Shelf life and biochemical changes of chilled Atlantic chub mackerel, Scomber colias, treated with extremophilic microbial films' extracts

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Research Article

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Abstract

To get rid of harmful effects of synthetic preservatives and develop new ones from natural sources, extracts of microbial films from extreme habitats of geothermal spring and hypersaline lake in Western Desert, Egypt, were used for chilled Atlantic chub mackerel, *Scomber colias*, preservations. The aqueous extracts of extremophilic microbial films showed considerable contents of total phenolic and flavonoid contents and good antioxidant activity. The cytotoxicity test of 2% w/v concentrations on normal cells of liver and colon showed very weak effects and the cell viabilities ranged between 95.09 and 98.14%, while the cell viability of carcinoma cells was in the range of 88.74–98.9%. We tested the effect of aqueous extracts of these films on microbial and biochemical characteristics changes of chilled Atlantic chub mackerel. Concentrations of 0.5, 1.0, 1.5 and 2.0% w/v were applied. Total plate count (TPC), pH, value and acid value (AV), total volatile basic nitrogen (TVB-N), trimethylamine nitrogen (TMA-N) content, thiobarbituric acid value (TBA) and free fatty acids (FFA) were investigated for 8 days of storage at 5°C. Principal component analysis (PCA) showed good understanding of the effect of extremophilic microbial films on biochemical features of chilled Atlantic chub mackerel and revealed that the best evaluated TPC and other biochemical characteristics were found at extremophilic microbial levels of 1.0 and 1.5% w/v till the 8th day.

Introduction

Atlantic chub mackerel, *Scomber colias*, is a popular pelagic fish widely distributed in the continental shelf of the Atlantic, the Mediterranean and its adjacent Seas- Aegean, Black and Adriatic Seas. The Mediterranean landings of chub mackerel had averaged 13,979 tons in 2016–2018 (FAO, 2020). In Egypt, the chub mackerel landings had an average of 806.667 tons in 2017–2018 (ICCAT, 2020). Atlantic chub mackerel is a semi-fatty fish, has high nutritional value not only due to its high protein contents (Bimbo, 2013) but also its high contents of essential amino acids (Motta et al., 2021) and poly unsaturated fatty acids, specifically DHA and EPA required for human nutrition (Ferreira et al., 2020). Moreover, *S. colias* characterized by compounds with major physiological roles as vitamin D, potassium, phosphorous and zinc (Bimbo, 2013). Despite, chub mackerel is mainly consumed as canned fish (Ferreira et al., 2020), fresh *S. colias* is considerably utilized.

Fresh fishes can easily deteriorate after being captured due to the endogenous enzyme and rapid microbial growth naturally present in fish and/or from contamination (Jiang et al., 2018). Changes in composition during fish decay led to protein degradation and lipid oxidation, as well as changes in fish odor, flavor, and texture (Shahidi & Zhong, 2010). Developing effective treatment methods to extend the shelf life of fish has become mandatory (McElhatton & Amaral, 2012). At the same time, fish industry is always looking for new preservation methods to provide consumers with the best quality in terms of sensory and nutritional levels (Rey et al., 2012).

As a result of increasing public awareness about the harmful effects of synthetic preservatives, the food industry has been prompted to develop new preservatives from natural sources. Natural preservatives are

available from a variety of sources including plants, animals, bacteria, algae, and fungi (Ghanbari et al., 2013; Hassoun & Coban, 2017). Algae and bacteria exhibiting antimicrobial and antioxidant activities could be served as potential natural preservatives as they can produce a wide range of bioactive compounds, including carotenoids, polyunsaturated fatty acids, polysaccharides, ormycosporine-like amino acids, and phenolic compounds (Rao et al., 2006; Li et al., 2007; Heller et al., 2016). Rodrıguez et al. (2014) studied the effect of the incorporation of spirulina on nutritional quality of dried pasta, concluding that these samples of pasta exhibited higher phenolic content and antioxidant capacity compared to control sample. Microbial films from extreme habitats in the Western Desert, Egypt, have not been studied yet for food preservations, although they have been reported as antimicrobial and antioxidant agents and good sources of bioactive compound (Abd El-Karim, 2014; Atwa, 2021). Moreover, dried microbial films from geothermal springs and hypersaline lakes could stimulate tilapia growth in indoor trials for three months experiment. The results indicated that the final body weight, weight gain, specific growth rate, and protein efficiency were improved by increasing microbial films' powder level in the experimental diets compared to the control diet (Abdelkarim et al., under prep). Moreover, the microbial films from geothermal springs and hypersaline lakes of Western Desert, Egypt, successfully increase artemia and daphnia growth rates in toxicity test experiment (Abdelkarim et al., under prep). This study aims at testing the quality and shelf life of chilled Atlantic chub mackerel, *S colias*, treated with extremophilic microbial films extracts from hypersaline lake and geothermal spring. Total plate count (TPC), pH, value and acid value (AV), total volatile basic nitrogen (TVB-N), trimethylamine nitrogen (TMA-N) content, thiobarbituric acid value (TBA) and free fatty acids (FFA) were investigated at time intervals of two days for 8 days of storage at 5°C.

Materials And Methods

Raw material

During August, 2020, a total of 30 kg of Atlantic chub mackerel, *S. colias,* caught from the Mediterranean Sea in Alexandria, Egypt, was directly purchased from local fishermen, then delivered under refrigerated conditions to the laboratory. *S. colias* averaged weight was 186g and average length was 20cm. Microbial films were collected from hypersaline lake (Zieton Lake) and geothermal spring (55°C), Western Desert, Egypt during summer 2020. All samples were kept in icebox till returning to the lab.

Extraction method

Microbial films were dried in an air circulated oven at 35 °C until reached a constant weight then grinded in an electric mill and sieved to a 63-mesh size and kept at -20 °C until use. For the proposed concentration, dried-sieved microbial powder was sonicated in deionized water for 30 minutes and was shaked overnight. All extracts were filtered on a Whatman filter paper (No. 1), centrifuged and stored in glass vials at −30 °C.

Estimation of total phenolic and flavonoid contents and the antioxidant activities

Total phenolic and flavonoid contents in concentration of 20% w/v of microbial films were determined using Folin-Ciocalteu's reagent method (Machu et al., 2015) and aluminum chloride colorimetric method (Magalhaes et al., 2012), respectively. Total phenolic concentrations were expressed as gallic acid equivalents mg GA/g, whereas flavonoid contents were expressed as quercetin equivalents mg QE /g, respectively. The free radical scavenging activity of the extracts was evaluated with DPPH by the method of Brand-Williams et al. (1995). The antioxidant activity was estimated as percentage of inhibition: % inhibition = [(Absorbance of control – Absorbance of sample)/(Absorbance of control)] X 100 (Yen & Duh, 1994).

Cytotoxicity evaluation

Cell lines and cell culture

Cell Line cells were obtained from American Type Culture Collection. Cell Line cells were cultured using DMEM (Invitrogen/Life Technologies) supplemented with 10% FBS (Hyclone), 10 µg/ml of insulin (Sigma), and 1% penicillin-streptomycin. All of the other chemicals and reagents were Sigma.

Cytotoxicity measurement

Hypersaline lake and hot spring microbial film concentrations of 2, 5,10, 15, 20% w/v, were prepared as mentioned above, and staurosporine (was used as reference compounds) were prepared in DMEM and sterilized. Cell viability was evaluated using a modified MTT colorimetric assay. Plate cells (cells density $1.2 – 1.8 \times 10^3$ cells/well) in a volume of 100µl complete growth medium per well in a 96-well plate were cultured for 24 hours. After treatment of cells with different concentrations of the compounds to be tested, plate cells were incubated for 48 h at 37ºC, then the plates were examined under an inverted microscope and proceed for the MTT assay. The absorbance was measured at a wavelength of 450 nm and at a background of 630 nm in the appropriate type of plate reader. The cell viability percentage for each concentration of the aqueous extracts of microbial films was calculated. The IC_{50}, concentration of extremophilic microbial film causing 50% inhibition of viable cell number, was determined graphically. For IC_{50} calculation, 5 different concentrations of 0.4, 1.6, 6.3, 25.0 and 100µg/ml of the extremophilic microbial films were prepared from 20% w/v concentration and were tested in triplicates in two independent experiments.

Technological methods

Fish samples were washed, drained under hygienic condition. Three fishes were analyzed for freshness and initial day parameters. The remaining fish samples were divided into nine equal lots, eight lots were soaked in the aqueous extracts of geothermal spring and hyper saline microbial films with concentrations of 0.5, 1.00, 1.5 and 2.00% o for 20 minutes under ambient temperature. the 9^{th} lot was soaked in distilled water and assigned as control. All treatments were packed in polyethylene bags and stored in refrigerator at 5±1ºC. Samples were analyzed at intervals of two days for quality parameters.

Analytical methods

Quality indices of preserved fish; pH value, acid value (AV) (AOAC, 2007), total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) (AMC, 1979), thiobarbituric acid value (Tarladgis et al., 1960) and free fatty acids (Egan et al., 1981) were determined. Microbial total plate count (TPC) was examined (APHA, 2001). All analyses and experiments were triplicated and mean with ±SD were given. One way ANOVA at significant level of $P \leq 0.05$ and PCA were performed using XLSTAT 2016.

