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Effect of Spices and Herb for Enhancing Microbial Quality and Shelf Life of Dried Indian Oil Sardine (sardinella longiceps) Fish during Storage at Room Temperature

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Abstract: *Dry fish is a low cost dietary source of protein but when dry fish is kept for a longer period it gets deteriorated. The key factor for the deterioration of the nutritional and sensory value of the dry fish is caused because of its tendency to absorb moisture from the surroundings. The main purpose of the study was designed to evaluate the effect of spices in improving the shelf life and organoleptic quality of the dry fish.*

This study was conducted on Indian oil sardine (sardinella longiceps) fish, the most common and low cost fish found in India. The study on fish sample was conducted in three different ways; Sample 1 in which the fish was treated with spices like turmeric, pepper, Ajwain, salt along with rice bran oil (FSO). Sample 2 in which the fish was treated only with spices and herb like turmeric, pepper, Ajwain and salt (FS) and sample 3 is the control sample treated with only salt (F).

The sample was then studied for a period of 9 months on the basis of their proximate composition, microbial and sensory evaluation. The result of the study shows that Samples (F) had very tough texture, high salt content, reduced moisture contents, dark discoloration; Oozing of fat and rancid smell during drying was very high. Hence the products were not acceptable and were not preserved for further study. Where as in case of sample (FSO) and (FS) moisture and ash value increased which resulted in the increase of microbial load with a decrease in fat and protein during the 9th month of storage period.

In the first month the initial percentage of moisture, protein, fat, ash and microbial load (TPC) of the sample 1 (FSO) was 12.98%, 10.07 %, 11.68 %, 3.92 % and 8.2x100 cfu/g and for sample 2 (FS) was 10.67%, 10.77 %, 6.05 %, 3.44 % and 3.3x100 cfu/g respectively. After the storage of 9^{th}month there was significantly ($p<0.05$) decrease in the percentage protein and fat whereas significantly ($p<0.05$) an increase in the moisture ash and microbial load was noticed. The percentage of moisture, protein, fat, ash and microbial load (TPC) of the sample 1 (FSO) and sample 2 (FS) are 17.88%, 5.97%, 3.57 %, 4.91 % and 4.8X1000 cfu/g and 15.26%, 7.08%, 4.21 %, 4.75 % and 1.2X1000 cfu/g respectively during the 9^{th} month of study. This study experimentally proved that the fish preserved with spices and herb alone FS has longer shelf life than FSO and is as an effective way of preservation.

Key words: *Indian oil sardine (sardinella longiceps), spices, herb, sun drying, proximate composition, microbial load and organoleptic study.*

I. Introduction

In the current scenario seafood has a considerable dietary importance owing to scientifically known beneficial effects of consuming aquatic foods, fats and oil. Fish provides a nutrition which has high biological value due to high protein retention in the body, low cholesterol level and presence of essential amino acids (Emikpe B.O et al., 2011). It has been widely accepted that fishes are good source of protein and other elements which helps in maintaining a healthy body (Ravichandran et al. 2012).

According to report of FAO (2004) 15% of the world supply of animal protein is derived from fish. Marine species of fishes are highly perishable and loose its quality very rapidly after the catch. Among the total fish landing in India, 32%are different species of sardines (CMFRI Newsletter, 2009) but due to the lack of considerable preservation facilities most of the fishes are converted into fertilizers. Fishes has to be preserved to avoid such wastage. One such common and most ancient method of preservation is sun drying because it preserves fish by inactivating enzymes and removes moisture which is necessary for the growth of bacteria and mold.

Sun drying is one of the least expensive methods of preservation (Balachandran, 2001) and mainly applied for small and lean fishes which are easily susceptible to spoilage one such fish is Indian oil sardine

(sardinella longiceps) which is mostly found in the northern region of Indian Ocean. Sun drying is one of the most important low cost methods of fish preservation and the products provide nutrients to all categories of people throughout the world especially in Indian tropical climates, under humid conditions. Heavy infestation of unsalted dried fish by blow flies may cause up to 30 % loss of the product (Wood 1981).

