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EFFECT OF SENSORY ENHANCEMENT ON YELLOWFIN TUNA (*THUNNUS ALBACARES*) MYOFIBRILS

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Abstract: Increasing the shelf life of fishes is one of the most difficult tasks in fish preservation. Time-temperature profile plays a significant role in affecting the textural characteristics of the fish. Loss of freshness is due to a complex combination of biochemical, chemical and physical processes, and is followed by muscle spoilage due to microbiological contamination. Marination is a process used to tenderize the structure and increase the taste of raw materials. Initial quality of raw materials, considering their freshness, microbiological load and physical damage are significant factors which influences the quality of the end product. Natural preservatives such as ginger, lemon and vinegar were used in the present study for increasing the shelf life and also increase textural and palatable acceptance. The species selected for present study is Yellowfin tuna (*Thunnus albacares*) because of its relevance and economic important in the current world market. A sensory panel analysis was done to understand the acceptability of the treatment. The effects of treatments on the myofibrils were carried out by SEM analysis.

***Key words*:** Tuna, Myofibrils, Marination, Muscle spoilage, Natural preservatives

INTRODUCTION

Yellowfin tuna; *Thunnus albacares* is an intensively exploited fish. Large quantities of yellowfin tuna are commercially used in canned and dry-salted products, and as sashimi, a delicate-tasting raw fish product popular in Korea and Japan (Elena Sanchez-Zapata *et al.,* 2007). The use of yellowfin tuna as sashimi is increasing in several other countries, with an annual worldwide production of 3,400,000 mt (Lee and Kim, 2008). Yellowfin tuna industrialization is increasing fishery by-products. The amount of by-products produced during fish processing can be as high as 75% of the total weight of fish. Some of the by-products are used to produce fish sauces and food products and also used in animal feed (Shahidi, 1994).

Fish muscle always contains two types of cells, white/light and red/dark, with the latter always present as the minor component. White muscle has an anaerobic metabolism and being dependent upon glycogen for energy, is well supplied with glycolytic enzymes which permit intense contraction during periods of flight. Red muscle on the other hand, has an aerobic metabolism and is rich in haemoglobin, myoglobin and mitochondria. In fatty species, particularly the surface swimming pelagic fish of which tuna, mackerel and herring are typical examples, red muscle is well-developed to provide energy for sustained high swimming speeds (Hall and Ahmad, 1997). There are many differences in the chemical composition of the two types of fish muscle, but the most important are the high fat and hemopigments content in the red or dark muscle. The higher lipid contents, less stable proteins, greater concentrations of heme proteins, lower ultimate pH values, and higher concentrations of sarcoplasmic proteins of dark muscle have been suggested to contribute to the difficulties in its industrialization and making high-quality products from raw material containing high contents of dark muscle. Because of this it is considered as a by-product of the tuna industry (Huss, 1998). However, the characteristics of yellowfin tuna dark muscle (DM) that make it not acceptable for these industries (strong dark colour and highly susceptible to lipid oxidation speeding up its de-

terioration) could give it a high nutritive value, even higher than that of light muscle. Dark muscle is also a high source of iron which is an essential mineral (Sanchez, 2011).

Fish muscle myofibrillar proteins are relatively unstable. The functional properties of protein are directly related to the quality of myofibrillar protein and the properties of this protein determine the quality of mince-based products. Aggregation and denaturation of myofibrillar protein occur during frozen storage, affecting the texture, as well as the gel-forming characteristics of the mince. The stability of fish myofibrillar protein is species dependent and there is evidence relating the stability of actomyosin to the habitat temperature. There are other constituents in the meat, such as free fatty acids, free amino acids and nucleotides that affect the stability of protein during frozen storage (Sankar and Ramachandran, 2005).

Fish muscle rapidly degrades during post mortem storage due to proteolytic enzymes acting probably both on muscle cells and connective tissue. These changes which are progressive during storage may have significant impact on the quality and consumer acceptance of the fish (Foegeding and Lanier Hultin, 1996). There are several proteolytic systems present in fish muscle tissue which may be involved in post mortem muscle degradation.