Results And Discussion

Antioxidant activity and chemical composition of microbial films extract

Total phenolic and flavonoid content of aqueous extract of microbial films were 1.5 and 4.23 mg GA /g and 6.23 and 5.62 mg QE /g for both geothermal spring and hypersaline lake, respectively (Table 1). The antioxidant activities were 26.87 and 39.97% for both geothermal spring and hypersaline lake, respectively. These amounts were lower than the values obtained by Atwa (2021). This may be due to that the author used polar and non-polar organic solvents.

Table 1. Phenolic compounds, flavonoids and antioxidant activity of water extracts of microbial film 20% w/v.

Microbial film	Phenolic contents (mg/g GAE)	Flavonoids contents (mg/g QE)	Antioxidant activity (%)
Geothermal Spring	1.5	6.23	26.87
Hypersaline Lake	4.23	5.62	39.79

Effect of aqueous extracts of microbial films on normal and carcinoma cells of liver and colon

The cytotoxicity of aqueous extracts of extremophilic microbial film from hot spring and hypersaline lake were examined on normal (THLE2 and FHC) and carcinoma cells (HepG2 and Caco2) of liver and colon, respectively. Cell viabilities of tested cells were concentrations-dependent (Fig. 1a and b). The least concentration of 2% w/v showed a nonsignificant decrease of cell viability compared to the control. Cell viability of normal and carcinoma cells under the effect of concentration of 2% w/v averaged 96.8% and 94.56%, respectively. On the other side, the highest concentration of 20% w/v exhibited a highly significant inhibition of cells viability; ranged from 55.072 to 37.066% for normal and carcinoma cells, respectively. The IC_{50} results showed that carcinoma cells of liver and colon were slightly susceptible compared with normal cells. The liver cells were highly resistant compared with the colon ones. IC_{50} of extremophilic microbial film against normal and carcinoma cells of the colon, except the effect of hypersaline lake against FHC, were weak (50-100 µgm/ml), while their effect against liver cells, HepG2 and THLE2, showed very weak effect (>100 µg/ml). The IC_{50} values of the aqueous extracts of hot spring microbial films against HepG2, Caco2, THLE2 and FHC cells were evaluated as 160.68±8.14, 52.06±3.0,

171.08±8.17 and 92.62±4.7 µg/ml, respectively, while they were 183.05±9.03, 74.27±4.06, and 203.9±10.7 and 112.7±6.49 µg/ml for the aqueous extracts of hypersaline lake's microbial films after 48h. Many cyanobacterial species showed very toxic effect regardless of their habitats. The IC_{50} of the methanolic extracts of freshwater cyanobacteria *Anabaena oryzae*, *Oscillatoria* sp and *Stigonema ocellatum* were 44.4, 130.7 and 59 µg/ml, respectively, against MCF-7 cell (Seddek et al., 2019). Soil cyanobacterial species; *Oscillatoria acuminata*, *O. amphigranulata* and *Arthrospira platensis* exhibited strong cytotoxicity activity (IC_{50}) in the range of 8.43-41.07 µg/ml against HepG2, HCT-116 and FHC (Gheda and Ismail, 2020). IC_{50} values were 8.03 µg/ml for marine cyanobacteria *Cyanobium* sp., and 17.17 µg/ml for *Synechocystis salina*, after 48 h (Freitas et al., 2016).

Effect of storage conditions (at 5±2°C) on physicochemical quality indices

pH value

pH value of the fresh Atlantic chub mackerel averaged 6.20. At the beginning of the experiment, the pH of the control was 6.65, whereas the treated fishes with extracts of geothermal spring and hypersaline lake were slightly lower; 6.51, 6.41, 6.37 and 6.81 and 6.67, 6.53, 6.43 and 6.71, respectively (Fig. 2). Decreasing of pH values after soaking in aqueous extracts could be explained by their organic acid contents. Microbial film extracts of hypersaline lake and geothermal spring contains many organic acids like; hexadecanoic, docosenoic, glutaranilic, chloropropanoic, methanesulfonylacetic and many others (Abd El-Karim, 2014; Atwa, 2021). At the highest treatments (%2 w/v), decreasing of Atlantic chub mackerel acidity compared with control may be due to the diminishing of bioactive compounds absorbance at high concentrations. Under different treatments, pH values progressively increased significantly ($P < 0.02$) with increasing time under refrigeration. Chilled Atlantic chub mackerel treated with microbial film extracts had lower pH values compared with control sample, which increased at rates higher than the permissible levels after four days. Increase of pH values may be due to the microbial growth which was suppressed in the treated samples by microbial film extracts. Microbial flora like *Pseudomonas*, *Alcaligenes*, *Vibrio*, *Serratia* and *Micrococcus* spp. (Gram and Huss, 2000) can grow and the enzymatic spoilage resulted in the accumulation of volatile basic compounds shifting pH from acidic to alkaline (Don et al., 2018). Increasing pH values relating to unpleasant and unacceptable sensory characteristics such as flavors, odor, color, and texture (Dalgaard et al., 2006; Apang et al., 2020).

Acid value (AV)

Throughout the experimental period, all extracted oils of control and different treatments exhibited acceptable values (0.21-0.53 mg KOH/g of oil) (Fig. 3), much lower than the permissible value (7-8 mg KOH/ml oil). Start values of AV of control and different treatments were < 0.3 mg KOH/ml oil. With the trial progress, control and different treatments increased significantly ($P < 0.004$, for all treatments) to attained highest values at the experimental end. Effect of different concentrations of geothermal spring microbial film extracts showed significant difference ($P < 0.01$), whereas the effect of hypersaline

microbial film extracts was non-significant (P = 0.7). At the end of storage period, the least AV values (0.27 and 0.39 mg KOH/ml oil) was found in Atlantic chub mackerel treated with geothermal spring microbial film extracts of 1.0 and 1.5%, respectively. Roy et al. (2022) suggested that the composition of the oil, extraction techniques, and the freshness of the raw materials, can affect the quality of fish oil. Increasing of acid value are mainly due to protein denaturation, formation of amino acids and free fatty acids, which were produced during the storage period (Shen, 1996).

Free fatty acids (FFAs) content

Figure (4) presents the effect of storage conditions on the free fatty acids (mg/100 ml oil) of Atlantic chub mackerel. The initial FFAs values were ≤ 0.21 for control and treated Atlantic chub mackerel with extremophilic microbial film extracts. FFAs values were gradually significantly ($P < 0.002$) increased for treated and untreated fishes with extending storage periods up to 8 days. The intermediate geothermal spring extract (1.0 and 1.5%) exhibited higher inhibitory effects ($p < 0.0001$) as compared to control and hypersaline microbial film extracts from day two to eight. Hwang and Regenstein (1993) reported that FFA and 1, 2- di acyl glycerol in minced mackerel increased during storage at 2-3ºC, particularly after 15 days of storage. The majority of lipolysis in most stored fish originates from endogenous enzymes, mainly phospholipase and triacyl lipase (Aryee et al., 2007). The exerted inhibitory effect of microbial film could be due to their high contents of polyphenols. Thus, polyphenols extracts have shown to inhibit pancreatic lipase during the development of in vitro experiments (McDougall et al., 2009; Gondoin et al., 2010). Similarly, Miranda et al. (2016) found that marine alga *Bifurcaria bifurcata* extracts implied an inhibitory effect on FFA formation in chilled megrim (*Lepodorhombus whiffiagonis*), and they attributed this effect to its contents of polyphenolic compounds.

Total volatile basic nitrogen (TVB-N) content

The content of total volatile bases is useful for estimating the freshness of lean fish. The upper limits suggested are 30-40 and 60 mgN/100gm (on fresh weight bases) for fresh water and marine fishes (Connel, 1990). Effect of storage conditions on the TVB-N values of Atlantic chub mackerel are shown in (Fig. 5). The results show that, TVB-N values increased significantly ($P < 0.001$) in all treatments with time at storage conditions. Initial values of TVB-N were 6.06, 6.06, 3.13, 3.13, 3.81, 4.95, 4.59, 4.55 and 4.90 mg/100g for control and different concentrations (0.5, 1.0, 1.5 and 2.0%) of geothermal spring and hypersaline microbial film extracts, respectively. Compared with control (53.88), the lowest TVB-N values (41.54, 42.13 and 42.69 mg/100g) were found in geothermal spring (1%) and hypersaline microbial films (1% and 0.5% treatments, respectively) by the experimental end. However, extremophilic microbial film extracts could control TVB-N (mg\100g) formation due to their potential antimicrobial activities. The increase in TVB-N of Atlantic chub mackerel might be due to increment of enzyme action and microbial content specify to fresh samples. These results are in accordance with those observed by Bennour et al. (1991) and Baohua et al. (2006). Many investigators found that extracts of terrestrial and aquatic plants can inhibit microbial growth and TVB-N. Arulkumar et al. (2018) found that the red alga *Gracilaria verrucose* extracts suppress TVB-N values in Indian mackerel. Apang et al. (2020) postulated that

Garcinia spp. extract can lower the levels of TVB-N in the treated Indian mackerel. Similarly, the ethanolic extract of *Mentha Aquatica* have the ability to inhibit the microbial activity, consequently lower the TVB-N in the rainbow trout (Amoli et al., 2019). Moreover, the phenolic compounds in rosemary extracts helped to lower TVB-N formation in sardine muscles (Ozyurt et al., 2012).

