Evaluation and Characterization of Postmortem Salmon Adhesion on PET Coatings.

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ABSTRACT

The study focused on the effects of biochemical changes of postmortem salmon on the coating and how muscle degradation inside the food can, resulting from the thermal treatment and storage favors adhesion to the food container. The experimental design consisted of a set of manufactured food cans stored which were submitted to chemical and physical tests of food-contact canning to evaluate the adhesion, and to treatment with urea solution to minimize the amount of muscle residues. The characterization of changes in the multilayer was performed by SEM and AFM. The degradation of the muscle–polymer system was evaluated by FT-IR, Raman, and Nuclear Magnetic Resonance analyses. The results showed the existence of denatured muscle proteins strongly adhered to the PET surface in time and the adverse effects of urea leading to chemical changes and molecular rearrangements in the polymer coating altering its functionality as a protective layer in canned salmon.

Keywords: adhesion; PET; salmon; characterization; freshness.

INTRODUCTION

In a supermarket where fish are offered in attractive and ready-to-cook units, color is
one of the major attributes that affect the consumer perception of quality (1). Minimization of the amount of product or residues adhering to the can wall after emptying of the can is one of the convenience requirements of consumers. This work evaluated the effect of the salmon farming processes on the performance of metal–polymer composites coated with low-permeability, homogeneous polymers employed in salmon canning.

The release of ammonia compounds, hydrogen sulfide and mercaptans (indole, skatole, and other sulfur-containing chemical compounds) are indicative of the microbiological spoilage of fish, and depends on the storage temperature (2-4). Therefore, we want to test the hypothesis that muscle adhesion to the container wall depends on the degree of salmon freshness, protein denaturation from the thermal treatment of the container, and the surface characteristics of the PET coating. Hence, it is essential to address the study on adhesion to proceed later with the effects, if any, of the muscle components on the polymer surface.

Long adhesion times of salmon muscle to the PET polymer pose questions on the possible interactions that may take place such as changes in the performance of the protective polymer, migration of products incorporated in the diet of farmed salmon into the environment, and limits to the recycling of PET as an input in new applications under parameters of sustainability (5-6).

To ensure sufficient shelf-life, canned food is heat-treated by steam, steam–air mixtures, water or spraying water. The amount of heating needed in the coldest spot in the packaged food is in the range 4–12 min at 121°C for some typical canned food products (7, 8-9). As a consequence of this heat treatment, some packed products can partly adhere to the can wall.

The glycogen (storage carbohydrate) or fats are oxidized in a series of reactions which finally produce carbon dioxide (CO2), water, and adenosine triphosphate (ATP). This type of respiration occurs in two stages: anaerobic and aerobic. The latter depends on the continuous presence of oxygen (O2), only available in the circulatory system (10). Postmortem glycolysis results in the accumulation of lactic acid with the concomitant decrease in muscular pH and increased aggressiveness of the products against the canning materials.

Several factors have been found to affect adhesion to lacquered can walls. It has been reported that proteins are the major cause of this adhesion (10-11). This study is focused on an integral view of the problems and considers new researches to
integrate every necessary aspect to optimize the use of multilayer materials in containers subject to changes due to the incorporation of new elements in order to improve their commercialization and enhance sustainable recycling processes (12). The present characterization will allow recommendations of the findings to be given to the manufacturing sector.

MATERIALS AND METHODS

The data provided indicate that the material consists of an electrolytic chromium coated steel (ECCS), formed by an average 0.20 mm thick laminate, protected by a layer generated by electrolytic deposition and consisting of a 0.01 μm thick chromium (Cr⁰) and chromium oxide (Cr₂O₃) with an average thickness of 0.01 μm, and a total chromium content of 101.33 mg/m². The chemical composition (% wt) of steel was as follows: 0.074% C, 0.260% Mn, 0.021% P, 0.016% S, 0.012% Si, 0.032% Al, 45 ppm N, and Fe (remaining percentage).

This ECCS steel plate had a 30 μm thick layer of polyethylene terephthalate applied to the surface to serve as a protective barrier against the canned food product and to prevent physicochemical interactions between the food and metal substrate.

