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Sathish Kumar Kannaiyan
Fish Processing Division, Central Institute of Fisheries Technology, Cochin - 682029, India

Nagalakshmi Kannuchamy
Fishery Resources, Harvest and Post-Harvest Management Division, Central Institute of Fisheries Education, Versova, Mumbai-400061, India

Venkateshwarlu Gudipati
Assistant Director General (EQR), Indian Council of Agricultural Research (ICAR), New delhi-110001, India

Correspondence
Sathish Kumar Kannaiyan
Fish Processing Division, Central Institute of Fisheries Technology, Cochin - 682029, India

Preservative effect of solvent free natural spice extracts on tuna fillets in chilled storage at 4 ºC: Microbial, biochemical and sensory attributes

Sathish Kumar Kannaiyan, Nagalakshmi Kannuchamy and Venkateshwarlu Gudipati

Abstract
The present study was carried out to extract solvent free natural spice extracts from three spices such as *Mentha arvensis* (Mint), *Anethum sowa* (Dill), *Capsicum annuum* (Chilli) and to evaluate their preservative effects on tuna fish fillets stored under chilled storage (4 ºC). Among the extracts treated fillets, chilli (20%) treated fillets showed lower total plate count (TPC) value of 8.07±0.03 log cfu/g, whereas control reached 8.67±0.03 log cfu/g at the end of storage period. The Thiobarbituric Acid (TBA) values of the spice extract treated fillets, dill 20% treated fillets had less value of 1.21±0.01 mg malondialdehyde (MDA)/kg, but control reached above rejection limit (8.41±0.01 mg MDA/kg) at the end of storage period. Sensory score of fillets dipped in 10% and 20% of spice extracts, showed acceptable level (>6), while control fillet was unacceptable (5.38±0.11) which indicates that the spice extracts could improve the shelf life of tuna fillets and could be used as solvent free natural preservative to replace synthetic antioxidant and antimicrobial agents.

Keywords: Tuna fillets, TPC, TBA, sensory analysis

1. Introduction
Tuna is a one of the commercial important fish species with high economic value and demand in international markets. It is consumed as fresh, canned and frozen. Though it comes under highly perishable commodity, gets spoiled mainly due to its high amount of polyunsaturated fatty acids (PUFAs), especially, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), which are highly prone to oxidation and also lost their nutritional quality due to microbial spoilage during storage [1-3]. The lipids get oxidised and undergo hydrolysis during processing, value addition, packaging and subsequent storage, which can affect the nutritional qualities, sensory properties and cause an impact on its demand and commercial value [4-6].
In food industry, lipid oxidation is one of the major concern and antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are the most commonly used as a synthetic preservatives to reduce the lipid oxidation [7, 8]. However, such substances are heat labile, volatile and unstable [9] and possibly affect human health by causing toxicity [10, 11]. Food spoilage due to contamination with microorganisms is another concern for food processors during food handling, processing and storage. Synthetic chemicals are widely used against these microorganisms; unfortunately they started to developing resistance to many antibiotics due to their indiscriminate use and sometimes cause allergic reaction and immunity suppression [12, 13]. Consumers are also concerned about the food safety aspects and started demanding of food products containing natural preservatives. Therefore, the food processers have turned and focusing to use of natural spice extracts having antioxidant and antimicrobial properties, potential health beneficial effects and environment friendly, for food preservation [14-18].
Spices and their essential oils are the most effective natural antioxidants and antimicrobial agents have been used to preserve food [19-21]. Based on the literature, the present study was aimed to extract the solvent free natural spice extracts and to evaluate their preservative effects on tuna fish fillets during chilled storage (4 °C), without adding any chemical substances.

2. Materials and Methods

2.1 Preparation of spice extracts

In this study, three spices namely mint (*Mentha arvensis*), dill (*Anethum sowa*) and chillies (*Capsicum annuum*) were purchased from local market and washed in distilled water. An amount of 100 gm of each spice was ground for 2 mins without adding water or organic solvents. The ground mixtures were sieved through a fine cotton cloth and the collected extracts were used for further analysis. All the chemicals and Media used in the study were of analytical grade and purchased from various companies *viz*. Sigma, Qualigens, Merck and HIMEDIA.

