

Application of Herbal Secondary Metabolite For Fish Preservation Under Laboratory Conditions



Fisheries

KEYWORDS : Phytochemicals, Punica granatum, Mastacembelus armatus, Proximate composition and shelf life.

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ABSTRACT

The Punica granatum (Pomegranate) peel extract were studied as a natural preservative for extending the shelf life of fresh water fish Mastacembelus armatus for its proximate composition at the per storage and post storage conditions under the laboratory conditions using the distilled water as a solvent for extraction. The preliminary screening of peel extract is showing the presence of some important secondary metabolites such as Alkaloids, tannin, Phenolic compounds, saponins; steroid, Flavonoids, Free amino acids, and vitamin C. Among these number of secondary metabolite were found in the sample of Punica granatum peel, these preliminary phytochemical screening confirming the presence of bioactive potential of that particular plant. Similarly the proximate composition of control sets and experimental sets were also carried showing the measurable changes due to herbal preservation. In the experimental group of fishes the maximum stability of important nutritive compounds were found than the control sets. The crude protein was observed in the range of 17.30-18.80 %, Carbohydrate was recorded in the range of 2.73-2.93% while lipids were estimated in the range of 2.16-2.26% during the storage period. The above estimated values were found more stable and higher than the control group of fishes. The protein breakdown and lipid oxidation of extract treated fishes were found significantly suppressed during the storage period. The moisture content was also estimated in both the sets of fishes. The moisture content was recorded in the range of 78.66-78.80%. During the storage period ph of both the samples were also recorded and the ph of control group of fishes found in the rapid increasing manner than the experimental group of fishes. Increasing ph of the samples may be due to microbial activity and basic nitrogenous and ammonia compounds. The overall acceptability of both the group of fishes were also determined by Hedonic scale method and the Quality index method (QIM) by recording the physical, chemical and biological parameters. The overall acceptability score of treated fishes were found much higher than the control fishes therefore the extract of Punica granatum peel may have the preservative potential in its content for extension of shelf life of fishes. All the results of experiments were statistically analyzed and found significant at <0.001 level of significance.

Introduction:

Fish is the very important item in the diet of many peoples. In addition to high percentage of protein it also contains various essential nutrients in the form of vitamins and minerals. It also contributes the major source of protein. Lunven (1982) stated that It consist of 10-15% protein of the global diet along with the vitamin A,B,E and K plus minerals. As like other animal meat fish also contains water, proteins, carbohydrates, lipids, minerals and vitamins. Proteins are the most important biomolecules made up of amino acids jointed together by peptide bonds and have grate rile in living system. The protein content of fish muscles ranges from 16-21%. The protein content varies with type of muscle. Dark muscles usually contains low level of moisture and protein compare to the white muscles. proteins are classified on the basis of shape, solubility and structure. Based on solubility in salt solution these are classified as sarcoplasmic protein, myofibrillar protein and stroma. The fish may contain 25-30% sarcoplasmic protein. Among the pelagic fishes found on the upper column of the sea have higher percentage of sarcoplasmic protein than the fishes living in the bottom of the sea. Myofibrillar protein is the structural proteins consist of 65-71% of total protein while stroma which are the proteins of connective tissue constitute approximately 3-10% of total proteins. Harlio and Yilmaz (2011) also reported the presence of polysaturated fatty acids (PUFA), Monounsaturated fatty acids (MUFA) and the saturated fatty acids (SFA) from the Mastacembelus, Similarly Goodnight et al.(1982) and philipson (1985) also reported the Omega-3 essential fatty acids(EEAs) reducing the risk of atherosclerosis by lowering the plasma triglyceride level. hence fishes are having important role in human diet.

Mastacembelus armatus is the fresh water fish commonly known as fresh water eel or fresh water spiny eel found in abundant quantities from freshwater resources of the study area the Marathwada region of Maharashtra state of India. Gandotra et al. (2012) stated the several methods of fish preservations such as freezing, refrigeration, salting, brining, icing, smoking, glazing, drying etc. refrigeration and freezing helps by lowering the temperature. At the low temperature microorganisms become inactive, enzymatic activity slows down and thus the spoilage decrease and fish shelf life is get extended. Begum et.al. (2012) stated that the high temperature of tropics, lack of basic infrastructure and the poor sanitation conditions are the drawback