The standard manufacturing protocol (13) was employed to determine the salmon adhesion to the PET coating of the container. Food cans with a net content of 114 g raw salmon were manufactured employing 50 mL of a 2.5% NaCl solution, sterilized at 120 ºC for 60 minutes, immersed in warm water bath in the range of 50-80 ºC prior to sterilization, and stored for 14 months at 20 ºC before opening. The residual amount of salmon was determined by calculating the weight difference between the portion attached to the polymer after emptying the cans and subtracting the weight of the cans after cleaning with detergent and water. 24 cans were randomly opened for each sampling period of 1, 2, 4, 11 and 14 months.

After the weight of the emptied can was determined, the cans were filled with 6 M urea solution and stored overnight. The cans were emptied again and the weight was recalculated. After cleaning and drying, the weight of the can was estimated. In addition, the experimental procedure employed 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to denature the proteins of the residues on the PET surface that persisted after the urea solution treatment to remove them. The peroxide index, which is directly related with the oxidation of fats,
and therefore it is also an indicator of the degree of freshness, was determined. The salmon samples were dissolved in glacial acetic acid and chloroform and then were treated with potassium iodide solution (14). The peroxides bound to unsaturated fatty acids can be measured by determining the amount of iodine which is formed by the reaction of peroxides with iodine ion to form free iodine, using 1% (w/v) soluble starch solution as indicator. Finally, the peroxide index was determined by titrating iodine with sodium thiosulfate 0.01 N.

The morphology of the composite’s constituting layers was characterized by SEM (LEO 420) and atomic force microscopy AFM (MFP 3D) to assess the degree of adhesion and degradation occurred. The characterization of the chemical and structural changes occurring in the PET coating of the container was performed by nuclear magnetic resonance (NMR) spectroscopy. Solid state NMR analyses were carried out with a Bruker 400WB spectrometer operating at a proton frequency of 400.13 MHz. NMR spectra were acquired with cross polarization (CP) pulse sequences under the following conditions: 13C frequency: 100.48 MHz, π/2 pulse 3.7 μs, decoupling length 6.3 μs, recycle delay: 5 s, 15k scans.

The changes in the multilayers were characterized by vibrational spectroscopy: FT-IR and micro-Raman. The experiment considered mainly the data gathered with the 785 nm spectrometer. This device consisted of a multi-mode fiber laser BWTEK BRM-OEM-785 (785 nm), a Raman head BWTEK BAC100-785E and an objective lens Zeiss Epiplan 50X/0.50 infinite/0 44 28 50 with a focal length of 6.5 mm. The analyses were applied to the same set of experimental samples.

**RESULTS AND DISCUSSION**

Salmon residues remained adhered to small areas of the PET coating, the percentage of adhesion in the different storage times for the samples analyzed is described in Figure 1a, where a slight increase in the percentage of salmon muscle adhering to the PET-coated can wall was observed with longer storage times.
The amount of muscle adhering to the can wall was determined after treatment with urea solution to unfold proteins bound to the container. This assessment was termed ‘post urea adhesion’ and showed the results indicated in Figure 1b. The percentage of residues was reduced down to 0.8-1.4% of total net weight. The variation seems non-significant; the occurrence of adhesion in time implies some sort of interaction between the PET polymer surface and the salmon muscle. Interaction has been suggested as the cause for the adhesion of proteins to PET. The carbonyl group in the ester bond of the PET coating is engaged to form hydrogen links with the amino group of the protein, which would explain the residues salmon on the container. On the other hand, the urea–PET interaction model performs likewise due to the structural similarity between urea and the amino acids of proteins; urea forms hydrogen bonding bridges with the PET coating by removing proteins due to its smaller size and greater electric charge density, reducing therefore the adhesion of proteins to the polymer (2, 15).

The peroxide values recorded for the analyzed samples were in the range between 45 mg O2/kg and 200 mg O2/kg; with regard to peroxide values and storage times evaluated, there was a 46% average decrease in the peroxide content (Fig. 2). Large variations in the peroxide ranges of sample batches were detected, but a decrease in the concentration was a common feature throughout the observation periods.
Figure 2. Peroxide levels (mg O2/kg) versus time (months) for 5 batch samples.

