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Exploting the Use of Mixed Pectin-Chitosan Film for the Shelf-life Extension of Tuna Fish Slices

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The effects of pectin-chitosan film on shelf life extension of tuna fish fillets during refrigerated storage (4°C) were evaluated over a period of 10 days. Fillet samples were previously treated by immersion in a solution containing 0.5 % ascorbic acid, 50 mM sodium citrate and 2% NaCl at pH 6 and after in edible film. Chemical, biochemical and microbiological assays were carried out to profile variations on fatty acid composition (UFA/SFA, MUFA/SFA and PUFA/SFA), Trimethylamine (TMA) content, protease activity, protein pattern on SDS-PAGE and total bacterial count.
The effect of lipid oxidation observed in control fillets as reduction of UFA/SFA, MUFA/SFA and PUFA/SFA ratios was reduced in pectin-chitosan treated tuna slices. Similarly, the amount of TMA per mg of treated sample resulted lower than the controls. Moreover, both microbiological and biochemical analyses provide evidences of an extension of the shelf-life extension of tuna fish slices after pectin-chitosan film deposition.
Thus, it could be hypothesized that such procedure could act preserving physical and chemical properties of tuna slice enabling the use of pectin-chitosan film for commercially important fish species.

1. Introduction

Tuna is one of the most widely traded and precious fish species with a global market of about $6 billion and annual amount nearby 4 million tonnes, (Garrett et al. 2010).
The yellow fin tuna (*Thunnus albacares*) is one of the pelagic fish species belonging to the Scombridae family. It was found in various marine environments as in tropical and subtropical waters including a few reports in Mediterranean sea (FAO, 2014). In addition to fresh consumption, it is stored frozen before consumption; while, it is usually commercially available in several format including processed, smocked and sliced.
Fish flesh represent the most perishable food products because of the occurrence of chemical reactions, enzymatic response, and microbial spoilage during processing and storage procedures (Gram and Huss, 1996, 2000; Shewan, 1971).The reduced shelf-life and the safety issues for human fish flesh consumption, gained the attention of Food Regulatory Agencies and Food Processing Industries. Thus, accurate processing and pre-treatment in addition to preservation techniques are known to improve the quality and food safety of fish products retarding deterioration.
Ice storage and cold chain in transport represents among the canonical methods to preserve fresh fish slowing deterioration events. Defects in such procedure usually resulted in contamination by bacterial pathogens and biotoxins and histamine fish poisoning (HFP) thus representing a problem for food safety and human consumption (Galaviz-Silva et al. 2009).
Tuna are known to possess elevated content of free histidine in their muscle tissues that in turn affects the decarboxylation events leading to histamine production (Lukton and Olcott, 1958; Taylor andEitenmiller, 1986).

During the years, several efforts have been made on the development of edible films and coatings with the aim to increase fish shelf life. Films based on cold water fish-skin gelatin and chitosan blends were reported for rainbow trout fillet packaging, showing antioxidant properties (Nowzari et al. 2013). Gelatin and chitosan were mixed to obtain antimicrobial films for cod fillet, which resulted in reduction of gram-negative bacteria growth (Gomez-Estaca et al. 2010). Similarly, trout fillets treatment with chitosan- skin gelatin films and grape seed extracts resulted in a shelf-life extension (Kakaei and Shahbazi, 2016).
Herein we report the development of an edible pectin-chitosan film (0.6% and 0.005% w/v, respectively) which in absence of any toxicity risks enabled an improved preservation of yellow fin tuna physical and chemical characteristics. In addition, the effects of ascorbic acid and sodium citrate pretreatment were evaluated on *T. albacares* fillets over a period of 10 days during refrigerated storage

2. Materials and Methods

2.1.Sample Processing

Aliquots (5 g) of edible tissue from fresh *T. albacares* were selected in order to obtain both red and white muscular fibrous components (Duun and Rustad, 2008).
Samples were pre-treated by soaking in an antioxidant solution based on 0.5% L-Ascorbic acid, 50 mM sodium citrate dehydrate, 2% sodium chloride, pH 6. For coating experiments, resulting in the formation of the edible film, samples were immersed for 3 min in 0.6% w/v pectin and and 0.005% w/v chitosan solution. Treated slices were allowed to drain at 10°C under sterile conditions. Control, pre-treated and coated samples were then stored at 4 °C for 10 days (d) while the recovery was performed at 0, 2, 4, 6, 8 and 10 (d)for processing and analyses.

