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PROOF RESEARCH PAPER

Biopreservation to Stabilize *Euthynnus affinis* Quality

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Abstract

Fishes are highly perishable and loose quality rapidly after catching due to enzymatic and bacterial spoilage. Plant-derived antimicrobial compounds have activity against many bacteria. In this study the antimicrobial activity of spice oleoresins and their sensory impact with fresh cooked tuna were investigated. Clove (5µl) produced a 20mm inhibition zone against *Escherichia coli* (MTCC 45). Oregano, cardamom and rosemary (10mm) showed moderate activity whereas the activity of garlic (8mm) was weak. Significant differences ($P<0.05$) were found in the microbial numbers with spices compared to the control samples. There was an 88% reduction in the microbial load for clove whereas the chemical antimicrobial, chlorine killed only 54% of the bacteria. Samples treated with 0.2% concentration of spice oleoresins showed significantly higher values for instrumental hardness than those with 0.1%. However, all other texture profile analysis parameters like springiness, stiffness, chewiness, and cohesiveness did not show any significant differences ($P\geq0.05$). The highest consumer acceptance was for cardamom (overall sensory score = 6 out of 7) followed by rosemary and clove (5.3 and 5.7, respectively). Garlic and oregano treated samples were poorly received by the panelists and were less acceptable than the control. A rosemary and cardamom blend was well received. The sensory scores of other combinations were also above the control. Although all the spices used for the experiment showed good antimicrobial properties, and did not have much effect on textural parameters except hardness. The experiment showed that spice combinations could play an important role in increasing the acceptability, safety and shelf-life of fresh tuna.

Keywords: *Euthynnus affinis*, biopreservation, *Escherichia coli*, clove, cardamom, microbial load, rosemary.

Introduction

Tuna in sushi was the main suspect in a *Salmonella bareilly* outbreak in 2012 that had sickened 116 people across 21 states in the USA (Alexandra, 2012). Still, tuna is in high demand and is harvested by nearly 80 nations. Tunas rank fourth in the international fish trade and contributes 7.6% by value to the fish trade (Bindu, 2009).

Since ancient times, spices have been used as food preservatives (Farag *et al.*, 1989) as well as for seasoning purposes. Sediek *et al.* (2012) showed that it was possible to produce safe and high-quality fresh sausages using natural antioxidants such as ginger extract (1.0%) to improve the quality and stability of frozen sausages. Alex and Eagappan (2016) found that sun dried Indian oil sardines (*Sardinella longiceps*) treated with salt, spices and herbs could be stored for 9 months without adversely affecting their quality.

If spice oleoresins are used at an acceptable level in tuna, they might inhibit the microbial activity and enhance the fish's sensory appeal. Consumer acceptance of the product is the limiting factor. Tuna products are available which use spices to impart flavour (e.g., Clover Leaf Seafoods, Markham, Ontario, Canada). In this paper the effects of spice oleoresin treatments on the textural parameters, organoleptic qualities, and microbiology of cooked tuna were studied.

Materials and Methods

Spice oleoresins: Six different spice oleoresins, i.e., rosemary (dried aerial parts of *Rosemarinus officinalis* L. (Family: Lamiaceae), item code ROS1001), garlic (crushed green bulbs of *Allium sativum* L. (Family: Liliaceae), item code GLC1001), cardamom (dried seeds of *Elettaria cardamomum* Maton (Family: Zingiberaceae), item code CAR1001), turmeric (crushed dry rhizomes of *Curcuma longa* L. (Family: Zingiberaceae), item code

TUR1001), oregano (dried aerial parts of *Origanum vulgare* L. (Family: Labiateae), item code ORE1001) and clove (dried buds of *Syzygium aromaticum* L. (Family: Myrtaceae), item code CLV1001) were obtained from M/s Synthite Industrial Chemicals Ltd. (Synthite Valley, Kolenchery, India). The spice extracts were diluted to the desired concentrations in purified ethanol (i.e., 95% ethanol that was double distilled).

Tuna: Whole un-eviscerated fresh tuna (*Euthynnus affinis*) were purchased from Vypeen Harbour (Kochi, India). They were caught by pole and line, packed in insulated boxes containing ice and delivered to the laboratory approximately 6-8 hr post-capture. The fish were about 60-70cm long and weighed 1-1.5Kg. The fish were beheaded, gutted and cut into chunks of 2x1x1cm using a sterile knife under aseptic conditions. This process was repeated after 3 wk to check the consistency of the sensory data.

Incubation: Fish chunks were divided into 8 lots. Among these, 6 lots were treated with an individual spice oleoresin, one with chlorine and one control. The duration of the dip treatment was 10 min with two different concentrations of spice extracts (0.1 and 0.2%). Control samples were untreated.

