

Preservative Efficiency of *Allium Sativum* on Shelf Life of Sea Water Fishes

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Research

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Abstract

Natural preservation techniques of food are associated with various naturally producing antibacterial property of various biologically active compounds obtained from plants, spices, animals and microbes. Garlic (*Allium sativum*) is the prominent biologically active source possessing greater antimicrobial property. Expansion of shelf life, declines the spoilage mechanism by the use of garlic. Garlic exhibits not only preservative properties but also has a biological activity viz. antibacterial, antimicrobial, anti-inflammatory, antiprotozoal, anti-helminthic, antifungal, wound healing, antitumor, and insecticidal. Allyl alcohol, allicin is an active biological compound present in garlic. Fish is highly beneficial and wholesome food as it contains high quantity of proteins and minerals as compared to other meat sources. Fresh fishes easily deteriorate due to high moisture and nutrient content. Spoilage of food begins as soon as fish dies. Hence, many preservation techniques are used to escalate the shelf life of fish. Enzymatic action of bacteria do not cause any variation in the metabolic activities of living organisms due to its existing immunity against it. As soon as the fish perishes, the enzymes associated with the catalytic activities in flesh and guts are now responsible for the autolytic reaction. Decomposition can be identified by its color change, foul odor and its texture. Various physiological analysis of these fishes were done with respect to their weight, color, texture, softness, and appearance. Further on microbial analysis were performed to identify and characterize the fish isolate, followed by its biochemical test. Various parameters were evaluated with respect to quality and shelf life of fishes with and without garlic as the natural preservative.

Introduction

Natural preservation process implies the implementation of naturally occurring antimicrobial compound obtained from plant, animals and other sources to avert deterioration of food caused by microbial activities and increased food borne pathogens (Delesa *et.al*, 2018).

A top-notch biological active known is garlic (*Allium sativum*) which has a pronounced antimicrobial activity. It act as a dormant inhibitor for food pathogens. Utilization of garlic intensifies shelf life and shrinks the spoilage of food (Muhammad *et.al*,2016).

Garlic prevalently belongs to Liliaceae family. It is well-known medicinal plant, food item and spice in different areas (Shivendu *et.al*,2012). This not only possess preservative attributes but also exhibit various biological activity such as antibacterial, antifungal, antimicrobial, antiprotozoal, anti-inflammatory, wound healing, anti-helminthic and antioxidant property. (Kumolu *et.al*, 2010).

Garlic is originally found in central Asia as a staple. It is morphologically a tall and bulbous covering 2–3 feet in height possessing pink or purple flowers (Kombat *et.al*, 2017).It has high content of protein, fats, sugar and various other minerals viz. calcium, potassium, phosphorous, sulphur, iodine and vitamins.

It inculcates broad range of activities like Hepatic protective activity, hematological activity, anti-neoplastic effects, anti-carcinogenic activity (Rana *et.al*, 2011).Antimicrobial activity of garlic is associated with Allinase which is triggered when it is crushed or cut into pieces (Rahman *et.al*, 2007).The foremost compound present in garlic is allicin, it is preferably known for its antimicrobial activity (Latifa *et.al*, 2017).

Fish is one of the most delicious food having large amount of proteins and minerals as compared to other sources of meat. It also act as good therapeutic apparatus in diagnosing multiple disease such as coronary disease, auto immune disease, protein energy malnutrition and anemia (Tairu *et.al.*, 2017). Due to high moisture and nutrient present in the fishes they rapidly deteriorate and starts spoiling as soon as fish dies. Living cell of a fish is not affected due to its natural protective mechanism. As the fish starts decomposing the enzymes performing metabolic activities now refracts its activity and carry out autolytic mechanism (Mukundam *et.al.*, 1986).Antiseptic activity of garlic was examined by Louis Pasteur in 1858 which resulted to the conclusion that fishes are highly sensitive to spoilage (Sharma *et.al.*, 2011).