of fish preservation in most of the developing countries and these will lead to loss of national economy. After the death of the fish synthesis of ATP as ETC and oxidative phosphorylation mechanism stop and resulting the depletion of ATP and creatine phosphate and the anaerobic conversion of glucose to lactic acids leads to drop the ph accelerate rigor mortis and protein denaturation. The drop in ph accelerates the action of cathop-sin and other photolytic enzymes, this lead to accumulation of various metabolites, flavor, and bacterial growth and ultimately spoilage thus all of the above workers have been presented the importance of fish in the diet as an essential nutritional requirement and its natural preservation. Presently the safe food preservation is an difficult task due to change in climatic conditions, pollution load, uncontrolled use of pesticides ,increasing the global temperature and use of harmful chemical preservatives beyond the permissible limits. Nowadays there are verities types of chemical preservative are available in the market such as Acetic acid, Ascorbic acid, Iso- ascorbic acid, sodium erythobate, benzoic acid, 4-Hexylresocinol, Benzoic acid, potassium benzoate, sodium benzoate and many other are being used by the food processing industries on large scale ignoring their harmful effect on the consumers therefore today there is a need of safe and natural food preservatives. The Punica granatum is one of the important medicinal plants and have been used in herbal therapy since ancient times. In the present study peel of Punica granatum commonly called as pomegranate is used as the fish preservative under the laboratory conditions. Similarly the freshwater fish Mastacembelus armatus commonly known as freshwater spiny eel is used for the extension of shelf life. This fish is abundantly available in the study area and have high commercial and medicinal values. Gami and Parabia (2010), Schubert et al.(2007) and Gil et al.(2005) has reported the presence of antioxidant in pomogranate. Hedge et al.(2012)has evaluated the antimicrobial properties ,phytochemical content and antioxidant properties from pomegranate. Maria et al.(2010)studied the biological properties of Punica gramtum. Begum et al.(2013) have evaluated the preservative potential of Tejpatta extract on dried Kachki under laboratory conditions. Similarly Bhandary et al. (2012) has screened the phytochemical of pomegranate for its preservative potential. Abou Taleb et al. (2007) have extended the shelf life of mullet fillets during the cold storage by using the extract of four different plants. Gulzar and Zuber (2006) have estimated the Omega-3 fatty acids from Mastacembelus armatus.

Elbandy and Ashoush (2012) have observed the effect of pomegranate seeds on Hypercholesterolemic rats. Mahida and Mohan (2007) have studied the antimicrobial potential of different plants extracts with some common microorganisms. Iheagwara et al. (2013) have studied Effect of Ginger Extract on Stability and Sensorial Quality of Smoked Mackerel (*Scomber scombrus*) Fish. Hossain, et al.(2012) have studied the Effects of 10% concentrations of Salt, Garlic and Coriander on the Quality of Smoked Hilsa Fish. Abbas et al.(2012) has reported the effect of different salt concentrations on chemical composition of *Hydrocynus* species. Begum et al (2012) focoused the Effect of sand garlic on the quality and microbial content of smoked Catfish *Pangasianodon hypophthalmus*.

Thus all above literature shows the medicinal importance of pomegranate and hence there is wide scope for the use of pomegranate as natural and safe fish preventives

Materials and Methods:

Proximate composition analysis:

Proximate composition analysis was determined using the method AOAC,1984. The moisture content of fresh and salted fish was determined by drying the meat in at oven 105c until a constant weight was obtained, crude protein content was calculated by converting the nitrogen content determined by Kjeldahl's method ,Fat was determined using the Soxhlet system

PH Measurement:

The PH was estimated by using Digital PH meter at room temperature. A little sample of both the fishes were blended with 10 ml distilled water, and stirred well with a magnetic stirrer, then centrifuged well, and supernatant was taken for the ph determination.

Aqueous extract

The peel of fresh fruit was safely removed and chopped in suitable size of pieces boiled distilled water for 15-20 minutes, kept in room temperature overnight and filtered. The filtrate was evaporated to dryness in hot air oven and stored in refrigerator. The condensed extracts were used for preliminary screening of Phytochemicals for the presence of secondary metabolites such as Steroids, Glycosides, Saponins, Alkaloids, Flavonoids, Tannins, Proteins and Free Amino Acids.

Organoleptic evaluation; Texture Colour ,Flavour, Taste and general acceptability of the fish samples were evaluated by expert panelist, selected from among students and staff of Department of Food Science and Technology of the V.N. Marathwada agriculture University Parbhani which is near by the present college. A 9- hedonic scale point method was used with 9 points for like extremely and 1point for dislike extremely. For the evaluation, the fried samples were served to the expert panelist after the allowing the particular code to particular dish without providing the details of the samples.