Trimethylamine nitrogen (TMA-N) content

Trimethylamine nitrogen (TMA-N) levels are used universally to determine microbial deterioration leading to fish spoilage. Initial TMA-N values were < 2.0 mg/100g in Atlantic chub mackerel for control and different treatments of extremophilic microbial films (Fig. 6). Although, a progressive significant ($P < 0.001$) formation of TMA-N values ensured in all treated Atlantic chub mackerel with microbial film extracts, these values were much lower than the control (22.64 mg/100g) and the critical limits reported by Malle and Poumeyrol (1989) after 6 days. Microbial film extracts, especially the intermediate concentrations (1.0 and 1.5%), led to a remarkable inhibition of TMA-N formation in Atlantic chub mackerel. Trimethylamine (TMA) is generally produced by the reduction of trimethylamine oxide (TMAO) possibly by endogenous enzymes in fish, but mainly by the enzyme activity of certain bacteria (*Aeromonas* sp., *Shewanella putrefaciens* and *Vibrio* spp.). In agreement with the present study, previous works showed that lowering of TMA-N could be improved due to incorporation of natural preservation in chilled storage of marine fish. These studies included the use of rosemary extracts during chilled storage of sardines (Ozyurt et al., 2012), and *B. bifurcata* extract applied during the chilled storage of megrim (Miranda et al., 2016), and the red alga *Gracilaria verrucose* (Arulkumar et al., 2018) and *Garcinia* spp (Apang et al., 2020) extracts used in the chilled storage of Indian mackerel.

Thiobarbituric acid (TBA) value

Thiobarbituric acid value was used as index for lipid oxidation in fish and fish products. Fish and fish products of good quality will have a TBA value less than 2. Fish with TBA number greater than two will probably smell and taste rancid (Bonnell, 1994). TBA values have been used to determine the secondary products of lipid oxidation, especially aldehydes and decomposition products of hydro peroxides (Thiansilakul et al., 2012). As shown in Figure (7), TBA amounts were significantly ($P < 0.002$) increased in all samples during the storage. However, the least TBA value was related to the treated samples with the intermediate cyanobacteria microbial film (1.0 and 1.5%) after the 4^{th} day, indicating that Atlantic chub mackerel treated with cyanobacteria microbial film extract had lower TBA levels as compared to control value. At the 8^{th} of storage, the highest amount of TBA was observed in the control and least concentration of cyanobacteria microbial film. These results are in agreement with Suthasinee et al. (2018) who reported that the TBA values in refrigerated little tuna slice treated by freshwater macro algae extracts increased slightly during the first 2 days of storage, thereafter, sharp increases in TBA were observed in the control, followed by a decrease at day 8 of storage ($P < 0.05$). Ice systems including oregano or rosemary extracts inhibited TBA formation in jack mackerel (*Trachurus murphyi*) (Quitral et al., 2009). The plant extract of *Portulaca oleracea* and the alcoholic extract of marine alga *Ulva intestinalis*

could inhibit TBA formation in fish sausages compared with the control treatments (Tanha et al., 2021; Jannat-Alipour et al., 2019).

Microbiological quality

Total bacterial count (TBC)

Maximum bacterial load of fresh fish specified for human consumption is 7 log CFU/g (ICMSF, 1986). Initial counts of TPC were < 5.0 $\log_{10}$ cfu/g for control and different extracts of geothermal spring and hypersaline cyanobacterial film extracts (Fig. 8). Surprisingly, TBC were significantly ($P < 0.0001$) different at day 0 between different treatments. This initial difference was mainly attributed to microbiological contamination of fish prior to laboratory arrival. Similar explanation was suggested by Arulkumar et al. (2018). Concerning microbiological quality of Atlantic chub mackerel, the increase in (TBC) showed a significant difference ($P<0.002$) between all samples during storage period. The presence of extremophilic cyanobacterial film extract give rise to a significant ($P < 0.001$) inhibition of microbial growth as compared with the control at day 6 at the intermediate concentrations (1.0 and 1.5% concentrations). The results of the present study corroborate many previous works. The extracts of *B. bifurcata* (Miranda et al., 2016), rosemary (Quitral et al., 2009), *Portulaca oleracea* (Tanha et al., 2021), marine red Alga *G. verrucosa* (Arulkumar et al., 2018) marine green alga *U. intestinalis* (Jannat-Alipour et al., 2019), *Amaranthus* leaf (Kanatt, 2020) could inhibit bacterial growth in fish muscles.

Principal component analysis

Principal component analysis (PCA) reduces confused and huge data to smaller groups, then reduce noise effect and increase our interpretation of the underlying data. Moreover, using of sample means make the resulting axes of PCs self-explanatory and increases the objectivity of the interpretation. The relationships of the PCAs for variables and observations are further observed through the effect of the microbial film concentrations of both geothermal spring and hypersaline lake, which showed the highest scores at PCA1 for observations and at PCA2 for the variables for.

Effect of geothermal spring microbial film

The first two eigenvalues of PCA for geothermal spring microbial film were 5.55 and 0.959, explaining 93.15% of the total variation. The first component (PC1) responsible for 79.32% while the second one (PC2) covered 13.83%. According to the PCA biplot, TVB, TMA, TBA, TBC, FFA and AV had high positive ($r = 0.90 – 0.99$) association with PC1, whereas the microbial film level had high positive ($r = 0.94$) correlation with PC2. The long line of the explanatory variable indicates that it is highly correlated with PC2 (Fig. 9a and b). As clearly revealed in the PCA biplot, the best biochemical characters and longest shelf-life of the Atlantic chub mackerel were those containing geothermal spring extracts at the levels of 1.0 and 1.5 g/100 g.

The first two eigenvalues of PCA for hypersaline lake microbial film were 5.62 and 1.05 and they explain 95.27% of the variation. The first component (PC1) responsible for 80.31% while the second one (PC2) connected to 14.96%. According to the PCA biplot, TVB, TMA, TBA, TBC, FFA and AV had high positive (r = 0.93 – 0.987) association with PC1, whereas the microbial film leveling had high positive (r = 0.986) correlation with PC2. The long line of the explanatory variable (hypersaline lake extract) indicates that it is highly correlated with axes 2. As clearly revealed in the PCA biplot (Fig. 10a and b), the best biochemical characters and longest shelf-life of the Atlantic chub mackerel were those containing geothermal spring extracts at the levels of 1.0 and 1.5 g/100 g.

Conclusions

Microbial films from extreme habitats of geothermal spring and hypersaline lake were used for chilled Atlantic chub mackerel, *Scomber colias*, preservation. Aqueous extract of microbial films from geothermal spring and hypersaline lake can control the growth of microorganisms in the chilled Atlantic chub mackerel for 6 days-storage. Accordingly, microbial films extract effectively increased the shelf life of chilled Atlantic chub mackerel up to 6 days. Microbial films extract was able to control the increase of pH and the measured biochemical changes; FFA, AV, TVB, TMA-N and TBA. PCA can extract useful information and offer an easy and talented way for the description of properties of chilled Atlantic chub mackerel samples. The best biochemical and shelf-life properties were observed in the samples containing 1% and 1.5% w/v extremophilic microbial films extract. This study highlights augmentations with natural effective preservatives from extremophilic microbial films in fisheries industry to exclude harmful effects of synthetic preservatives and develop new preservatives from natural sources to achieve a healthier diet.

Declarations

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

The authors declare that the manuscript has not been published previously.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Competing Interests

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Funding Declaration

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Author's contribution

Mohamed Ibrahim and Mohamad Abdelkarim conceptualized, designed and performed the experiments and wrote the manuscript. Mohamad Abdelkarim analyzed the data. Soaad Sabae and Kamel Abo-Zeid sharing the experimental parts and the final editing and revision of the manuscript. All authors read, reviewed and approved the final manuscript.

References

1. Abd El-karim, M. S. (2016). Chemical composition and antimicrobial activities of cyanobacterial mats from hyper saline lakes, Northern western desert. Journal of applied Science, *16*(1), 1–10. https://doi.org/10.3923/jas.2016.1.10
2. AMC (Analytical Methods Committee) (1979). Recommended General Methods for the Examination of Fish and Fish Products. (Report prepared by the fish products sub-committee). Analyst, *104*, 434–450.
3. Amoli, Z. A., Tooraj, M., Hossein, T., & Maryam, A. (2019) Shelf-life extension of refrigerated, vacuumpacked rainbow trout dipped in an alginate coating containing an ethanolic extract and/ or the essential oil of Mentha Aquatica. Chemical Papers, *73*, 2541–2550. https://doi.org/10.1007/s11696-019-00808-8
4. AOAC (2007). Official Methods of Analysis of the Association of Official Analytical Chemistry. 18th ed., Gaithersburg, Maryland, USA.
5. Apang, T., Xavier, K., Lekshmi, M., Nagalakshmi, K., Porayil, L., & Amjad, K. B., (2020). Garcinia spp. extract incorporated icing medium as a natural preservative for shelf-life enhancement of chilled Indian mackerel (*Rastrelliger kanagurta*). LWT- Foods Science and Technology *133*, 110086. https://doi.org/10.1016/J.LWT.2020.110086.
6. APHA (2001). American Public Health Association Compendium of Methods for Microbiological Examination of Foods. 4th Ed., Washington, USA.
7. Arulkumar, A., Sadayan, P., & Jose, M. M. (2018). Combined Effect of Icing Medium and Red Alga *Gracilaria verrucose* on Shelf-Life Extension of Indian Mackerel (*Rastrelliger kanagurta*) Food and Bioprocess Technology, *11*, 1911–1922. https://doi.org/10.1007/s11947-018-2154-x
8. Aryee, A. N., Simpson, B. K., & Villalonga, R. (2007). Lipase fraction from the viscera of grey mullet (*Mugil cephalus*) isolation, partial purification and some biochemical characteristics. Enzyme and Microbial Technology, *40*, 394–402. https://doi.org/10.1016/j.enzmictec.2006.07.009