Indian oil sardine as its name suggest has high fat content thus this fishes cannot be preserved for longer period as it undergoes rancidity and gives off- flavor and off odor. Mainly hot smoking method of preservation is done for Indian oil sardine (Moorjani and Vasantha, 1972) but the shelf life is only for 2 to 3 weeks in room temperature and so it was suggested that addition of chemical preservatives like sodium propionate to enhance shelf life and addition of butyl hydroxyl anisole (BHA) to prevent rancidity (Muraleedharan et al. 1976) which are very harmful for the human consuming it. The present study was carried out in three different ways in first study the fish sample was treated with the salt, rice bran oil and spices and herbs like Turmeric, pepper and Ajwain.

The other study was with the same spices and herb alone and the third study was carried out with the normal sundried Indian oil sardine as the control sample. The report showed that the second study with the addition of spices and herb alone like Turmeric, Pepper, salt and Ajwain were able to prevent the fish from rancidity and microbial load to an acceptable level without the addition of any artificial preservatives for 9 months. The spices used in the study like black pepper(Piper nigrum) is rich in glutathione peroxidase and glucose-6-phosphate dehydrogenase (Karthikeyan and Rani, 2003) Black pepper has a well-known antioxidant and radical scavenging properties which have been well documented (Gülcin, 2005). Khalaf et al. (2008) and showed that piperine can increase the absorption of selenium, vitamin B complex, beta-carotene and curcumin as well as other nutrients.

Apart from this, Pepper is considered as an active alkaloid modulate benzopyrene metabolism through cytochrome P450 which is essential for metabolism and transport of xenobiotics and metabolites (Reen et al., 1996), enhances thermogenesis of lipid (Malini et al., 1999), and increases the flow of digestive juice (Moorthy et al., 2009).Similarly Turmeric (Curcuma longa) is a most extensively used spice, food preservative and coloring agent which has very good biological actions and medicinal applications (Burt, 2004). Curcumin is the main active ingredient found in turmeric, which was found to have antioxidant (Karami et al., 2011) as well as antibacterial activities (Negi et al., 1999). Additionally, Soni et al. (1997) proved the protective effect of turmeric as feed additives on aflatoxin induced mutagenicity and hepatocarcinogenicity. Another herb used is Ajwain, which is common called as omum in Indian languages, contain about 50% thymol, a well-known antibacterial essential oil, and along with thyme can be used to enhance the immune system to ward off colds and flu and other viral infections (S. N. Dwivedi et al. 2012).Fishes are very much susceptible to pathogenic bacteria but when anti-oxidant and anti-bacterial rich spices and herbs were applied they prevented the growth of microbes and thus helped in increasing the shelf life of the product.

Hence, in the present exploratory analytical study, we aim to use spices and herb mixture which has been very effective in controlling rancidity of Indian oil sardine (sardinela longiceps) as well as reduced microbial growth and adds value to the dried fish. The broad objective of the study is to determine the efficiency of spice and herb mixture in extending the shelf-life by retarding lipid oxidation and microbial growth and providing a good organoleptic quality to the dried fish. Similarly, potentials of solar radiation which appears abundant, convenient and cheapest form of preservation informed the conduct of this study.

II. Materials And Methods

Sample Collection

For the present experiment Indian oil sardine (sardinella longiceps) fish was chosen for long-term preservation. Pure common salt, turmeric, pepper, Ajwain, Rice bran oil and Indian oil sardine fishes were used as raw materials for this study. The raw fishes were purchased from the local market of Kerala in the early hours of the day and the fishes were brought to the Laboratories, to conduct the research activities.

The fish was collected from the harbor of Alappuzha early in the morning. Fresh mature fish samples were transported to laboratory in sterile polythene to avoid any type of microbial contamination. The fish samples were washed thoroughly to remove sand and slime, and were eviscerated. The fishes were cleaned and dipped in 10% sodium chloride solution for 15minutes for blanching which helps in making the fish softer in texture and also helps in removing raw flavor and blood strains. After blanching the fishes were drained and divided into 3 batches.

