LWT - Food Science and Technology xxx (2014) 1–7

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Characterization of fish protein films incorporated with essential oils of clove, garlic and origanum: Physical, antioxidant and antibacterial properties

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ARTICLE INFO

Article history:
Received 9 February 2012
Received in revised form
16 May 2012
Accepted 12 April 2014
Available online xxx

Keywords:
Hake muscle proteins
Biodegradable films
Active packaging
Bioactive properties
Physical properties

ABSTRACT

Consumers are demanding high quality foods using environmental-friendly packaging systems and natural preservatives, like edible/biodegradable films or coatings with bioactive properties. In this context, films prepared with Cape hake by-products proteins were combined with three essential oils (garlic, clove, and origanum) and characterized in terms of physical, mechanical, antioxidant, and antibacterial properties. Control films, without essential oils, were homogeneous, transparent, slightly yellow, and mechanically resistant. The incorporation of garlic, clove, and origanum essential oils in this film significantly decreased thickness, water solubility, breaking force and elongation, whereas increased the free radical scavenging activity of films. Particularly, clove films showed lower water vapour permeability than control films, and the highest antibacterial activity (against *Shewanella putrefaciens*). Garlic films were the most yellowish and had the highest antioxidant activity. Finally, origanum films were rather similar to the control, particularly in colour, transparency and reducing power. In conclusion, proteins recovered from Cape hake by-products combined with essential oils have adequate properties with applicability in new preservation food packaging systems.

1. Introduction

In recent years, edible and biodegradable films and coatings prepared with proteins, polysaccharides, and lipids have received increasing attention. Some examples of their commercial applications are: collagen casings for sausages, confectioner's glaze made from shellac, corn zein and gelatin-based coatings for pharmaceuticals, and waxes on various fruits (Gennadios, Hanna, & Kurth, 1997). Factors contributing to the interest in films and coatings development include: consumers demand for high quality foods and natural preservatives; food processors' needs for new storage techniques; environmental concerns over disposal of nonrenewable food packaging materials; and opportunities for creating new market outlets for under-utilized film-forming ingredients (Gennadios et al., 1997).

Edible and biodegradable films must meet a number of specific functional requirements, including colour, appearance, barrier properties, mechanical, and rheological characteristics, which are dependent on the type of material used and type of application (Guilbert, Gontard, & Gorris, 1996). Films primarily composed of proteins usually have suitable mechanical and optical properties, but show poor water vapour barrier properties because of their hydrophilic nature (Guilbert et al., 1996). Active compounds like essential oils can be added to films to improve their functional properties, such as water vapour permeability, as well as antimicrobial and antioxidant properties (García, Martino, & Zaritzky, 2000; Oussalah, Caillet, Salmiéri, Saucier, & Lacroix, 2004; Seydim & Sarikus, 2006).

In the seafood processing industry, a substantial amount of by-products are generated that can be used to recover proteins to prepare films and restructured seafood products. Protein films have been successfully prepared using fish proteins, including

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URL: http://www.ipma.pt/en/investigacao/index.jsp

http://dx.doi.org/10.1016/j.lwt.2014.04.024
0023-6438/

myofibrillar and sarcoplasmic proteins (e.g. Benjakul, Artharn, & Prodpran, 2008; Cuq, Gontard, & Guilbert, 1997). Essential oils of aromatic plants like clove, garlic, and origanum show strong antimicrobial and antioxidant properties (e.g. Benkeblia, 2004; Bounatirou et al., 2007; Misharina & Samusenko, 2008). Therefore, incorporation of essential oils in films can improve functionalities of films. In this context, the aim of the current work was to study the physical, mechanical, antioxidant, and antibacterial properties of films prepared with fish proteins recovered from Cape hake by-products and essential oils from aromatic plants (clove, garlic and origanum).

