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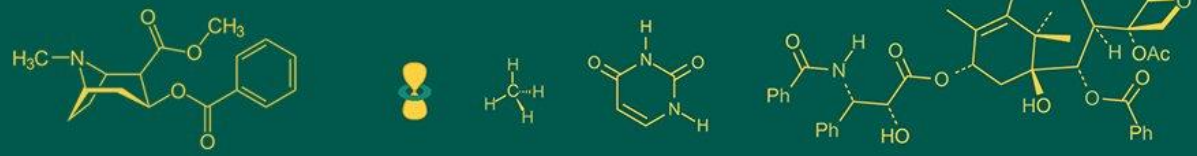

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Effect of *Garcinia indica*, Kokum extract on shelf life of Indian Mackerel, *Rastrelliger kanagurta* (Cuvier, 1816) during chilled storage

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Abstract
Fish has been a vital source of food for humanity across the globe since ancient times. Although fish is an important constituent of human diet which provides protein, vitamins, minerals, fats, etc. but being perishable, gets decomposed if not stored and handled properly. Natural plant-based products have been widely studied and used to extend the shelf life of food products and improve their microbiological quality. The study investigated the effect of kokum, *Garcinia indica* extract as a natural preservative on the shelf life of Indian mackerel, *Rastrelliger kanagurta* packed in Low density polyethylene bags during chilled storage (0-4 °C). Study focused on standardizing the concentration of kokum extract (0%, 5%, 10%, and 15). Quality assessments included proximate composition, biochemical parameters (pH, Total volatile base nitrogen, trimethyl amine nitrogen, free fatty acid and peroxide value), microbiological counts, and sensory attributes. Results indicated that kokum extract-treated fish exhibited significantly lower increases in pH, TMA-N, TVB-N, FFA, PV, and microbial counts compared to untreated controls, thereby delaying spoilage. The proximate composition of fresh Indian mackerel (*Rastrelliger kanagurta*) meat had 76.65±0.94%, 18.58±0.07%, 3.44±0.08% and 1.17±0.01% of moisture, protein, fat and ash respectively. The proximate composition showed good retention in treated samples as compared to the control samples. Sensory evaluation indicated that T_3 retained higher overall acceptability and extended up to 18 days compared to the control sample. Among the concentrations tested, 15% kokum extract showed the most effective preservation. Overall, kokum extract demonstrated significant potential in enhancing the shelf life and quality of chilled mackerel, supporting its application as a natural preservative in fish preservation.

Keywords: Kokum, natural preservative, Indian mackerel, LDPE, chilled storage, sensory evaluation, shelf life

Introduction
Fish has been a vital source of food for humanity across the globe since ancient times. Its significance as a provider of high-quality, well-balanced, and easily digestible protein, along with essential vitamins and polyunsaturated fatty acids, is now widely recognized (Pawar *et al*. 2013) [17]. One of the most important components of fish is its healthy fats, which are high in omega-3 fatty acids, including α-linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Pal *et al*. 2018) [16]. Fish is a highly perishable food that is very prone to microbial and oxidative deterioration. Therefore, to mitigate these alterations in fish, effective preservation methods must be utilized to ensure the extended quality and safety of the fish products (Walayat *et al*. 2023) [26]. Various methods have been explored to maintain seafood quality by preventing spoilage, slowing down enzymatic activity, and controlling harmful pathogens. Among these, the use of natural products to extend seafood shelf life and inhibit foodborne pathogens has gained significant attention. (Baptista *et al*. 2020) [5]. Natural plant-based products have been widely studied and used to extend the shelf life of food products and improve their microbiological quality (Tayel *et al*. 2017) [23].
Garcinia indica, also known as Kokum, Brindon or Bhirand, Anslil, (Marathi) Murgal (Tamil), Punarpulli (Tulu), is the most underrated fruit species and is a rich source of anthocyanin, a natural flavonoid and water soluble pigments present in the epidermal cell of flowers and plants.

In India, *Garcinia indica* is mainly distributed among the Western Ghats of south India as well as north eastern part of India. More than 200 species of *garcinia* are available all over the world and out of which 30 are reported to be found in India (Nayak *et al*. 2010) [15]. The ancient Indian medical system of Ayurveda has traditionally used kokum. Because of their sour, astringent, and digestive qualities, its leaves and fruits are said to help treat intestinal problems, rheumatic pain, and inflammatory diseases. Fruit is also said to be good for intestinal worm removal and heart health. (Baliga *et al*. 2011) [4]. Kokum (*Garcinia indica*) has shown potential as an alternative antibacterial agent. Aqueous extracts of kokum rind inhibit the growth of bacteria such as *E. coli*, *B. subtilis*, *E. aerogenes*, and *S. aureus*. Organic extracts (hexane and benzene) from spent kokum rinds and its active compound, garcinol, also display antibacterial activity (Murthy *et al*. 2011) [13]. Another important compound found in Kokum is garcinol which acts as the strong antioxidant due to the presence of phenolic hydroxyl groups and diketone moiety. It also acts as a free radical scavenger and thus is very important in the pharmaceuticals. Compounds like furfural and its derivatives, cyanidin-3-glucose, which are present as anthocyanins, are responsible for the antimicrobial activity of Kokum. (Sutar *et al*. 2012) [22].