However, considering that the reference value of 60 mg O2/kg was surpassed since the first month on average, indicating loss of freshness, it is clear that the canned product began to deteriorate even after the application of heat treatment. The micrographs represent the morphological, Fig.3 with SEM, showing different conditions of muscle adhesion and including only significant cases of general occurrence in the other samples, which relate this adherence with the polymer.

Figure 3. (a) Gaping in slit of central portion of muscle adhered to the PET; b) surface macrograph of PET surface showing areas of muscle adherence after the urea treatment c) morphology of pore on the PET after muscle removal from the coating; d) condensation particle by lyophilization of muscle residues (SDS-PAGE).

Figure 3a shows the morphology of muscle portions naturally adhered to the PET coating; it is possible to see large surface portions with varied amounts of adhered salmon tissue, mostly on localized spots. Figure 3b shows the muscle volume loss and partial detachment from the polymer surface after the urea treatment. Most part
of the polymer surface shows no adhesion, which relates to that shown in Figure 1; and the areas with numbers have some degree of adhesion to the PET; these areas are points that were also characterized by AFM and spectroscopy. The muscle, in general, adheres perpendicularly to the lamination of the steel plate, a feature related with the deep drawing manufacturing process. Figure 3c characterizes a micropore revealed after muscle detachment from the PET surface by the urea solution, where small amounts of residues inside the pore and larger portions of muscle strongly adhered to the pore edge by protein fibrils can be observed. Open pores are not common on the PET surface of cans; we have recorded in other works average pore sizes between 0.2-10 µm by the BET method (16). Figure 3c shows this severe condition of surface defect. A higher pore density will adversely influence the surface functionality of the protective polymer, affecting its permeability and the corrosion protection of the base steel.

Figure 3d shows greater detail of particulate solid residues after lyophilization of the PET surface, indicative of the isolation of some compound from the surface. The SEM of its morphology closely resembles a compound with amyloid proteins (folding of proteins), that is self-generated proteins or peptides. The AFM of samples allowed us to measure the degree of adhesion of the muscle to the PET, as well as to evaluate the protective polymer surface. Figure 4 represents samples treated with urea solution to unfold the adhered proteins.

![AFM Image](image)

Figure 4. 3D characterization by AFM of PET surface with nanoportions of muscle.

The image depicts a 3D raised-relief image of the PET polymer by AFM with nanosized zones of salmon proteins adhered (protrusions) on the surface. It shows that the proteins are in intimate physical and chemical contact with the polymer, being capable of sticking after emptying the food can and the urea treatment.
In a segment 8 μm wide on the sample surface it is possible to detect the adhesion of salmon muscle proteins with heights reaching 135 nm. Figure 5 shows the results of the FT-IR and ATR analyses of the salmon muscle not attached to the PET, where its relevant spectral characteristics are depicted. This pattern allows for its detection in the PET coating when adhered, after the removal attempts either mechanical or with urea solution to eliminate the surface residues.

![Figure 5](image)

Figure 5. FT-IR spectra of canned muscle not adhered to PET polymer surface.

Figure 6 details the regions for comparison. The different spectral features between the attached and non-attached muscle to the PET (spectra 1, 2, and 3, respectively) occur mainly at 1030, 1202 and 1336 cm\(^{-1}\). There are differences in intensity for band ν(C–H) at 1744 cm\(^{-1}\), even though it appears in all parts of the muscle.

![Figure 6](image)

Figure 6. FT-IR spectral comparison between the PET coating in areas of salmon adhered, number 1; and muscle not adhered, numbers 2 and 3 to the polymer.

The protein infrared spectra of adhered muscle show wider bands than those of non-adhered salmon, and the band amide II with a 3D structure (17-18) at 1544 cm\(^{-1}\) shifts clearly to 1525 cm\(^{-1}\) in the salmon adhered to the coating. This is indicative of denaturation of the salmon muscle in contact with the PET-coated ECCS plate due to the pasteurization or of other physicochemical interaction during the canned.
It is interesting to relate this FT-IR finding with the amyloid protein characterized by SEM and resulting from the lyophilization of the muscle adhered to the PET coating, a feature indicative of some change in the protein structure in areas of adhesion. Figure 7 shows the Raman spectrum obtained from a point in the PET coated sample with remains of adhered muscle after the urea treatment in contrast with the spectrum from a muscle not attached. The coincidences and differences between spectra confirming the results obtained by FT-IR in the range 600-1800 cm⁻¹.

