Preservative Effects of Mustard Marination on Indian Mackerel Fish

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Abstract: ***Fish has high nutrient value especially in terms of protein content and are more prone to spoilage. Marination of fish with mustard paste is a traditional method used for taste and flavor development in addition to its nutrient content. The antimicrobial effect of mustard indicates its potential to be used as natural preservative. The main objective of the present investigation is to study the role of mustard marination in fish preservation. Indian Mackerel (Rastrelliger kanagurta) fish was selected for the study. The fish procured from the local fish market were divided into two groups, one group as control (unmarinated) and second group as experimental (marinated with mustard paste). The entire set of fish was kept at laboratory temperature for 10 days and the preservative effects of mustard paste marination were studied with respect to organoleptic and biochemical parameters. After marinating the fish for 10 days, the organoleptic parameters such as colour, texture, smell, stiffness are normal and similar to control (0 day's unmarinated fish). The protein content of the muscle of the fish is however reduced up to 4% and 7% after 5 days and 10 days of marination respectively. The organoleptic and biochemical study of mustard marinated fish revealed the preservative effects of mustard paste and henceforth strongly recommends the use of mustard as a good preservative for the fish to be used for human consumption.***

Index Terms**: Mackerel, mustard paste, marination, natural preservative**

I. INTRODUCTION

Marination, as described in the research conducted by Kiyoung *et al*. (2018), involves treating meat or flesh with a combination of vinegar, salt, and herbs like mustard, along with oil, before cooking. This method enhances various aspects of the meat, including its quality and sensory attributes, such as nutritional value, tenderness, moisture retention, and flavor. Additionally, it contributes to prolonging the shelf life of the meat by reducing bacterial growth, primarily due to the low pH level and the presence of salts and herbs. The antioxidants and antimicrobial properties of natural preservatives help in increasing the shelf life of meat products (Reza *et al*., 2015). The medicinal plant, *Brassica nigraie*, mustard has been known as a natural preservative (Thompson *et al*., 2013). Mustard seeds exhibit a spherical, black appearance with a diameter of approximately 1mm. These seeds are characterized by their piquant flavor and a distinct, aromatic nutty fragrance. They belong to a weedy plant that is deliberately grown for its seeds, primarily serving as a spice in various culinary applications. Indian mustard is an oilseed crop belonging to family Brassicaceae. It contains natural antimicrobial compound allylisothiocyanate (AIT), a volatile compound and has been known to be effective against the growth of many pathogenic bacteria (Clarissa *et al*., 2009). Antimicrobial activity of mustard has been reported to retard the growth of food-borne pathogenic bacteria. (Min-Suk Rhee *et al*., 2003).

Fish represents the vital source of protein and other nutrients including omega 3 fatty acids known for anticancer property. The fish however, are very perishable and thus easily spoiled by microorganisms (Petronilla. *et al*, 2014). Numerous microorganisms find their way into the seafood processing chain due to insufficient process oversight, subpar hygiene and sanitation standards in processing facilities, and contamination during improper handling or storage after production (Eze, E. *et al*., 2011). These microorganisms can pose health risks to humans.

The Indian Mackerel fish (*Rastrelliger kanagurta*), which serves as the animal model in the current study, is readily accessible for human consumption throughout the year in Indian local fish markets. Mackerel, locally known as Bangda is a deep water sea fish. Studies have revealed that the main factor in fish spoilage is the growth of harmful microbes associated with fish and its surrounding environment. Fish are vertebrate animals characterized by intricate chemical compositions within their cell membranes, including compounds like phospholipids. Additionally, their muscle cells contain proteins such as actin and myosin, while their bones consist of collagen and hydroxyapatite (Singh *et al*., 2016). According to Sebastian and Stefan (2011), the functional roles of these proteins are diverse and complex, with amino acids serving as pivotal constituents of muscle tissue. Moreover, the degradation of amino acids can give rise to volatile compounds that function as indicators of fish spoilage.