The *Rastrelliger* genus belongs to the family Scombridae, which includes the epipelagic fishes found in tropical and subtropical regions. (Anonymous. 2023) [1]. This study mainly includes investigation of use of natural preservatives in enhancing the shelf life of the fish without the use of any artificial preservatives. The aim of the research is to study the effect of kokum *Garcinia indica* on the shelf life of Indian mackerel *Rastrelliger kanagurta* during chilled storage.

Material and methodology

Fresh fish Indian mackerel, *Rastrelliger kanagurta* (Cuvier, 1816) were collected from Mirkarwada jetty Ratnagiri, Maharashtra. The fresh Indian mackerel (*Rastrelliger kanagurta*) of a length b/w in the range of around 15 to 25 cm and weight b/w of nearly 200-300 g were used for the experiment purpose. Dry *Garcinia indica* fruit kokum/mangosteen fruit rinds were procured from Ratnagiri market.

Preparation of kokum stock solution

The kokum extract was prepared using the method given by Apang *et al*. (2020) [1]. Dried kokum rinds were procured from the local market. The aqueous extract of Kokum was prepared from dry rinds of the fruit in the ratio of 1:5 (W/V) (kokum: water). For this, 100 g kokum and 500 ml water in 1:5 w:v ratio were added and kept overnight. This mixture was stirred and boiled for 15 minutes. Then this mixture was centrifuged and then filtered using Whatman no. 1 filter paper. This kokum extract stock solution was stored in air tight glass bottles at room temperature for further use.

Preparation of kokum extract dilutions

The dilutions of kokum extract were prepared using potable water. The concentrations of the extract were decided on the basis of the results of the previous trial studies carried out which showed the optimum range of the requirements. The extract dilutions were prepared for different concentration of 0%, 5%, 10% and 15%, where T_0 (control), T_1 by adding 250 ml stock solution in 5000 ml water, T_2 by adding 500 ml stock solution in 5000 ml water and T_3 by adding 750 ml stock solution in 5000 ml water.

Chilled storage of Indian mackerel

The fish were washed thoroughly and cleaned properly. Then beheading and gutting of fish was carried out. Four separate lots were prepared according to the different concentrations i.e, T_0, T_1, T_2 and T_3. These fish were dipped in the tanks with different concentrations for 15 minutes. Then the fish were packed in LDPE bags and kept in chilled storage at 4 °C. During the storage study, the sampling was carried out at an interval of 3 days from each treatment. The samples were subjected to organoleptic evaluation, proximate composition, biochemical analysis as well as microbiological evaluation by following appropriate methods.

Organoleptic evaluation

The physical attributes such as appearance, colour, texture, odour, and overall acceptability were evaluated. by a group of 10 panellists. A '0 to 9' point hedonic scale was used during the period of chilled storage study until the quality of fish was unfit for human consumption (Hough G. 2010) [8]. The scores given by panellists for each of the attributes were collected and average scores were recorded.

Proximate composition

The proximate composition of mackerel fish i.e. moisture, crude protein, crude fat, and ash were carried out as per the methods given by AOAC (2005) [2].

Biochemical analysis

The pH of the samples was measured using filtrate was recorded using a digital pH meter (EQUIPTRONICS brand, MODEL EQ 610) according to the AOAC (2005) [2] technique. The PV, TVB-N, TMA-N FFA of the samples was detected using AOAC (2005) [2] method.

Microbiological analysis

The glassware needed for analytical work was used after being properly sterilised for 15 minutes at 15 psi in an autoclave. Microbiological analysis was carried out to enumerate TPC total plate count (Maturin and Peeler 2001) [11] *E-coli* and *staphylococcus aureus* USFDA (2022) [22].

Statistical Methods

Using the proper statistical techniques, the findings of the quality examination of fish samples were statistically analysed (Snedecor and Cochran 1967) [19]. A two-way ANOVA and post-hoc analysis using the "Tukey test" were used to identify any significant differences between the treatments.

Results and Discussion

Proximate composition of fresh Indian mackerel (*Rastrelliger kanagurta*) fish meat

In the present study, the proximate composition of Indian mackerel meat was determined to be 76.65±0.94% moisture, 18.58±0.07% protein, 3.44±0.08% fat, and 1.17±0.01% ash. These findings are consistent with those reported by several other researchers. Apang *et al*. (2020) [1] observed comparable values, reporting 75.65±0.94% moisture, 19.58±0.07% protein, 3.54±0.08% fat, and 1.07±0.01% ash

in mackerel. Similarly, Aneesh *et al*. (2012) recorded 76.17±0.26% moisture, 19.2±0.18% protein, 2.64±0.05% fat, and 1.99±0.04% ash. Viji *et al*. (2015) [24] also reported proximate values in line with these findings, noting 74.05±1.65% moisture, 21.75±1.23% protein, 2.64±0.35% lipids, and 1.03±0.07% ash. It is important to note that the chemical composition of fish can vary not only between species but also within the same species, influenced by factors such as age, sex, environmental conditions, and seasonal changes (Reza *et al*., 2009) [17].