At first, the raw fishes were blanched and weighed. The weight of fish before drying was 0.27g and after cleaning it weighed 0.20g. Then three samples of the raw fishes were taken for three different analysis.

Sample Preparation

Following three samples were prepared for the study:

Sample 1- Fish with Spices and Oil (FSO): Here the cleaned fish with a mixture of dry salt, pepper, turmeric, Ajwain and rice bran oil was added to the fish gently and evenly. The total ratio of total fish composition= Fish

weight: salt: turmeric: pepper: Ajwain: Rice bran oil= 50: 3.75: 1.2: 1.2: 1: 8 respectively. They were dried in sun for 14 hours.

Sample 2- Fish with Spices (FS): Here the fish samples were treated with only spices and no oil was used. Fish was cleaned and a mixture of spices like pepper, turmeric, Ajwain and salt was added and dried in sun for 14 hours. Here the total ratio of total fish composition= Fish weight: salt: turmeric: pepper: Ajwain = 50: 3.75: 1.2: 1.2: 1 respectively.

Sample 3- Normal Sun Dried Fish as Control (F): Here the fish was normally sun dried where the fish was enrolled in dry salt and the extracted salt solution was removed and dried. Thus the ratio of total composition= Fish weight: salt= 3:1

The processed and preserved fish were kept in drier box and was netted to prevent any external contamination (mainly insects and pest).The fishes were dried for about 14 hours.

Fish Storage

The processed and preserved sun dried fishes were stored in Zip Lock polyethylene bags and stored at room temperature for a period of 9 months. Samples were subjected to visual observation, chemical analysis, microbiological analysis and sensory evaluations.

Chemical Analysis

In this study the quality of the products were assessed on the basis of proximate compositions of fish which includes moisture, fat, protein and ash content of dried products according to A.O.A.C. (1995) procedure.

Microbiological Analysis

The samples were analyzed for the total bacterial load using plate count agar by spread plate technique. 10 g of the sample was mixed with 90 ml saline water and appropriate dilutions of fish homogenate were spread on plate count agar and incubated at 37°C for 24-48 hours and the colonies were counted for total Plate count and the count was expressed as cfu/g (AOAC, 1990).

Organoleptic Assessment

The Subjective evaluations of product quality were carried out by an experienced panel composed of 10 people. Coded samples accompanied by questionnaires were presented to the panelists. Panel members scored all factors on a 5-point Hedonic Scale as depicted on the score sheet below on the basic quality attributes studied which include appearance, taste, texture, saltiness, rancidity (off flavor) and general acceptability (Martinsdottir et al., 2001).

Table 1: 5 Hedonic Scale Rating

Product Quality	Sensory Score
Like Very Much	5
Like Slightly	4
Neither Like Nor Dislike	3
Dislike Slightly	2
Dislike Very Much	1

Statistical Analysis:

Statistical data were analyzed by using SPSS for windows-20 statistical program. Significance was established at ($P< 0.05$) [Sokal R. R et al., 1962)

III. Results And Discussion

The comparative study between the qualities of the three samples of fishes treated by Three different methods of preservation were studied on the basis of several parameters such as proximate composition, shelf life, sensory evaluation and microbiological analysis. The proximate composition of fresh Indian oil sardine used for the study had protein 16.38%; fat 16.20%; moisture 66.00% and ash 2.02 %. In this study, the sample (F) was considered as the control group to study the effectiveness and preservative action of spices and herb treatment on sample (FSO) and (FS). The proximate composition (moisture, protein, fat and ash) and microbial load of Indian oil Sardine (sardinella longiceps) from 1stmonth to 9th month on all the three samples, the fish treated with spice and oil (FSO), the fish treated with spice alone (FS) and the control dried fish sample (F) was determined. Fat and protein are used as a source of energy, decreasing progressively during storage while moisture and ash content increases significantly ($P<0.05$) due to water loss.

