PhD THESIS

Active packaging material to extend the shelf-life of fresh fish

(SUMMARY OF Ph.D. THESIS)

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SUMMARY

Introduction

Fish plays an important role in the nutritional and balanced diet, and for a long time, its consumption has been associated with certain health benefits. This product is an important source of omega-3 long-chain polyunsaturated proteins and fatty acids (such as eicosapentaenoic acid-EPA and docosahexaenoic acid-DHA). However, fish is a perishable food product due to the high level of free water (aw) activity, neutral pH, low connective tissue content and the presence of autolytic enzymes that cause rapid appearance of unpleasant odors. Therefore, appropriate treatment is needed to maintain the quality of this vulnerable product and to reduce its spoilage for as long as possible.

In recent years the interest in using active packaging materials for fish and fish products increased. The traditional role of food packaging continues to evolve in response to market needs. As defined by Regulation (EC) 450/2009, the active materials are " *materials that are intended to extend the shelf-life or to maintain or improve the condition of packaged food; they are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food*". Although extensive research is underway on active packaging technologies, many of them have not been yet implemented in commercial food packaging systems. Popularize their benefits in food applications will facilitate the successful development and market introduction.

Food loss and waste is a global challenge. According to the Food and Agriculture Organization of the United Nations (FAO), about a third of all food produced worldwide is lost or wasted during the "farm-to-consumer" circuit. The lack of modern processing technologies, poor storage and handling practices, long distances to markets and the absence of market infrastructure are frequently cited as causes of fish losses and waste in the agri-food chain.

The quality of the fish is affected by several factors, such as species, age, chemical composition, fishing area, season and nutritional status of the fish. However, the freshness of the fish is considered the most important quality parameter, as it is directly related to the sensory attributes perceived by consumers, such as appearance, texture, smell and taste. Freshness is essential for the quality of the product as this quality parameter is affected by several factors, such as rigor mortis, autolysis processes and post-mortem microbiological alteration.

Assessment of the quality of fish can be performed by instrumental and sensory methods. The physical, chemical and biochemical methods used to evaluate their quality are based on the determination of pH, electrical conductivity (EC), texture analysis; determination of total volatile basic nitrogen (TVB-N) and other volatile amines (such as ammonia, dimethylamine-DMA and trimethylamine-TMA), biogenic amines (such as histamine, putrescine, agmatine, cadaverine, tyramine, tryptamine and 2-phenylethylamine), nucleotide catabolites (such as inosine monophosphate-IMP, inosine-Ino, hypoxanthine-Hx), ethanol, peroxide index (PV) as well as thiobarbituric acid reactive substances (TBARS). In terms of sensory methods, the most widely used

methods for determining the freshness of fish have traditionally been applied to determine some attributes (including appearance, color, odor, aroma, texture and taste) perceived by the human senses.

This doctoral thesis addresses the problem of spoilage of fish during storage under refrigeration. Due to its biological composition, fresh fish is a very perishable food. When stored under normal refrigeration conditions, its shelf life is limited by enzymatic and microbiological alteration. Fresh fish stored on ice, under aerobic conditions, has a short shelf-life. Psychotrophic bacteria dominate the spoilage flora of fish kept on ice, *Pseudomonas* spp. and *Shewanella putrefaciens* being specific spoilage bacteria. Globally, fish losses due to spoilage account for about 10% (10 to 12 million tonnes per year) of total fisheries and aquaculture production. Post-harvest fish losses caused by microbial spoilage are a major problem for the fishing industry. Food waste caused by deteriorating fish quality leads to huge economic losses for fish traders and retailers.

A more efficient method of preserving fresh fish would reduce the amount of waste and costs in the supply chain. The research of this doctoral thesis is intended to find a technological solution capable of retarding the development of spoilage microorganisms in refrigerated fish during storage.

Research and objectives

The aim of this doctoral thesis was to develop an active packaging material that would extend the shelf-life of fresh fish. In this regard, the researches of the thesis had two objectives for which two independent studies were designed:

(01) Development of an antimicrobial film based on whey protein isolate and tarragon essential oil-UET.

This study aims a comparative characterization of whey protein isolate (WPI) films obtained from heat-treated (HT) and untreated (UNT) film-forming solutions, with different concentrations of UET incorporated [0.5 (F0), 1.0 (F1), 1.5 (F1.5), 2.0 (F2) and 2.5% (F2.5), m/m).

(02) Investigation of effectiveness of the developed film in maintaining the quality and extending the shelf life of refrigerated brook trout.

In the second study, films were prepared from thermally denaturated whey protein isolate solutions, with (WPIf + 2.5% UET) and without tarragon essential oil (WPIf). These were used to cover the brook trout samples, before being stored at 4°C for 15 days.

The results of this thesis were published in a review article (ISI journal with FI 2.330 - Coatings), two original research articles (ISI journals with FI 3,426 - Polymers, and FI 4.092 – Foods).

General methodology

This doctoral thesis comprises an extensive literature study, presented in the current state of art, and provides up-to-date information regarding the development of active packaging materials and their effectiveness when applied on fresh fish.