The spoilage of the fish is often reflected in terms of protein degradation and change in organoleptic characteristics of the fish. The change in colour, texture, stiffness and odour of the fish may be the easiest ways for the fish traders and especially the fish eaters to identify the degree of fish spoilage and hence unsuitability of the fish for human consumptions.

The present work emphasizes the role of mustard paste as a fish preservative suitable for storing the fish for longer time before cooking. The study is also helpful in extrapolating the mustard and its products at least in Indian kitchen as flavoring and preservative agent.

II. MATERIALS AND METHODS

A. Collection and maintenance of fish

Indian mackerel fish (*Rastrelliger kanagurta*) (weight 90.5 ± 1.17 g and length 19.56 ± 0.09) were selected for the present study assuming that the fish are easily available and are more preferred by the local community. The fish were brought to the laboratory in an ice pack, washed with clean water and gills and intestines were removed immediately to minimize the chances of growth of harmful microorganisms.

B. Seed collection and preparation of paste

For marination, black unpolished mustard seeds were collected from spice market of Kalyan, Thane, Maharashtra, India. Mustard seeds, once collected, were thoroughly cleaned, subjected to oven drying, and subsequently transformed into a fine powder. Mustard paste was made by mixing 50 ml of distilled water to 100 g of mustard powder. The collected washed fish were then marinated. 12 fish were marinated with mustard paste and 12 were kept without marination as control at 4°C.

C. Experimental design

The fish were divided into two groups each with 12 fish. One group of fish was marinated with mustard paste in such a way the entire fish body was covered with a thin layer of the paste. The paste was also inserted into the empty space formed by the removal of gills and alimentary canal of the fish. Both the marinated and unmarinated fish were kept in fridge at 8 0C temperature for 10 days. After expiry of 0 day, 5 days and 10 days, the fish were analyzed for organoleptic alterations and simultaneously the fish muscle in between the lateral line and 1st dorsal fin was removed and processed for histochemical and biochemical analysis of proteins.

C. Organoleptic and protein analysis

Marinated and unmarinated fish (4 each) were used for organoleptic and protein analysis on each 0, 5th and 10th day of experimentation. The organoleptic parameters were studied with respect to change in colour, texture, stiffness and smell. Protein histochemical analysis was done by using mercury bromophenol blue method (Mazia et al., 1953) on thin (5 µm) paraffin sections. Protein quantification was done using Folin – Ciocalteau Phenol reagent (Lowry *et al*. 1951). 10 % fish muscle homogenate was centrifuged at 5000 rpm for 10 minutes. The supernatant was used for quantification of protein using colorimeter at 750 nm.

III. RESULTS AND DISCUSSION

Aquatic animal-based foods, in comparison to terrestrial counterparts, offer a wealth of protein with lower caloric density. They are also notable for their high content of omega-3 long-chain polyunsaturated fatty acids (n-3 LC PUFA) (Tacon and Metian, 2013). According to Lund (2013), the consumption of fish is not only attributed to its elevated levels of n-3 LC PUFA but also to its rich array of other nutrients. These include a well-balanced amino acid

composition, high proportions of taurine and choline, as well as vitamins A, D3, and B12. Furthermore, fish provides essential minerals such as calcium, phosphorus, iodine, selenium, iron, and zinc, all of which have beneficial effects on human health.

Fish is a highly perishable commodity, with spoilage beginning immediately after harvesting due to autolytic enzymes and bacterial activity. It's worth noting that roughly one-fourth of the world's fish supply, and 30% of landed fish, are lost solely due to microbial activity. Preserving fish has become crucial to store and transport this valuable food source to meet the needs of the growing global population. Additionally, it aims to extend its shelf life while preserving its nutritional value, texture, and flavor (Ayuba *et al*., 2015). Numerous preservation techniques, such as salting, drying, smoking, fermentation, and canning, have been employed effectively to thwart fish spoilage and prolong its storage duration. Furthermore, in response to consumer preferences for texture, appearance, and taste, methods like cooling, freezing, and chemical preservation have been embraced. Within the fish processing industry, the essential approaches involve low-temperature treatments and the application of chemicals to control enzymatic, oxidative, and microbial spoilage.

