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RESEARCH ARTICLE

PRESERVATIVE EFFECT OF HERBS AND SPICES ON THE MICROBIAL AND SENSORY QUALITIES OF SCOMBEROMORUS GUTTATUS AND LUTJANUS GIBBUS

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Abstract

In the present study, the effect of natural preservatives on the microbial, biochemical and sensory qualities of economically important marine fishes Scomberomorus guttatus and Lutjanus gibbus were evaluated. Fish fillets were treated with 5% concentration of medicinal herbs rosemary (Rosmarinus officinalis) and thyme (Thymus vulgaris) and spices, ginger (Zingiber officinale - rhizome) and garlic (Allium sativum - bulb). Food spoilage bacterias such as Salmonella typhimurium, Eschericia coli, Staphyloccoccus aureus, Enterobacteriaceae aerogens, Shigella dysenteriae, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus mirabilis and Streptococcus faecalis were isolated from treated and non-treated samples. The biochemical parameters pH, salt, moisture, protein, carbohydrate and fat contents and sensory qualities such as appearance, odour, flavor, taste and texture of were also tested. The best preservative effect in terms of lowest pH and microbial growth was observed in 5% of Z. officinale extract treated fishes. Next to ginger, thyme was also an efficient in preservation of the fishes compared to control fishes stored at room temperature. Best sensory qualities were recorded in Z. officinale treated cooked fish, which was followed by thyme, garlic and rosemary treated fish fillets.

Introduction:-

Fishes are perishable by nature and require protection from spoilage during their preparation, storage and distribution to give them desired shelf life. The greater consumer awareness and concern regarding synthetic chemical additives, foods preserved with natural additives have become more popular. This has led researchers and food processors to look for natural food additives with a broad spectrum of antimicrobial activity. Antimicrobial compounds present in foods can extend shelf-life of unprocessed or processed foods by reducing microbial growth rate or viability. Spices and herbs, originally added to change or improve taste, can also enhance shelf-life because of their antimicrobial nature. Thyme, rosemary, ginger and garlic are economically important plants which are used to improve the sensory characteristics, extend the shelf-life of foods due to their natural antimicrobial and antioxidant properties.

Ginger and garlic are spices, in addition to contributing taste and aroma to foods, also contain a variety of bioactive substances which are of considerable use from the standpoint of food science and technology. These may be used

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singly or in combination, and some act synergistically to control spoilage of foods, and herbs and spices can be used as bio-preservative (Yanishlieva et al., 2006). The antioxidant and antimicrobial activities of rosemary and thyme extracts are mainly because of a group of phenolic diterpenes and other phenolic compounds such as rosmarinic and caffeic acids (Bicchi et al., 2000). Natural products can be used effectively due to their preservative properties for enhancing the microbiological safety and extending the shelf-life of seafood (Rafaela et al., 2020). Natural antimicrobial agents that inhibit bacterial and fungal growth for better quality and shelf life have been of considerable interest in recent years. Natural antimicrobials are mainly extracted and isolated as secondary metabolites of plants, animals, and microorganisms. Plants, especially herbs and spices, are given more attention as a source of natural antimicrobials (Teshome et al., 2022). The main scope of this research was to analyse and evaluate the effect of natural preservatives on the microbial population and various other quality index of the selected fishes.

Materials and Methods:-

Sample collection and sample preparation

Fresh marine fishes Scomberomorus guttatus and Lutjanus gibbus were collected from local whole sale fish market in Coimbatore, Tamil Nadu and brought to the laboratory in an ice cold box, washed thoroughly in running water, weighed and then aseptically cleaned and filleted into pieces then they were subjected to various analysis.

Collection of preservatives and extract preparation

Fresh leaves of rosemary and thyme were collected from home garden in Ooty, Tamil Nadu. 25 gms of rosemary and thyme leaves were steam distilled in 100ml of distilled water for 4 hours at 120^{o} C then the extract was cooled at room temperature and the extraction was filtered through Whatman filter paper. Similarly ginger and garlic extract were obtained by steam distillation of twenty five gram of each spices in two separate flask with 100 ml distilled water boiled at 60^{o} C for 2 hours, cooled and then filtered through Whatman filter paper.

Dip Treatment

S. guttatus and L. gibbus of each weighing 50 gm were cut into fillets and were subjected to dip treatment in all preservative extracts (5%) for 15 minutes and then drained and stored in room temperature for 5 hrs and then subjected to microbial and sensory analyses.