Recommended levels of available chlorine for different sanitation purposes are <2ppm for an EU approved plant's process water, glaze water and ice (European Communities Council Directive (98/83/EC); Mukundan 2007). Therefore, chlorine treated tuna samples (2ppm) were also studied. Sodium hypochlorite was used as the chlorine source in this experiment. Total available chlorine was determined using an iodometric method (APHA, 1998) (Acetic acid 5 ml and potassium iodide 1g were added to the chlorine solution and titrated against sodium thiosulphate until the colour turned straw yellow). A stock solution of 100ppm chlorine was prepared. The required concentration of chlorine (2ppm) was prepared by diluting the stock solution with sterile distilled water. The dip treated samples were stored in a refrigerator at 4ºC for one hr.

Spices receiving sensory scores above 6 (out of 7, i.e., clove, cardamom and rosemary) were further analyzed using different combinations. The individual spices were added in such a way that the final spice concentration was not more than 0.2% (0.1 + 0.1% for a combination of two and 0.5 + 0.5 + 0.5% for a combination of three spices).

Bacteriological Enumeration and Antimicrobial Activity

After incubation, the fish chunks were aseptically crushed in a sterile mortar and pestle with sterilized 0.1% normal saline (Surendran *et al*., 2006), diluted and inoculated on duplicate plates of tryptone glucose beef extract agar (Himedia, Mumbai, India). Colonies were counted after incubating at 37°C for 48 hr. The antimicrobial susceptibility testing of *E. coli* (MTCC 45) was done using the disc diffusion method of Kirby-Bauer (Bauer *et al.,* 1966) as per the modifications of Islam *et al*. (2008). All the tests were done on Mueller-Hinton agar (Surendran *et al*., 2006). The surface was lightly and uniformly inoculated with bacterial suspension using a sterile cotton swab and the plates allowed to dry for five min. Using sterile forceps sterilized paper discs (Surendran *et al*., 2006) were placed on the plate. Using aseptic conditions, the antimicrobial agents (2, 3, 4 and 5µl) were added to the paper discs using a micropipette. Following overnight incubation at 37ºC, the diameter of the zone of inhibition was measured using a vernier caliper (Dintis Technologies Pvt. Ltd., Ernakulam, Kerala, India). An ethanol dipped paper-disc and an untreated disc served as controls.

Cooking Procedure

The cooking was done according to the procedures of Stoneham *et al*. (2000) and Verlinden *et al*. (2000). The uniform sized fish chunks were wrapped in 0.25mm thick aluminum foil (Stoneham *et al*., 2000) and placed in a wire-mesh basket, which was immersed in a thermostatic water bath, maintained at 100 ± 1°C and cooked for 20 min. After cooking, the samples were immediately cooled in water containing ice for about 3 min and subsequently equilibrated at room temperature before doing the mechanical tests as well as sensory analysis. The cook loss was calculated using the following equation.

Cook loss (%) = $(I_0 - I_1) * 100/I_0$

Where, I_0 = weight of sample before cooking and I_1 = weight of sample after cooking (after drying with a paper towel).

Yield (%) = 100 – Cook loss.

Texture Analysis

Instrumental texture profile analyses of samples were done using a texture analyzer (Lloyd Materials Testing, Bognor Regis, West Sussex, United Kingdom). The method was modified from Bourne (1978). A small flat-faced cylindrical 50mm diameter probe compressed a bite-size sample of cooked fish (2 cm^3 before cooking). Samples were compressed twice to 40% of their original height. The probe test speed and trigger force were maintained at 15mm/min and 0.5N, respectively. From the force-time curve various textural parameters like hardness, cohesiveness, springiness and stiffness (Bourne *et al*., 1978) were calculated by the software included with the instrument. Five replicates were taken for each treatment.

Organoleptic Evaluation

Panelists were selected from the staff and postgraduate students at CUSAT, Cochin, India based on their interest, availability and previous experience in sensory evaluation and familiarity with eating fish. A prescreening exercise was done for 20 candidates where they were evaluated for normal sensory acuity through basic taste, odour and intensity ranking tests (using the hardness scale) as described by ASTM (1981). The panelists who passed the prescreening test were selected for further training. A series of tests were designed to train the selected panelists to have a higher acuity in their taste, odour and aroma perceptions, and to make judgments on the intensity and quality (ASTM, 1981) of key attributes. The panelists were also familiarized with the different test methods that would be used in the sensory evaluation.

The panelists judged the samples individually. A piece of fish (28±2°C) wrapped in aluminum foil was served in a dish coded with a random three-digit number. Consecutive samples were served after a 1 min rest. Mineral water was served to clean the mouth between samples and to eliminate residual mouth-coating effects. Tests were done at around 28°C with normal white fluorescent illumination.

The sensory panelists recorded their sensory descriptions using a 7-point hedonic scale. To check the consistency and reproducibility of their ratings, the panelists repeated the exercise 3 wk later without being informed that they were rating similarly treated fish that they had assessed before.