Spoilage of food products is due to chemical, enzymatic or microbial activities. Food preservation becomes necessary in order to increase its shelf life and maintain its nutritional value, texture and flavour. Proper handling, pre-treatment and preservation techniques can improve the quality of fish and fish products and increase their shelf life. Fish spoilage results from three basic mechanisms: enzymatic autolysis, oxidation and microbial growth. Low temperature storage and chemical techniques for controlling water activity, enzymatic, oxidative and microbial spoilage are the most common in the industry today (Ghaly *et al.,* 2010).

Marinating process is one of the oldest methods of preservation of fish. Marinated fish are typically inspired as ready-to-eat products with no heat treatment (Gramm and Huss, 1996). Marination is also used to tenderise or to change taste, textural and structural properties of raw material. Initial quality of raw materials, considering their freshness, microbiological load and physical damage are significant factors which influences the quality of the end product (Fusselli *et al.,* 1994). Keeping qualities depend largely upon storage temperatures. Several methods have been successfully applied to preserve fish and to extend the shelf life of fish products but none has been tested to use in DM. In the present study, ginger, lemon and vinegar were added in order to analyse which one will improve the taste and textural quality of the fish.

Ginger has been used as a spice and as natural additives for more than 2000 years (Bartley and Jacobs, 2000). As a spice, ginger has a geographical spread that covers every part of the globe and it is consumed whole as a delicacy, used in traditional oriental medicine, or as spice in foods, such as fish and meat. Ginger contains spectra of biologically active compounds, such as curcumin, 6-gingerol, 6-shagaols, zingiberene, bisabolene and several other types of lipids that confer on it, the properties of being pungent and a stimulant. These compounds are responsible for the unique aroma and flavour of ginger and account for about 1-3% of the weight of fresh ginger (Akram *et al.,* 2011).

The juice of the lemon is about 5% to 6% citric acid. It is used in marinades for fish, where its acid neutralizes amines in fish by converting them into non-volatile ammonium salts, where the acid partially hydrolyzes through collagen fibres, tenderizing the meat, but the low pH denatures the proteins, causing them to dry out when cooked. Lemon juice contains plenty of vitamin C, also known as ascorbic acid, which is a powerful antioxidant that prevents spoilage and rotting. Similar to salt, lemon juice draws out water content, balancing the pH factor and natural acids in food. 5% to 6% citric acid, which is used in beverages, foods, cosmetics and pharmaceuticals for preserving colour, flavour and taste.

The preservative action of vinegar is based upon its acetic acid content. The application of vinegar as a food preservative is a traditional method of preventing fish spoilage. Vinegar is an effective acidulant that causing depression of pH below the growth range of many bacteria (Jay, 2000).

MATERIALS AND METHODS

Fresh sample of yellow fin tuna, Thunnus albacares weighing about 3 kg was collected from the Thoppumpady harbour (Ernakulam District, Kerala). The collected samples were preserved at<=4°C. The fish sample was divided into 4 lots and 5% w/v of Vinegar, Lemon, and Ginger were added to first three lots and the last lot was kept as Control. Ginger extract was prepared by crushing and squeezing fresh ginger rhizome. 5% w/v (weight/volume) of ginger extract was used per kg fish sample. Citric acid extract was produced by squeezing lemon without adding water. 5% w/v of lemon extract was used per kg fish sample. A bottle of prepared vinegar was used. 5% w/v was applied to the fish samples and Control was also kept without any treatment.

The treated sample was stored at d" 4 °C. The duration of storage study was for 6 months. Sample was analyzed every 2 months for change in pH and moisture. The analysis was done in triplicate.

A team of 7 panel members performed the sensory evaluation of the samples and a hedonic scale of 7-point was used for assessment (Borderias *et al.,* 1983). The selected characteristics were tested as defined by Jowitt (1974). The Proforma for the sensory evaluation is given in Table 1. The sensory panel recorded the sensory descriptions of the sample (odour, colour, flavor, texture and overall acceptability scoring) using 7-point hedonic scale. Five replicates of each sample were considered.

