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PROTEIN LOSS DURING SPICE TREATMENT OF FISH

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ABSTRACT

Spices are used for stabilizing quite a few food items from deterioration and are an integral part of Indian cuisines. The effect of spice treatment (0.2%) in combination with cooking/frozen storage on the quality of protein lost from tuna was analyzed using SDS-polyacrylamide gel electrophoresis. The protein band in the range of 20.1KDa remained intact in the control and clove treated samples while the protein band of 29 KDa remained intact only in control. Additional bands were observed to appear between the range 29-43KDa for cardamom and garlic treated samples. The low molecular weight bands (20.1 to 3 KDa), which were present in all the treatments, was absent in garlic treated tuna. The electrophoretic pattern of thaw-drip protein is very similar in all the spice treated samples and closely resembles control. Only low molecular weight proteins were present (<29 kDa) in thaw-drip, whereas, a wide range of protein bands (66KDa-3KDa) were observed in cook -water. The experiment shows that, freezing after marinating fish does not have much effect on the quality compared to cooking fish after marination with spices. Marination leads to loss of more type of protein when compared to untreated sample, even though there is no significant difference in their total amount. It is evident that spice treatment itself does not effect the tissue protein. Hence, it is advisable to expand value added fish products containing spices because, it increases their shelf life with antimicrobial/antioxidant properties, without tampering with the yield of the product.

Keywords: SDS-PAGE, Tuna, Thaw-drip, Cook-water, Electrophoresis.

I. INTRODUCTION

Spices are indispensable components of Indian cuisines since ancient times. These are used in minuscule amounts to impart flavour, taste and aroma in food preparation to improve their palatability (Nair and Chanda, 2006). Spices are also used for stabilizing quite a few food items from deterioration (Kizil and Sogut, 2003). Spices are considered as source rich in bio-active antimicrobial compounds (Das *et al*., 2012). The typical Indian spices and herbs like cumin, black cumin, mustard, fenugreek, ajowain, curry-leaf, nutmeg and henna are usually used in curries, pickles, sauces etc. These spices are also known to have some ethno-medicinal or anti-microbial properties (Singh *et al*., 2002). Plants traditionally used for medicinal purpose in different parts of the world have been screened for possible antimicrobial action by several workers (Bonjar, 2004). Antibacterial activities of extracts of different plants against various microorganisms have been reported by many scientists (Shan *et al*., 2007; Chaudhury and Tariq, 2008; Gutierrez *et al*., 2008).

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Israti *et al*. (2011) found that the addition of thyme, marjoram and horseradish in marinade resulted in more pronounced decrease of total mesophilic aerobic bacteria compared to the marinade without spices and seasoning plants. The results of that study indicate that marination with marinades consisting of lime-tree honey, spices and seasoning such as thyme (*Thymus vulgaris)*, marjoram (*Majorana hortensis*), garlic (*Allium sativum*) and horseradish (*Armoracia rusticana*) can be used as an effective and natural preservation method. But, how the spice treatment effects the thaw-drip and cook-water has not been analysed.

Freezing is a commonly employed method for the preservation and maintenance of the nutritional quality of fish by retarding the biochemical and microbiological reactions in the tissue. The freeze induced physico-chemical changes in the colloidal structure of fish protein create several technological problems like exudation of drip from thawed fish. The toughness of fish muscle increases with prolonged storage and results in economic loss and reduced consumer acceptability. The effect of spice treatment in combination with cooking/frozen storage on the quality of protein present in tuna is analyzed in this paper.

II. MATERIALS AND METHODS

Raw material collection and sample preparation

Fresh whole tuna (*Euthynnus affinis*) was collected and divided in to 8 groups. Each group of fish was subjected to dip treatments of the specific spice extracts (0.2%) namely, clove, cardamom, garlic, oregano, rosemary and turmeric. A commercial antimicrobial, chlorine (2ppm) was also used. Control sample was not subjected to any sort of treatments. The duration of dip treatment was 10 minutes with two different concentrations of spice extract (0.2% and 0.1%). The dip treated samples were stored in a refrigerator at 4ºC for one hour before cooking. To analyze the proteins present in thaw-drip, the treated samples were stored at -30°C for six months.