Statistical Analysis

Data obtained were subjected to statistical analysis using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Mean value, standard deviation, regression, correlation, ANOVA and the post-hoc Turkey tests were done to analyze the results. Statistical acceptance level was set at $P<0.05$.

Results

Bacterial Load and its Reduction

Antimicrobial susceptibility of bacteria showed that all spice oleoresins showed some degree of antibacterial activity against the test organisms. Clove oleoresin (5µl) gave a 20mm clear inhibition zone against *E. coli* (Figure 1). At 3 and 4µl it showed moderate activity of 16-17mm. Activity of turmeric was nil for *E. coli.* Oregano, cardamom and rosemary showed moderate activity (12, 10 and 10mm, respectively) whereas the activity of garlic was weak. Significant differences ($P<0.05$) were observed for the microbial numbers of spice treated and control samples. The antimicrobial activity between the treatments were as follows: clove > oregano > cardamom > rosemary > garlic > turmeric ($P<0.05$). Inhibitory zones produced by combination of spices were lower than those of the individual spices having the maximum antimicrobial effect in the combination (Figure 2).

There were significant differences between treatments and the resultant total plate count. The effect of spice extracts on raw fish media is shown in Table 1. There was a reduction of 88% with 0.2% clove compared to a 70% reduction with 0.1% ($P<0.05$). Turmeric showed the least activity but still killed 32% of the viable bacterial load at 0.2%. The rest of the spices at 0.2% killed at least 50% of the bacterial load ($P<0.05$).

Cook Loss

There were no significant differences between the cook losses and yields of the samples (Table 2).

Texture Profile Analysis

Samples treated with 0.2% concentration of spice extracts showed higher hardness values than those treated with 0.1% concentration (Table 3 and Table 4). But, there was no significant difference between concentrations ($P\geq0.05$) in the cohesiveness, springiness, gumminess, chewiness and stiffness (resistance to deformation) values.

Sensory Evaluation

The panel evaluation of the samples for the suitability of flavour and taste is given in Table 6 and 7. The panel rated the control tuna samples 4.3 for overall acceptability. Addition of spices significantly influenced the sensory properties of the tuna although differences between 0.2 and 0.1% were not significant for any spice ($P\geq0.5$).

The tuna chunks with cardamom at 0.2% showed a significant higher odour intensity compared to the other treatments and the control ($P<0.05$). Garlic at 0.2% had an odour score of 2 due to its pungent aroma. All other spices were rated above the control showing that addition of these spices improved odour (Table 6).

The 'appearance' of cardamom differed significantly from that of control ($P<0.05$). Cardamom treatment also differed from garlic and oregano ($P<0.05$). Turmeric's appearance was low due to a yellow stain produced by the curcumin (Table 6). There was no difference in 'taste' score between clove, cardamom, turmeric and rosemary treated tuna and that of control ($P\geq0.05$). The control differed significantly ($P<0.05$) from tuna treated with oregano and garlic. As with other sensory parameters, oregano and garlic had the lowest scores (Table 6).

The highest overall score of 6 was obtained with cardamom treated tuna. Garlic and oregano treated sample were least preferred by the panelists due to their pungency. Rosemary and clove (0.2%) were also well received with an overall rating of 5.3 and 5.7, respectively. Among the treatments, cardamom, rosemary and clove showed higher values compared