Marination is a common method used for different durations in order to develop the flavor and to enhance the taste of fish. There are various marinade solutions that often contain natural dried ingredients including the spices, herbs and other extracts. Spices and herbs added marinades significantly enhance meat structure and minimize lipid oxidation (Vlahova and Dragoev, 2014). The Indian mackerel (*Rastrelliger kanagurta*) fish holds the distinction of being the predominant and widely consumed edible fish in the area. It stands out for its affordability, nutritional richness, and consistent availability in the local fish market throughout all seasons. In fish markets and during transportation, mackerel fish remain stored in ice and are sold to consumers without maintaining proper hygienic conditions that in turn provide greater opportunity for growth of microbes on fish body surface. In studies, it has been established that the Microbial load associated with surface of the fish plays important role in its spoilage. Petronillah R. *et al*., (2014) have isolated various pathogens like *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Vibrio cholerae* and *Shigella dysenteriae* from the skin surface of fish. Mackerel fish, the animal used in the present study has already been reported to be associated with *E. coli*, *Salmonella sp*., *Vibrio* sp. (Ahire K. and Khade R, 2018), *Staphylococcus aureus*, *Escherichia coli* and *Lactobacillus plantarum*. Eze, E. I. Echezona *et al*., (2010). Durlu-Ozkaya *et al*., (2001) have reported the role of *E. coli* in fish protein degradation.

The mackerel fish collected for the present study were fresh and silver grey in color with normal fishy smell. The texture was rough, firm and hard. In experimental fish having mustard paste, the organoleptic characteristics such as smell, colour, texture and stiffness were not significantly changed compared to unmarinated fish (**Table 1**). Role of mustard in fish preservation was evaluated over a period of 10 days at 4^0 C. The unmarinated fish when analyzed for various organoleptic characteristics over a period of 10 days showed marked difference in the appearance and stiffness compared to marinated fish. Not much difference was observed in the colour and smell of the marinated fish that showed thick brown slime layer on the surface along with loss in the tissue firmness. The autolytic enzymes play important role in reduction of textural quality during early stages of deterioration. Autolytic degradation, as noted by the FAO (2005), has the potential to curtail the shelf life and overall quality of a product, even when the presence of spoilage organisms is relatively low.

Histochemical analysis was performed in order to study the effect of mustard paste on structural integrity of fish muscle and also with respect to protein content of the fish. After 10 days, the sections showed more muscle fiber loosening in unmarinated fish than in mustard marinated fish (Figure 1). The

Table 1: Physiological examination of marinated Mackerel fish.

Parameters	Storage period					
	0 Day		5Days		10 Days	
	Marinated	Unmarinated	Marinated	Unmarinated	Marinated	Unmarinated
Color	SG SG SG SG	SG SG SG SG	SG SG SG SG	BYL BYL BYL BYL	SG SG SG SG	BYL BYL BYL BYL
Texture	R R R R	R R R R	R R R R	S S S S	R R R R	S S S S
Stiffness	H H H H	H H H H	H H H H	Soft Soft H H	H H H H	Soft Soft Soft Soft
Smell	NF NF NF NF	NF NF NF NF	NF NF NF NF	F F F F	NF NF NF NF	F F F F

Key - SG- Silver grey; R- Rough;H- hard; NF- fishy; F– Fow;BYL-Brownish yellow layer

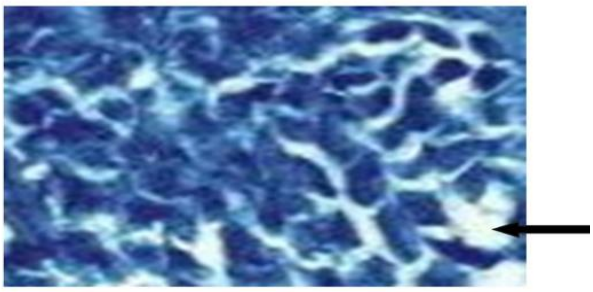

Figure 1: Alteration in texture of muscle fibres and staining intensity of protein of Indian Mackerel fish (Unmarinated) after exposure of 10 days.
Note wide gaps due to loosening of muscle fibres.