Cooking Procedure

The cooking was done according to the procedures of Stoneham *et al*. (2000). The uniform sized fish fillets were wrapped in 5mm thick aluminum foil and placed in a wire-mesh basket. It was immersed in a thermostatic water bath, maintained at 100 ± 1°C and cooked for 20 min. After cooking, the cook water of each sample was collected in separate vials for further analysis.

Thaw-drip

The treated samples were placed in polythene bags and stored at -30°C for a period of six months. At the end of the storage period the samples were thawed and the accumulated thaw-drip was transferred to labeled vials for further analysis.

Electrophoretic analysis of cook water/thaw-drip

Single dimension SDS/PAGE was performed on a vertical slab gel electrophoresis system according to Laemmli (1970) to analyse proteins lost in cook water in treated and control tuna samples. The running gels were 10% and sample wells were made using a 20 well template. Sample solubilized in 0.1N NaOH were digested with an equal volume of SDS in sample buffer at 100°C for 5 to 10 minutes. The gels were stained with CBB R-250 (coomassie brilliant blue R-250). A mixture of 7% acetic acid and 20% methanol in water was used as destain.

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III. RESULTS AND DISCUSSION

Several quantitative analytical techniques have been utilized to study the structure of protein molecule with its amino acid sequences. Electrophoresis is one of the mostly preferred methodologies for the quantitative and qualitative fractionation of the muscle protein. The most common technique used for the separation of protein samples is polyacrylamide gel electrophoresis (PAGE) in the presence of strong ionic detergent such as sodium dodecyl sulphate (SDS). This provides separation of denatured protein subunit partially on the basis of charge but principally on the basis of molecular size. The resolution and sensitivity is balanced by choice of the amount of the gel matrix.

Cook loss

During cooking, water soluble sarcoplasmic proteins in the range of 40 KDa to 70 KDa are lost along with the cook water. ANOVA for total amount of protein in treated samples and control did not show any significant change ($p<0.05$) though the mean value was higher for control (Fig 1).

Qualitative analysis of cook water

SDS-polyacrylamide gel electrophoresis was performed for assessing the effect of spice treatment in the proteins present in cook water. The gels were visualized after staining with commassie brilliant blue. The broad range molecular markers (HMWM 205 KDa to 3 KDa) consisted of the following proteins : Myosin (205 KDa), Phosphorylase B (97.4 KDa),

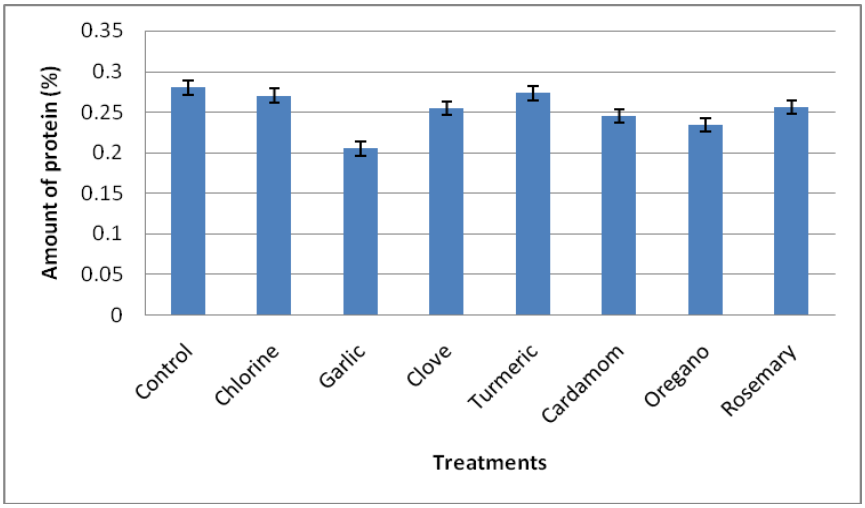

Fig 1. Amount of protein lost in cook water in spice treated tuna samples.

Bovine serum albumin (66 KDa), Ovalbumin (43 KDa), Carbonic anhydrase (29 KDa), Soyabean trypsin inhibitor (20.1 KDa), Lysozyme (14.3 KDa), Aprotinin (6.5 KDa) and Insulin (3 KDa). Plate 1 shows the electrophoretogram of proteins present in the cook water of control and spice treated tuna muscles in 10% SDS-PAGE gel. Comparision of the protein bands in the gels with their Rf values showed a similar pattern and hence the bands were compared visually. The corresponding molecular weights of the protein bands are mentioned on the plate.