Organoleptic score of mackerel fish treated with different concentration of kokum extract during chilled storage

In the present study, overall acceptability scores were assessed over a 21-day storage period. The shelf life of fish samples was determined based on the point at which the acceptability score approached the rejection threshold (score < 5). The results indicated that sample T_0 reached a score of 5.6 on day 12, T_1 scored 5.4 on day 15, T_2 scored 5.6 on day 15, and T_3 scored 5.2 on day 18. Accordingly, the maximum shelf life for samples T_0, T_1, T_2, and T_3 was established as 12, 15, 15, and 18 days, respectively. These findings align with those reported by Apang *et al*. (2020) [1], who observed similar trends in the organoleptic evaluation of chilled mackerel, with rejection occurring on day 12 for the control and on days 15 and 18 for treated samples. A score below 5 was used as the criterion for rejecting samples based on overall acceptability.

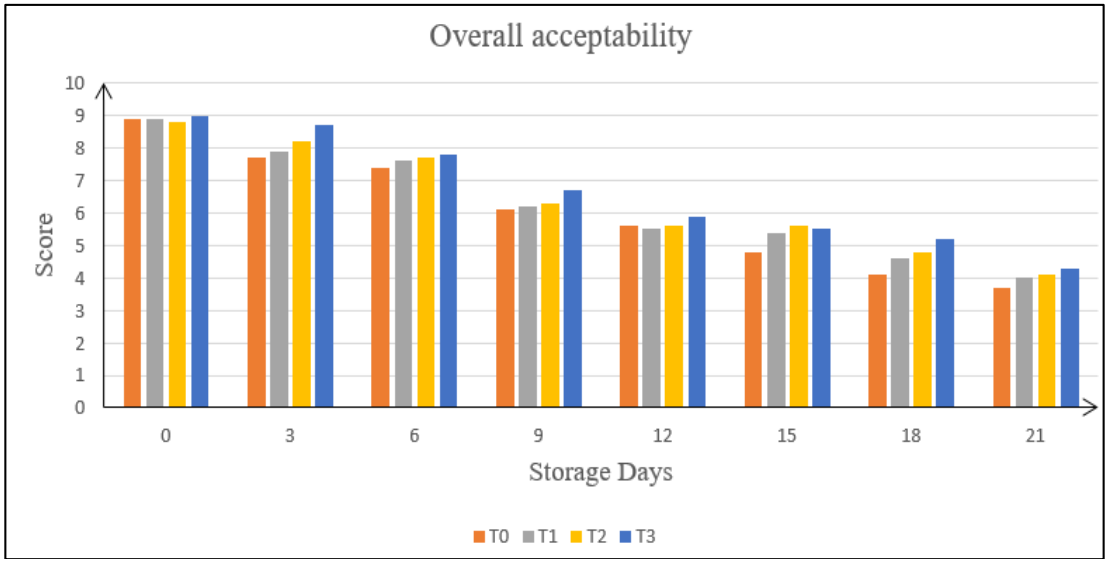

Fig 1: Organoleptic score for overall acceptability of mackerel fish treated with different% of kokum extract during chilled storage

Proximate composition of mackerel treated with different concentration of kokum extract during chilled storage

The proximate composition—comprising moisture, protein, ash, and fat—of mackerel treated with kokum extract and stored under chilled conditions was monitored over a 21-day period. The analysis revealed minor fluctuations in these parameters throughout the storage duration.

Moisture

On the first day of chilled storage, the moisture content of mackerel samples was recorded as 76.76% for T_0, 76.59% for T_1, 76.52% for T_2, and 76.21% for T_3. By the 21st day, moisture levels had increased to 81.32% in T_0, 79.26% in T_1, 79.25% in T_2, and 78.91% in T_3. Among all treatments, T_3 exhibited the lowest moisture content at the end of the storage period. The present study demonstrated a consistent rise in moisture content across all samples during chilled storage. Similar findings were reported by Sharifian *et al*. (2011) [20], who observed an upward trend in moisture levels in grouper fish during chilled storage. Reza *et al*. (2009) [17] also noted that increased moisture content during ice storage could be attributed to water absorption over time. Their study on the proximate composition of various fish species—including ribbon fish, Bombay duck, and Chinese pomfret—revealed a comparable pattern of moisture increase throughout the chilled storage period.

Protein

At the beginning of chilled storage, crude protein levels in mackerel were recorded as 17.75% for T_0, 17.49% for T_1, 17.49% for T_2, and 17.54% for T_3. By the 21st day, a decline in protein content was observed across all treatments, with T_0 dropping to 14.59%. However, samples treated with kokum extract showed better protein retention: 15.75% in T_1, 15.82% in T_2, and 16.05% in T_3. A similar downward trend in protein content during chilled storage was reported by Sharifian *et al*. (2011) [20] in their study on grouper. Reza *et al*. (2009) [17] attributed the reduction in protein levels during ice storage to the leaching of water-soluble protein fractions from fish muscle, a phenomenon observed across various species including ribbon fish, Bombay duck, and Chinese pomfret.

Fat

The initial fat content on day 0 was recorded as 3.86% for T_0, 3.90% for T_1, 3.86% for T_2, and 3.75% for T_3. By the 21st day of chilled storage, these values had declined to 2.24% in T_0, while T_1, T_2, and T_3 exhibited slightly higher levels at 2.43%, 2.56%, and 2.65%, respectively. This indicates a consistent downward trend in fat content across all treatments during the chilled storage period. A similar pattern was observed by Gandotra *et al*. (2012) [6] in their study on chilled storage of mackerel. Additionally, Mahboob *et al*. (2018) [10], in their research on Clarias gariepinus and Cyprinus carpio, attributed the reduction in

lipid content to the oxidative degradation of polyunsaturated fatty acids in fish tissues, resulting in the formation of secondary products such as aldehydes, free fatty acids, ketones, and peroxides.