![Raman spectrum of muscle adhered and not adhered](image)

**Fig7. Raman spectra of muscle adhered and not adhered, number 4 and 5.**

The spectra in Figures 8 and 9, control PET coating, are compared with those observed in Figures 5, 6 and 7 relating to the adhesion of muscle. The band at 960 cm⁻¹ can be attributed to the symmetrical vibration of the phosphate group (Fig. 7, spectrum 4). The most likely source in the sample may be ascribed to the lipid content of the muscle, which consists in a high percentage of phospholipids.

![Raman spectral distribution of the virgin PET](image)

**Figure 8. Raman spectral distribution of the virgin PET at different positions (points 2, 3, 4, 5 and 6) that evidence the microstructural homogeneity at the molecular level.**

To validate the experimental characterization and to detect changes in the material,
the PET from the samples was analyzed by NMR, Fig. 9. The spectra differentiate the samples with and without salmon adhesion and the effect of urea in protein denaturation to disrupt the bond between the polymer and salmon flesh. The typical shifts of PET (numbers 1, 2, 3 and 4) can be identified by the spectra from the samples analyzed. The three samples produced similar carbon spectra showing the expected PET resonances, labeled and indicated in Fig. 9a. Methylene, carbonyl and aromatic peaks are visible in Figure 9b. Moreover, there is a broad and low resonance in the range 20-40 ppm, accounting usually for –CH2- peaks.

Figs.9a) Molecular structure of PET. The typical shifts identified by the NMR spectra; b) spectra. S01: shows no biological or organic change, virgin layers; S02: sample with urea to remove muscle; S03: salmon flesh attacked on the surface; no chemical attack.

Samples S02 and S03 show a small sharp peak at 32.8 ppm, possibly of bio-organic origin, which may be attributed to the changes of postmortem degradation of muscle. The presence of urea cannot be excluded, since its C=O peak could have been masked by the C=O of PET. Peaks marked (*) are spinning sidebands. Importantly, the application of urea to the PET coating was mainly focused on achieving the salmon muscle detachment by denaturation of the proteins, making urea compete for the hydrogen bonds that link the muscle to the polymer surface. The common practice is to apply hydrophobic products such as organosilanes (14).

From the results it is possible to say that the polymer was affected by urea and the muscle adhered to the surface as indicated by the FT-IR, Raman and NMR analyses. The sources of these alterations are associated with various factors related with quality control aspects, such as the degree of freshness of the salmon, the thermal treatment applied, the storage time, and surface quality of the protective polymer.

CONCLUSIONS

The heat treatment of containers was directly related with the loss of freshness of
salmon as indicated by the peroxide measurements, which since the first month surpassed the accepted levels, being a factor that favors salmon muscle adhesion. The application of urea solution was not capable of completely detaching the residues and revealed the presence of stronger surface bonds with the polymer. The muscle adhesion to the PET polymer depended on the storage time, it increased by the physicochemical interactions, and by the surface discontinuities on the protective polymer produced during the deep drawing manufacturing process of the container as evidenced by the SEM. The low-dimension AFM showed the adhesion with phase changes on the polymer surface due to the presence of denatured proteins deposited on the PET chains inferring changes of the surface energy. In turn, the FT-IR and Raman spectroscopy shows that the spectra of zones with adhered muscle or degraded surface were different for samples with and without urea treatment. Urea and salmon residues after treatment showed minor wavelength differences by Raman, implying changes or molecular rearrangements. The NMR analyses showed also the PET changes caused by the salmon adhesion and by the urea solution treatment to denature proteins, detecting the presence of bio-organic compounds that may be associated to the postmortem degradation of the salmon muscle, and which require further analyses for their identification.

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REFERENCES