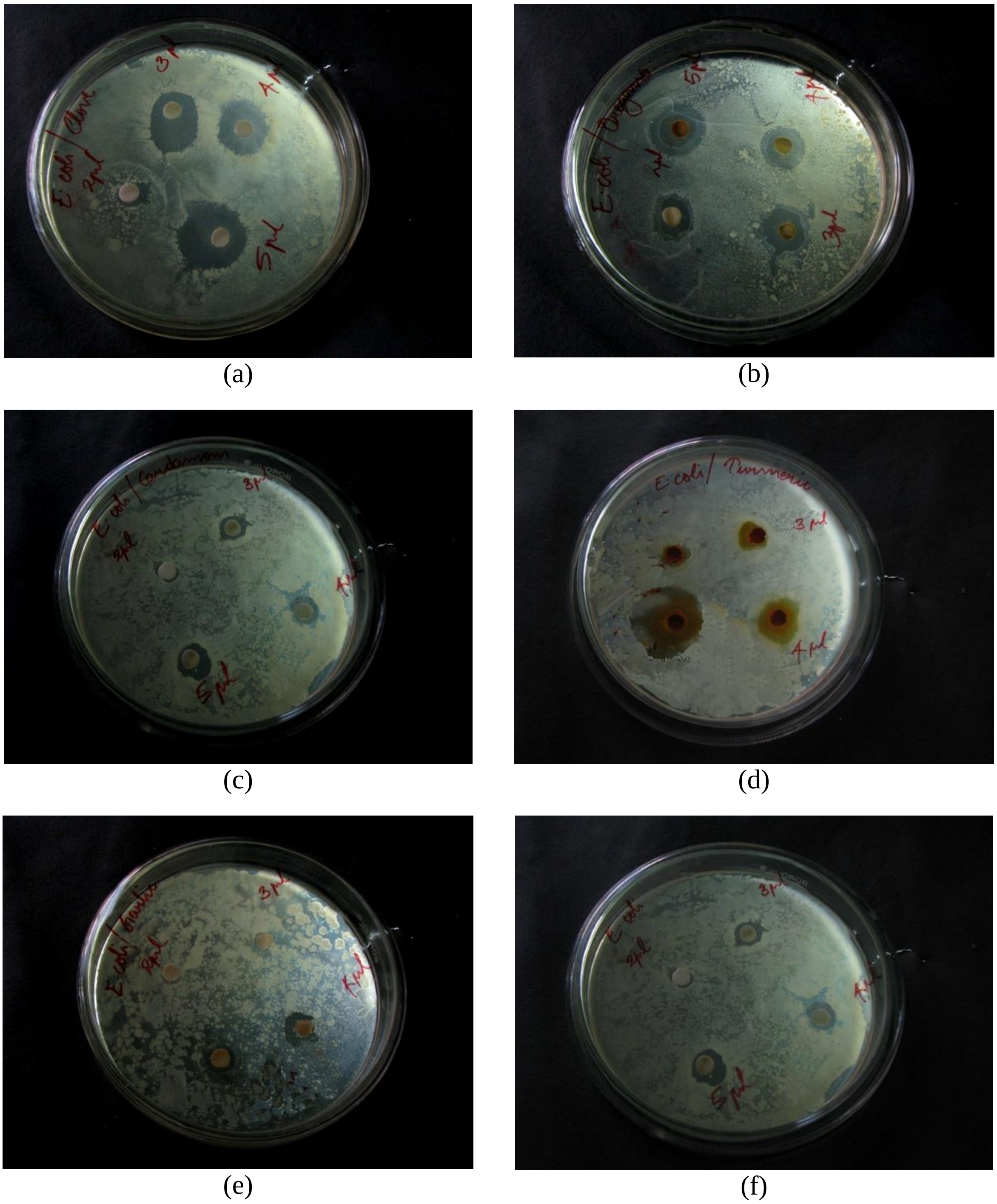

Figure 1. Inhibitory zones produced by spices on a bed of *E. coli:* a - clove, b - oregano, c - cardamom, d - turmeric, e – garlic and f- rosemary.

to control in the order of: cardamom > clove > rosemary > turmeric > control > oregano > garlic. It may be assumed that addition of spices even at 0.2% resulted in improvement of taste and odour for some spices. The most significant attributes in the sensory profiling of the tuna meat were taste and odour. The highest correlation was found between overall score and taste (r = 0.907). It was followed by odour (r = 0.887) and appearance (r = 0.876). These results suggested that taste is the chief component in determining the overall score of the product followed by odour. The sense of smell is routinely regarded as the least important of the five which in fact is responsible for 80% of food flavour (Martin, 2004, Michaels, 2010). Though all parameters had a high positive correlation with each other, the lowest correlation was found between taste and appearance (r = 0.746).

The effects of a combination of the three most preferred spices (i.e., cardamom, clove and rosemary)