There are many mouth-watering, nutritious and affordable fishes such as Tuna (*Thunnini*), Mackerel (*Rastrelliger kanagurta*)and Anchovy india (*Stolephorus indicus*) for consumers (Kannaiyan *et.al*, 2014). These fishes are usually recommended by people even in off season so to make it available its shelf life is extended using garlic paste (Nahid *et.al.*, 2017).

Hence, the main perspective of the research is to evaluate the antimicrobial activity of garlic and its efficiency to increase the shelf life of fishes.

Material And Methods

Collection of fish sample

Different species of fish sample such as Tuna (*Thunnini*), Mackerel (*Rastrelliger kanagurta*) and Anchovy india (*Stolephorus indicus*) were purchased from local market. Two sample of each species having weight and total length in the range of 75-100gm and 19-22cm respectively were selected for study.

Processing of sample

Scales and guts of all 6 fresh fish samples were removed and the fish were thoroughly cleaned under running tap water.

Collection of Garlic

About 1 kg of the garlic used for the study was also purchased from local market.

Processing of Garlic

The garlic was peeled, washed with running tap water and crushed into paste using blender and mortar pestle.

Pretreatment of the fish with Garlic paste

Three species of each one sample were uniformly smeared with garlic paste including internal and external parts. The remaining three fishes were not treated and served as ac control for the experiment. The treated and untreated fresh fish samples were placed in sealed plastic bags and stored in laboratory at room temperature for 5 days.

Antimicrobial activity of garlic paste

Fish samples such as Tuna, Mackerel, Anchovy india were coated with and without garlic paste respectively. Control was kept without coating. They are then incubated for 45 minutes at room temperature so as to stick evenly. Now these fish samples are then transferred into 6 different zip lock pouches for preservation.

Isolation and characterization of fish isolates

Suspension of each fish samples were prepared and a loopful of it is used for streaking on various selective and differential media, it is then incubated at 37°C for 24 hrs. Fish isolates were characterized on the basis of their cultural and biochemical test and same procedure is repeated after 5 days.

Results

Collection of sample

Two samples of each species of Tuna, Mackerel, Anchovy india were collected from local market of Bhiwandi .Fresh fish sample were brought to laboratory in zip lock bag to avoid microbial contamination. Physiological analysis was done on the basis of weight, color, softness, texture, appearance and smell before preservation.

Processing of sample

Scales and guts of al fish samples were removed and the fishes were cleaned under tap water.

Collection of garlic

One kilogram of garlic was purchased from the local market of Bhiwandi and bulbs were peeled and cleaned.

Processing of garlic

Cleaned garlic cloves were crushed and small pieces were finely crushed using mortar pestle it was then transferred into clean air tight container and used immediately.

Treatment of fish with garlic paste

Each one sample of three fishes were uniformly smeared with garlic paste, ensuring that every part of the body was covered including internal and external parts. Remaining three fishes were not smeared and it served as control for the experiment. The treated and untreated fish samples were then sealed placed on separate trays in laboratory at room temperature for 5 days.

Antimicrobial activity of garlic paste on fish

Samples such as Tuna, Mackerel and Anchovy india were coated with and without garlic paste respectively. Control was kept without coating and incubated at room temperature for 45 minutes, so that garlic sticks

uniformly. These coated and uncoated samples were then transferred into zip lock bags for preservation.

Isolation and identification fish isolates

Microbiological analysis was performed by taking inside and outside swabs of each fish samples in a sterile tube for identification and characterization of fish isolate. Streaking was done on various differential and selective media and incubated at 37°C for 24 hrs. Identification of isolated organisms on first day i.e. before preservation and on fifth day i.e. after preservation was performed by different biochemical tests such as Sugar fermentation, IMViC, Urease, PPA, Oxidase, TSI, Catalase and LD, after performing it the total number of isolates found were shown in table 1 and table 2 respectively.