9. Atwa, K. M. (2021). Studies on Cyanobacterial Mats as a Bioremediator and a Source of Antimicrobial Compounds. M.Sc. Thesis, Faculty of Science, Mansoura university.
10. Baohua, A. K., Yoyling, L., Kenneth, R.X., Linda, S. T., Muzinic, A. M., & Webster, D. C. (2006). Influence of gender and spawning on meat quality of Australian, red claw crayfish (*Cherax quadricarinatus*) stored at 2C°. Journal of Foods Science, *71* (6), 320–25. https://doi.org/10.1111/j.1750-3841.2006.00117.x
11. Bennour, M., El-Marrakchi, A., Bouchriti, N., Hamama, A., & El-Quadaa, M. (1991). Chemical and microbiological assessment of mackerel (*Scomber scombrus*) stored in ice. Journal of Food Protection, *54*, 789. https://doi.org/10.4315/0362-028x-54.10.784
12. Bimbo, A. P. (2013). Sources of omega-3 fatty acids. Food Enrichment with Omega-3 Fatty Acids, Woodhead Publishing, Cambridge, UK, pp. 27–107, https://doi.org/10.1533/9780857098863.1.27
13. Bonnell, A. D. (1994). Quality assurance in seafood processing: A Practical Guide. Academic Press, New York, USA.
14. Brand-Williams, M. W., Cuvelier, E., & Berset, C. (1995). Use of a Free Radical Method to Evaluate Antioxidant Activity. LWT-Food Science and Technology, *28*, 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5
15. Connel, J. J. (1990). Control of Fish Quality. Cambridge, UK.
16. Dalgaard, P. Madsen, H. L. Samieian, N. & Emborg, J. (2006). Biogenic amine formation and microbial spoilage in chilled garfish (Belone belone) effect of modified atmosphere packaging and previous frozen storage. Journal of Applied Microbiology, *101*, 80–95. https://doi.org/10.1111/j.1365-2672.2006.02905.x
17. Don, S., Xavier, K. M., Devi, S. T., Nayak, B. B., & Kannuchamy, N. (2018). Identification of potential spoilage bacteria in farmed shrimp (*Litopenaeus vannamei*): Application of Relative Rate of Spoilage models in shelf life-prediction. LWT-Food Science Technology, *97*, 295–301. https://doi.org/10.1016/j.lwt.2018.07.006
18. Egan, H. S., Kirk, R. & Sawyer, R. (1981). Pearson's chemical analysis of food. 8th Ed, Churchill Living Stone, Edin burgh.
19. FAO, The State of Mediterranean and Black Sea Fisheries (2020). General Fisheries Commission for the Mediterranean. Rome. https://doi.org/10.4060/cb2429en
20. Ferreira, I., Gomes-Bispo, A., Lourenço, H., Matos, J., Afonso, C., Cardoso, C., Castanheira, I., Motta, C., Prates, J. A., & Bandarra, N. M. (2020). The chemical composition and lipid profile of the chub mackerel (*Scomber colias*) show a strong seasonal dependence: contribution to a nutritional evaluation. Biochimie, *178*, 181–189. https://doi.org/10.1016/j.biochi.2020.09.022.
21. Freitas, S., Rosario, M., Alexandre, C., Joana, A., Hugo, O., Margarida, C., Piedade, B., Vitor, V., Ralph, U. (2016). Insights into the potential of picoplanktonic marine cyanobacteria strains for cancer therapies – Cytotoxic mechanisms against the RKO colon cancer cell line. Toxicon 119, 140–151. http://dx.doi.org/10.1016/j.toxicon.2016.05.016

22. Ghanbari, M., Jami, M., Domig, K. J., & Kneifel, W. (2013). Seafood biopreservation by lactic acid bacteria—A review. LWT-Food Science Technology, *54*, 315–324. https://doi.org/10.1016/j.lwt.2013.05.039
23. Gheda, S., & Ismail, G. (2020). Natural products from some soil cyanobacterial extracts with potent antimicrobial, antioxidant and cytotoxic activities. Annals of the Brazilian Academy of Science, 92(2), e20190934. DOI 10.1590/0001-3765202020190934
24. Gondoin, A., Grussu, D., Steward, D., & McDougall, G. (2010). White and green tea polyphenols inhibit pancreatic lipase in vitro. Food Research International, *43*, 1537–1544. https://doi.org/10.1016/j.foodres.2010.04.029
25. Gram, L., & Huss, H. H. (2000). Fresh and Processed Fish and Shellfish. In: B. M. Lund, A.C. Baird-Parker & G.W. Gould (Eds.), *The Microbiological Safety and Quality of Foods*. (pp: 472–506).
26. Hassoun, A., & Coban, O. E. (2017). Essential oils for antimicrobial and antioxidant applications in fish and other seafood products. Trends in Food Science & Technology, *68*, 26–36. https://doi.org/10.1016/j.tifs.2017.07.016
27. Heller, M. I., Wuttig, K., & Croot, P. L.)2016(. Identifying the sources and sinks of CDOM/FDOM across the mauritanian shelf and their potential role in the decomposition of superoxide (O^{-}_{2}). Frontiers in Marine Science, *3*, 132. https://doi.org/10.3389/fmars.2016.00132
28. Hwang, K. T., & Regenstein, J. M. (1993). Characteristics of mackerel mince lipid hydrolysis. Journal of Food Science, *58*, 79–83. https://doi.org/10.1111/j.1365-2621.1993.tb03216.x
29. ICCAT, International Commission for the conservation of Atlantic Tunas, 2020. Annual Report of contracting parties, Madrid, Spain.
30. ICMSF, International Commission on the Microbiological Specifications for Foods, (1986). Microorganism in foods, sampling for microbiological analysis: Principles and specific applications. University of Toronto Press.
31. Jannat-Alipour Hakimeh, Masoud Rezaei, Bahareh Shabanpour, Mehdi Tabarsa, Fereidoon Rafipour, 2019. Addition of seaweed powder and sulphated polysaccharide on shelf_life extension of functional fish surimi restructured product. Journal of Food Science and Technology, *56*(8), 3777–3789. https://doi.org/10.1007/s13197-019-03846-y
32. Jiang, D., Liu, Y., Jiang, H., Rao, S., Fang, W., Wu, M., Yuan, L., & Fang, W. (2018). A novel screen-printed mast cell-based electrochemical sensor for detecting spoilage bacterial quorum signaling molecules (N-acyl-homoserine-lactones) in freshwater fish. Biosensors and Bioelectronics, *15*, 102: 396–402. https://doi.org/10.1016/j.bios.2017.11.040
33. Kanatt, S. R. (2020). Amaranthus leaf extract for shelf-life extension of chicken/fish during chilled Storage. Food Packaging and Shelf Life, *24*, 100506. https://doi.org/10.1016/j.fpsl.2020.100506
34. Li, H. B., Cheng, K.W., Wong, C.C., Fan, K.W., Chen, F., & Jiang, Y. (2007). Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chemistry, *102*(3), 771–776. https://doi.org/10.1016/j.foodchem.2006.06.022