For electron microscopic evaluation, 10g of sample was taken from each lot. 1cm cubes were cut out of the fish using a sharp razor. The sections were fixed in 25% of glutaldehyde. Thin slices of sample were obtained by microtome. Each small sample was treated with an alcohol series for dehydration (70%, 80%, 90% and 100% of alcohol). Sample in 100% alcohol was kept in a dessicator till the ethanol had fully evaporated. It was carried in 2 ml vials to the SEM unit were it was sputter coated with platinum.

For SEM analysis sample was analyzed using JEOL- Scanning Electron Microscope at 500X and 5000X at Sophisticated Test and Instrumentation Centre (STIC), CUSAT. The present study was carried out during July 2010 to January 2011.

Table 1. Proforma for Sensory Evaluation

HEDONIC SCALE		Sensory parameters
7	Out standing	Texture
6	Excellent	Odour
5	Very good	Taste
4	Good	Appearance
3	Fair	Over all
2	Acceptable	
1	Not acceptable	

RESULTS AND DISCUSSION

Sensory panel was selected after an initial screening of 20 candidates. The candidates were tested for their sensory accuracy by providing them with known concentrations of salt and sugar solutions. 7 of the personnels were selected as the sensory panel since they gave a correct scoring for maximum number of tests.

The mean values in texture shows a high score for lemon treated samples with ginger very close behind whereas, vinegar scored the lowest. In the case of 'odour', control and lemon was favored followed by ginger. But when taste is taken as the significant parameter in a sensory test, ginger treated samples gains the maximum value on the 7 point hedonic scale. The mean value for 'appearance' had the highest score for the untreated control/sample.

In Vinegar treatment, the Texture, Taste and Overall score recorded were similar for all the panelists (SD = 0.487). The appearance showed largest SD (0.976) when compared to other qualities. This shows a significant variation between the data and the statistical average (Table 2).

The Texture (0.487) Taste (0.377) and Overall (0.534) does not show much variation for SD in Ginger treated sample. The SD recorded zero value for odour. The Appearance, which has the greater value, recorded 1.46 for SD. This denotes that there is no significant variation from the mean value (Table 3).

The SD value for Texture and Overall recorded zero for lemon treated sample. For the Odour and Taste, the SD values were recorded as 0.377 and 0.487 respectively. This shows no significant variation from the mean. The Appearance recorded greater value (1.06) for SD and showed significant variation from the mean (Table 4).

Sensory analysis of treated tuna

Table 2. Sensory data of Vinegar Treated Tuna

	SAMPLE NUMBER								
TREATMENTS	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**MEAN**	**SD**
TEXTURE	5	5	4	5	5	5	4	4.71	0.487
ODOUR	3	3	3	4	4	4	3	3.42	0.534
TASTE	3	3	3	3	2	3	2	2.71	0.487
APPEARANCE	2	2	4	4	4	4	3	3.42	0.976
OVERALL	3	3	3	3	3	2	3	2.71	0.487

Table 3. Sensory data of Ginger treated tuna

	SAMPLE NUMBER								
TREATMENTS	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**MEAN**	**SD**
TEXTURE	6	6	5	6	6	6	5	5.7	0.487
ODOUR	4	4	4	4	4	4	4	4	0
TASTE	3	3	3	4	3	3	3	3.14	0.377
APPEARANCE	5	2	4	5	2	2	2	3.14	1.46
OVERALL	5	5	4	5	4	4	4	4.42	0.534

Table 4. Sensory data of Lemon treated tuna

	SAMPLE NUMBER								
TREATMENTS	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**MEAN**	**SD**
TEXTURE	6	6	6	6	6	6	6	6	0
ODOUR	4	4	5	4	4	4	4	4.14	0.377
TASTE	3	3	2	3	3	2	3	2.71	0.487
APPEARANCE	2	4	2	4	4	4	2	3.14	1.06
OVERALL	4	4	4	4	4	4	4	4	0

