.000	0.171	0.707	0.445	0.433	0.284	0.380
day 10										
CONTROL	10	15	14	0 **b**	2	20	6	15	9 **b**	13
CHITOSAN	8	15	13	0 **b**	2	21	7	7	23 **a**	21
GARCINIA	7	18	11	28 **a**	4	31	5	9	3 **b**	11
Ch-GARCINIA	7	15	11	26 **a**	3	19	5	7	5 **b**	10
p-value	0.759	0.300	0.707	0.000	0.433	0.369	0.883	0.076	0.011	0.428
day 12										
CONTROL	13	6	16	0 **b**	1	14	11	13	14	32 **a**
CHITOSAN	14	11	16	0 **b**	0	9	1	3	8	7 **b**
GARCINIA	8	7	10	17	2	10	4	11	12	14 **b**
Ch-GARCINIA	7	4	10	30 **a**	1	14	9	9	5	13
p-value	0.527	0.377	0.470	0.023	0.369	0.817	0.129	0.418	0.763	0.041
day 14										
GARCINIA	14	14	9	15	3	22	13	19	9	21
Ch-GARCINIA	9	15	16	22	3	23	18	11	8	25
p-value	0.062	0.534	0.448	0.474	1.000	0.819	0.188	0.247	0.767	0.320

Appendix 1: **The experimental trial**

The fish fillets were immersed in the treatment solutions for 15 seconds and then placed on metal racks. Subsequently, the racks were left to dry at 4ºC for 1.5 hours.

Figure 3: After immersion of the fillets in treatment solutions.

Figure 2: Drying of fish fillets at 1-2ºC for 1.5 hours.

Figure 1:Storing of fish fillets at 4ºC.