35. Machu, L., Ladislava, M., Jarmila, V., Ambrozova, J., Orsavova, J., Mlcek, J. S., & Tunde, J. (2015). Phenolic Content and Antioxidant Capacity in Algal Food Products. Molecules, *20*, 1118–1133; https://doi.org/10.3390/molecules20011118
36. Magalhães, L. M., Almeida, M. S., Barreiros, L., Reis, S., & Segundo. M. A. (2012). Automatic Aluminum Chloride Method for Routine Estimation of Total Flavonoids in Red Wines and Teas. Food Analytical Methods, *5*, 530–539. https://doi.org/10.1007/s12161-011-9278-1
37. Malle, P., & Poumeyrol, M. (1989). A new chemical criterion for the quality control of fish; Trimethyl amine/Total volatile basic Nitrogen (%). Journal of food protection, *52*(6), 419–423. https://doi.org/10.4315/0362-028X-52.6.419
38. McDougall, G., Kulkarni, N., & Stewart, D. (2009). Berry polyphenols inhibit pancreatic activity in vitro. Food Chemistry, 115, 193–199. https://doi.org/10.1016/j.foodchem.2008.11.093
39. McElhatton, A. & Amaral, S. P. (2012). Novel Technologies in Food Science: Their Impact on Products, Consumer Trends and the Environment. Springer; New York, NY, USA. https://doi.org/10.1007/978-1-4419-7880-6
40. Miranda, J., Ortiz, J., Barros-Velázquez, J., & Aubourg, S. P. (2016). Quality enhancement of chilled fish by including alga *Bifurcaria bifurcata* extract in the icing medium. Food Bioprocess Technology, 9, 387–395. https://doi.org/10.1007/s11947-015-1626-5
41. Motta, C., Regoa, A., Cardoso, C., Coelho, I., Gomes-Bispob, A., Afonsob, C., Pratesd, M., Castanheiraa, I., & Bandarra, N. M. (2021). Seasonality as experienced in the market and the resulting variation in the amino acid and elemental composition of chub mackerel (*Scomber colias*). Journal of Food Composition and Analysis, 104, 04151 https://doi.org/10.1016/j.jfca.2021.104151
42. Ozyurt, G., Kuley, E., Balikci, E., & Kacar, C. (2012). Effect of the icing with rosemary extract on the oxidative stability and biogenic amine formation in sardine (*Sardinella aurit*a) during chilled storage. Food and Bioprocess Technology, 5(7), 2777–2786. https://doi.org/10.1007/s11947-011-0586-7
43. Quitral, V., Donoso, M. L., Ortiz, J., Herrera, M. V., Araya, H., & Aubourg, S. P. (2009). Chemical changes during the chilled storage of Chilean jack mackerel (*Trachurus murphyi*): Effect of a plant extract-icing system. LWT-Food Science Technology, 42, 1450–1454. https://doi.org/10.1016/j.lwt.2009.03.005
44. Rao, A. R., Sarada, R., Baskaran, V., & Ravishankar, G. A. (2006). Antioxidant activity of *Botryococcus braunii* extract elucidated in vitro models. Journal of Agricultural and Food Chemistry, 54(13), 4593–4599. https://doi.org/10.1021/jf060799j
45. Rey, M. S., Garcíasoto, B., Fuertesgamundi, J. R., Aubourg, S., & Barrosvelázquez, J. (2012). Effect of a natural organic acid-icing system on the microbiological quality of commercially relevant chilled fish species. LWT-Food Science Technology, 46, 217–223. https://doi.org/10.1016/j.lwt.2011.10.003
46. Rodrıguez, D. S. Steffolani, E. M., Martínez, C. S., & Leon, A. E. (2014). Effects of spirulina biomass on the technological and nutritional quality of bread wheat pasta. LWT–Food Science Technology, 8(1), 102–108. https://doi.org/10.1016/j.lwt.2014.02.054

47. Roy, V. C., Jin-Seok, P., Truc, C. H., & Byung-Soo, C. (2022). Lipid Indexes and Quality Evaluation of Omega-3 Rich Oil from the Waste of Japanese Spanish Mackerel Extracted by Supercritical CO_2. Marine Drugs, 20(70), 1–13 https://doi.org/10.3390/md20010070
48. Seddek, N., Fawzy M., El-Said W. & Ahmed M. (2019). Evaluation of antimicrobial, antioxidant and cytotoxic activities and characterization of bioactive substances from freshwater blue-green algae. Global NEST Journal, 21(3), 328–336. https://doi.org/10.30955/gnj.002949
49. Shahidi, F., & Zhong, Y. (2010). Novel antioxidants in food quality preservation and health promotion. European Journal of Lipid Science and Technology, *112*(9), 930–940. https://doi.org/10.1002/ejlt.201000044
50. Shen, L. (1996). Amperometric determination of fish freshness by a hypoxanthine biosensor. Journal of the Science of Food and Agriculture, 70, 298–302. https://doi.org/10.1002/(SICI)1097-0010(199603)
51. Suthasinee, Y., Benjakul S., & Senphan, T. (2018). Antioxidant Activity of the Extracts from Freshwater Macroalgae (*Cladophora glomerata*) Grown in Northern Thailand and Its Preventive Effect against Lipid Oxidation of Refrigerated Eastern Little Tuna Slice. Turkish Journal of Fisheries and Aquatic Sciences, 19(3), 209–219. https://doi.org/10.4194/1303-2712-v19_03_04
52. Tanha, L. D., Zhaleh, K., & Mohammad, H. A. (2021). Application of edible coating made of sturgeon gelatin and Portulaca oleracea extract for improving the shelf life of fish sausages. Journal of Food Measurement and Characterization, *15*, 4306–4313 https://doi.org/10.1007/s11694-021-01013-6
53. Tarladgis, B. G., Watts, B. M., Younathan, M. T. & Dugan, J. L. (1960). A distillation method for the quantitative determination of Malonaldehyde in rancid foods. Journal of the American Oil Chemists' Society, *37*, 44–48. https://doi.org/10.1007/BF02630824
54. Thiansilakul, Y., Benjakul, S., Grunwald, E. W., & Richards, M. P. (2012). Retardation of myoglobin and haemoglobin-mediated lipid oxidation in washed bighead carp by phenolic compounds. Food Chemistry, *134*(2), 789 https://dx.doi.org/10.1016/j.foodchem.2012.02.182.
55. Yen, G. C., & Duh, P. D., (1994). Scavenging effect of Methanolic extracts of peanut hulls on free radical and anti-oxygen. Journal of Agricultural and Food Chemistry, *42*, 629–632. https://doi.org/10.1021/jf00039a005

Figures

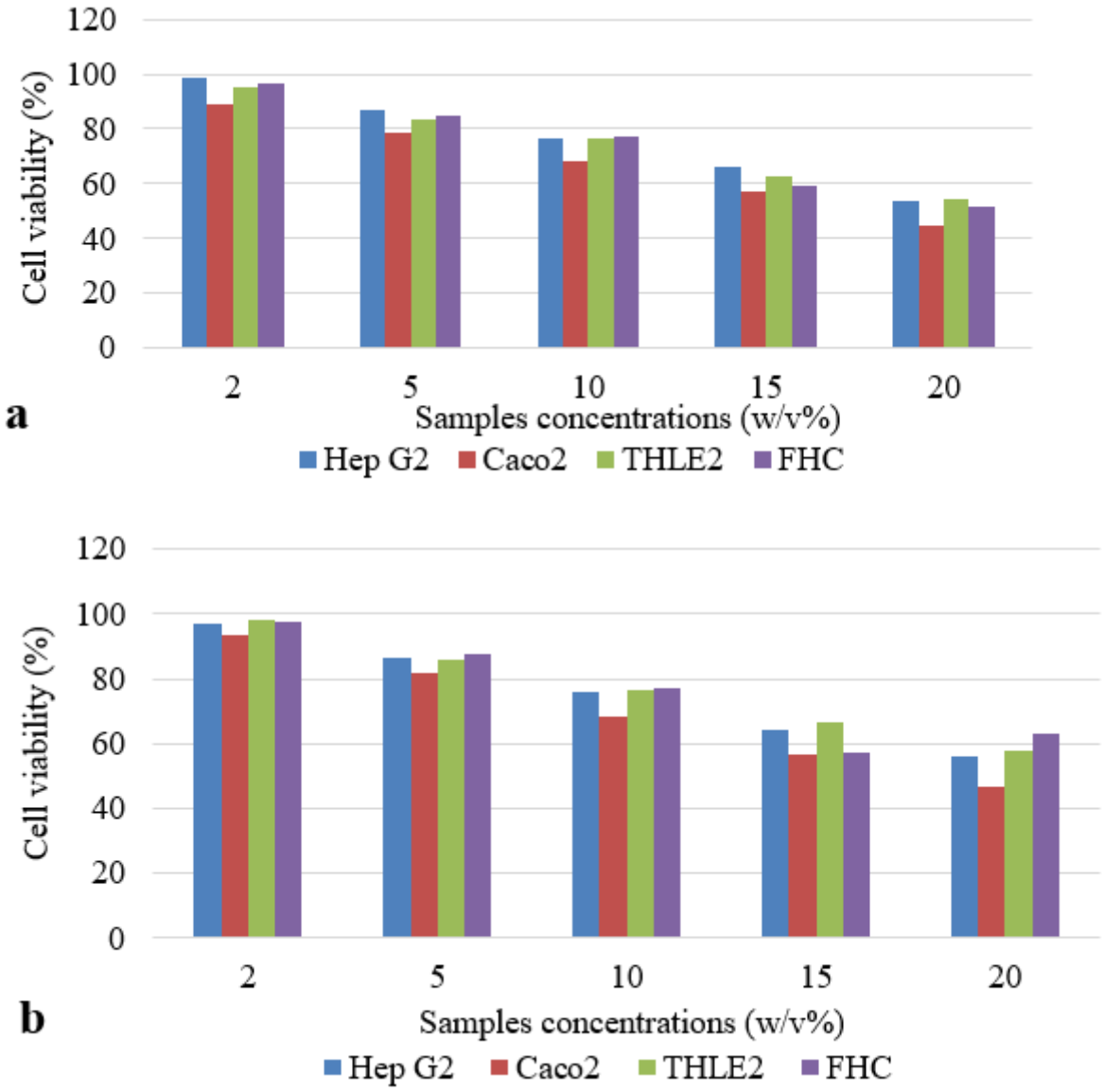

Figure 1

Effect of aqueous extracts (w/v) of extremophilic microbial film, a) hot spring and b) hypersaline lakes on normal and carcinoma cells of liver and colon.