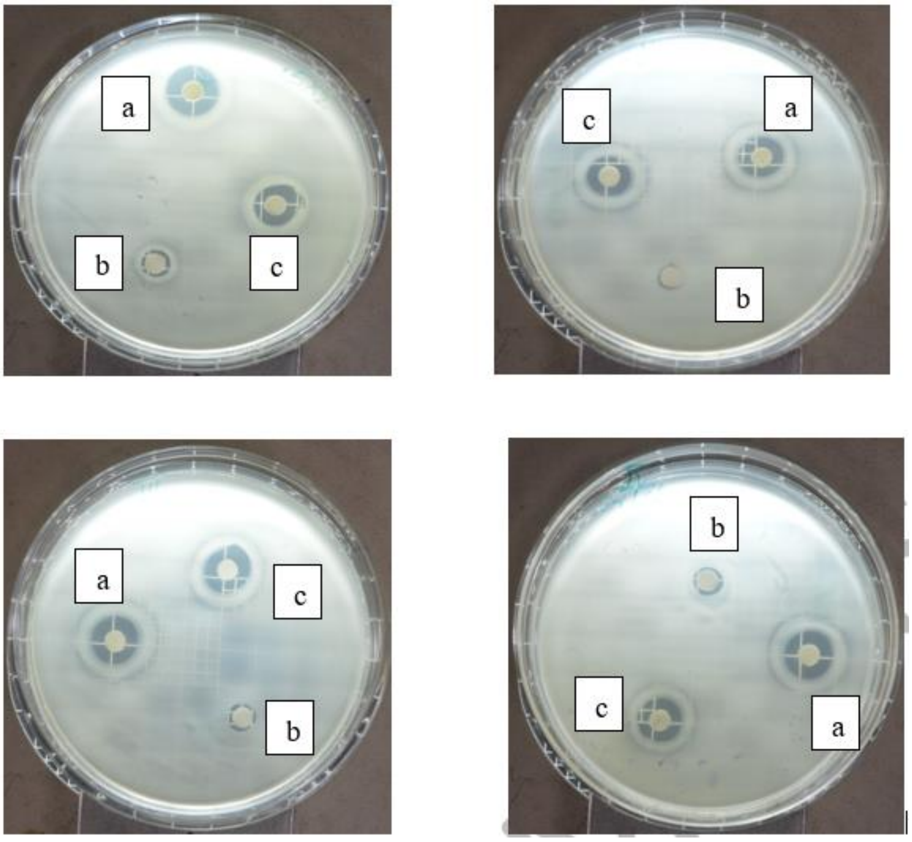

Figure 2. Inhibitory zones produced by individual and combined spices on a bed of *E. coli:* (i) 1µl, (ii) 2µl, (iii) 3µl and (iv) 4µl (a - clove, b - cardamom, c – clove + cardamom 1:1)

Table 1. Total plate count of fish sample containing spice oleoresins (n=3).

Treatment	0.2% spice extract		0.1% spice extract	
	TPC	% reduction	TPC	% reduction
Control	2.1±0.1 × 10^6_a	NA	2.1±0.1 × 10^6 $_a$	NA
Garlic	8.4±0.1 × 10^5 $_b$	60.6	1.2±0.1 × 10^6 $_a$	43.7
Clove	2.5±0.02 × 10^5 $_b$	88.2	6.38±0.02 × 10^5 $_b$	70.1
Turmeric	1.45±0.05 × 10^6_a	31.9	1.8±0.1 × 10^6 $_a$	13.6
Cardamom	1.0±0.2 × 10^6 $_a$	51.6	1.81±0.06 × 10^6 $_a$	15.0
Oregano	2.7±0.2 × 10^5 $_b$	79.2	7.12±0.05 × 10^5 $_b$	66.6
Rosemary	8.2±0.03 × 10^5 $_b$	61.5	9.6 ±0.1 × 10^5 $_b$	54.9
Chlorine (2ppm)	9.8±0.2 × 10^5 $_b$	54	9.8±0.2 × 10^5_b	54.0

*values are given as Mean±SD (values with different subscripts in a column differ significantly at P<0.5)

Table 2. Effect of 0.2% spice treatments and cooking at 100° C on texture of tuna meat (n=3)

Treatment	Hardness1 (kgf)	Cohesiveness	Springiness (mm)	Gumminess (kgf)	Chewiness (kgf.mm)	Stiffness (kgf/mm)
Control	0.75±0.03$_a$	0.26±0.05	2.73±0.07	0.28±0.01 $_a$	0.65±0.07 $_a$	0.35±0.06
Chlorine (2ppm)	0.88±0.08$_b$	0.24±0.02	2.53±0.01	0.21±0.00 $_a$	0.73±0.05 $_a$	0.81±0.03
Clove	1.3±0.1$_c$	0.28±0.04	3.2±1.0	0.35±0.09 $_a$	1.12±0.04 $_b$	0.34±0.01
Cardamom	0.78±0.06$_a$	0.29±0.05	2.65±0.0 3	0.22±0.07 $_a$	0.60±0.1 $_a$	0.36±0.00
Garlic	0.79±0.1$_a$	0.26±0.01	2.16±0.04	0.25±0.02 $_a$	0.63±0.07 $_a$	0.26±0.00
Turmeric	1.1±0.1$_c$	0.32±0.09	3.3±0.1	0.32±0.01 $_a$	1.07±0.01 $_b$	0.25±0.04
Oregano	0.89±0.04$_b$	0.25±0.01	2.6±0.1	0.10±0.00 $_b$	0.64±0.01 $_a$	0.28±0.05
Rosemary	0.71±0.04$_a$	0.28±0.07	2.83±0.03	0.19±0.00 $_b$	0.64±0.08 $_a$	0.18±0.02

*values are given as Mean±SD (values with different subscripts in a column differ significantly at P<0.5)

Table 3. Effect of 0.1% spice treatment and cooking at 100°C on texture of tuna (n=3)