2.2 Storage Study

2.2.1 Treatment of tuna fillets with spice extracts

Little tuna (*Euthynnus affinis*) were purchased from versova landing center, Mumbai and the fishes were made into a fillets with mean weight about 100-150 g. Then the fillets were dipped in 10% and 20% of each spice extracts for 10 mins and the excess liquid was drained off, then the fillets were placed in sterile polythene bags and stored at refrigerated temperature (4 ºC). Periodically (0, 4, 7, 10 and 13 days), samples in triplicates were randomly removed from each treatment, and to evaluate the preservative of spice extracts on tuna fillets.

2.2.2 Total Plate Count

The total plate count was estimated by spread plate technique [22]. Fish sample (10 g) was weighed and homogenized aseptically with 90 ml saline in a Stomacher (Seward Stomacher 80) for 60 seconds. Using a sterile pipette, 1 ml of the supernatant was aseptically transferred into a 9 ml saline tube and mixed well using Vortex mixer. Similarly further dilutions were prepared. Aliquots of 0.1 ml each of the appropriate dilutions was pipetted and spread plated. The plates were incubated at 37 °C for 24 hrs. The individual bacterial colonies were counted and the results were recorded. The average counts of the triplicates were taken and the counts were calculated as cfu/g of the sample.

cfu/g = (Average count X Dilution factor X 10) / Weight of the sample

2.2.3 Determination of Thiobarbituric Acid (TBA) value

TBA of fish sample was estimated by standard procedure as given by [23] with slight modification. Fish sample (10 gm) was blended with 50 ml of distilled water using a pestle and mortar. The mixture was transferred into a 250 ml flat bottom flask with 47.5 ml distilled water and 2.5 ml of 4N HCl was then added. The flask was then connected to a distillation unit and heated by an electric mantle in such a way that 50 ml of the distillate was collected in 10 mins. The distillate (5 ml) was taken in a stoppered tube and 5 ml of TBA reagent was added. The tubes were then placed in boiling water for 35 mins. Blank was prepared by using 5 ml distilled water and 5 ml TBA reagent. After cooling the optical density was measured at 538 nm by a UV-spectrophotometer. TBA value is expressed as mg malondialdehyde (MDA)/ kg sample.

2.2.4 Sensory evaluation

Sensory evaluation was carried out by method described by [24]. The spice extract treated tuna fillets were introduced to panellists for sensory evaluation. The evaluation was based on characterization and differentiation of the various sensory characteristics such as appearance, odour, flavour, texture, consistency and overall acceptability. Score was given on a 10-point hedonic scale by sensory panel members consisting of 6 regular members, as per the guidelines given in IS: 6273[II] -1971. A general 'freshness score' was calculated as an average of all grades. According to the freshness score, over all acceptability was determined as having a fresh score of over 6.

2.3 Statistical analysis

Statistical analysis was performed using SPSS software, version 16. The comparison of flesh quality indicators during storage were tested using Tukey HSD multiple range test (95% confidence interval) with one-way ANOVA.

3. Results and discussion

3.1 Total Plate Count

At the initial time of storage, the total bacterial count was very low because of the sterile nature of the fish flesh. However, after death, bacteria invade the flesh [25]. The initial TPC counts (4.07±0.03 log cfu/g) of all fillet samples indicates that fish is acceptable, considering the proposed upper limit for aerobic plate count of 5 x 10^5 cfu/g (5.70 log cfu/g) for fresh fish [26]. However, the values increased steadily during storage (Table 1). TPC value of tuna fillets dipped in 10% chilli, dill and mint extracts increased from the initial level of 4.07±0.03 log cfu/g to 8.17±0.03 log cfu/g, 8.18±0.04 log cfu/g and 8.33±0.03 log cfu/g respectively by the end of the storage period. Tuna fillets treated with 20% chilli, dill and mint extracts had TPC value 8.07±0.03 log cfu/g, 8.13±0.03 log cfu/g and 8.20±0.00 log cfu/g respectively, whereas TPC of control reached a maximum count of 8.67 ±0.03 log cfu/g respectively by the end of 13 days storage and among the spice extract at 20% concentration, chilli extract (8.07±0.03 log cfu/g) had better activity. The control tuna fillets reached TPC value of 7.42±0.01 log cfu/g at 7th day of storage; whereas all spice treated fillets (10% and 20%) reached (7.30±0.02 to 7.43±0.03 log cfu/g) on 10th day of storage. This finding has clearly shown that the spice extract extended the shelf life of tuna fillets by 3 days (table 1). From the results it is clear that the tuna fillets treated with spice extracts significantly delayed the rate of microbial spoilage and extended the shelf life during the chilled storage.