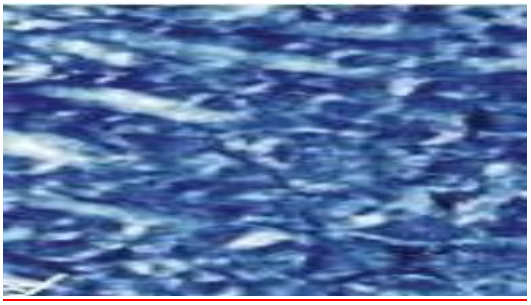

Figure 2: The texture of muscle fibres and staining intensity of protein of Indian Mackerel fish marinated with mustard paste after exposure of 10 days.

staining intensity of proteins in marinated fish was more indicating less degradation perhaps due to preservative effects of mustard paste. Protein contents of the mustard marinated fish appeared darker than control.

The presence of gaps among the muscle fibers in control (unmarinated) fish samples were more than in the marinated fish (Figure 1 and 2). The gaps thus formed corroborate the protein reduction which was more significant in unmarinated fish. In studies it has been revealed that bacterial putrefaction is a well-known cause of protein reduction in the fishes (Shewan, 1962).

Fish are highly nutritious especially with respect to proteins, as they are rich in essential amino acids. A post-harvest loss of proteins in fish muscles occur owing to poor hygienic and storage conditions that in turn lead to microbial growth on fish body surface. The percentage reduction in the protein content of the marinated and unmarinated fish has been shown in Figure 3. The reduction in protein content of

unmarinated fish was prominent as compared to mustard marinated fish. The protein reduction of unmarinated fish after 5 days of storage at 4^0C was found to be 6% while it was only 4% with mustard marination. After 10 days, the reduction in protein content almost doubled in unmarinated fish. The mustard marination was found to be more effective as it retained 93% of the fish protein even after 10 days of storage.

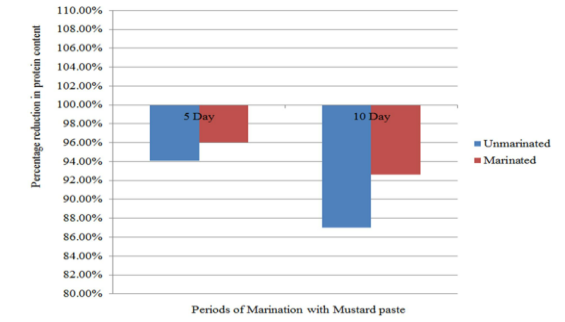

Figure 2: The percentage reduction in protein content of Indian Mackerel after marination with mustard paste.

The present study concludes that marination of fish especially the Indian mackerel with mustard paste helps in maintaining the integrity of muscle fibers and also the protein amount in the muscles. Apart from these, the mustard paste also preserves the actual organoleptic characteristics and imparts good flavor and taste to the fish.

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V. CONTRIBUTIONS

The author, Ms. Khushali Ahire performed this experiment as a part of M.Sc. Part 2 dissertation work in the subject of Microbiology under the guidance of Dr. Ranjana Khade, Associate Professor, Department of Microbiology, R. K. Talreja College of Arts, Science & Commerce, Ulhasnagar-3, Thane (M. S.), India. Dr. Ajai Kumar Singh and Ms. Parimita P. Sharma of the Department of Zoology of the College collectively helped Ms. Khushali in handling, maintaining and processing of fish for histochemical and biochemical analysis of protein. They (Dr. Ajai Kumar Singh and Ms. Parimita P. Sharma) also did final editing of the manuscript, tables and figures and placing them (table and figures) appropriately into the text.

REFERENCES

[1] Ahire, K and Khade R. (2018), Antimicrobial activity of mustard against Mackerel fish pathogens, *International journal of trend in scientific research and development*, 2(2): 2456-6470.