Treatments	Hardness1 (kgf)	Cohesiveness	Springiness (mm)	Gumminess (kgf)	Chewiness (kgf.mm)	Stiffness (kgf/mm)
Control	0.75±0.02$_a$	0.29±0.05	3.2±1.1	0.23±0.04$_a$	0.74±0.04$_a$	0.31±0.005
Chlorine (2ppm)	0.86±0.06$_b$	0.33±0.02	2.4±1.0	0.32±0.00$_a$	0.76±0.03$_a$	0.65±0.04
Clove	1.06±0.05$_c$	0.25±0.07	3.50±0.03	0.27±0.04$_a$	0.95±0.09$_b$	0.29±0.02
Cardamom	0.71±0.05$_a$	0.33±0.06	3.13±0.02	0.31±0.02$_a$	0.97±0.06$_b$	0.64±0.06
Garlic	0.77±0.09$_a$	0.30±0.05	2.70±0.03	0.27±0.01$_a$	0.73±0.03$_a$	0.39±0.01
Turmeric	1.1±0.1$_c$	0.28±0.02	3.1±0.1	0.36±0.09$_b$	1.1±0.2$_b$	0.27±0.07
Oregano	0.86±0.06$_b$	0.28±0.07	2.63±0.05	0.24±0.09$_a$	0.65±0.05$_a$	0.25±0.09
Rosemary	0.70±0.07$_a$	0.28±0.01	2.9±0.1	0.24±0.02$_a$	0.16±0.02$_c$	0.18±0.06

*values are given as Mean±SD (values with different subscripts in a column differ significantly at P<0.5)

Table 4. Cook loss of treated samples cooked at 100°C (n=3**)**

Sl.no	Treatments	Yield (%)	Cook loss (%)
1	Control	87±1$_a$	13±1
2	Chlorine (2ppm)	88±1$_a$	12±1
3	Clove	87±1$_a$	13±1
4	Cardamom	88±1$_a$	13±1
5	Garlic	85±1$_a$	15±1
6	Turmeric	85±1$_a$	15±1
7	Oregano	87±3$_a$	13±3
8	Rosemary	86±2$_a$	14±2

*values are given as Mean±SD (values with different subscripts in a column differ significantly at P<0.5)

Table 5. Cook loss of spice combination treated samples cooked at 100°C (n=3)

Sample Number	Treatment	Yield (%)	Cook loss(%)
1	Clove+rosemary	80±1$_a$	20±1
2	Clove+cardamom	83±2$_a$	17±2
3	Cardamom+Rosemary	80±1$_a$	20±1
4	Clove+cardamom+rosemary	81±1$_a$	19±1
5	Control	80±1$_a$	20±1

*values are given as Mean±SD (values with different subscripts in a column differ significantly at P<0.5)

Table 6. Effects of 0.1% spice treatments on tuna (n=3, values are given as Mean±SD).

Sensory characters	Concentration	Treatments							
		Clove	Cardamom	Garlic	Turmeric	Oregano	Rosemary	Chlorine 2ppm	Control
Odour	0.10%	5.7±0.6$_{aA}$	6±0.5$_{aA}$	2±0.4$_{bB}$	5.3±0.4$_{aA}$	4.3±0$_{cC}$	5.7±0.5$_{aA}$	4.3±0.6$_{cC}$	4.3±0$_{cC}$
	0.20%	6±0.2$_{aA}$	6.2±0.3$_{aA}$	2±0.5$_{bB}$	5.4±0.2$_{aA}$	4.4±1$_{cC}$	6±0.5$_{aA}$	4.3±0.6$_{cC}$	4.3±0$_{cC}$
Appearance	0.10%	5.7±0.4$_a$	6±0.7$_a$	3±0.6$_b$	4.7±0.1	3.3±0.5	5.3±0.7$_a$	4.7±0.4	4.7±0.3$_c$
	0.20%	5.7±0.4$_a$	6±0.5$_a$	3±0.1$_b$	4.7±0.5	3.3±0.9	5.3±0.6$_a$	4.7±0.4	4.7±0.3$_c$
Taste	0.10%	5±0.0$_a$	5±0.3$_a$	3±1$_b$	5±0.9	3±0.8	5±0.9$_a$	5±0.5	5±0.8$_a$
	0.20%	5.2±0.5$_a$	5.2±0.0$_a$	3.1±0.3$_b$	5.1±0.8	3.1±0.5	5.2±0.1$_a$	5±0.5	5±0.8$_a$
Overall	0.10%	5.7±0.5$_{aA}$	6±0.5$_{aA}$	2.7±0.9	5±0.2$_{aA}$	3±0.2	5.3±0.5$_{aA}$	4.3±0.2$_{bA}$	4.3±0.3$_{bA}$
	0.20%	5.2±0.9$_{aA}$	6±0.2$_{bA}$	2.1±0.5	5.1±0.4$_{aA}$	3.5±0.7	5.4±0.6$_{aA}$	4.3±0.2$_{cA}$	4.3±0.3$_{cA}$

(values with different small letter subscripts in a row differ significantly at P<0.5, values with different capital letter subscripts in a column differ significantly at P<0.5)

on the sensory attributes were investigated. Spice blending significantly influenced the sensory properties of the tuna. It is clear from Figure 4 that all of the combinations gave better result for odour than the control. The highest rating for odour was given by the combination of all three spices with a 1:1:1 ratio, and clove and cardamom with a 1:1 ratio. The combination with rosemary and cardamom was also well received by the members of the panel and it scored a 5 for overall acceptability on the hedonic scale (Figure 4).