Table 1: Changes in TPC of tuna fillets dipped in spice extracts during storage at 4 °C

TPC (log cfu/g)					
Treatment	**0 th day**	**4th day**	**7th day**	**10th day**	**13th day**
Control	4.07±0.03^{aA}	6.80±0.00^{bE}	7.42±0.01^{cF}	7.97±0.03^{dD}	8.67±0.03^{eE}
Chilli 10%	4.07±0.03^{aA}	6.43±0.01^{bD}	7.05±0.02^{cE}	7.41±0.01^{dC}	8.17±0.03^{eCD}
Chilli 20%	4.07±0.03^{aA}	6.31±0.01^{bBC}	6.93±0.02^{cDE}	7.34±0.02^{dC}	8.07±0.03^{eC}
Dill 10%	4.07±0.03^{aA}	6.20±0.01^{bAB}	6.92±0.01^{cC}	7.37±0.03^{dC}	8.18±0.04^{eCD}
Dill 20%	4.07±0.03^{aA}	6.10±0.01^{bA}	6.77±0.03^{cB}	7.30±0.02^{dBC}	8.13±0.03^{eC}
Mint 10%	4.07±0.03^{aA}	6.39±0.04^{bCD}	6.88±0.04^{cBCD}	7.43±0.03^{dC}	8.33±0.03^{eD}
Mint 20%	4.07±0.03^{aA}	6.15±0.02^{bA}	6.80±0.01^{cBC}	7.32±0.02^{dC}	8.20±0.00^{eCD}

Results are mean ± standard error (n=3), values with different letters within a row (a-e) and values with different letters within a column (A-C) are significantly different (p<0.05) in one way ANOVA followed by Tukey HSD test.

3.2 TBA

The TBA value is widely used for measuring lipid oxidation in fish and fish products [27]. The present study revealed that the TBA value of control tuna fillet significantly increased during storage days compared to the fillets treated with spice extracts (Table 2). In tuna fillets dipped in 10% chilli, dill and mint extract, the TBA values increased from 0.81±0.00 to 1.73±0.03 mg MDA/kg, 1.69±0.02 mg MDA/kg and 1.72±0.02 mg MDA/kg respectively and chilli 10% extract had lowest (p<0.05) TBA value, among the 10% extracts after 13 days of storage. The TBA value of treated tuna fillets with 20% chilli, dill and mint extracts were in the range of 0.81±0.00 to 1.56±0.03 mg MDA/kg, 1.21±0.01 mg MDA/kg and 1.43±0.03 mg MDA/kg respectively (p<0.05). Among the 20% extracts, dill extract had the lowest TBA value at the end of storage period. The fillets treated with spice extracts had lower TBA values throughout the storage, ranging from 0.81±0.00 to 1.73±0.03 mg MDA /kg muscle after 13 days of storage, whereas control fillets TBA value increased to 4.42±0.01 and 8.41±0.01 mg MDA /kg muscle on Day 6 and Day 12, respectively. There was an increase in TBA values during the storage period, followed by either a decrease in these values. Decrease in TBA values may be caused by interaction between MDA and proteins, amino acids, glycogen and also natural spice extracts which can act an antioxidant, resulting in lower amount of free MDA. This observation is in agreement with results reported by several authors [28-32]. In our study, TBA values were below the threshold level of 3 mg MDA/kg fish muscle throughout the storage period [33] in agreement with [34, 35] stored sword fish in various packaging conditions.

The TBA value of food products, less than 3 mg MDA/kg is considered as "perfect material" [33, 36]. TBA values of all extract treated fillets were almost in the limits of perfect material at the end of the study but the control fillets reached > 3 mg of MDA/kg on 6th day of storage, indicating rancidity of the products and spoilage of fillets.