The highest microbial growth was found on the control of all three fish samples such as *E.coli*, *S.aureus*, *K.pneumoniae*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Shigella spp*, *Vibrio spp*, *Vibrio parahaemolyticus* after preservation, whereas garlic paste effectively delayed the microbial growth on all three preserved fish samples hence there is no growth of any pathogenic bacteria. This proved the efficacy of the garlic treatment as a preservative (Kombat *et.al*, 2017) found similar result.

CHANGES OBSERVED DURING FISH PRESERVATION

Table 3 and table 4 respectively represents changes in physical characteristics in garlic untreated (control) and garlic treated (test). Tuna (*Thunnini*), Mackerel (*Rastrelligerkanagurta*), Anchovy india (*Stolephorus indicus*) respectively, during laboratory storage condition with respect to days.

Discussion

Antibacterial properties of garlic leads to the inhibition of spoilage of sample sea water fishes. Microbial analysis of fish before preservation projected high count of bacteria and enteric pathogen too after incubation as this was similar to the observation (Kannaiyan et.al., 2014). While the sample which was coated with garlic paste acted as a preservative and hence no growth of microbes were observed. This proved the efficiency of garlic (*Allium sativum*) as a preservative and the results were similar (Kombat et.al., 2017)

Conclusion

Naturally many sea fishes are perishable they easily deteriorate. This study has demonstrated and confirmed the efficiency of garlic paste as natural preservatives to enhance the shelf life fish. From this study it is concluded that all fishes contain various pathogenic organisms in them. Garlic has antibacterial property, it kills all the organisms and no new organisms develop inside the body of seawater. Anchovy fish decays more rapidly than tuna fish which decays in atleast five days. More the production of garlic oil on preserved fish indicates the fish has natural ability to decay slower if kept unpreserved. Along with preservation flies are also inhibited if fish is treated with garlic paste and if not treated it doubles the number of pathogenic organisms inside and on the fishes.

To determine antibacterial property of garlic extract using different solvents such as ethanol, aqueous and methanol on different bacterial isolates can be studied. Isolation and characterization of the larvaecidal principle of garlic can be tested for purification of water. The synergistic interaction of garlic with commercial antibiotics can be checked.

Abbreviations

IMViC – Indole test, Methyl Red test, Voges Proskauer test, Citrate test. TSI – Triple sugar ion slant. LD – Lysine Decarboxylase agar. PPA – Phenyl alanine deaminase agar.

Declarations

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Author's contribution :

I, Miss Dhanya Poojary have performed by the experiment and wrote the article, Miss Malika Ahuja guided me in writing and in proofreading.

Availability of data and materials :

All data generated or analyzed while performing the experiment are included in this article.

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Tables

Table 1: Total number of isolates before preservation

	ANCHOVY				MACKEREL				TUNA				Total Isolates
	Test		Control		Test		Control		Test		Control		
	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	
<i>E.coli</i>	+	+	+	+	+	+	+	+	+	+	+	+	12
<i>S.aureus</i>	+	+	+	+	+	+	+	+	+	+	+	+	12
<i>K.pneumoniae</i>	-	+	+	+	-	+	+	+	+	+	+	+	10
<i>V.cholera</i>	+	+	+	+	+	+	+	+	+	+	+	+	12
													46
Key:(+) Growth, (-) No growth, In(Inside), Out(Outside)													