Sensory analysis

Sensory evaluation of the fish fillets treated with preservatives were performed and were conducted by ten panel members who asked to evaluate appearance, odour, flavor, taste and texture of the cooked samples. According to the scoring table, a total score of sensory attributes of 10 indicates first quality, scores from 9.9 to 8 indicate second quality, 7.9 to 6.0 indicate third quality and 5.9 to 4.0 indicate fourth quality and 3.9 or less corresponds to unfit for consumption.

Physicochemical and Bacteriological Examination

The pH content, moisture, salt, protein, carbohydrate, lipid and ash contents were estimated in the preservatives treated and non-treated fish samples after five years of storage (AOAC, 2005).

Total Viable Count

Ten grams of muscle tissues from each fish samples were aseptically homogenized in a sterile homogenizer flask with 90 ml of sterile peptone for 2 minutes. Further, tenfold dilutions were made and then 100 µl of each dilution were spread on agar plates. The bacterial colonies were counted after the plate's incubation at 37°C for 48 hours. The bacterial numbers were then expressed as log colony forming unit (Cfu/gm).

Isolation of food spoilage bacteria

Food spoilage bacterias such as S. typhimurium, E. coli, S. aureus, E. aerogens, S. dysenteriae, P. aeruginosa, K. pneumonia, P. mirabilis, S. facecalis were isolated from non-treated and preservatives treated fish fillets using suitable media.

Results and Discussion:-

The present study was aimed to study quality of marine edible fishes, S. guttatus and L. gibbus in relation to the influencing factors like physicochemical parameters and microbial population. The

efficacy of traditional preservatives like spices (Z. officanlis and A. sativum) and herbs (R. officinalis and T. vulgaris) on the preservation of the fishes from spoilage was observed. Non-treated fishes stored at room temperature for 5 hours served as control to know the effective of preservatives on the fish samples stored at room temperature.

The p^H values were estimated at 6.12 and 5.98 of the freshly collected samples of S. guttatus and L. gibbus from local fish market in Coimbatore (Table 1). After 5 hours of storage at room temperature there was a gradual increase in p^H in non-treated samples and it ranged up to 6.90 and 6.71 in S. guttatus and L. gibbus. The p^H of selected preservative such as Z. officanlis and A. sativum spices treated fish fillets were 6.5 and 6.3, the herb R. officinalis and T. vulgaris fish muscles showed 6.7 and 6.4 respectively (Table 2). The increase in p^H are relate to the accumulation of alkaline compounds such as ammonia mainly derived from microbial activity during fish muscle storage (Huss et al., 1995). The above result was in agreement with the findings of Libata et al., (2010) tested the effect of different concentrations of ginger on the quality of smoke dried catfish. Hasan et al., (2013) found that the phytochemical constituent zingiberene and gingerol in Z. officinale and thymol in T. vulgaris has the ability to retard or sustain the growth of microbial activity in fishes. The present study proved that there was a gradual decrease in p^H of the preservative treated fish muscles when compared to the non-treated fish muscles stored at room temperature for 5 hours.

Moisture determination is one of the most important and widely used measurements in the processing and testing of foods. The moisture content of freshly collected S. guttatus and L. gibbus was 62.52% and 50.32% which drastically increased to 73.82% and 83.42% in fish samples stored at room temperature for 5 hours (Table 1 and 2). Moisture contents of the preservatives treated fish samples were lower than the control. The moisture content were 69.66% and 53.95% in ginger treated fish samples, 74.74% and 60.10% in garlic treated fishes (Samples treated with thyme were 78.37% and 63.10% and samples treated with rosemary were 62.82% and 60.03% respectively (Table 1 and 2). Among the treatments ginger treated fishes showed decreased moisture content (69.66% and 53.95%) in both the fishes when compared to the non-treated fishes stored at room temperature for 5 hours, which is followed by rosemary treated samples (63.10% and 60.03%) in S. guttatus and L. gibbus. The progressive moisture reduction observed in fish samples may be due to penetration of active preservative ingredients of Z. officinale (gingerol) and rosemary (carvasol) into fish muscles preventing the binding of water molecules. The present study results were similar to the study conducted by Kinn-Kabari (2011) on effects of extracts from three Indigenous Spices on the chemical stability of smoke-dried catfish (Clarias lezera) during storage, he observed that among Piper guinensis, Myristica monodora and Xylophia aethiopicum, Piper guinensis extract treated fishes showed decreased moisture content.