Ash

On day one, the ash content was recorded as 1.63% for T_0, 1.55% for T_1, 1.74% for T_2, and 1.63% for T_3. By the end of the chilled storage period, these values had increased to 1.73% (T_0), 1.94% (T_1), 1.96% (T_2), and 1.85% (T_3), respectively. This upward trend in ash content aligns with findings by Mahboob *et al*. (2018) [10], who reported a similar increase in *Clarias gariepinus and Cyprinus carpio* during chilled storage. Sharifian *et al*. (2011) [20] also documented a comparable rise in ash content in grouper fish under chilled storage conditions.

Biochemical analysis

Changes in pH of kokum treated samples

In the present study, all kokum-treated samples exhibited a slight increase in pH during chilled storage. Specifically, the pH of T_0 rose from 6.40 to 6.94, T_1 from 6.31 to 6.64, T_2 from 6.29 to 6.75, and T_3 from 6.22 to 6.68 by the end of the 21-day storage period. These findings are consistent with the observations of Apang *et al*. (2020) [1], who reported a general increase in pH over time during chilled storage. However, a relatively lower pH was noted in samples with higher concentrations of kokum extract, suggesting its moderating effect.

The rise in pH is attributed to bacterial proliferation and enzymatic spoilage, which lead to the formation of volatile basic compounds that shift the pH from acidic toward neutral. This increase negatively impacts sensory attributes such as odour, colour, and texture. Mugahi *et al*. (2022) [13] similarly reported a pH increase during storage in their study on the effects of turmeric, cinnamon, and lemon extracts on the shelf life of carp. They noted that the elevation in pH was likely due to the accumulation of volatile nitrogenous compounds resulting from protein degradation, which creates an alkaline environment. Their findings also emphasized that maintaining a lower pH helps inhibit bacterial and enzymatic spoilage, thereby enhancing the preservation of fish.

Changes in Peroxide value (PV) of kokum treated samples

In the present study, the peroxide value (PV) of all kokum extract-treated samples exhibited a progressive increase during chilled storage. Specifically, PV in T_0 rose from 1.63 to 15.13 meq O_2/kg, T_1 from 1.56 to 13.63 meq O_2/kg, T_2 from 1.57 to 12.34 meq O_2/kg, and T_3 from 1.55 to 11.64 meq O_2/kg by the end of the 21-day storage period. These findings align with those reported by Apang *et al*. (2020) [1], who observed a significant rise in PV values over time. However, their study also highlighted that increasing concentrations of kokum extract led to a notable reduction in PV, indicating its antioxidative effect.

Saju *et al*. (2022) [18] similarly investigated the impact of tamarind leaves and Malabar tamarind on the shelf life of vacuum-packed sardine, concluding that PV—being a primary indicator of lipid oxidation—tends to increase with prolonged storage. Mugahi *et al*. (2022) [13] also reported comparable results, noting a significant rise in peroxide values over time. However, their findings emphasized that treated samples showed markedly lower PVs compared to the untreated control, underscoring the protective role of natural extracts in mitigating oxidative spoilage.

Changes in Total volatile base-nitrogen (TVB-N) of kokum treated samples

In the present study, the total volatile base nitrogen (TVB-N) content of kokum-treated samples stored under chilled conditions exhibited a consistent upward trend over the 21-day period. Specifically, TVB-N levels increased from 5.85 to 46.45 mg/100 g in T_0, from 5.61 to 37.45 mg/100 g in T_1, from 5.65 to 34.26 mg/100 g in T_2, and from 5.58 to 33.86 mg/100 g in T_3. These results are in agreement with the findings of Apang *et al*. (2020) [1], who reported a significant rise in TVB-N values with extended storage duration. However, their study also demonstrated that higher concentrations of kokum extract led to a marked reduction in TVB-N levels.

This decrease was attributed to the lower pH and antimicrobial properties of kokum extract, which effectively suppressed microbial growth and thereby reduced spoilage. Mugahi *et al*. (2022) [13] also observed a similar pattern, noting that while TVB-N values increased over time, the treated samples exhibited significantly lower levels compared to the untreated control, further supporting the preservative potential of kokum extract.

Changes in Trimethyl amine nitrogen (TMA-N) of kokum treated samples

In the present study, the trimethylamine nitrogen (TMA-N) content of kokum-treated fish samples stored under chilled conditions showed a progressive increase over the 21-day period. TMA-N levels rose from 1.66 to 16.13 mg/100 g in T_0, from 1.56 to 14.44 mg/100 g in T_1, from 1.54 to 13.23 mg/100 g in T_2, and from 1.52 to 11.73 mg/100 g in T_3. These findings are consistent with those reported by Heising *et al*. (2014) [7], who explained that TMA-N is produced through microbial reduction of trimethylamine oxide (TMAO) during anaerobic respiration.