Statistical analysis

The analysis was carried out in replicates as R1,R2 and R3 and data obtained were analyzed using the analysis of variance (ANOVA) method for Standard Error Mean (SEM) Critical Difference (CD) Coefficient variation (CV) and (F-values) proved significant.

Phytochemical analysis:

Test for Glycosides:

Bromine water test - Test sample was dissolved in bromine water and observed for formation of yellow precipitation confirms the presence of glycosides

Test for Saponins:

Foam Test – Test sample was mixed with water and shaken and

observed for the formation of froth, which are table for 15 minutes for a positive result.

Test for Alkaloids:

Hager's Test – Test solution was treated with few drops of Hager's reagent for Formation of yellow precipitate would show a positive result for the presence of alkaloids

Test for Flavonoids:

Ferric chloride test – Test solution when treated with few drops of Ferric chloride solution would result in the formation of blackish red color indicating the presence of flavonoids.

Alkaline reagent Test – Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow color which would become colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids

Test for Tannins:

Gelatin Test – Test solution when treated with gelatin solution would give white precipitate indicating the presence of tannins.

Test for Free Amino Acids:

Ninhydrin Test – Test solution when boiled with 0.2% solution of Ninhydrin, would result in the formation of purple color indicating the presence of free amino acids

Test for Vitamin C: DNPH Test – Test solution was treated with Dinitrophenyl hydrazine dissolved in concentrated sulphuric acid. The formation of yellow precipitate would suggest the presence of vitamin C.

Result and Discussion:

The proximate composition of fish is directly depends on the available food, seasonal changes, reproductive cycles, migratory pattern, water quality, pollution load, presence of predators, population density and number of physiological process like digestion, feed conversion ration. Similarly it may be different from species to species, inter specific and intra specific with geological distributions. In other words proximate composition depends up on physical ,chemical and biological parameters of the habitat.

Phytochemical analysis:

The phytochemical screening of *Punica grantum* was carried out and found positive for the presence of some important secondary metabolites as, tannin, alkaloids Flavonoids Steroids, Free Amino acids, Vitamin C and Glycosides and saponins was found negative in the sample.

Proximate composition:

In the present investigation crude protein of control and experimental sets were estimated. In the control set it was observed decreasing from 16.66 to 15.23% and in experimental group it was also found little decreasing from 18.80 to 17.30% from day zero to sixth day of the storage period. The decreasing protein during the storage may be due to leaching effect of amino acids and water soluble proteins. The proteins exposed to oxidizing environment are very susceptible to chemical modifications such as amino acid destruction, peptide scission and formulation of protein lipid complexes that results in decreasing protein content. The crude carbohydrates of control group was also found to be decreasing from 1.66 to 0.86% while in experimental group was recorded as 2.93 to 2.73% from day zero to day sixth of storage period. Similarly crude lipid were also found decreasing order as 1.41 to 1.05 % in control group while in experimental sets it was observed as 2.46 to 2.26% from day zero to day sixth of storage period. The moisture is found in greater percentage of composition in both the sets. In control set it was recorded in decreasing order from 78.86 to 78.66 % and in experimental sets

it was recorded as 78.86 to 78.66% from day zero to day sixth of storage period. The PH of both the sets were also observed and recorded in increasing manner from 6.18 to 7.03 while in experimental set it was also slightly increasing from 6.18 to 6.83 from day zero to day sixth of storage period. The increasing of the pH during the storage period may be due to basic nitrogenous compound which are produced as a result of some microbial activity in the sample. Such type of increasing pH during storage is also reported by Taleb et al.(2013). Mohajira et al.(2013) have evaluated proximate composition of the dried Kachki under the Tej-patta extract treatment, Gandotra et al.(2012) have estimated the proximate composition of labeo rohita under the low temperature storage. Nadakatu et al.(2010) have examined the proximate composition of *Oreochromis niloticus* under the various salt treatments and smoking treatments and the findings of the present work is found close to the above workers.