The comparative study of the proximate composition (Fig.1) & (Fig.2) of the Indian oil sardine (sardinella longiceps) cured in three different method showed that the Control Samples 3 (F) had very tough

texture, high salt content, reduced moisture contents, dark discoloration, Oozing of fat and had very high microbial growth during drying. As per organoleptic quality it was marked as unacceptable and highly rancid. Hence the sample (F) was not acceptable and was not preserved for further study. Whereas sample (FS) that is fish treated with spice and herb alone contained the highest protein (7.08%) and fat (4.21%) content and lowest moisture (15.26 %) and ash (4.75%) content compared to sample (FSO) which contain (5.97%) protein, (3.57%) fat, (17.88%) moisture and (4.91%) ash at the end of 9th month.

The results of Proximate and microbiological evaluation of both the sample (FSO) and (FS) till 9thmonth of storage at room temperature are shown in Table 2.

Table 2

PERIOD	PROXIMATE ANALYSIS								MICROBIAL ANALYSIS	
Month	Moisture (%)		Protein (%)		Fat (%)		Ash (%)		TPC (cfu/g)	
	FSO	FS	FSO	FS	FSO	FS	FSO	FS	FSO	FS
First	12.88	10.67	10.07	10.77	11.68	6.05	3.92	3.44	8.2X100 cfu/g	3.3X100 cfu/g
Third	15.1	12.03	8.83	9.57	8.72	5.28	4.27	3.97	1.6 X1000 cfu/g	6.9X100 cfu/g
Sixth	16.03	13.77	7.04	8.62	5.21	4.88	4.81	4.12	2.1X1000 cfu/g	8.6X100 cfu/g
Ninth	17.88	15.26	5.97	7.08	3.57	4.21	4.91	4.75	4.8X1000 cfu/g	1.2X1000 cfu/g

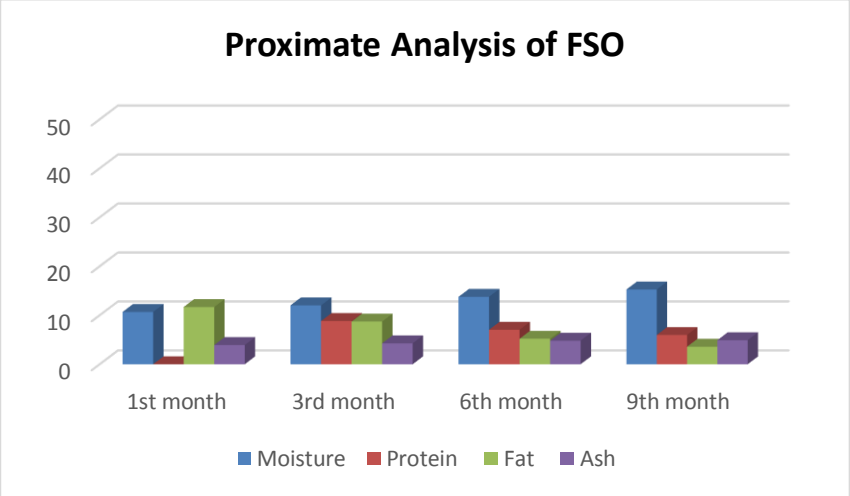

Fig. 1 Changes in Proximate Composition of sun dried Indian oil sardine fish treated With spices and oil (FSO)