Apang *et al*. (2020) [1] also observed a similar trend, noting that TMA-N levels increased with prolonged storage. However, their study highlighted a significant reduction in TMA-N content with higher concentrations of kokum extract. This reduction was attributed to kokum's antimicrobial properties, which likely inhibited bacterial activity responsible for converting TMAO to TMA-N. Murthy *et al*. (2018) [12] further supported these observations, reporting a steady rise in TMA-N levels in squid during chilled storage

Changes in Free Fatty Acid (FFA) of kokum treated samples

In the present study, the free fatty acid (FFA) content of kokum-treated fish samples stored under chilled conditions exhibited a consistent upward trend over the 21-day period. FFA levels, expressed as oleic acid, increased from 1.85% to 6.26% in T_0, from 1.77% to 6.04% in T_1, from 1.76% to 5.84% in T_2, and from 1.73% to 5.35% in T_3. These results align with the findings of Viji *et al*. (2015) [24], who investigated the use of mint leaf and citrus peel extracts to extend the shelf life of chilled Indian mackerel. Their study emphasized that FFA values reflect the extent of lipid hydrolysis occurring during spoilage and showed a similar increasing trend, with relatively lower FFA levels in

samples treated with higher concentrations of natural preservatives.

Gandotra *et al*. (2012) [6] also reported a rise in FFA content during chilled storage of *Mystus seenghala*, attributing the accumulation of FFAs to the enzymatic activity of lipase and phospholipase present in the fish's digestive organs. These enzymes accelerate lipid breakdown, contributing to spoilage over time.

Microbiological analysis

Total plate count

The value of TPC (log cfu/g) was found to increase during the storage period at a chilled temperature. The total plate count for T_0 increased from 2.53 to 8.86 log cfu/g, T_1 increased from 2.49 to 7.55 log cfu/g, T_2 increased from 2.46 to 6.93 log cfu/g and T_3 increased from 2.38 to 6.56 log cfu/g at the end of 21th day during chilled temperature. These results show a similar increasing trend like that of Gandotra *et al*. (2012) [6] who have studied on the *Mystus seenghala* fish. The study has also stated that at 4ºC there is a rapid proliferation of microorganisms which leads to increase in their count throughout the chilled storage period.

Escherichia coli

In the present study, the pathogenic bacterium *Escherichia coli* was not detected in any of the samples treated with varying concentrations of kokum extract during chilled storage. These findings are consistent with those reported by Binsi *et al*. (2015) [5], who observed a similar absence of *E. coli* in vacuum-packed freshwater catfish (*Ompok pabda*) under chilled conditions. Their study also emphasized that strict adherence to best management practices was maintained throughout the experimental process. Likewise, Kedar *et al*. (2016) [9] reported no detection of *E. coli* in vacuum-packed *Catla* steaks stored under chilled conditions, further supporting the effectiveness of hygienic handling and preservation methods.

Staphylococcus aureus

In the present study, the pathogenic bacterium *Staphylococcus aureus* was not detected in any of the samples treated with varying concentrations of kokum extract during chilled storage. This observation aligns with the findings of Kedar *et al*. (2016) [9], who also reported the absence of *S. aureus* in vacuum-packed *Catla* steaks stored under chilled conditions, indicating effective microbial control and hygienic handling throughout the preservation process.

Table 1: Proximate composition of fresh mackerel fish meat

Sr no	Parameters	Composition%
1	Moisture	76.65±0.09%
2	Protein	18.58±0.07%
3	Fat	3.44±0.08%
4	Ash	1.17±0.01%

Table 2: Changes in proximate composition of mackerel fish treated with different% of kokum extract during chilled storage