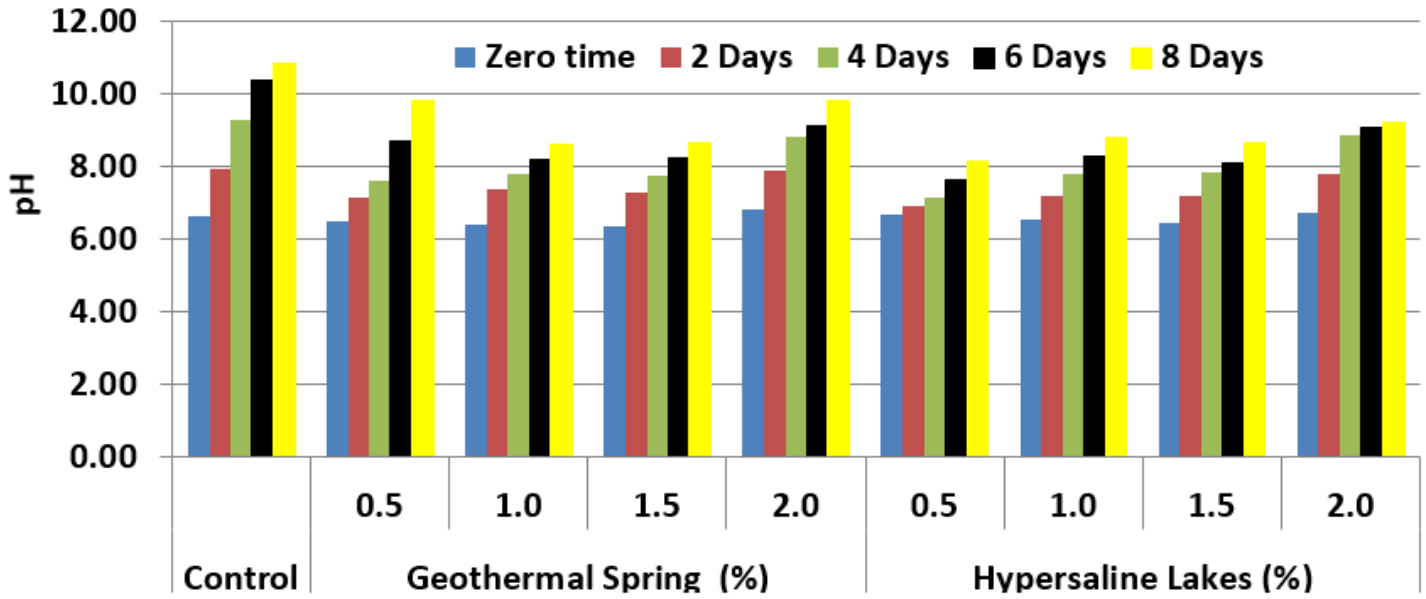

Figure 2

Effect of different levels of extremophilic microbial film extracts on pH value of refrigerated Atlantic chub mackerel (5±1°C).

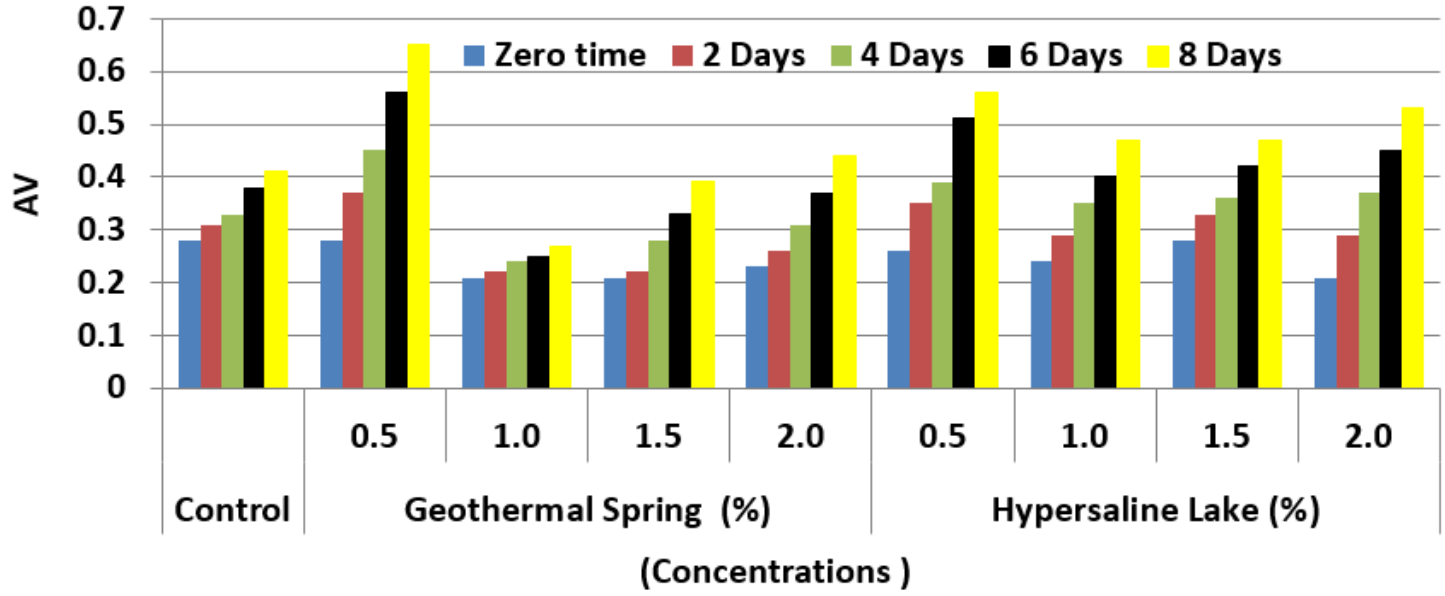

Figure 3

Effect of different levels of extremophilic microbial film extracts on acid value (AV, mg KOH\ml oil) of refrigerated Atlantic chub mackerel (5±1°C).

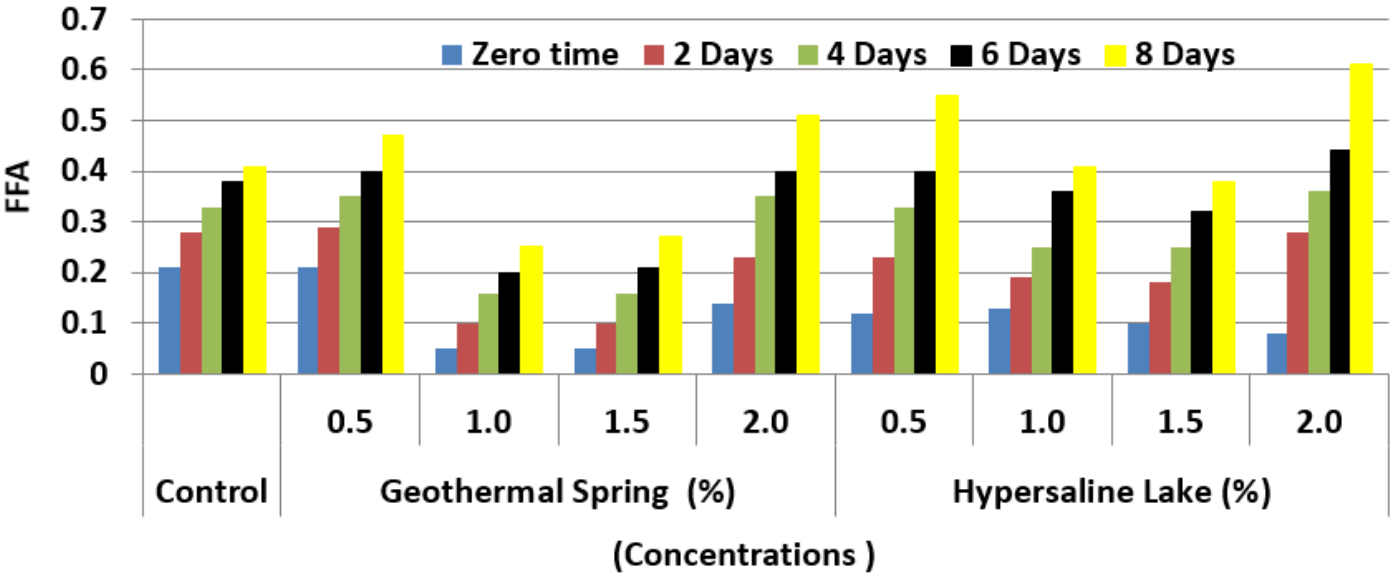

Figure 4

Effect of different levels of extremophilic microbial film extracts on free fatty acids (FFAs, mg \100ml oil) of refrigerated Atlantic chub mackerel (5±1°C).