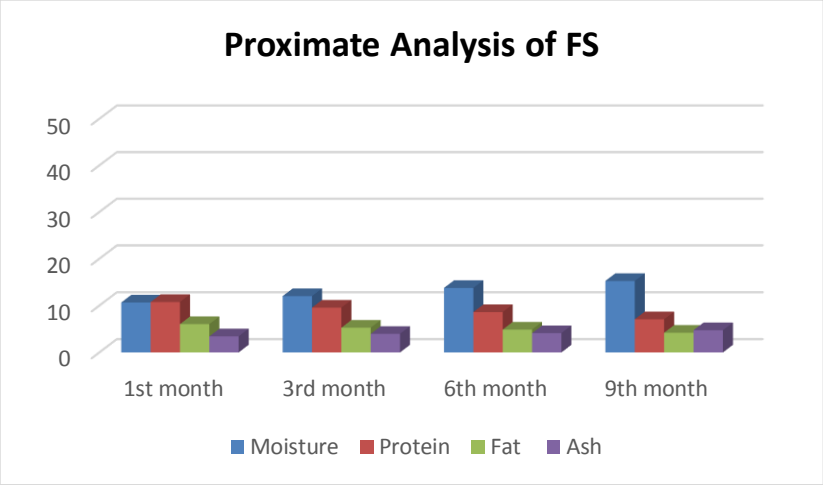

Fig: 2 Changes in proximate composition of Indian oil sardine fish treated with spices and herb (FS)

Moisture (%)

The moisture content is used to determine the rate at which deterioration in fish occur. Drying combined with salt and spices which has duel effects such as the lowering of the water activity (aw) level and a specific

inhibitory effect on the growth of some species of microorganisms. In this study Samples (F) showed very tough texture, high salt content, reduced moisture contents, dark discoloration and Oozing of fat during drying was very high. Hence the products were not acceptable and were not preserved for further study. In the present study it was found that sample (FSO) had more moisture content of 17.88% compared to that of sample (FS) having15.26% % by the end of 9th month of study. In sample (FSO) where the spices along with oil when dried showed lower drying rate. This is because of the fact that different arrangements of fish muscles which gets bound to water along with the fat content of the fish as well as the external oil, spices and herb cause reduction in the drying rate and lead to increase in moisture content during storage and this results in the growth of microbes. Where as in case of (FS) sample which was treated only with the dry spices and herbs lead to reduction in the moisture effectively. The main cause of decrease in moisture was due to osmotic migration of salt and spices into and water out of the fish (Horner, W.F.A. 1997). Decrease in moisture lead to increase in spice content and consequently extend shelf life of the product (Lopez, A. 1987) and (Itou, K et al., 2000).

Protein (%):

The product with highest protein and fat content is referred as a nutritious food. Normally dried fish contains more nutrients than fresh fish (khuda M.A.A 1962). Protein is considered as one of the major desirable component for a growing child and similarly low lipid is equally desirable to reduce oxidation and rancidity in the fish products which is responsible for the cause of off- flavor and bad taste in fish products (Oparaku et al., 2013). Fish is a major supplier of nutrient factor contributed by animals to human diet. With passing time protein starts to decompose (Ghezala S 1994). Since Fish is a highly bio-perishable product; so it is very essential to preserve it, in the present study three different methods are applied to preserve both the intrinsic and extrinsic qualities of the fish soon after the harvest to ensure and find the better microbiological quality and shelf life at room temperature.

In this study Samples (F) showed very tough texture, high salt content, reduced moisture contents, dark discoloration and Oozing of fat during drying was very high. Hence the products were not acceptable and were not preserved for further study. There was a significant ($P<0.05$) decrease in the protein level was found. Protein (%) was found to vary from 10.07% (1st month) to 5.97% (9th month) in (FSO), where as in case of (FS) it was 10.77% (1st month) to 7.08% (9 month). It was found that degradation of protein was more in (FSO) than (FS). This could be due to the fact that in (FSO) high lipid content during storage may have leached out some extractable soluble protein fraction by the hydrolysis of some of the lipid fractions. Thus in storage condition, the protein content starts to decrease significantly with the time because water soluble protein diffused out to the surrounding for exosmosis (Hassan, M.N. et al., 2013). The main reason behind this is due to gradual degradation of initial crude protein to more volatile products such as total volatile bases, hydrogen sulfide and ammonia (Eyo, A. A. 2001).