Sample	Storage days	0 day	3 day	6 day	9 day	12 day	15 day	18 day	21 day
T_0	Moisture	76.76±0.03^{a,w}	77.07±0.05^{a,v}	77.76±0.06^{a,u}	78.64±0.07^{a,t}	79.16±0.06^{a,s}	79.84±0.06^{a,r}	80.74±0.08^{a,q}	81.32±0.04^{a,p}
	Protein	17.75±0.06^{a,w}	17.22±0.05^{a,v}	16.88±0.03^{b,u}	16.62±0.06^{b,t}	16.17±0.04^{c,s}	15.76±0.08^{c,r}	14.92±0.04^{c,q}	14.59±0.07^{c,p}
	Fat	3.86±0.03^{a,q}	3.74±0.05^{ab,t}	3.45±0.04^{b,s}	3.15±0.03^{b,s}	2.94±0.03^{c,r}	2.82±0.06^{c,q}	2.53±0.03^{b,p}	2.24±0.07^{c,p}
	Ash	1.63±0.02^{b,r}	1.66±0.02^{b,qr}	1.76±0.02^{a,p}	1.75±0.03^{b,p}	1.74±0.02^{b,p}	1.74±0.02^{b,p}	1.74±0.02^{b,p}	1.73±0.02^{c,p}
T_1	Moisture	76.59±0.07^{b,w}	76.87±0.05^{b,v}	77.54±0.06^{c,u}	77.86±0.05^{c,t}	78.19±0.07^{c,s}	78.52±0.05^{c,r}	78.94±0.02^{c,q}	79.26±0.05^{b,p}
	Protein	17.49±0.0^{b,w}	17.26±0.04^{a,v}	17.07±0.04^{a,u}	16.84±0.04^{a,t}	16.68±0.03^{b,s}	16.26±0.04^{b,r}	15.88±0.03^{b,q}	15.75±0.04^{b,p}
	Fat	3.90±0.03^{a,v}	37.5±0.04^{ab,u}	3.65±0.04^{a,t}	3.25±0.04^{b,s}	3.08±0.02^{b,r}	2.95±0.03^{b,q}	2.83±0.02^{a,q}	2.43±0.05^{b,p}
	Ash	1.55±0.03^{c,t}	1.65±0.02^{b,s}	1.76±0.02^{a,r}	1.86±0.02^{a,q}	1.84±0.02^{a,q}	1.83±0.02^{a,qr}	1.83±0.01^{a,qr}	1.94±0.02^{a,p}
T_2	Moisture	76.52±0.08^{c,w}	76.81±0.07^{c,v}	77.18±0.07^{d,u}	77.58±0.04^{d,t}	77.83±0.08^{c,s}	78.18±0.03^{d,r}	78.65±0.07^{d,q}	79.25±0.06^{c,p}
	Protein	17.49±0.04^{b,u}	17.27±0.05^{a,u}	17.05±0.04^{a,t}	16.91±0.03^{a,s}	16.70±0.05^{b,r}	16.31±0.07^{b,r}	15.93±0.04^{b,q}	15.82±0.03^{b,p}
	Fat	3.86±0.04^{a,v}	3.81±0.03^{a,u}	3.64±0.04^{a,t}	3.39±0.02^{a,s}	3.14±0.03^{b,r}	2.96±0.04^{ab,q}	2.84±0.03^{a,p}	2.56±0.04^{ab,p}
	Ash	1.74±0.02^{a,r}	1.77±0.02^{a,r}	1.65±0.02^{b,s}	1.63±0.02^{c,s}	1.65±0.02^{c,s}	1.74±0.02^{b,r}	1.86±0.02^{a,q}	1.96±0.02^{a,p}
T_3	Moisture	76.21±0.08^{ab,v}	76.40±0.04^{b,u}	76.90±0.03^{b,t}	77.33±0.08^{b,s}	77.81±0.03^{b,r}	77.89±0.05^{b,r}	78.42±0.06^{b,q}	78.91±0.06^{b,p}
	Protein	17.54±0.05^{b,v}	17.30±0.05^{a,u}	17.03±0.03^{a,t}	16.93±0.03^{a,s}	16.81±0.02^{a,r}	16.51±0.02^{a,r}	16.27±0.05^{a,q}	16.05±0.03^{a,p}
	Fat	3.75±0.03^{b,v}	3.66±0.04^{b,u}	3.56±0.02^{a,t}	3.42±0.05^{a,s}	3.25±0.04^{a,r}	3.07±0.02^{a,q}	2.91±0.02^{a,pq}	2.65±0.03^{a,p}
	Ash	1.63±0.02^{b,r}	1.66±0.02^{b,r}	1.65±0.02^{b,r}	1.67±0.02^{c,r}	1.65±0.02^{c,r}	1.63±0.01^{c,r}	1.74±0.02^{b,q}	1.85±0.01^{b,p}

Mean ±SD^{a-d} in the same column Mean ±SD^{p-w} in row followed by different superscript is significantly different (p<0.05) (n=3) with respect to sampling days. T_0 (Control): fish treated with 0% kokum extract; T_1: fish treated with 5% kokum extract; T_2: fish treated with 10% kokum extract; T_3: fish treated with 15% kokum extract

Table 3: Changes in pH of mackerel fish treated with different% of kokum extract during chilled storage

Storage period	pH			
	T_0	T_1	T_2	T_3
0	6.40±0.03^{a,t}	6.31±0.03^{b,v}	6.29±0.02^{bc,v}	6.22±0.02^{c,u}
3	6.40±0.02^{a,t}	6.36±0.02^{a,uv}	6.33±0.03^{ab,uv}	6.28±0.03^{b,tu}
6	6.53±0.03^{a,s}	6.42±0.03^{b,tu,}	6.40±0.03^{bc,tu}	6.33±0.02^{c,st}
9	6.57±0.03^{a,rs}	6.49±0.02^{ab,st}	6.46±0.02^{b,st}	6.36±0.04^{c,s}
12	6.64±0.03^{a,r}	6.56±0.02^{b,rs}	6.55±0.02^{b,rs}	6.45±0.02^{c,r}
15	6.76±0.02^{a,q}	6.62±0.03^{b,qr}	6.60±0.03^{bc,qr}	6.53±0.02^{c,qr}
18	6.85±0.04^{a,p}	6.68±0.03^{b,q}	6.64±0.03^{bc,q}	6.57±0.03^{c,q}
21	6.94±0.02^{a,p}	6.84±0.04^{b,p}	6.75±0.04^{c,p}	6.68±0.03^{c,p}

Mean ±SDa-c in the same column and Mean ±SD^{p-w} in row followed by different superscript is significantly different (p<0.05) (n=3) with respect to sampling days. T_0 (Control): fish treated with 0% kokum extract; T_1: fish treated with 5% kokum extract; T_2: fish treated with 10% kokum extract; T_3: fish treated with 15% kokum extract

Table 4: Changes in PV of mackerel fish treated with different% of kokum extract during chilled storage