The rosemary and clove combination had a rating lower than the control tuna sample with respect to appearance. This discrepancy could not be

Sensory characters	Treatments				
	R+Cl	Cl+Cr	Cr+R	Cr+R+Cl	Control
Odour	5.3±1.2$_a$	6.3±1.2$_a$	6.0±0.0$_a$	6.7±1.0$_b$	4.3±1.0$_c$
Appearance	5.3±1.0	6.0±1.0	6.0±1.0	6.3±1.0	6.0±0.0
Taste	6.0±0.0	6.3±1.0	6.0±0.0	6.0±0.0	5.7±1.0
Overall	5.3±1.0$_a$	5.7±1.0$_a$	6.0±1.0$_a$	5.7±2.0$_a$	4.3±1.0$_b$

Table 7. Effects of spice combinations treatment (0.2%) on sensory scores of tuna samples (n=3, values are given as Mean±SD)

Sensory characters	Treatments				
	R+Cl	Cl+Cr	Cr+R	Cr+R+Cl	Control
Odour	$5.3\pm1.2_a$	$6.3\pm1.2_a$	$6.0\pm0.0_a$	$6.7\pm1.0_b$	$4.3\pm1.0_c$
Appearance	5.3±1.0	6.0±1.0	6.0±1.0	6.3±1.0	6.0±0.0
Taste	6.0±0.0	6.3±1.0	6.0±0.0	6.0±0.0	5.7±1.0
Overall	$5.3\pm1.0_a$	$5.7\pm1.0_a$	$6.0\pm1.0_a$	$5.7\pm2.0_a$	$4.3\pm1.0_b$

R+Cl=rosemary+cardamom (1:1), Cl+Cr=clove+cardamom (1:1), Cr+R=cardamom+rosemary (1:1) and Cr+R+Cl=cardamom+rosemary+clove (1:1:1).
(values with different subscripts in a row differ significantly at P<0.05)

explained but all other combinations had sensory score equal to or higher than 5. The results of the sensory panel scaling for taste open an opportunity for evolution of recipes to improve the characteristics and overall acceptability of tuna products for the Indian market. All the experimental combinations had higher scores for taste compared to the control. This is also reflected in the overall acceptability score due to their high positive correlation.

The cardamom-rosemary-clove mix was the most acceptable with the highest overall score followed by clove-cardamom and cardamom-rosemary. In the individual spice treatment, cardamom treated tuna chunks had the highest rating.

Discussion

The control of microbial growth is a primary step towards ensuring seafood spoilage and safety. Deans and Ritchie (1987) had found that clove shows antibacterial affects against 23 genera of bacteria. The antimicrobial properties of oregano are due to its ability to damage the membrane integrity of bacterial cells, which further affects the pH homeostasis and equilibrium of inorganic ions (Lambert *et al*., 2001). The activity of garlic was lower than oregano and clove in this experiment, but it still significantly reduced the bacterial load.

Dellaquis and Mazza (1998) described antimicrobial properties of isothiocyanate derived from onion and garlic. Assays done by Agaoglu *et al*. (2005) indicate that cardamom seed has inhibitory activity with *Staphylococcus aureus*, *Micrococcus luteus* and many other bacterial strains. Curcumin at concentrations of 2.5-50.0mg/100ml have been reported to inhibit *S. aureus* (Shankar and Srinivasamurthy, 1979). In addition to antimicrobial properties, some spices like oregano, rosemary and thyme also show antioxidant properties (Kacaniova *et al*., 2012) which can be an additional advantage in processed lipid rich foods and fatty fishes like mackerel (Sulochanan, 2008).

During cooking, chemical and physical reactions take place that improve or impair the nutritional value of foods (Finot, 1997; Bognar, 1998). March (1984) and Deman (1999) found that cooking of fish led to solubilization of proteins and hence leads to loss of proteins from the final product. In this study there was an average yield of 87% after cooking with no significant differences between the spices and control.