Fat (%)

The present study showed that fat content decreased significantly ($p<0.05$). Fat (%) were found to vary from 11.68% (1st month) to 3.57% (9 month) in (FSO) and, 6.05% (1st month) to 4.21% (9 month) in case of (FS). Fat content is usually influenced by moisture and are inversely related to each other (FAO 1999) and this is one of the major causes of rancidity which leads to off-odor and off-flavor. This inverse relationship has been well defined in this experiment. Thus it is clear from the present study that fat content decreased significantly in both the sample while at the same time sample (FS) maintained the quality due to the preservative action of spices and herb but in case of (FSO) there was a drastic decrease in the fat content which leads to oxidative deterioration, thereby affecting lipid extraction. A decrease in the level of crude protein and fat of (FSO) sample was found due to the addition of rice bran oil as a result the fish became more fatty and vulnerable to lipid oxidation which create severe quality problems such as unpleasant (rancid) taste and smell, and also produce alterations in nutritional value, on storage at room temperature. The various reactions involved in the lipid oxidation are either non-enzymatic or catalyzed by microbial enzymes or by intracellular or digestive enzymes from the fish themselves.

Ash (%)

Ash (%) was found to vary from 3.92% (1st month) to 5.01% (9thmonth) for (FSO), 3.44% (1stmonth) and 4.75% (9th month) for (FS) sample. The change in ash content during storage occurs due to absorbance of moisture and loss of protein (Hassan et al, 2013). Smaller the fish species higher will be its ash content due to the higher bone of flesh ratio (Daramola, et al, 2007). Significant statistical differences between the initial product and end product was found ($P < 0.05$) after storage period.

Microbiological Analysis

There was a steady increase in bacterial count as storage period progresses in all the treatments. However, sample (FS) showed lower microbial count of 1.2X1000 cfu/g compared to (FSO) having a 4.8X1000 cfu/g of TPC by the end of 9th month of storage at room temperature. A combination of spices and herb treatment with solar drying in (FS) sample resulted in the decrease of microbial levels because this spices and herb has a significant antimicrobial effect against different bacteria which prevents the growth of micro-organisms (Abhishek S. 2011), whereas in case of (FSO) sample due to the presence of oil lead to lipid oxidation in the fish and resulted in the rancidity and off flavor.

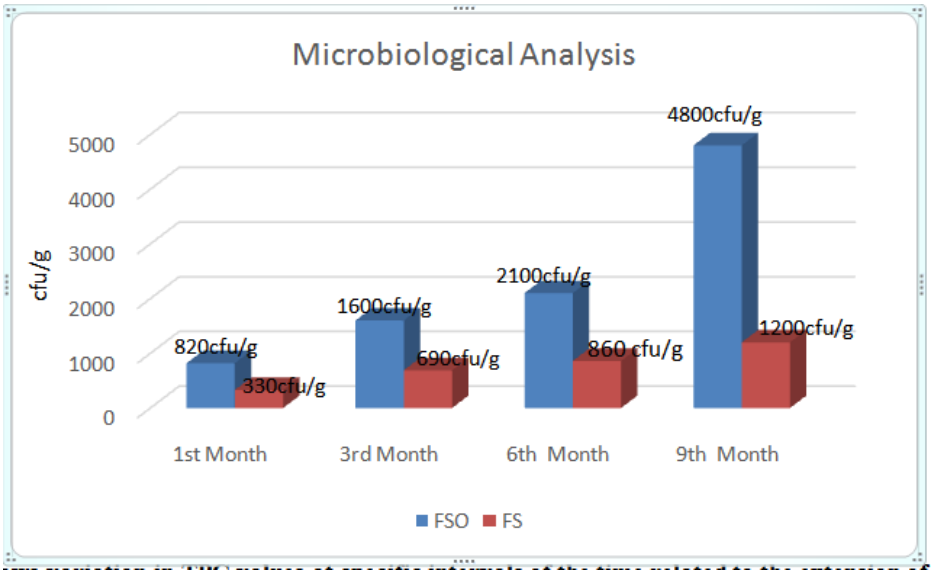

Fig: 3. Shows variation in TPC values at specific intervals of the time related to the extension of the shelf-life of FSO and FS sample.