Table 2: Total number of isolates after preservation

	ANCHOVY				MACKEREL				TUNA				Total Isolates
	Test		Control		Test		Control		Test		Control		
	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	
<i>E.coli</i>	-	-	+	+	-	-	+	+	-	-	+	+	06
<i>K.pneumoniae</i>	-	-	+	+	-	-	+	+	-	-	+	+	06
<i>S.aureus</i>	-	-	+	+	-	-	+	+	-	-	+	+	06
<i>Shigellaspp</i>	-	-	+	+	-	-	+	+	-	-	+	+	06
<i>V.cholera</i>	-	-	+	+	-	-	+	+	-	-	+	+	06
<i>V.parahaemolyticus</i>	-	-	+	+	-	-	+	+	-	-	+	+	06
<i>S.paratyphi A</i>	-	-	+	+	-	-	+	+	-	-	+	+	06
<i>S.paratyphi B</i>	-	-	+	+	-	-	+	+	-	-	+	+	06
<i>Proteus mirabilis</i>	-	-	+	+	-	-	+	+	-	-	+	+	06
													54
Key: (+) Growth, (-) No growth, In (Inside), Out (Outside)													

Table 3: Changes observed in seawater fishes not treated with garlic during storage

Chara		Flesh	Gills	Skin	Pupil	Fish odor	Maggot	Flies
Day1	A	Soft	Pale	Bright grey	Bright	Fresh	-	-
	M	Soft	Pale	Bright grey	Bright	Fresh	-	-
	T	Soft	Pale	Bright grey	Bright	Fresh	-	-
Day2	A	Slightly elastic	Pinkish	Dark grey	Dark	Pungent	+	+
	M	Slightly elastic	Pinkish	Dark grey	Dark	Pungent	++	+
	T	Slightly elastic	Pinkish	Dark grey	Dark	Pungent	+	+
Day3	A	Flabby	Grey	Dark grey	Flat white	Fetid	++	++
	M	Flabby	Grey	Dark grey	Flat white	Fetid	+	+
	T	Flabby	Grey	Dark grey	Flat white	Fetid	+	+
Day4	A	Elastic	Grey	Creamy	Flat cloudy	Fetid	+++	++
	M	Elastic	Grey	Creamy	Flat cloudy	Fetid	++	+
	T	Elastic	Grey	Creamy	Flat cloudy	Fetid	++	+
Day5	A	Decay	Dark grey	Creamy	Flat cloudy	Very bad	++++	+++
	M	Decay	Dark grey	Creamy	Flat cloudy	Very bad	+++	++
	T	Decay	Dark grey	Creamy	Flat cloudy	Very bad	+++	++
Key: Chara: Characteristics; (A): Anchovy india ; (M): Mackerel ; (T): Tuna ; (-): No growth; (+): Growth;								

Table 4: Changes observed in seawater fishes treated with garlic during storage

Chara.		Flesh	Gills	Skin	Pupil	Garlic odor	Garlic oil	Garlic color
Day1	A	Firm	Red blood	Bright grey	Bright	Fresh	-	-
	M	Firm	Red blood	Bright grey	Bright	Fresh	-	-
	T	Firm	Red blood	Bright grey	Bright	Fresh	-	-
Day 2	A	Dense	Red blood	Bright grey	Dark	Strong	++	+
	M	Dense	Red blood	Bright grey	Dark	Strong	+	+
	T	Dense	Red blood	Bright grey	Dark	Strong	+	+
Day3	A	Soft	Red blood	Bright grey	Flat white	Strong	++	++
	M	Soft	Red blood	Bright grey	Flat white	Strong	++	+
	T	Soft	Red blood	Bright grey	Flat white	Strong	++	+
Day 4	A	Elastic	Red blood	Bright grey	Flat cloudy	Strong	+++	++
	M	Elastic	Red blood	Bright grey	Flat cloudy	Strong	+++	+
	T	Elastic	Red blood	Bright grey	Flat cloudy	Strong	+++	+
Day 5	A	Elastic	Red blood	Dark grey	Dark	Piquant	+++	+++
	M	Elastic	Red blood	Dark grey	Dark	Piquant	+++	++
	T	Elastic	Red blood	Dark grey	Dark	Piquant	+++	++

Key: Chara; Characteristics; (A): Anchovy india; (M): Mackerel; (T): Tuna; (-): No growth; (+): Growth; (++): More growth; (+++): High growth

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