[2] Aiysha Thompson, Dilruba Meah, Nadia Ahmed, Rebecca Conniff-Jenkins, Emma Chileshe, Chris O Phillips, Tim C Claypole, Dan W Forman and Paula E Row.(2013). Comparison of the antibacterial activity of essential oils and extracts of medicinal and culinary herbs to investigate potential new treatments for irritable bowel syndrome. *BMC Complementary and Alternative Medicine*, 13: 338-357.

[3] Amir RazaShaviklo. (2012),Development of fish protein powder as an ingredient for food applications: a review, *Food Sci.Technol*,52(2): 648–661

[4] Ana Clarissa dos Santos Pires, Nilda de Fatima Ferreira Soares, Nelio Jose de Andrade, Luis Henrique Mendes da Silva, GeanyPeruchCamilloto, Patrícia Campos Bernardes. (2009). Increased preservation of sliced mozzarella cheese by antimicrobial sachet incorporated with allylisothiocynate, *Brazilian Journal of Microbiology* 4(10): 1002-1008.

[5] Ayuba, V.O., Okomoda, V.T., Ikape, S.I. (2015). Suitability of corn husk and cow dung as alternatives to fuel wood for smoking fish. *Journal of food and Health science.* 2: 149-163.

[6] Durlu O. and Ayhank V. N. (2003). Biogenic amines produced by Enterobacteracae isolated from meat products. *Meat science* 58:163-166.

[7] Eze. E.I., Echezona B. C. and Uzodinma E. C. (2011).Isolation and identification of pathogenic bacteria associated with frozen mackerel fish (Scomberscombrus) in a humid tropical environment. *African Journal of Agricultural Research*, 6(8): 1947-1951.

[8] KiyoungJeong, Hyeonbin O, So Yeon Shin, and Young-Soon Kim. 2018 Effects of Different Marination Conditions on Quality, Microbiological Properties, and Sensory Characteristics of Pork Ham Cooked by the Sous-vide Method. *Korean J. Food Sci. An.*38 (3):506-514.

[9] Lowry O.H., Rosebrough N.J., Farr A. L., Randall R.J. (1951). Protein measurement with the folin-phenol reagents. *Journal of Biological Chemistry*193: 265–273.

[10] Lund, E. K. (2013). Health benefits of seafood; Is it just the fatty acids? *Food Chem*., 140: 413–420.

[11] Mazia, D., Brewer, P.A., Alfert, M., (1953). The cytochemical staining and measurement of protein with mercuric bromophenol blue. *Biol. Bull*. 104:56-67.

[12] Min Suk Rhee. (2003). Antimicrobial Effects of Mustard Flour and Acetic Acid against Escherichia coli O157:H7, Listeria monocytogenes, and Salmonella entericaSerovarTyphimurium, *Applied and Environmental Microbiology*, 69(5): 2959–2963.

[13] PetronillahR.,Robert K., Gono J., Muzvondiwa v., and Nyani S. (2014), Isolation and Identification of Pathogenic Bacteria in edible fish:A case study of rural aquaculture projects feeding livestock manure to fish in Zimbabwe. *International Journal of current Microbiology and Applied Science* 3(1): 897-904.

[14] Sebastian Paczkowski and Stefan Schütz. (2011). Post-mortem volatiles of vertebrate tissue. *Appl. Microbiol. Biotechnol*. 91:917–935.

[15] Singh A. K., Sawale S. and Chavan S. (2016). Glycoconjugates study in surface secretions of fresh water edible catfish, Clarias gariepinus in response to clove oil anaesthesia. *International journal of chemical and biochemical science*: 2(5): 34-41.

[16] Shewan J. M. (1962). The bacteriology of fish and spoiling fish and some related chemical changes. *Recent Adv. Food Sci*. 1, 167-193.

[17] Tacon, A. G. J., and M. Metian. (2013). Fish matters: importance of aquatic foods in human nutrition and global food supply. *Rev. Fisher. Sci*. 21: 22–38.

[18] Vlahova V. D and Dragoev D. (2014). Marintion: effect on meat safety and human health. A review. *Bulgarian Journal of Agricultural Science*, 20(3): 503-520