Total viable count (TVC) is the most common microbiological method aimed to detect and enumerate high proportion of microbial population as possible. The microbial analysis such as TVC and identification of spoilage microbes in fresh and after 5 hours storage in room temperature were observed, preservative treated fishes stored at room temperature for 5 hours were also analysed to study the effect of traditionally used spices and herbs on selected fishes. From the observed TVC analysis, the control fish muscles showed highest TVC of $34X10^5$ and $28X10^5$ cfu/g in S.guttatus and L.gibbus than the fresh fishes samples ($4X10^5$ and $2X10^5$ cfu/g respectively) (Table 3 and 4) which is within the permitted limit ($7X10^5$) by FDA (2022), whereas the control fishes samples exceeded the max limit. Fish in its natural environment has its own micro flora in the slime on its body, in its gut and in its gills. The microorganisms and enzymes present in the tissues of the fish, which brings about putrefactive changes in fish when it dies. TVC values in preservatives treated fishes S. guttatus and L.gibbus were $2X10^5$ and $0.8X10^5$ cfu/g of Z. officinale, $10X10^5$ and $7X10^5$ cfu/g for A. sativum treated fishes, while rosemary and thyme treated S. guttatus and L.gibbus were $9X10^5$ and $8X10^5$ cfu/g and $6X10^5$ and $4X10^5$ cfu/g respectively (Table 3 and 4). It is observed that the control fish samples which are not subjected to preservative treatment stored at room temperature for 5 hours revealed highest viable counts than the fresh and preservative treated fishes. All the preservative treated samples revealed TVC values which is within the permissible limit except rosemary which showed a slight exceeded level, this may be due the least concentration of extract used in the treatment.

Spoilage microbes like E.coli, Enterobacteriaceae aerogens, Staphylococcus, Shigella dysenteriae, Pseudomonas aureginosus, Proteus mirabilis, Streptococcus faecalis, Klebsiellia pneumoniae and Salmonella typhimurium were isolated (Table 5) from fresh fish fillets, preservatives treated and control samples stored at room temperature for 5 hours. E.coli was not detected in any of the treated samples. From the findings, it was clear that there were no

presence of any spoilage bacteria's in the fresh samples of both the fishes; only Staphylococci and Pseudomonas were detected. The antimicrobial activity of the preservatives was known by its inhibitory effect of the bacterial species. Among the preservatives used 5% of ginger (Z.officinale) extract showed vast inhibitory effect against E.coli, E. aerogens, Staphylococcus, P. aeruginosa, K. pneumonia, S. dysenteriae and Salmonella (Table 6). Similarly 5% of thyme also showed inhibitory effect against 5 species of bacterial strains except E.coli. Present study results were also in agreement with the findings of Attouchi and Sadok (2009) who found that dipping of carp fillets in thyme solution (1%) not only reduced the total viable count but also extended the shelf life from 4 days to at least 12 days at 5^{o} C. The microbial populations for all the preservative treated fishes observed in this study E. coli, Salmonella and Staphylococcus were within the recommended limits for good quality fish product according to International Commision on Microbiology Safety for Food.

Sensory evaluation is reported to be highly subjective and lack objectivity. In fishes the present study sensory criterion were taken into consideration to account the effect of preservatives on the quality, taste and appearance of the fishes (Table 6). The sensory qualities such as flavor, taste, odour, colour and overall acceptability were observed for the selected fishes (S. guttatus and L. gibbus) treated with spices (Z. officinale and A. sativum) and herbs (R. officinalis and T. vulgaris) tested individually. From the observed evaluation the two fish samples treated with Z. officinale (Ginger) were considered as first quality in all the sensory quality parameters, A. sativum and T. vulgaris treated fish samples were ranked as second quality and R. officinalis treated fish samples were ranked third quality by the scores given by judging panel members. Similar results were observed by Rathod and Pagarkar (2013) in fish cutlets, made from Pangassisus fish (Pangasianodon hypophalamus), during storage in refrigerated display unit at 15- 18^{o} C. Biochemical contents such as protein, carbohydrate, lipid and ash contents of fresh fishes were 3.65, 1.04, 2.65 and 1.12(%) in Scomberomorus guttatus and 2.16, 0.56, 2.06 and 0.65(%) in Lutjanus gibbus. There were no changes in biochemical contents observed during storage period in the preservative treated samples.

Table 1:- Physico-chemical Parameters of control and Preservative treated Scomberomorus guttatus.

S.No	Parameters	Fresh Fish	After 5 hours of storage				
			Non-treated	Preservative treated samples			
				Spices		Herbs	
				Ginger	Garlic	Rosemary	Thyme
1	pH	6.12	6.90	6.81	6.67	6.78	6.93
2	Moisture (%)	62.43	73.82	74.74	69.66	78.37	63.10
3	Salt (%)	0.12	0.07	0.06	0.08	0.04	0.06

Table 2:- Physico-chemical Parameters of Non-treated and Preservative treated Lutjanus gibbus.