Sensorial evaluation:

The sensorial evaluation of fish is the main tool of consumer for quality assessment of fish on the spot in the market and their previous experience regarding the taste and flavor at the time of purchasing the fish. In the present study the sensorial assessment of both the groups of fishes were carried out by 9 point Hedonic scale method by the expert panel. 1 score point is for highly disliked fishes and 9 point score for highly liked fishes. During the storage period of six days the texture of the fish of control group was found decreasing from 4.66-2.66 points during the storage while in treated group is recorded as 9.00-8.20 points. The color of the fishes was also observed and found as 5.58-2.66 in control and 9.00-8.63 in the treated fishes. The taste of the both the group was also detected by the panelist by serving the fried samples and scored 5.00-2.33 points in decreasing order for the control group while the treated groups were shown good taste with 9.00-8.20 points confirming the good taste of fried samples. The flavor of the control group was scored decreasing points as 4.66-2.33 while in treated group it was 9.00-9.00 maintain the level of flavor. The overall acceptability of both the groups were also determined and it is found decreasing in the control group from 4.66-2.66 while the treated group scored the 9.00 -8.00 points maintain the overall acceptability by the treatment of *Punica granatum* extract. Thus the above results show that this treatment is supporting the sensorial quality of treated fishes. The decreasing quality of acceptability may be due to rancidity and the lipid oxidation may affecting the texture, taste, colour, flavor and acceptability of the sample. The storage time duration is may be inversely proportional to the acceptability.

Conclusion:

The present finding reveals that, the *Punica granatum* peel extract contains some important bioactive compounds in the form of secondary metabolites with anti-oxidative, antimicrobial properties, which may be found useful for the fish preservation to extent the shelf life of fishes under laboratory conditions.

Table-01 Phyto-chemical analysis of *P. garantum* peel and proximate composition *M. armatus* (muscles)

Phyto constituents of <i>P.grantum</i> Peel		Proximate composition of Fresh <i>M.armatus</i> (muscles) %	
Tanin	+	Protein	18.80
Alkaloids	+	Carbohydrate	2.85
Phenols	+	Lipid	2.26
Saponin	-	Moisture	78.86
Steroids	+	PH	7.03
Flavonoids	+		
Glycosides	+		
Free Amino acids	+		
Vitamin C	+		

Table-02 Post-storage proximate composition of *P. granatum* peel extract treated fishes Solvent distilled water

Days/ chemical composition	Solvent distilled water							
	Protein %		Carbohydrate%		Lipid%		Moisture%	
	control	test	control	test	control	test	control	test
Day 0	16.66	18.80	1.66	2.93	1.41	2.26	79.56	78.86
Day 2	16.54	18.20	1.25	2.86	1.33	2.16	79.33	78.73
Day 4	15.23	18.00	1.12	2.73	1.23	2.16	79.20	78.83
Day 6	15.66	17.30	0.86	2.73	1.05	2.26	78.14	78.66
SEM	0.1087		0.4534		0.6055		0.1084	
CD	0.3543		0.1477		0.1973		0.3534	
CV	1.0608		2.9824		4.8857		0.2391	
< 0.001	**		**		**		**	

Table -3 Sensorial Quality of post storage fishes under treatment of pomegranate peel extract

Solvent	Parameters	control	Experimental
Distilled water	Texture	4.66-2.66	9.00-8.20
	Color	5.58-2.66	9.00-8.63
	Taste	5.00-2.33	9.00-8.88
	Flavor	4.66-2.33	9.00-9.00
	Acceptability	4.66-2.66	9.00-8.80

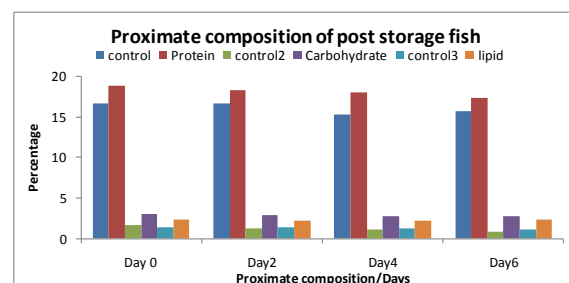


Fig 1-Proximate composition of post storage fish under treatment of *P. granatum* peel

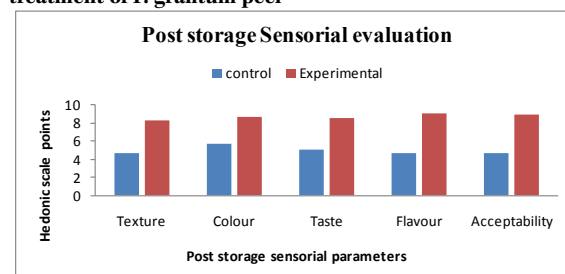


Fig-2 Post storage sensorial analysis

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