Cook water of the treated clove samples had fewer protein bands compared to control samples. Rosemary and cardamom treated samples showed narrow banding compared to that of other spices. The protein band in the range of 20.1KDa remained intact in the control and clove treated samples while the protein band of 29 KDa

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remained intact only in control sample. This may be due to the cleavage of higher molecular weight proteins into lower molecular proteins by the action of spices.

Additional bands were observed to appear between the range 29-43KDa for cardamom and garlic treated samples. This could be the result of protein aggregates formed by the addition of spice. Consequently the protein band toward the origin became thicker with the protein having formed aggregates that were too large to enter the gel during electrophoresis. The low molecular weight bands (20.1 to 3 KDa) which are present in all the treatments including control, is found absent in garlic treated tuna.

Qualitative analysis of thaw drip

Plate 2 show the electrophoretic pattern by SDS-PAGE of thaw drip of tuna subjected to different spice treatments. Lane at the left extreme (M) end and right extreme end (M') show the broad range protein marker and high range protein marker, respectively. Visual comparision of protein bands in the sample lanes with the R_f values of protein markers showed protein bands ranging from 29 KDa to 3.5KDa.

The electrophoretic pattern of thaw-drip protein is very similar in all the spice treated samples. The pattern also closely resembles to that of control (untreated sample). No distinct differences in the number of bands was observed but, there was slight variation in the intensity of the bands among the spice extract treated samples. Thus, the treatments did not appear to have any observable effect on the denaturation of proteins in the concentration range of spice extract used for treatment.

In the electrophoretic pattern of thaw drip only low molecular weight proteins were present (4 bands with molecular weight less than 29 kDa), whereas, in the electrophoretic analysis of cook -water a wide range of protein bands (66KDa-3KDa) were observed (Plate 1). The low molecular weight bands similar to thaw-drip electrophoretic pattern were more intense in the cook- water pattern (<3 KDa). Additional bands in high molecular weight range was also seen in the latter but, they were less intense.

SDS-PAGE has been used for identification of different muscle proteins and their subunits in fresh muscle and also to estimate the effects of storage and processing on the stability of proteins (Bechtel and Parrish, 1983). All the bands in oregano and turmeric treated sample showed more intensity compared to others. The content of the ~20.1KDa protein fraction increased for turmeric, oregano and clove treated fish compared to that of control. It was probably obtained from breakdown of a larger protein such as myosin (205kDa). In support to this finding, extensive proteolysis was revealed in the eletrophoretic pattern of muscle proteins treated with ginger extract in a study conducted by Naveena *et al*. (2004).

Cook water of the clove treated tuna samples had fewer protein bands compared to control samples. Comparing clove treated and control samples, the protein band in the range of 20.1KDa remained intact in the control and clove treated samples while the protein band of 29 KDa remained intact only in control sample. This can be due to the cleavage of higher molecular weight proteins into lower molecular proteins by the action of spices. Preferential degradation of myofibrils in the I-bands as well as of the collagen as a result of treatment with ginger was reported by Lee *et al*. (1986a) and Thomson *et al*. (1973), respectively.

Additional bands were observed to appear between the range 29-43KDa for cardamom and garlic treated samples. This could be the result of protein aggregates formed by the addition of spice. Consequently the protein band toward the origin became thicker with the protein having formed aggregates that were too large to enter the gel during electrophoresis.