Among texture attributes, hardness, has been associated with fish freshness and is closely associated with human visible acceptability of fish products (Cheng *et al*., 2013). This attribute depends largely on the structure of connective tissue (Cheng *et al*., 2013; Casas *et al*., 2006). Some studies (Johnston *et al*., 2000; Thompson 2002; Purslow 2005) have indicated that textural attributes have a significant positive correlations with the density of muscle fibers. Sun-dried Indian oil sardine (*Sardinella longiceps*) treated with salt, spices and an herb combination could be stored for 9 months without adversely affecting quality in terms of lipid oxidation, microbial quality, colour and nutritional quality (Alex and Eagappan, 2016). Løjeand *et al*. (2007) investigated the effect of chilled storage on the structure of smoked salmon fish, and the results showed that the cells of a smoked salmon sample were more firmly constrained than those of the raw fish control. Roy *et al*. (2012) measured the changes of structure and ultrastructure of cultured Pacific bluefin tuna muscle slices during chilled storage and indicated that the changes of the fish muscle slices were due to loss of myofibers and to myofiber adhesion, detachment of the sarcolemma, increases of intermyofibrillar spaces, and adjustment of hexagonal arrangement of thick compared with thin contractile myofilaments in myofibrils.

Spices are known to enhance the flavour and colour of foods (Eluyode and Akpa, 2007) and hence contribute to the consumer acceptance of a food product. The aroma and flavour principles in spices modified the flavour, taste and colour, thus making the tuna samples more acceptable in some cases than the untreated samples.

Spices are sometimes used for the purpose of deodorizing or masking the smell of raw materials. Fish smells can be deodorized by soaking in spices or lemon juice/vinegar. The deodorizing mechanism involves neutralizing alkali trimethylamine to a nonvolatile compound (Takemasa and Hirasa, 1998). The essential oils of spices are most effective in suppressing the odour of trimethylamine (Nakatani *et al*. 1989). The trimethylamine level in fish is an inverse indicator of seafood freshness (Shimizu and Hibiki, 1957). Suppression of the odour of trimethylamine can result in increased sensory scores for seafood. In a similar study, the deodorizing effect of rosemary was found to be 6 times stronger than sodium copper chlorophyllin (Hirasa and Takemasa, 1998). Sodium copper chlorophyllin is a chemical deodorizer and is often used in breath-freshening chewing gums and toothpastes. The effective deodorizing compounds of rosemary are carnosol and rosemanol. These also have antioxidant functions.

Takemasa and Hirasa (1998) had looked at 40 spices that appear in recipes worldwide and analyzed statistically their frequency of appearance in foods according to nation, cooking ingredient and cooking technique. All the spices used in the current experiment, namely garlic, oregano, turmeric, clove, cardamom and rosemary, were also found to be suitable for use with seafood in a detailed worldwide consumer survey (Takemasa and Hirasa, 1998).

Apart from the sensory enhancement, addition of these spices has other advantages. Kharb and Ahlawat (2010) studied the effect of precooking and a spice mix on chemical and physico-chemical properties of dehydrated spent hen meat mince during storage at ambient temperature (27±2°C). Raw and precooked meat mince (with and without the spice mix) were dehydrated at 60°C for 12 and 9 hr, respectively. They concluded that the mince treated with 2% spice mix retained the most desirable physico-chemical properties up to 60 days of storage.

Statistically, there are very few cases where only one kind of spice is used for cooking (Takemasa and Hirasa, 1998). In most cases, a spice is used in combination with one or more spices. Even a pleasant flavoured spice can have a medicinal-like smell or taste if used in excess (Takemasa and Hirasa, 1998). However, if a spice is grouped with other spices, the total combination will have a more delicate flavour than when each is used individually. This is called the spice-blending effect (Takemasa and Hirasa, 1998). However, Shamsuddeen (2009) reported that individually the extracts of the spices are more active against microorganisms than when they were combined. This finding limits their antibacterial activity in the product, although the sensory properties are found to be amplified.

Leistner (1978) introduced the hurdle concept. The microbial safety, stability, sensorial, and nutritional qualities of foods are based on the application of combined preservative factors that microorganisms present in the food are unable to overcome. Using an adequate mix of hurdles is not only economically attractive; it also serves to improve microbial stability and safety (Leistner, 2000). The present study showed that apart from the microbial safety being improved with spices, the sensory acceptability of the product was also improved.

Conclusion

Cook loss was negligible for all samples cooked at 100°C irrespective of the dip treatment. Samples treated with 0.2% of spice oleoresins showed significantly higher values for hardness than those treated with 0.1%. Spice at 0.2% had the additional advantage of keeping the *E. coli* population in check. The highest acceptance by the trained panel occurred for cardamom. Garlic and oregano treated sample had a negative impact. Rosemary and clove were also well received. Evaluation of the suitability of spice blending showed that rosemary and cardamom blending was well received by the members of the panel. Clove-cardamom and clove-rosemary combinations also had acceptable sensory scores. To conclude, adding spices in tuna products at a controlled rate can reduce the bacterial load as well as enhance its consumer acceptance. Though spices show good antimicrobial properties, their contribution to textural parameters seems to be limited. The experiment also showed that spice combinations may play an important role in increasing the product acceptability, although it may reduce the anti-microbial value of the system.

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