Organoleptic Assessment

The Statistical analysis showed that there was a significant difference between the 5 attributes (Appearance, texture, taste, saltiness and rancidity) on their acceptability. The mean score by the panelist for both sample the (FSO) and (FS) during a 9 month storage period showed that the (FS) samples received highest panel scores than compared to (FSO) samples with regards to appearance, rancidity, saltiness, texture and general acceptability. The (FS) remained acceptable (sensory score 3.5) for 9 months of storage. So sample (FS) has longer shelf-life than that of the other cured fish products. Lipid oxidation in the (FSO) samples was responsible for the development of rancidity which affected general acceptability of the samples. So (FS) Samples treated with spice and herb recorded highest acceptability.

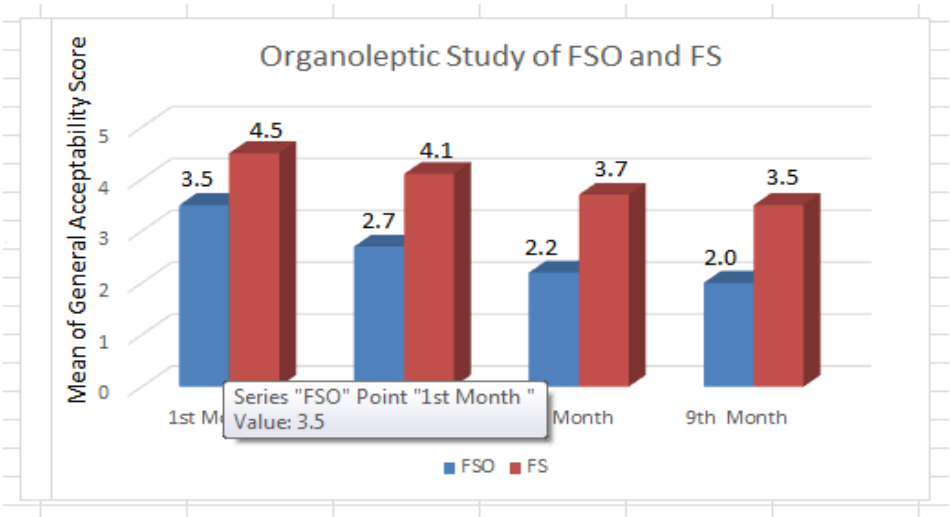

Fig: 4 Sensory score values of the sample FS and FSO pinpointing the level of acceptability by the panel judges.

IV. Conclusion

This study gives a clear perception on the variation of physio chemical and bacterial content of both the sample (FSO) and (FS). The study revealed that sample (FS) where treatment of fish with salt, spices and herb combination in the ratio = Fish weight: salt: turmeric: pepper: Ajwain = 50: 3.75: 1.2: 1.2: 1 respectively can be used as natural preservatives in sun dried Indian oil sardine (sardinella longiceps) without adversely affecting quality in terms of lipid oxidation, microbial quality, color and nutritional quality throughout a period of 9 months of storage. Indian oil sardine when treated in combination with salt, Turmeric, Ajwain and black pepper had a significant decrease in microbial load, along with shelf life extension during the storage. The samples exhibited shelf stability and the microbiological load fell within acceptable level stipulated by microbiological standards. This indicates that the samples were safe for consumption throughout the period of storage.

The present research revealed that the application of salt, pepper, turmeric and Ajwain treated Indian oil sardine that is (FS) sample was an acceptable process and was more effective in preserving fish products as they retained more beneficial nutrient property, higher sensory score, higher resistance against bacterial and enzymatic activity and had longer shelf-life than that of control sample (F) and the sample treated with spices and oil (FSO). This is because the spices used in this study had high active phenolic antioxidant property which is used to inhibit the free-radical mediated damages like lipid oxidation thus preventing oxidative rancidity (Khatun et al. 2006). Apart from this the research study also revealed that the correct combination of spices and herb on sardinella longiceps in the given ratio helped to increase protection against insect, pests, bacteria, fungus and other pathogens and acted as a natural preservative and a taste enhancing ingredient. The general salting methods used to remove water from fish body and thus made the fishes to dry whereas spice treated fish helped not only to remove water from fish body but also added some nutrients that prevent the growth of micro-organism due to the formation of an unfavorable growth medium. So, this process can be recommended for the preservation use in large scale food production.

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