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Based on the differences in the physico-chemical properties, proteins are classified as sarcoplasmic and fibrillar proteins. The sarcoplasmic proteins form approximately 20-30% of the total proteins (Dyer and Dingle, 1961). They are generally soluble in water and buffers of low ionic strength. Most of these are low molecular weight proteins in the range of 40KDa to 70 KDa. The fibrillar proteins constituting salt soluble and insoluble fractions contribute about 60-80% of the total proteins that have molecular weight in the range of 400-600 KDa. The protein lost during cook loss are water soluble and it was seen that only faded banding was present in the 43-66KDa range, implying that only negligible loss of

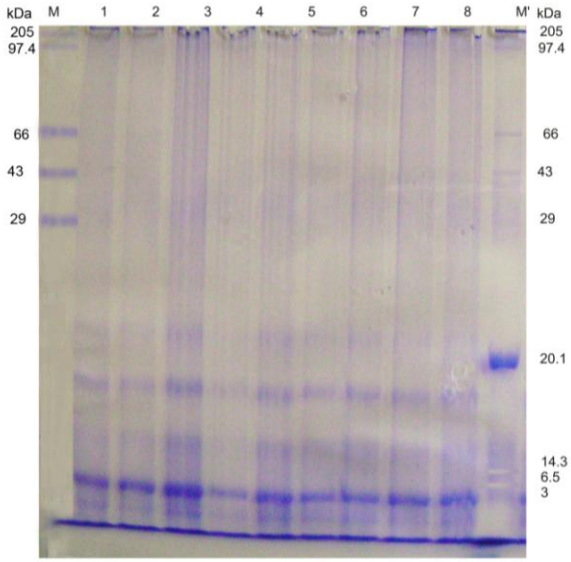

Plate 1. Electrophoretic analysis of cook water of treated tuna samples

(M- High range marker, 1-Chlorine, 2-Rosemary, 3-Cardamom, 4-Garlic, 5-Turmeric, 6-Oregano, 7-Clove, 8-Control, M'- Protein marker Broad range)

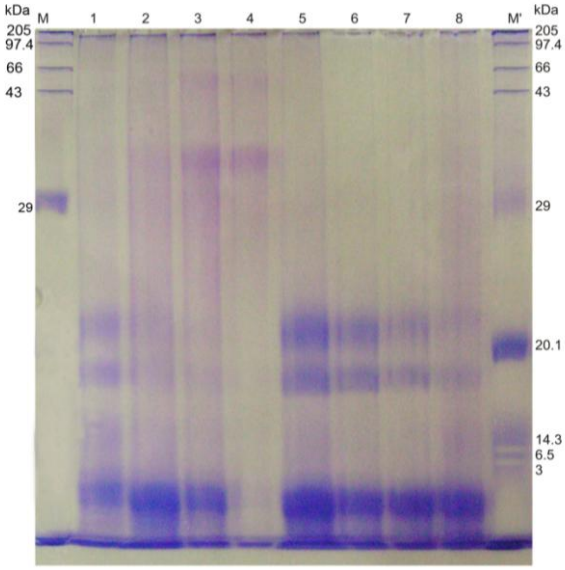

Plate 2. Electrophoretic pattern of thaw drip of tuna subjected to different spice treatments.

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M-High range marker; 1-Control; 2-Clove; 3-Turmeric; 4-Garlic; 5-Oregano; 6-Cardamom; 7-Rosemary; 8-Chlorine;M'-Broad range marker.

sarcoplasmic protein occurred during cooking of tuna. The basic aim of the fish processor and food technologist is to control the changes in the functional properties of tissue protein, and thus to preserve and improve the quality of the meat. Cooking tuna does not contribute much to the loss of sarcoplasmic proteins. But, loss of smaller peptides <29KDa is evident from the electrophorogram. The protein lost during thawing was of similar molecular weight for all treated and untreated samples. Therefore, it is evident that spice treatment itself does not effect the functional properties of tissue protein.

IV. CONCLUSION

Cook water of the treated clove samples had fewer protein bands compared to control samples. Rosemary and cardamom treated samples showed narrow banding compared to that of other spices. The protein band in the range of 20.1KDa remained intact in the control and clove treated samples while the protein band of 29 KDa remained intact only in control sample. Additional bands were observed to appear between the range 29-43KDa for cardamom and garlic treated samples. The low molecular weight bands (20.1 to 3 KDa) which are present in all the treatments including control, is found absent in garlic treated tuna.

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The experiment shows that, freezing after marination of fish have less effect on the protein quality compared to cooking fish after marination with spices. Marination leads to loss of more type of protein when compared to untreated sample, even though there is no significant difference in their total amount. Hence, it is advisable to expand value added fish products with spices because it increases their shelf life with antimicrobial/antioxidant properties and does not contribute much to the yield loss.

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