Table 2: Changes in the TBA values in tuna fillets dipped in spice extracts during storage

TBA in mg MDA/100g					
Treatment	**0thDay**	**4thDay**	**7th Day**	**10th day**	**13th Day**
Control	0.81 ± 0.00^{aA}	1.92 ± 0.01^{bF}	4.42 ± 0.01^{cF}	6.64 ± 0.02^{dG}	8.41 ± 0.01^{eG}
Chilli 10%	0.81 ± 0.00^{aA}	1.18 ± 0.00^{bBC}	1.42 ± 0.01^{cCD}	1.51 ± 0.01^{dCD}	1.73 ± 0.03^{eD}
Chilli 20%	0.81 ± 0.00^{aA}	1.14 ± 0.02^{bB}	1.31 ± 0.00^{cBC}	1.41 ± 0.00^{dBC}	1.56 ± 0.03^{eC}
Dill 10%	0.81 ± 0.00^{aA}	1.25 ± 0.02^{bC}	1.40 ± 0.03^{cCD}	1.52 ± 0.01^{dD}	1.69 ± 0.02^{eD}
Dill 20%	0.81 ± 0.00^{aA}	0.96 ± 0.03^{abA}	1.05 ± 0.07^{bcA}	1.06 ± 0.02^{bcA}	1.21 ± 0.01^{cA}
Mint 10%	0.81 ± 0.00^{aA}	1.23 ± 0.02^{bBC}	1.41 ± 0.01^{cCD}	1.63 ± 0.03^{dE}	1.72 ± 0.02^{dD}
Mint 20%	0.81 ± 0.00^{aA}	1.03 ± 0.01^{bA}	1.21 ± 0.01^{cB}	1.33 ± 0.03^{dB}	1.43 ± 0.03^{dB}

Results are mean ± standard error (n=3), values with different letters within a row (a-e) and values with different letters within a column (A-C) are significantly different (p<0.05) in one way ANOVA followed by Tukey HSD test.

3.3. Sensory evaluation

The changes in quality of fish during storage were assessed by organoleptic examination. During storage of tuna fillets dipped in 10% and 20% spice extracts, a gradual decreases in the overall acceptance were observed (Figure 1). Among the spice treated fillets, dill (20%) had highest sensory value (6.51±0.10) followed by mint (20%) (6.48±0.09), whereas the control fillet sample reached below the unacceptable limit (5.38±0.11) at the end of the storage period because of their bitterness, decomposition and off flavour production. The main reason is lipid oxidation causes production of undesirable rancid off-flavour and toxic products, which leads to the qualitative deterioration of fish [37]. After 13 days of storage, all spice extracts treated sample had sensory score above the acceptable limit (>6), whereas control sample had sensory value below the acceptable limit (<6) (Figure 1). So this study, clearly evident that all spice extracts could be used to increase the shelf life of tuna fillets effectively.

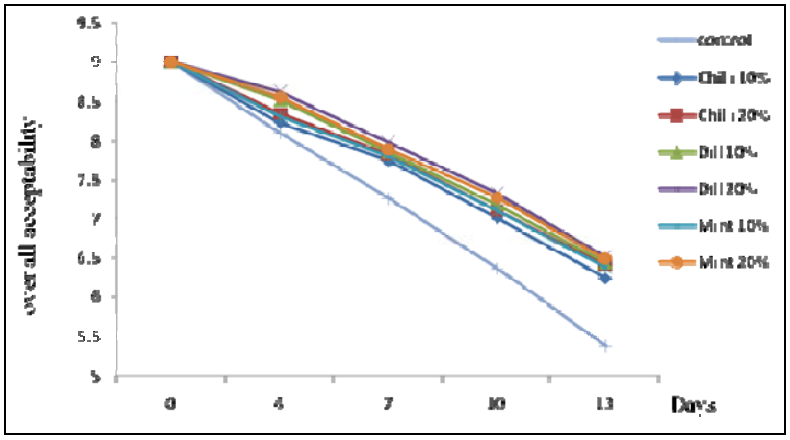

Fig 1: Changes in sensory evaluation of tuna fillets dipped in 10% and 20% spice extracts during storage.

4. Conclusion

The study concluded that, the dip treatment in solvent free spice extracts (at 10% and 20%) extended the shelf life of tuna fillets very effectively with good sensory acceptance and has potential to use as natural preservative to extent the shelf life of fish and fishery products. It can be used as a replacement for synthetic antioxidant and antibiotic to prevent the antioxidation and microbial deterioration of food products.

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