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Application of fungal chitosan incorporated with pomegranate peel extract as edible coating for microbiological, chemical and sensorial quality enhancement of Nile tilapia fillets

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ABSTRACT

Fish are generous sources for providing man with his essential nutritional requirements, but the extreme susceptibility to quality deterioration hinders their optimal usage and storage. Natural derivatives are always the perfect alternatives for food preservation. The application of fungal chitosan (Ch), from *Aspergillus niger*, and pomegranate peel extract (PPE), in coating films for Nile tilapia (*Oreochromis niloticus*) fillets preservation and maintaining their microbiological, chemical and sensorial quality during cooled storage at 4 °C for 30 days, was investigated. Fish fillet were coated with Ch (2%) and combined Ch + PPE, at PPE percentages of 0.5, 1.0, 1.5 and 2.0%. Fillets coating resulted in sharp decrease of the entire microbial counts during storage; the increased concentrations from PPE strengthened coating film antimicrobial activity. Additionally, fillets coating could retard the chemical spoilage parameters increasing, i.e. nitrogen volatile base (TVB-N), peroxide value (PV) and reactive substances of thiobarbituric acid (TBARS), during storage period. The sensory evaluation indicated higher preferences for the odor, texture, color and overall quality of coated samples. Fish fillets coating with Ch and Ch + PPE could be recommended for shelf life extension and maintaining the microbiological, chemical and sensorial quality through the application of safe preservatives from natural origins.

1. Introduction

Fish were always regarded as the potential ideal sources to overcome food shortage worldwide due to their highly nutritional value and containment of most essential nutrients for man, but because of their perishable nature, fresh fish is also considered from the fastest susceptible foods to spoilage and quality loss [1]. Fish muscle spoilage is frequently resulted from diverse biological reactions arising from microorganisms metabolic activities and fish's own enzymes, to cause lipid oxidation and protein denaturation, ending by a very short shelf-life of fisheries and seafood products [2].

Nile tilapia (*Oreochromis niloticus*) is from the most prosperous fish that lives in tropical and subtropical waters; tilapia could grow in a wide range of salinity, temperature and oxygen concentration [3]. The global production of tilapia was tripled through the last decade due to its importance and quality that increased the acceptance and preference of human consumers to the whole fish or its products [4].

Chitosan is a derivative from chitin after its deacetylation; it was commercially produced from marine crustaceans' wastes, but during the last decade, chitosan could be promisingly produced from the grown mycelia of many fungal species, with comparable bioactivity to standard chitosan [5–7]. Fungal chitosans, due to their influential antimicrobial activity, were successfully applied for the preservation of many foodstuffs through the formation of edible coatings [8,9], or via blending with minced animal muscles [7,10].

The plant kingdom generously provided man with his needs from trustworthy, effective and precious compounds that could be very safely applied for his consumption and curing [11].

Pomegranate (*Punica granatum*) is a blessed fruit from the paradise that has many nutritional and pharmacological uses [12]. The peels of pomegranate were reported to have many bioactive phytochemicals that could be safely utilized for various human applications, e.g. gastrointestinal disorders treatment, as antimicrobial agents, as food disinfectant and biopreservatives, infectious diseases treatment . . .etc. [12–14].

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The exploration and application of natural derivatives for the formation of preservative coating films, which have high antimicrobial, antioxidant and quality enhancing properties, is highly desirable for the biopreservation and shelf life prolongation of fishery products [1,10].

Therefore, the present study intended to evaluate the application of natural derivatives, i.e. fungal chitosan and pomegranate peel extract, in coating films for Nile tilapia fillets preservation and maintaining their microbiological, chemical and sensorial quality during cooled storage.

2. Materials and methods

2.1. Preparation of fish fillet

Alive fresh water Nile tilapia fish (*Oreochromis niloticus*), with a 600–650 g average weight, were harvested from the aquaculture research farm and immediately transported, in a water path at 25 °C, to the fish processing plant, at distance of 15 m from the farm, in the Faculty of Aquatic and Fisheries Sciences- Kafrelsheikh University. Fish were manually decapitated, skinned, deboned and filleted. The average fillet pieces weight was about 100 ± 2 g.

2.2. Coating materials extraction and preparation

Grown mycelia, in potato dextrose broth, from *Aspergillus niger* (ATCC-16404), was used as a source for chitosan extraction [10]. The molecular weight of fungal chitosan was 29,000 Da and its deacetylation degree was 91%.

Pomegranate fruits (*Punica granatum* L.), were obtained from the research farm, GEBRI-USC. Fruits were manually washed and peeled; the peels were disinfected using sodium hypochlorite solution (5%), washed and dried by hot air for 48 h at 50 °C. Plant extraction was performed as previously described [15], using 70% ethanol as a solvent. Filter sterilized extract, with 20% concentration, was used for further experiments.