2.2. Gas Chromatographic Fatty Acids Analysis

The Bligh and Dyer (1959)method with minor modifications was used to separate the lipids from the matrices in samples containing 80% water. An aliquot (5 g) of edible muscle was homogenized in chloroform-methanol (1:2; v/v) and extracted by Accelerated Solvent Extraction. The homogenate was filtered through Whatman n.1 filter paper. The polar and non-polar phases were allowed to separate in separating funnel.
The lower clear phase was drained into round-bottom flask and concentrated with a rotary evaporator at 40°C. multivapor TM Buchi b-12 . The extracted lipids were kept in amber glass bottles and flushed with nitrogen. The bottles were stored immediately at −20°C for future analyses.
The base-catalyzed transesterification was used to analyse the amounts of fatty acids methyl ester (FAMEs) into fish matrix (Eder, 1995). Methyl esters were prepared by trans-methylation using 2M potassium hydroxide (Merck, Darmstadt, Germany) in methanol (Sigma-Aldrich, Steinhein, Germany).
Approximately 20 mg of oil was dissolved in 1 ml hexane, added with 0.2 ml of 2M KOH in methanol. The tubes were vortexed at 25 °C for 1 min at ambient temperature; after phase separation the hexane layer was recovered for GC analyses. The analysis of FAMEs was carried out on a capillary gas cromatograph (Gas Cromatography Thermo Electron Corporation Focus GC) equipped with Spectrometer (Thermo Electron Corporation DSQ II) with TRACE TR-FAME (100 m x 0.25 mm i.d x 0.22 µm film thickness) GC column. Injection temperature was set 250 °C with split system (10:1). Septum flow to split vent was 1.5 ml/min and purge flow to splint vent was 10 ml/min. Oven temperature was programmed at the beginning 100 °C (hold time 2 min), increased to 250 °C with a 2 °C/min increment (hold time 15 min) and finally reached to 255 °C with a 40 °C/min increment (hold time 5 min). The mass spectrum was acquired using Xcalibur Data System ver. 1.4. Peaks were identified by comparison with standards Supelco™ Component FAME Mix 37 (Sigma Aldrich) and using the NIRST mass spectral database. Mix FAMEs including saturated, monounsaturated and polyunsaturated FAMEs. A further comparison was made from the literature for the values of m/z of saturated, monounsaturated and polyunsaturated respectively to 74, 55, 67 and 79 m/z (AOCS Lipid library). Fatty acids composition were calculated by percentage (% weight) of wet weight on the total lipid content.

2.3.TMA Analysis

A 5% trichloroacetic acid solution and a basic aqueous solution consisting in potassium carbonate and picric acid was used to recover trimethylamine (TMA). The detection was done by means of spectrophotometric UV-VIS measures setting the wavelength at 410 nm using the standard calibration curve.

2.4 SDS-PAGE Analysis

Samples were suspended in an 20 mM Tris-HCl pH 7.5 and homogenized. After centrifugation at 10000 g for 15 min at 4°C for removal of debris, the soluble fraction was analysed on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions to separate the myofibrillar protein and evaluate the total protein pattern

2.5. Microbiological Analysis

The sample preparation for microbiological analysis was performed by depositing 5 g from each sample in 50 ml of sterilized 0.9% NaCl solution and homogenizing it. Serial decimal dilutions were prepared and plated into appropriate microbiological media. Each experiment was performed in triplicate on Plate Count Agar (PCA) according to ISO 4833-1:2013 standardized test method.
The equation used to obtain the ufc/g was:

$$N = \frac{\Sigma C}{V * 1.1 * d}$$

C = amount of the colonies counted in all the plates (considered of two successive dilutions), and at least one of the plates must contain a minimum of 10 colonies;
d = dilution corresponding to the first dilution considered
V = inoculum volume used in each plate (mL)