Storage period	PV (meq of O_2/kg)			
	T_0	T_1	T_2	T_3
0	$1.63\pm0.03^{a,w}$	$1.56\pm0.02^{a,w}$	$1.57\pm0.01^{a,w}$	$1.55\pm0.04^{b,w}$
3	$3.85\pm0.02^{a,v}$	$3.54\pm0.03^{b,v}$	$3.55\pm0.04^{b,v}$	$3.47\pm0.01^{b,v,}$
6	$5.67\pm0.02^{a,u}$	$4.96\pm0.04^{b,u}$	$4.86\pm0.02^{c,u}$	$4.71\pm0.04^{d,u}$
9	$7.44\pm0.02^{a,t}$	$6.54\pm0.03^{b,t}$	$6.45\pm0.02^{c,t}$	$6.15\pm0.03^{d,t}$
12	$9.73\pm0.02^{a,s}$	$7.95\pm0.02^{b,s}$	$7.56\pm0.02^{c,s}$	$6.45\pm0.03^{d,s}$
15	$10.64\pm0.03^{a,r}$	$9.53\pm0.02^{b,r}$	$9.35\pm0.03^{c,r}$	$7.44\pm0.03^{d,r}$
18	$12.35\pm0.03^{a,q}$	$11.65\pm0.03^{b,q}$	$11.42\pm0.02^{c,q}$	$9.93\pm0.02^{d,q}$
21	$15.13\pm0.02^{a,p}$	$13.63\pm0.02^{b,p}$	$12.34\pm0.02^{c,p}$	$11.64\pm0.03^{d,p}$

Mean ±SDa-d in the same column and Mean $\pm SD^{p-w}$ in row followed by different superscript is significantly different ($p<0.05$) (n=3) with respect to sampling days. T_0 (Control): fish treated with 0% kokum extract; T_1: fish treated with 5% kokum extract; T_2: fish treated with 10% kokum extract; T_3: fish treated with 15% kokum extract.

Table 5: Changes in TVB-N of mackerel fish treated with different% of kokum extract during chilled storage

Storage period	TVB-N (mg/100 g)			
	T_0	T_1	T_2	T_3
0	$5.85\pm0.04^{a,w}$	$5.61\pm0.07^{b,w}$	$5.65\pm0.04^{b,w}$	$5.58\pm0.03^{b,u}$
3	$10.33\pm0.02^{a,v}$	$10.17\pm0.01^{b,v}$	$9.86\pm0.03^{c,v}$	$9.64\pm0.03^{d,v}$
6	$19.73\pm0.02^{a,u}$	$15.44\pm0.03^{b,u}$	$14.95\pm0.02^{c,u}$	$14.65\pm0.02^{d,u}$
9	$24.66\pm0.02^{a,t}$	$18.76\pm0.02^{b,t}$	$18.15\pm0.01^{c,t}$	$17.93\pm0.02^{d,t}$
12	$29.63\pm0.02^{a,s}$	$23.53\pm0.02^{b,s}$	$21.33\pm0.02^{c,s}$	$20.93\pm0.02^{d,s}$
15	$34.35\pm0.03^{a,r}$	$28.64\pm0.04^{b,r}$	$25.12\pm0.01^{c,r}$	$24.83\pm0.02^{d,r}$
18	$39.53\pm0.02^{a,q}$	$32.63\pm0.02^{b,q}$	$31.44\pm0.02^{c,q}$	$29.46\pm0.02^{d,q}$
21	$46.45\pm0.03^{a,p}$	$37.45\pm0.02^{b,p}$	$34.26\pm0.02^{c,p}$	$33.86\pm0.02^{d,p}$

Mean $\pm SD^{a-d}$ in the same column and Mean $\pm SD^{p-w}$ in row followed by different superscript is significantly different ($p<0.05$) (n=3) with respect to sampling days. T_0 (Control): fish treated with 0% kokum extract; T_1: fish treated with 5% kokum extract; T_2: fish treated with 10% kokum extract; T_3: fish treated with 15% kokum extract.

Table 6: Changes in TMA-N of mackerel fish treated with different% of kokum extract during chilled storage

Storage period	TMA-N (mg/100 g)			
	T_0	T_1	T_2	T_3
0	$1.66\pm0.02^{a,w}$	$1.56\pm0.03^{b,w}$	$1.54\pm0.01^{b,w}$	$1.52\pm0.02^{b,w}$
3	$5.76\pm0.02^{a,v}$	$3.94\pm0.03^{b,v}$	$3.83\pm0.02^{c,v}$	$3.65\pm0.01^{d,v}$
6	$6.14\pm0.02^{a,u}$	$5.96\pm0.02^{b,u}$	$5.85\pm0.01^{c,u}$	$5.14\pm0.04^{d,u}$
9	$8.93\pm0.02^{a,t}$	$6.83\pm0.02^{b,t}$	$6.67\pm0.02^{c,t,}$	$6.15\pm0.04^{d,t}$
12	$9.47\pm0.01^{a,s}$	$8.83\pm0.02^{b,s}$	$8.77\pm0.01^{b,s}$	$8.21\pm0.08^{c,s}$
15	$11.63\pm0.02^{a,r}$	$9.94\pm0.02^{b,r}$	$9.67\pm0.03^{c,r}$	$9.34\pm0.03^{d,r}$
18	$14.85\pm0.02^{a,q}$	$12.85\pm0.02^{b,q}$	$10.93\pm0.03^{c,q}$	$9.94\pm0.02^{d,q}$
21	$16.13\pm0.02^{a,p}$	$14.44\pm0.03^{b,p}$	$13.23\pm0.02^{c,p}$	$11.73\pm0.02^{d,p}$

Mean $\pm SD^{a-d}$ in the same column and Mean $\pm SD^{p-w}$ in row followed by different superscript is significantly different ($p<0.05$) (n=3) with respect to sampling days. T_0 (Control): fish treated with 0% kokum extract; T_1: fish treated with 5% kokum extract; T_2: fish treated with 10% kokum extract; T_3: fish treated with 15% kokum extract.