The coating solution was constituted according to Tayel et al. [9]; sterilized chitosan solution (2% w/v in 1% acetic acid) was complemented with 1% glycerol, as a plasticizer, and well stirred. Pomegranate peels extract (PPE) was added to the coating solution, with concentrations of 0.5, 1.0, 1.5 and 2.0%, aseptically homogenized and used for fish fillet coating.

2.3. Fish fillet treatment

Fish fillet samples were equally divided into six lots; each contained 25 pieces of 6 × 15 cm and thickness of ~2.5 cm. The first was the uncoated (control) group, and the remaining groups were treated with coating solution contained either chitosan alone (Ch) or chitosan with the addition of each PPE concentrations. For coating experiments, fish fillets were immersed for 1 min in the coating solution and allowed to drip the excess solution for 5 min then were re-immersed, for 30 s, in coating solution. Treated fillets were then allowed to drain on a metal net at 10 °C for 4 h, under aseptic conditions, and the entire samples were then stored at 4 ± 1 °C for the consequent quality assessment. Microbiological, chemical and sensory evaluations of samples were conducted, during storage period (30 days) at 5-day intervals, to assess the fish overall quality.

2.4. Analysis of proximate composition

The moisture content and crude ash were determined at 105 °C and 550 °C, respectively, using an electric oven, until recording a constant weight. Crude protein content was evaluated using Kjeldahl's method described by the Association of Official Analytical Chemists [16], whereas the lipid content was appraised according to Bligh and Dyer [17].

2.5. Microbiological analysis

The sample preparation for microbiological analysis was performed by depositing 10 g from each sample in 90 ml of sterilized saline solution (0.85% NaCl) and homogenizing them in a stomacher. Serial decimal dilutions were then made and plated onto appropriate microbiological media. The entire microbiological counts were performed in triplicate and their means were recorded as $\log_{10}$ CFU/g.

Different standard methods, for microbiological analyses, were applied to assess the effectiveness of edible coating treatment in protecting fish fillet from microbial contamination. The examined microbial groups and procedures were as follow, according to standardized test methods:

- Enumeration of aerobic colony count at 30 °C, ISO 4833:2003 [18].
- Enumeration of total microbial psychrotrophs, ISO 17410:2001 [19].
- Yeasts & molds enumeration, ISO 21527-1:2008 [20].
- Coliforms enumeration, ISO 4832:2006 [21].
- Enterobacteriaceae detection & enumeration, ISO 21528-2:2004 [22].
- *Escherichia coli* enumeration (β-glucuronidase-positive), ISO 16649-2:2001 [23].
- Enumeration of *Salmonella* spp., ISO/TS 6579-2:2012 [24].
- *Staphylococcus aureus* enumeration, ISO 6888-1:1999 [25].

2.6. Chemical analyses

The estimation of the total nitrogen volatile base value (TVB-N) was expressed as N (mg)/100 g fish, as described in the microdiffusion method by Malle and Poumeyrol [26]. The peroxide value (PV) was estimated from peroxide content in the total lipid extracts by the Pearson's method, while the reactive substances of thiobarbituric acid (TBARS) were colorimetrically determined according to the Porkony & Dieffenbacher's method [27]; the PV results were expressed as oxygen (meq)/lipid, whereas TBARS value was calculated from the absorbance at 530 nm and expressed as the equivalents mg of malonaldehyde/kg fish muscle.

2.7. Sensory evaluation

Treated raw fish fillets, and control samples, were sensory evaluated by a trained panelist team (10 members), using a 5 points scale, according to Ojagh et al. [1]. The examined sensory attributes and their grades were: odor (1, extremely off-odors/unacceptable; 5, extremely desired); texture (1, very soft; 5, compacted); color (1, extremely discolored; 5, no discoloration); and overall quality and acceptability (1, extremely unacceptable; 5, extremely desirable). Shelf-life benchmarks presumed that the sample rejection would arise with the decline of sensory attributes below 4.0.

3. Results

The mean proximate composition values (%) of fish fillets, including moisture content, ash, lipid and protein, were 79.6 ± 0.3, 0.78 ±0.03, 1.81 ± 0.05 and 17.56 ± 0.71, respectively.

The influence of fish fillet coating with chitosan and combined chitosan + PPE, at percentages of 0.5, 1.0, 1.5 and 2.0% from PPE, on the growth of total aerobic microbial count, psychrotrophic bacteria, enterobacteriaceae, coliform, *Salmonella*, *E. coli*, yeast & molds and *S. aureus*, during refrigerated storage (at 4 °C) for 30 days, is