3. Results and Discussions

3.1.Fatty acid changes

In the last years, it has been a global increase in the use of polymer-based film originated from natural materials for coatings which protect food from external bacterial or toxin contamination, delaying deterioration, thus extending its shelf-life. Herein, we produced an edible film formulation for yellow fin tuna consisting in a combination of two biopolymers, namely pectin and chitosan at defined ratio. Such blend confers mechanical and water resistance associated to the possibility to forming a cohesive three-dimensional matrix (Gupta et al. 2014; Benbettaïeb et al. 2016;).
It is well known that alteration of flesh structure due to natural changes during storage usually result in changes in lipid fraction and fatty acids compositions (Ozden, 2005; Yang et al.1981). Concurrently, oxidation events strongly affect quality and shelf life, mainly because of the increase in the total amount of saturated fatty acids (SFA) and the decrease in the total amount of unsaturated fatty acids (UFA). Thus, to control the changes in fatty acid composition during the storage, an antioxidant solution was hypothesised. Prior to coating samples were exposed to on 0.5% L-Ascorbic acid, 50 mM sodium citrate dehydrate, 2% sodium chloride,pH 6.
The ratios between unsaturated and saturated fatty acids (UFA/SFA), monounsaturated fatty acids/saturated fatty acids (MUFA/SFA) and polyunsaturated fatty acids/saturated fatty acids (PUFA/SFA) were used as a tool to evaluate changes in to storage and oxidation events (Voldrich et al. 1991).
Figure 1 reported changes in fatty acids ratios in the yellow fin tuna muscle during a period of 8 days and a decrease in all the proxy used was observed in control samples. Similar UFA/SFA and MUFA/SFA profiles were retrieved both in treated and coated samples. Conversely, PUFA/SFA remained at stable levels in pre-treated and coated samples. Thus, it could be hypothesised that antioxidant processing specifically act maintaining the amount polyunsaturated fatty acids, while coating enable to improve such phenomenon.

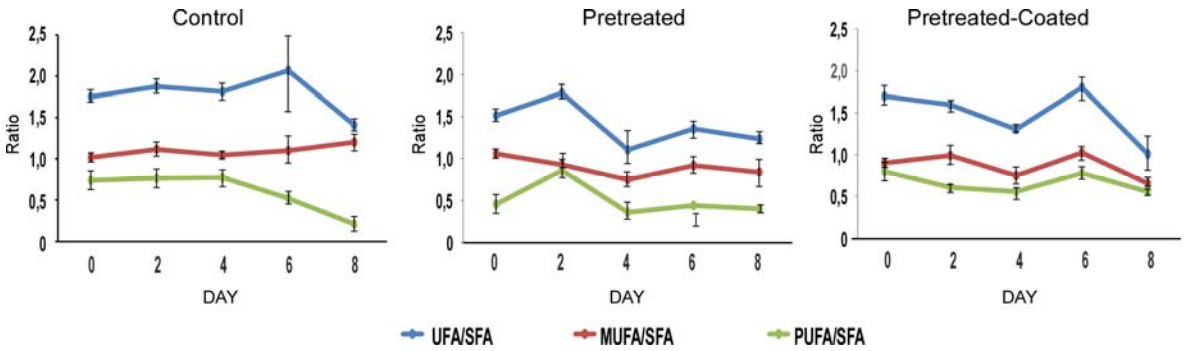

Figure 1: Fatty acid changes over a period of 8 days as UFA/SFA and MUFA/SFA and PUFA/SFA ratios in muscles of yellowfin tuna. Untreated samples, samples treated with 0.5% L-Ascorbic acid, 50 mM sodium citrate dehydrate, 2% sodium chloride,pH 6 and coated samples are shown. Data are expressed as means ± standard deviation (SD) oftriplet measurements of at least three independent experiments.