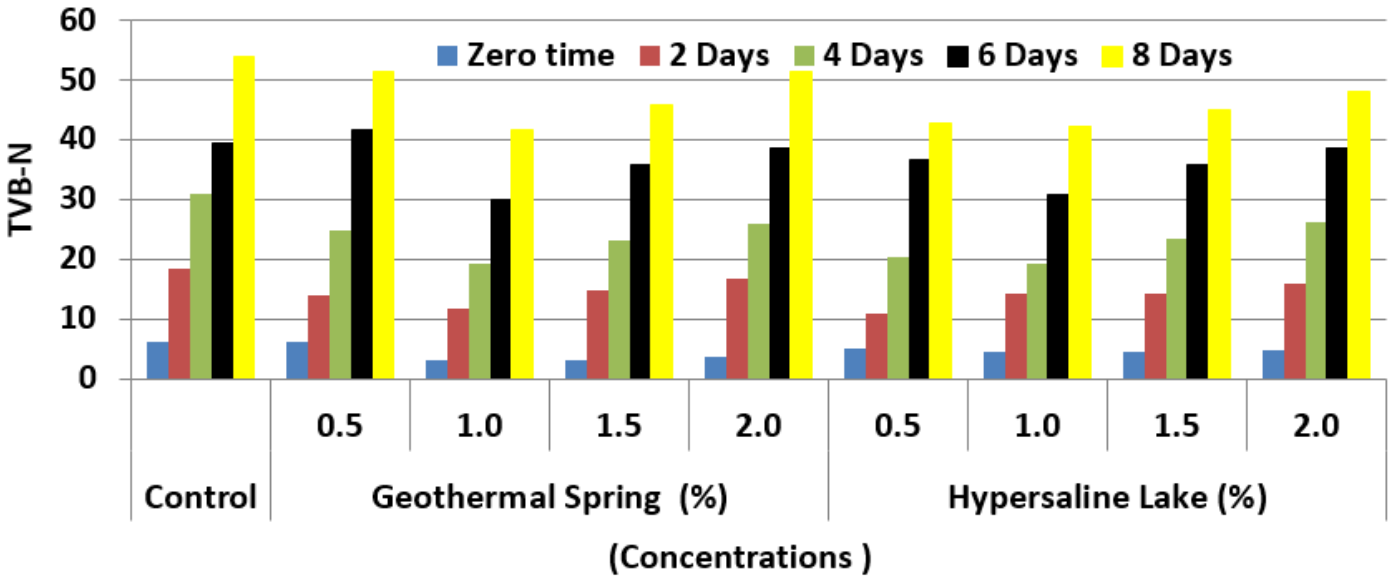

Figure 5

Effect of different levels of extremophilic microbial film extracts on TVB-N (mg\100gm) content of refrigerated Atlantic chub mackerel (5±1°C).

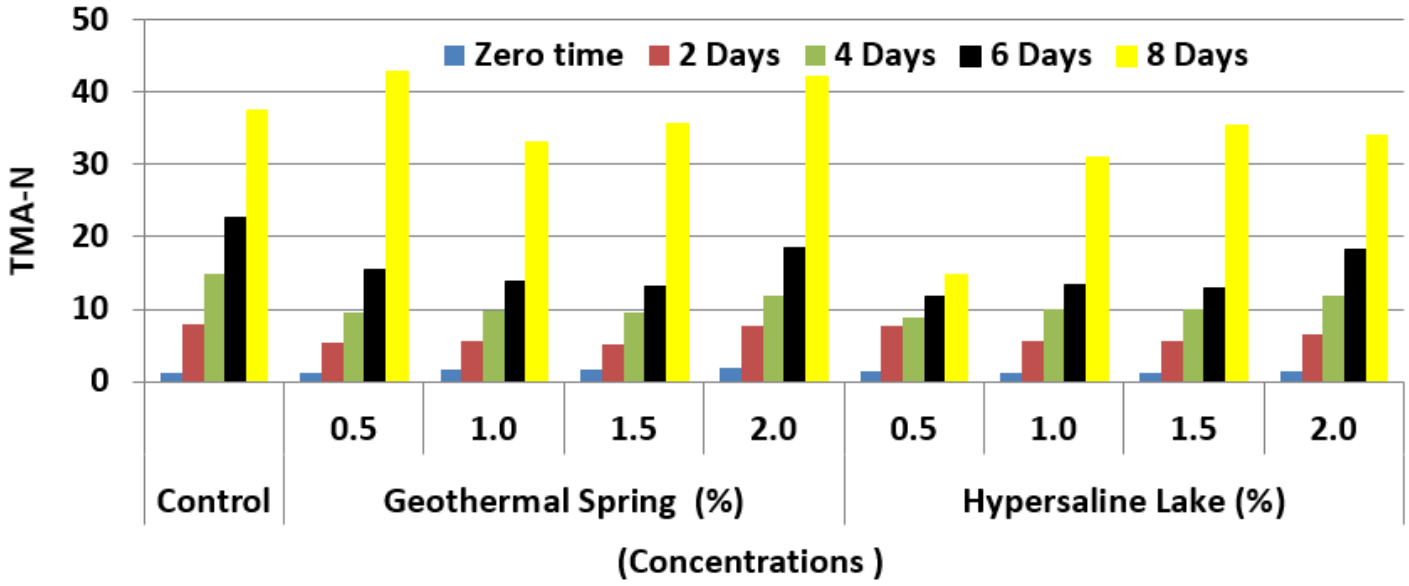

Figure 6

Effect of different levels of extremophilic microbial film extracts on TMA-N (mg\100gm) content of refrigerated Atlantic chub mackerel (5±1°C).

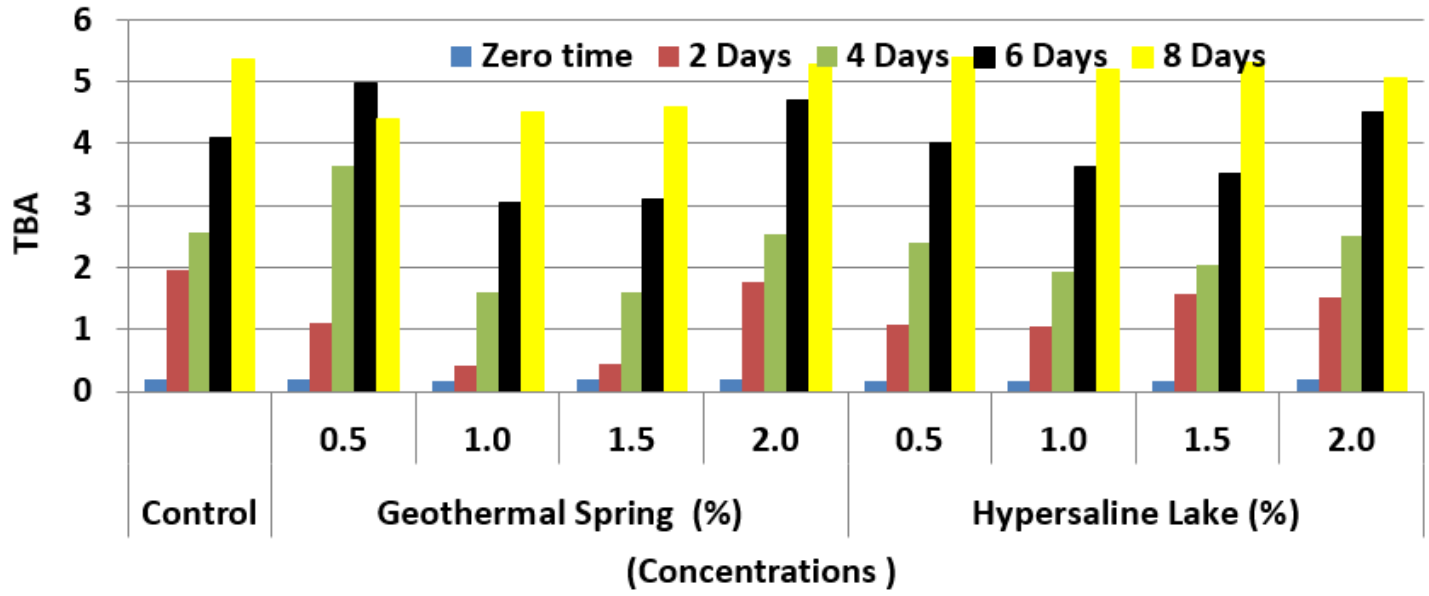

Figure 7

Effect of different levels of extremophilic microbial film extracts on Thiobarbituric acid (TBA, mg MDA\kg sample) value of refrigerated Atlantic chub mackerel (5±1°C).

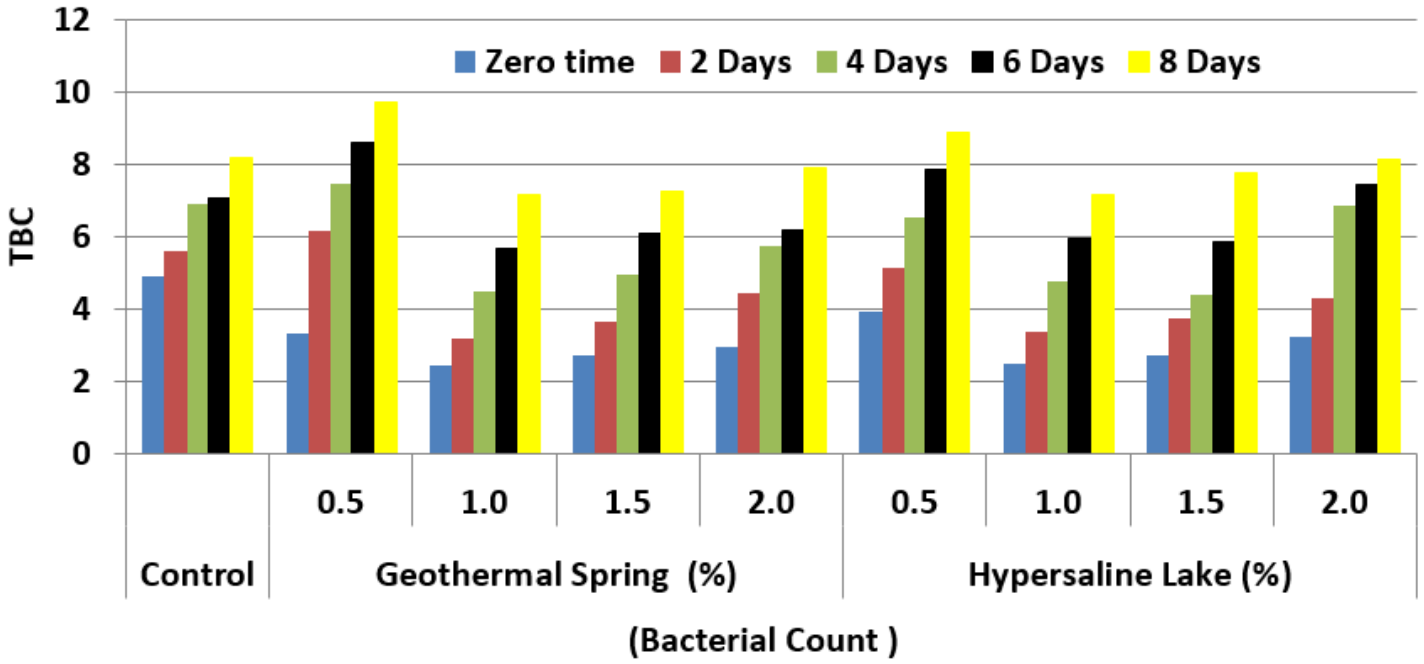

Figure 8

Effect of different levels of extremophilic microbial film extracts on Total bacterial count (TPC Log cfu/g) of refrigerated Atlantic chub mackerel (5±1°C).