S.No	Parameters	Fresh fish	After 5 hours of storage				
			Non-treated	Spices		Herbs	
				Ginger	Garlic	Rosemary	Thyme
1	P^{H}	5.98	6.32	6.96	6.74	6.94	6.71
2	Moisture (%)	80.44	83.42	60.10	53.95	60.03	62.82
3	Salt (%)	0.5	0.02	0.04	0.12	0.02	0.06

Table 3:- Total Viable Count (cfu/g) of non-treated and preservative treated Scomberomorus guttatus and Lutjanus gibbus.

S. No	Fish samples	Fresh sample	Non-treated sample	Preservative treated sample			
				Spices		Herbs	
				Ginger	Garlic	Rosemary	Thyme
1	**Scomberomorus guttatus**	4×10^5	34×10^5	$2 X10^5$	$10 X 10^5$	$9 X 10^5$	$6 X 10^5$

2	**Lutjanus gibbus**	2 X 10^5	28 x 10^5	0.8 X10^5	7 X 10^5	8 X 10^5	4X 10^5

Table 4:- Microbial population in fresh and preservative treated Scomberomorus guttatus.

S.No	Bacterial species	Scomberomorus guttatus					
		Fresh sample	Non-treated sample	Spices treated sample after 5 hours		Herbs treated sample after 5 hours	
				Ginger	Garlic	Rosemary	Thyme
1	E.coli	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
2	Salmonella	**+**	**+**	**ND**	**+**	**+**	**ND**
3	Staphylococci	**ND**	**+**	**ND**	**+**	**ND**	**ND**
4	Enterobacter aureginosa	**ND**	**+**	**ND**	**+**	**+**	**ND**
5	Pseudomonas aureginosa	+	+	ND	+	+	+
6	Klebsiella pneumonia	**ND**	**+**	**ND**	**+**	**+**	**ND**
7	Shigella dysenteriae	**ND**	**+**	**ND**	**ND**	**+**	**ND**
8	Proteus mirabilis	**ND**	**+**	**ND**	**ND**	**+**	**ND**
9	Streptococcus facealis	**ND**	**+**	**ND**	**ND**	**+**	**+**

ND – Not detected + - Detected

Table 5:- Microbial population in fresh and preservative treated Lutjanus gibbus.

S.No	Bacterial species	Lutjanus gibbus					
		Fresh sample	Non-treated sample	Spices treated sample after 5 hours		Herbs treated sample after 5 hours	
				Ginger	Garlic	Rosemary	Thyme
1	E.Coli	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
2	Salmonella	**ND**	**+**	**ND**	**ND**	**ND**	**ND**
3	Staphylococci	**ND**	**+**	**ND**	**+**	**+**	**ND**
4	Enterobacter aureginosa	**ND**	**+**	**ND**	**ND**	**+**	**ND**
5	Pseudomonas aureginosa	**+**	**+**	**ND**	**+**	**+**	**+**
6	Klebsiella pneumonia	**ND**	**+**	**ND**	**+**	**ND**	**ND**
7	Shigella dysenteriae	**ND**	**+**	**ND**	**ND**	**+**	**ND**
8	Proteus mirabilis	**ND**	**+**	**ND**	**ND**	**+**	**ND**
9	Streptococcus facealis	**ND**	**+**	**ND**	**ND**	**+**	**+**

ND – Not detected + - Detected

Table 6:- Sensory quality of Scomberomorus guttatus and Lutjanus gibbus treated with different preservatives.

Fish samples	Fish fillets treated with preservatives	First quality	Second quality	Third quality	Fourth quality	Unfit for consumption
Scomberomorus guttatus	Ginger	10	-	-	-	-
	Garlic	-	9.7	-	-	-
	Rosemary	-	-	6.0	-	-
	Thyme	-	9.9	-	-	-
Lutjanus gibbus	Ginger	10	-	-	-	-
	Garlic	-	9.9	-	-	-
	Rosemary	-	-	6.0	-	-
	Thyme	-	9.6	-	-	-

Conclusion:-

Preservatives are the substances, which are used to prevent food spoilage by inhibit the growth of microorganisms like bacteria and fungi. The dipping of fish fillets in an effective concentration of natural preservatives such as spices (Z. officinale and A. sativum) and herbs (R. officinalis and T. vulgaris) has beneficial effects on the overall quality of the final products. Preservation with

the natural preservatives will not only reduce the substantial losses associated but would also increase the rate of turn over as consumers would now find increased satisfaction with the naturally processed fish as indicated by the sensory quality of the product. This would substantially improve fish protein intake and reduce protein malnutrition and its associated problems in the country and also confirms the consumer safety.

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