Table 7: Changes in FFA of mackerel fish treated with different% of kokum extract during chilled storage

Storage period	% as oleic acid			
	T_0	T_1	T_2	T_3
0	$1.85\pm0.01^{a,w}$	$1.77\pm0.02^{b,w}$	$1.76\pm0.01^{b,w}$	$1.73\pm0.03^{b,v}$
3	$2.33\pm0.01^{a,v}$	$1.95\pm0.02^{b,v}$	$1.93\pm0.02^{c,v}$	$1.88\pm0.01^{c,u}$
6	$3.55\pm0.02^{a,u}$	$2.94\pm0.03^{b,u}$	$2.84\pm0.02^{c,u}$	$2.76\pm0.02^{d,t}$
9	$3.95\pm0.02^{a,t}$	$3.16\pm0.02^{b,t}$	$2.94\pm0.04^{c,t}$	$2.84\pm0.03^{d,t}$
12	$4.24\pm0.03^{a,s}$	$3.54\pm0.02^{b,s}$	$3.45\pm0.03^{c,s}$	$3.14\pm0.03^{d,s}$
15	$5.56\pm0.03^{a,r}$	$4.84\pm0.03^{b,r}$	$4.55\pm0.04^{c,r}$	$3.56\pm0.04^{d,r}$
18	$5.94\pm0.01^{a,q}$	$5.34\pm0.02^{b,q}$	$5.16\pm0.04^{b,q}$	$4.55\pm0.04^{c,q}$
21	$6.26\pm0.01^{a,p}$	$6.04\pm0.03^{b,p}$	$5.84\pm0.03^{c,p}$	$5.35\pm0.04^{d,p}$

Mean $\pm SD^{a-d}$ in the same column and Mean $\pm SD^{p-w}$ in row followed by different superscript is significantly different ($p<0.05$) (n=3) with respect to sampling days. T_0 (Control): fish treated with 0% kokum extract; T_1: fish treated with 5% kokum extract; T_2: fish treated with 10% kokum extract; T_3: fish treated with 15% kokum extract.

Table 8: Changes in TPC value of mackerel fish treated with different% of kokum extract during chilled storage

Storage period	TPC log cfu/g			
	T_0	T_1	T_2	T_3
0	$2.53\pm0.05^{a,w}$	$2.49\pm0.03^{a,w}$	$2.46\pm0.01^{b,w}$	$2.38\pm0.04^{c,w}$
3	$3.21\pm0.01^{a,v}$	$2.62\pm0.02^{a,v}$	$2.51\pm0.04^{b,v}$	$1.96\pm0.02^{c,v}$
6	$3.94\pm0.01^{a,u}$	$2.94\pm0.03^{b,u}$	$2.82\pm0.02^{c,u}$	$2.56\pm0.02^{d,u}$
9	$4.81\pm0.02^{a,t}$	$3.84\pm0.04^{b,t}$	$3.53\pm0.02^{c,t}$	$3.24\pm0.03^{d,t}$
12	$5.65\pm0.03^{a,s}$	$4.55\pm0.04^{b,s}$	$4.15\pm0.04^{c,s}$	$3.54\pm0.04^{d,s}$
15	$6.38\pm0.05^{a,r}$	$5.54\pm0.04^{b,r}$	$5.34\pm0.03^{c,r}$	$4.25\pm0.04^{d,r}$
18	$7.64\pm0.03^{a,q}$	$5.96\pm0.02^{b,q}$	$5.85\pm0.03^{c,q}$	$5.25\pm0.04^{d,q}$
21	$8.68\pm0.05^{a,p}$	$7.55\pm0.04^{b,p}$	$6.93\pm0.03^{c,p}$	$6.56\pm0.03^{d,p}$

Mean $\pm SD^{a-d}$ in the same column and Mean $\pm SD^{p-w}$ in row followed by different superscript is significantly different ($p<0.05$) (n=3) with respect to sampling days. T_0 (Control): fish treated with 0% kokum extract; T_1: fish treated with 5% kokum extract; T_2: fish treated with 10% kokum extract; T_3: fish treated with 15% kokum extract.ed with 15% kokum extract

Conclusion

The results of the present study revealed that the Indian mackerel treated with different concentrations of kokum extract showed enhanced shelf life as compared to the controlled samples. According to the organoleptic evaluation, the fish samples with 15% kokum extract showed overall acceptability upto 18 days as compared to 12 days for the controlled fish at the end of the storage period. Thus form the results of the present study, it was concluded that the mackerel fish treated with 15% kokum concentration dipped for 15 minutes improved the shelf life of the fish upto 18 days during the chilled storage.

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