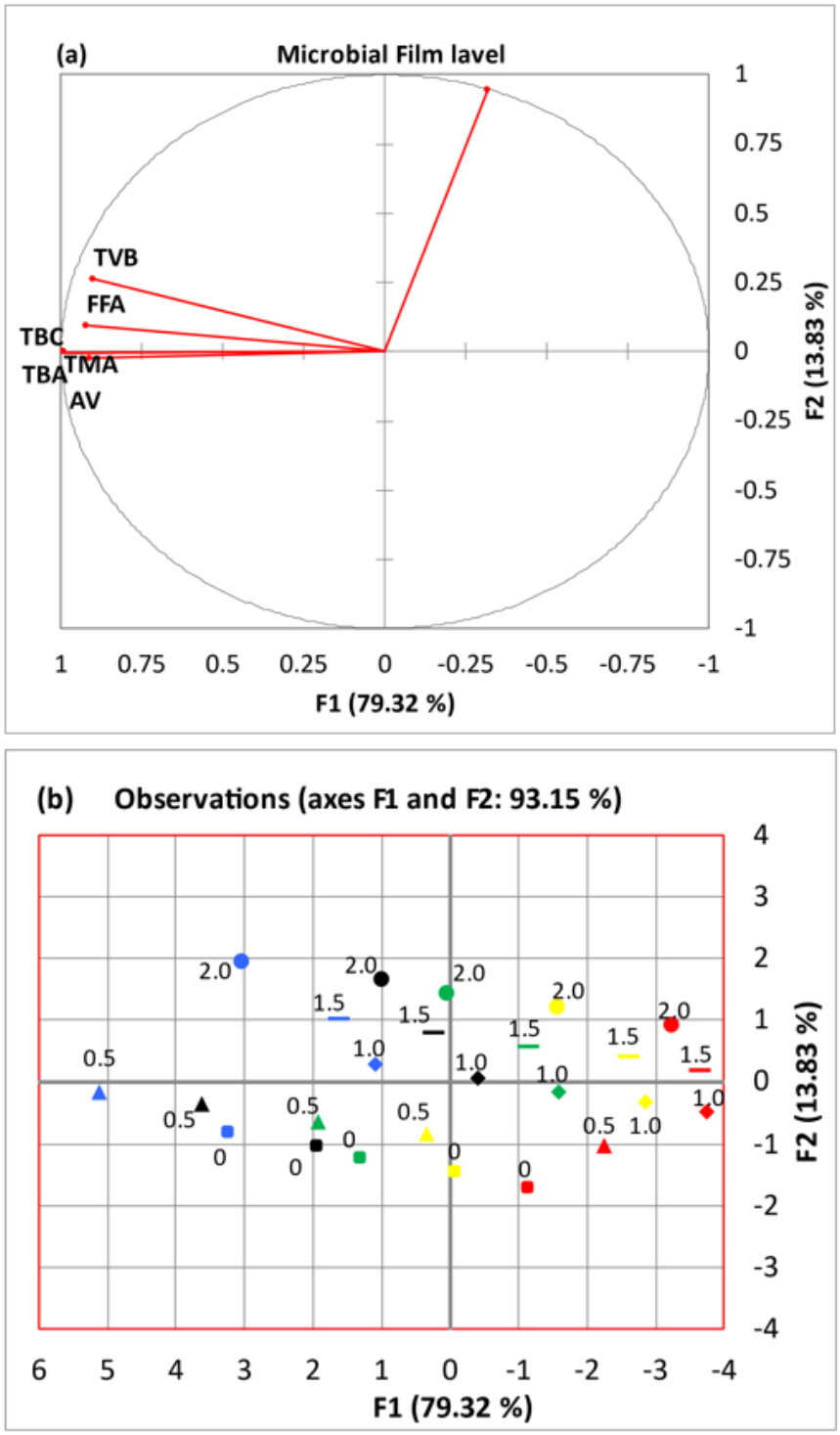

Figure 9

PCA showing properties of shield Atlantic chub mackerel containing different levels of geothermal spring extremophilic microbial film extracts; the numbers next to the symbols on **(9b)** show the geothermal spring microbial film level.

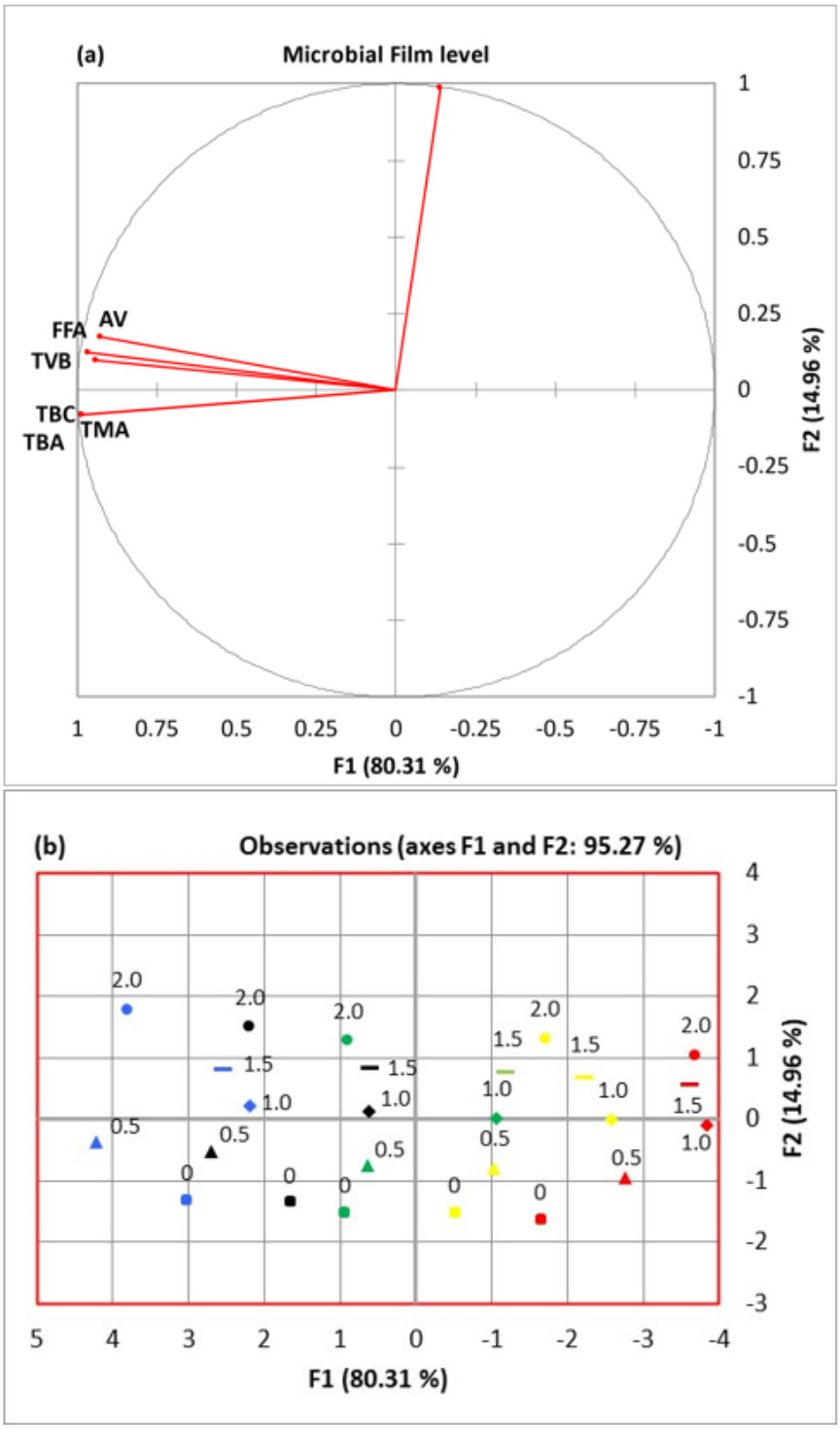

Figure 10

PCA showing properties of shield Atlantic chub mackerel containing different levels of hypersaline lake microbial film extract; the numbers next to the symbols on (10b) show the hypersaline lake microbial film level.