Table 5. Sensory data of Control Sample

	SAMPLE NUMBER								
TREATMENTS	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**MEAN**	**SD**
TEXTURE	4	6	5	5	6	6	5	5.2	0.755
ODOUR	4	4	4	4	4	4	5	4.14	0.377
TASTE	4	3	3	2	3	3	4	3.14	0.694
APPEARANCE	5	2	4	6	5	5	6	4.7	1.38
OVERALL	2	2	4	4	4	4	2	3.14	1.069

By analyzing the SD of sensory data for control, the Texture (0.755), Odour (0.377) and Taste (0.694) showed not much deviation when compared with each other. Appearance (1.38) and Overall (1.069) treatments showed much deviation in the controlled samples when comparing to other quality parameters (Table 5).

In the present analysis, the quality parameters in control samples recorded high values than that of other treatments and it is evident that in all 3 quality tests, the Appearance quality showed much dominance than the other quality parameters.

In the scanning electron micrograph, vinegar treated samples showed the least difference from the control. Tuna chunk that underwent 5% w/v ginger extract treatment showed stretching of the myofibrils and this can be explained by the proteolytic activity exhibited by gingerol, an active constituent in ginger extract. Lemon treated

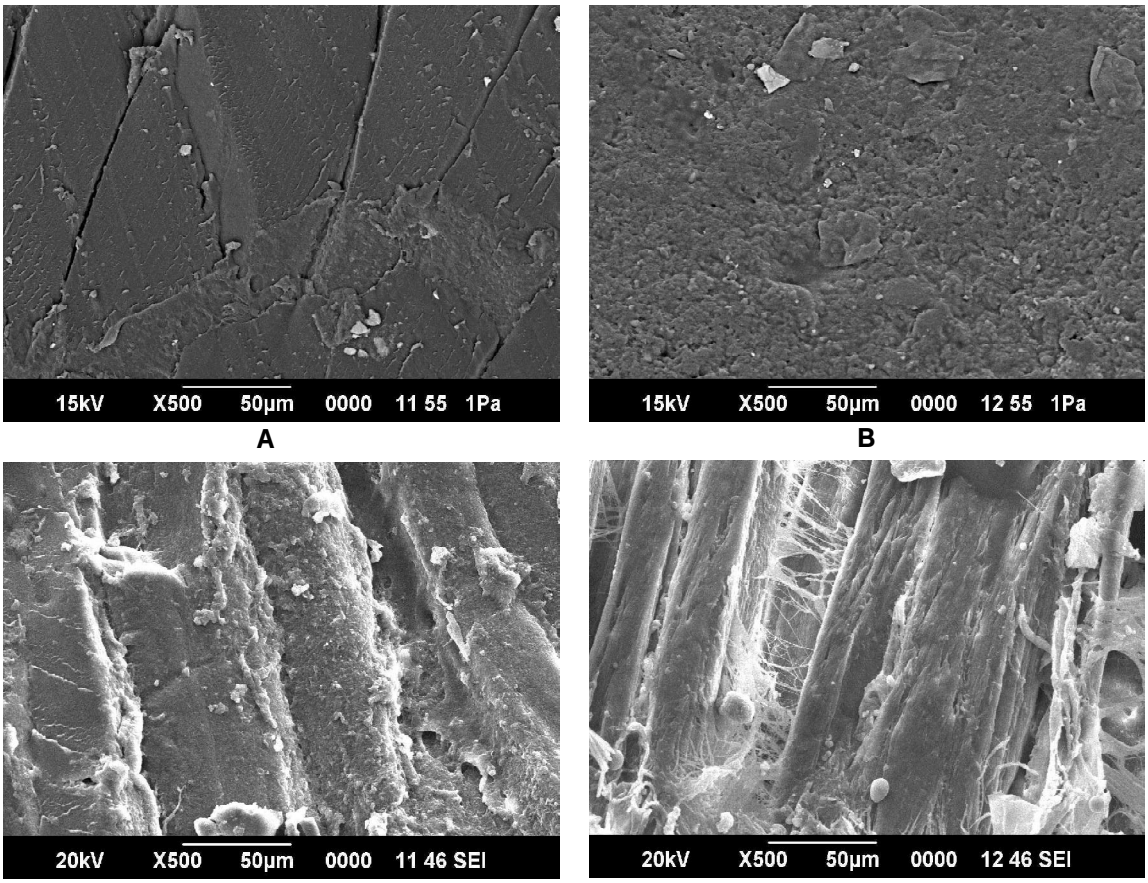
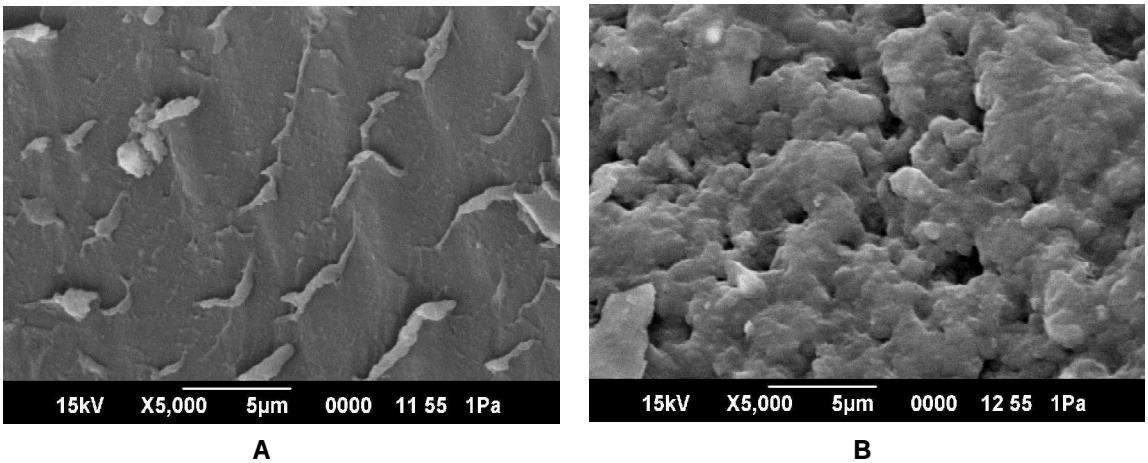
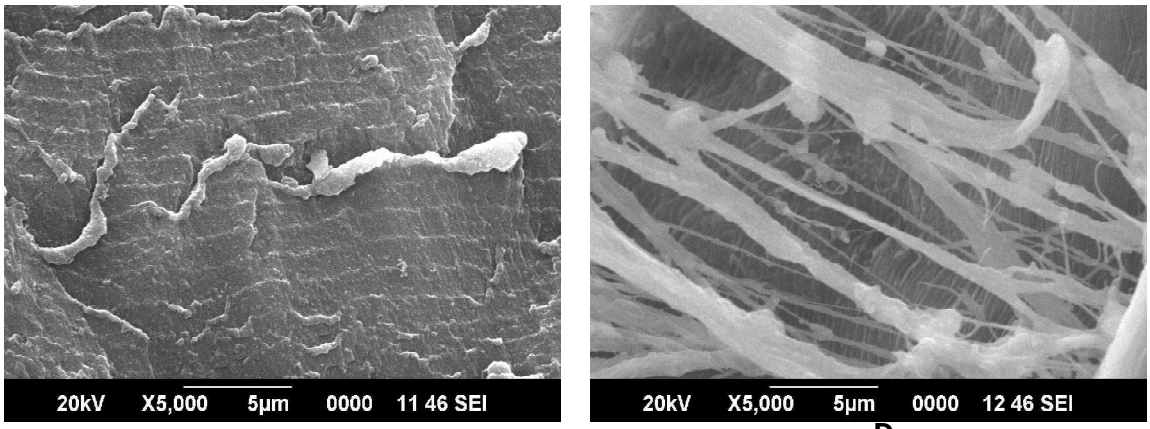


Fig.1. Electron microscopic views of muscle fibres of tuna sample at 500X

A- Control, **B**- Lemon treated, **C**- Vinegar treated and **D**- Ginger treated