3.2.Trimethylamine (TMA)

Trimethylamine oxide (TMAO) is a major constituent of the non-protein nitrogen fraction in marine fishes usually exerting osmoregulatory activity. In absence or in conditions characterised by low level O_2, TMAO serves as a terminal acceptor for anaerobic respiration thus converting in TMA (Boskou andDebevere, 1997). Values reaching 10-20 mg 100 g^{-1} TMA has been proposed acceptability limit for yellow fin tuna (Huss, 1996
The changes in TMA content in yellow fin tuna samples stored under refrigeration at 4 °C in control, pre-treated and coated experiments are shown in Figure 2 TMA levels increased during the storage time. Although the lower TMA content at day 0 (9,98 ± 0.15 mg of TMA/100 g fish muscle) indicates that the fillet samples are similar to TMA levels observed in fish with incipient state of alteration, they resulted lower than acceptability limits proposed elsewhere.
Untreated samples reach such level at day 2; while, experimental slices exceed the 20 mg 100 g^{-1} limit starting from day 4, thus suggesting that the antioxidant pre-treatment and coating will work delaying the TMAO reduction in TMA.

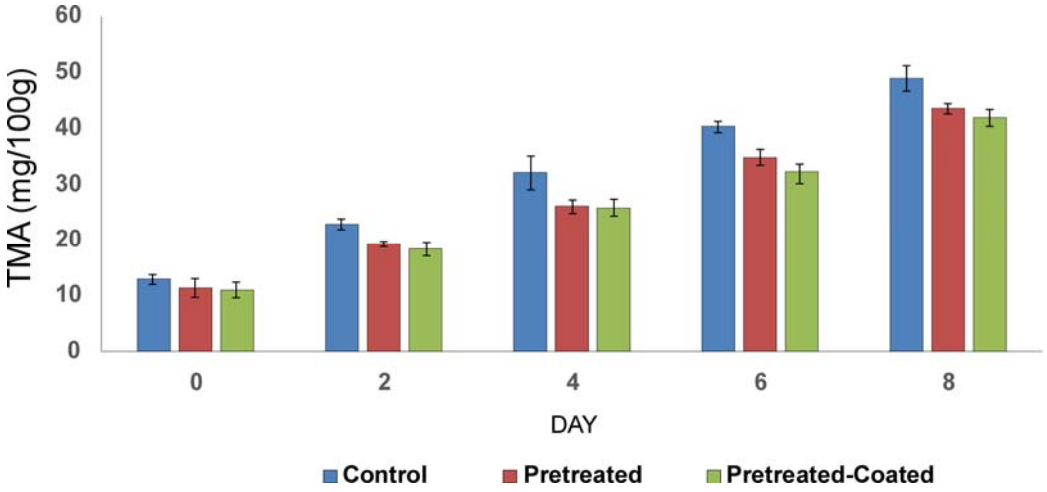

Figure 2: Changes in trimethylamine (TMA) of untreated samples (blue bar), samples treated with 0.5% L-Ascorbic acid, 50 mM sodium citrate dehydrate, 2% sodium chloride, pH 6 (red bar) and coated samples (green bar) are shown. Data are expressed as means ± standard deviation (SD) of triplet measurements of at least three independent experiments

3.3. Microbiological Analyses

Total microbial count of in control, treated and coated yellow fin tuna slices during storage at 4 °C for 10 days is shown in Figure 3.
According to the culture-dependent methods, all batches presented a relatively similar starting conditions. The initial number of bacteria in trout samples was 1.2/1.5 *10^2CFU/g, indicating that the fish used in this study had good microbiological quality. Similar low initial number have been reported for rainbow trout (Özogul and Özogul, 2004), rainbow trout fillets (Frangoset al. 2010) and yellow fin tuna (Silbande et al. 2016)

Table 1: Microbial changes observed in untreated samples, samples treated with 0.5% L-Ascorbic acid, 50 mM sodium citrate dehydrate, 2% sodium chloride, pH 6 and coated samples

Day	CFU/g		
	Control	Pretreated	Coated
0	$1.2*10^2$	$1.4*10^2$	$1.5*10^2$
2	$1.6*10^3$	$1.1*10^3$	$1.8*10^2$
6	$1.8*10^6$	$1.3*10^5$	$7.1*10^2$
10	$9,8*10^6$	$1.5*10^6$	$3.9*10^6$

standard error below 1 %

The CFU/g never exceeded the value of 7 log cfu/g, which is considered the upper acceptability limit for fresh water and marine species as defined by ICMSF (2002) during the experiments. The number gradually

increase in all the samples while it remains below 10^3 in coated slices at day 6. Therefore, pre-treating and coating procedures represent useful tools for inhibition of aerobic bacteria growth.