Personal contributions consisted of: (1) development of a whey protein isolate-based film incorporated with different amounts of tarragon essential oil (C-control film; F0.5-film with 0.5% tarragon essential oil; F1-film with 1% tarragon essential oil; F1.5-film with 1.5% tarragon essential oil; F2-film with 2% tarragon essential oil; F2.5-film with 2.5% tarragon essential oil), identifying the film with the best physical and mechanical properties, and (2) application of the most promising film on fresh fish to evaluate its effectiveness in retarding the enzymatic activity and inhibiting the development of microorganisms.

The first study investigated the effects of heat treatment and the addition of tarragon essential oil on physical and mechanical properties of films prepared with 5% whey protein isolate (WPI) and 5% glycerol. It was focused on (1) the characterization of the essential oil extracted from dried tarragon leaves by determining its total phenolic content, volatile constituents, antioxidant capacity and antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteritidis*, respectively *Listeria monocytogenes*, and (2) characterization of the films obtained by determining the thickness, moisture content, swelling degree, solubility in water, water vapor permeability (WVP), color, light transmittance, transparency, puncture resistance (PR) and puncture deformation (PD).

Heat treatment of the film-forming solution caused increases in thickness, moisture content, swelling degree, water vapor permeability (WVP), b^*-value, ΔE^*-value, transmittance values in the 200–300-nm region, transparency, and puncture resistance of the film, but decreases in water solubility, L^*-value, a^*-value, transmittance values in the 350–800-nm region, and puncture deformation. When incorporated with tarragon essential oil, heat-treated films have the potential to be used as antimicrobial food packaging. The addition of tarragon essential oil in film-forming solution caused increases in moisture content, solubility in water, WVP, a^*-value, b^*-value, ΔE^*-value, and transparency of the film; decreases in transmittance values in the range of 600–800 nm; and variations in swelling degree, L^*-value, transmittance values in the range of 300–550 nm, puncture resistance, and puncture deformation. Nevertheless, different tendencies were noticed in UNT (untreated) and HT (heat-treated) films with regards to transparency, light transmittance, puncture resistance, and puncture deformation.

The second research project studied the effectiveness of edible films prepared from thermally denatured whey protein isolate solutions, with (WPIf + 2.5% TEO) and without tarragon essential oil (WPIf) on the quality and the shelf-life of brook trout samples during storage at 4°C. Fish samples (covered and uncovered) were evaluated periodically (every 3 days) during the 15 days of storage for physico-chemical [pH, electrical conductivity (EC), total volatile basic nitrogen (TVB-N), thiobarbituric acid reactive substances (TBARS)], microbiological [total viabile count (TVC), psychrotrophic count (PTC), lactic acid bacteria (LAB), H_2S-producing bacteria] and sensorial properties (color discoloration; odor; overall acceptability). The WPIf+2.5%TEO has proven enhanced quality preservation effects compared to WPIf by

showing lower values for physicochemical parameters, lower microbial loads, and higher sensory scores in the fish sample. All these effects have led to an extension of the sample's shelf-life.

General conclusions and recommendations

Fish represent one of the most-traded segments of the world food sector. Therefore, there is a great demand for the packaging of this good. Industrial production and commercialization of antimicrobial packaging materials for fresh fish could be an exploitable sector by the packaging industry.

According to the first study, focused on achieving the first goal, the essential oil of tarragon has shown to possess both antioxidant and antibacterial activities. The WPI-based edible film was affected by heat treatment of the filmogenic solution. HT film showed improved physical and mechanical properties, being more transparent, less soluble in water, more light protective in the range of 350–800 nm, and more resistant to mechanical penetration. Therefore, it is more suitable for certain end-use applications.

The second objective concerning the effectiveness of the film in maintaining the quality and extending the shelf life of brook trout, The WPI-based film incorporated with 2.5% tarragon essential oil has proven to be effective during storage at 4°C. The tarragon essential oil from its matrix has caused delays of chemical reactions and microorganisms growth in the fish sample, leading to retention of desirable sensory attributes for a longer period. At the same time, due to the low level of incorporation tarragon essential oil didn't negatively affect the organoleptic properties of the fish sample. In summary, this active packaging material has good industrial application potential to extend the shelf-life of fresh fish.

The whey protein isolate-based film incorporated with tarragon essential oil developed following the research undertaken by this doctoral thesis has a high production potential at the industrial level. To obtain the antimicrobial and antioxidant effect demonstrated here, it is recommended to add a minimum of 2.5 g of tarragon essential oil to 100 g of film-forming solution. It is important to note, however, that higher concentrations of essential oil in film may impart the taste and smell of tarragon to the product to which it is applied. Given the good barrier properties in the field of visible light, it could be successfully used for packaging products with a high lipid content. However, the high water solubility of the film limits its application to food matrices with high moisture content.

Originality and personal contributions

The results of this research can be considered useful both for fish processors and traders as well as for food packaging producers.

This doctoral thesis includes an extensive literature study on films and coatings capable of inhibiting the enzymatic activity and development of microorganisms in fresh fish, providing up-to-date information on the development of active packaging materials.

The comparative characterization of the films based on whey protein isolate obtained from untreated and heat-treated film-forming solutions, with different concentrations of incorporated tarragon essential oil, showed that the heat treatment of the film-forming solution considerably improves the physicochemical and mechanical properties of the film.

Investigation of the effectiveness of the film based on protein isolate incorporated with 2.5% tarragon essential oil in maintaining the quality of refrigerated brook trout showed an extension of the shelf-life by 3 days. At the industrial level, this means a significant reduction in fish losses and waste, therefore a major impact on production costs.