C D

Fig. 2. Electron microscopic views of muscle fibres of tuna sample at 5000X
A- Control, **B**- Lemon treated, **C**- Vinegar treated and **D**- Ginger treated

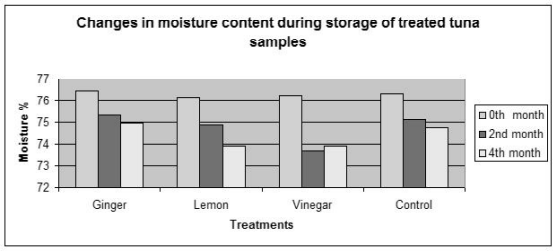


Fig. 3. Changes in moisture content during storage of treated tuna

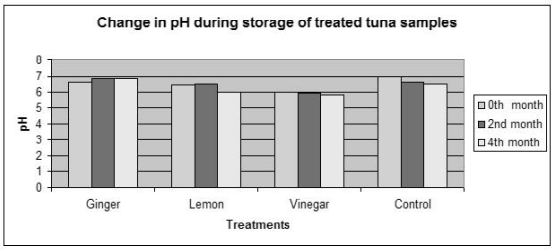


Fig. 4. Changes in pH during storage of treated tuna

samples also showed deep dentations due to the action of citric acid. This may have resulted in the flavour and texture which was most favoured by the sensory panel. Hence, the electron microscopic analysis revealed the reason for the sensory acceptability of ginger and lemon .The similarity in the score sheet of vinegar and control samples indicated that there was not much difference between the vinegar treated and the control samples.

The moisture content in ginger treated samples showed an elevated value compared to the other treatments. This may be due to the water binding property of active components of ginger (Ali *et al*., 2008; Zhao *et al*., 2009). Irrespective of the treatments, the moisture content showed a decreasing trend with the advancement of storage. Vinegar and lemon treated tuna chunks showed an acidic nature throughout the storage period while the ginger treated sample showed a reverse trend.

The myofibrillar protein particularly myosin of many species may be altered by the interaction with different types of lipids or lipid oxidation products during frozen storage (Saeed *et al.,* 1999). This interaction caused considerable changes in some functional properties and in the texture of fish muscle (Howell, 1999). This can contribute to changes in moisture and pH of the fish muscle.

Lipids, especially oxidized lipids, may affect the hydrogen bonds and hydrophobic interactions in the proteins of frozen fish. The fatty acid character of lipid molecules exerts a surfactant effect on protein surfaces, leading to hydrophobic interaction and protein unfolding, thus exposing interior groups for reaction. Furthermore, the carbonyl groups of oxidized lipids may participate in covalent bonding, leading to the formation of stable protein-lipid aggregates. The softening seen in the electron microscopic photograph can be related to naturally occurring proteolytic en-

zymes, and gelation could be due to the covalent intermolecular cross linkages between proteins. Incorporation of spices which has a tenderizing effect on texture is brought out by ginger cannot be over looked.

'Over all acceptability' score was highest for ginger treated tuna followed by lemon treated samples. Vinegar treatment alone produced a low score on sensory evaluation. Scanning electron micrographs revealed the changes in myofibrils leading to the textural properties of the sample. The protease activity of ginger was pronounced in the muscle tissue treated with 5% w/v ginger extract. Lemon extract treated sample was also favored by the sensory panel mostly due to its odour and texture. Water binding property of ginger was evident and revealed by the results obtained during the storage study.

Spoilage of food products is due to chemical, enzymatic or microbial activities.One-fourth of the world's food supply and 30% of landed fish are lost through microbial activity alone. With the ever growing world population and the need to store and transport the food from one place to another where it is needed, food preservation becomes necessary in order to increase its shelf life and maintain its nutritional value, texture and flavour. Proper handling, pre-treatment and preservation techniques can improve the quality fish and fish products and increase their shelf life (Ghaly *et al.*, 2010). This study analyses the action of natural preservatives on tuna muscle. These preservatives can also prevent the spoilage and increase the shelf life of Tuna meat by their anti-microbial action.

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