Protein degradation

Electrophoretic protein pattern was examined in control and experimental samples and at different time intervals during the 8 days of storage at 4C (Fig. 3). In control band intensities corresponding to heavy meromyosin-myosinheavy chain (HMM-MHC) (150 KDa), light meromyosin (LMM) (75 kDa) and Actin (A) (45 kDa) were reduced. Additionally more intense bands below the 40 kDa region appeared, likely due to hydrolysis of high molecular weight proteins(Cortes-Ruiz et al.2008; Blanco-Pascual et al.2013). Conversely, in pre-treated and coated yellow fin tuna slices these band retained their intensities even after 8 days. Thus, the electrophoretic pattern of muscle proteins provide evidence for delayed deterioration and extended shelf-life.

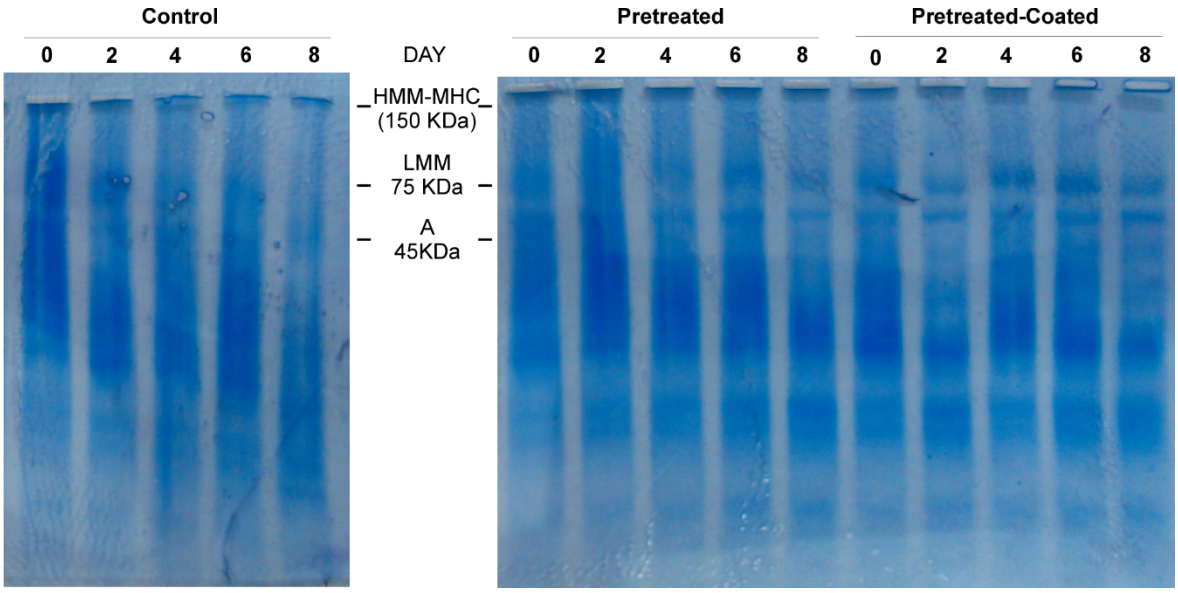

Figure 3: Electrophoretic profiles of muscular proteinin untreated samples, samples treated with 0.5% L-Ascorbic acid, 50 mM sodium citrate dehydrate, 2% sodium chloride, pH 6 and coated samples. Heavy meromyosin-myosin heavy chain (HMM-MHC), light meromyosin (LMM) and Actin (A) are reported.

4. Conclusions

In this study we developed of an edible pectin-chitosan film associated to a pretreatment procedures enabling increased shelf-life of yellow fin tuna taking advance on the hydrocolloid properties of the coating materials. This represent a first step for the assessment of novel systems ensuring food safety and quality. In addition, the use of materials with natural origin, such as pectin and chitosan, the fast and low-cost procedures represent the main advantages of this treatments and a starting point for future product shelf-life improvements in fisheries.

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