2. Material and methods

2.1. Chemicals

Essential oils of clove from *Eugenia* spp. (C8392; lot 116K1861; buds distillation; origin: Indonesia; Sigma–Aldrich), garlic (biological source not specified; W250317; lot 04712 EE-148; bulbs synthetic organic material; origin: Mexico; Sigma–Aldrich), and origanum from *Thymus capitatus* (W282812; lot 21417CL-214; dried flowering herb steam distillation; origin: Spain; Sigma–Aldrich), glycerol, α,α-diphenyl-β-picrylhydrazyl (DPPH), sodium bromide, trizma hydrochloride (Tris–HCl), sodium azide, and potassium hexacyanoferrate III were obtained from Sigma–Aldrich (Sigma Aldrich Chemie GmbH, Steinheim, Germany); phosphate buffer, ferric chloride, trichloroacetic acid, and ascorbic acid were purchased from Fluka (Buchs, Germany); tryptic soy agar and plate count agar from Merck (Darmstadt, Germany); brain heart infusion broth and maximum recovery diluent from Oxoid (Basingstoke, Hampshire, England); ethylene diamine tetracetic acid (EDTA calibration sample) from LECO (LECO corporation, St. Joseph, USA); ethanol had a purity grade of 99% and the water used was Milli-Q purified and distilled.

2.2. Film preparation

Fish proteins were recovered from frozen by-products resulting from the portioning (fish 'sawdust' and cut offs) of Cape hake (*Merluccius capensis*) by alkaline solubilisation following a methodology previously described (Batista, Pires, Nelhas, & Godinho, 2006). Recovered proteins (90 g/100 g protein content) were freeze-dried, packed under vacuum conditions, and stored at −30 °C until utilisation.

Hake protein powder (30 g) was added to water (2 L) and homogenized (5000 rpm, 1 min) using a Polytron homogenizer. The pH was adjusted to 11 with 1 mol/L sodium hydroxide and mechanically stirred, followed by centrifugation (10,000 *g*, 15 min, 5 °C) to remove insoluble material. The protein concentration of the soluble fraction was determined (see methodology below), glycerol was added at 59 g/100 g of protein, and the mixture was gently stirred (30 min). Afterwards, the essential oils of clove, garlic, and origanum were added to protein film forming solutions, emulsified in a Polytron homogenizer (13,500 rpm, 2 min) and the emulsion was degassed under vacuum (20 min) and casted on plates to obtain films with 4 mg of protein and 1 μL of essential oil per cm^2 when present. Control films had the same amount of protein per surface area. The plates were placed on levelled surfaces to obtain films with homogeneous thickness, dried in a ventilated drying chamber (30 °C, 50% relative humidity, 20 h), peeled off, and stored at room temperature at 57% relative humidity in desiccators with saturated solutions of sodium bromide. Four types of films were prepared (treatments): a) without essential oils; b) with clove essential oil; c) with garlic essential oil; and d) with origanum essential oil.

The protein content of the soluble fraction was determined using a FP-528 LECO nitrogen analyser (LECO, St. Joseph, USA), calibrated with EDTA (carbon − 41.07 ± 0.17, hydrogen − 5.55 ± 0.02, nitrogen − 9.57 ± 0.03), according to the Dumas method (Saint-Denis & Goupy, 2004). All determinations were performed in triplicate.

2.3. Colour

Films colour parameters (L^*, a^* and b^*) were measured ($n = 6$ for each film) using a colorimeter (CR-410, Konica Minolta Camera, Co, Ozaka, Japan) with a measure cell opening of 50 mm. For measurements, films were placed on a white standard plate. Chroma (C^*), hue (h^*) and whiteness (W) were estimated using the following equations, accordingly to Atarés, Bonilla, and Chiralt (2010):

$$C^* = \sqrt{(a^*)^2 + (b^*)^2}$$

$$h^* = arctg\frac{b^*}{a^*}$$

$$W = 100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2}$$

Colour was expressed as the difference of colour (ΔE^*) in the different parameters, accordingly to the equation (García & Sobral, 2005):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^*, Δa^* and Δb^* correspond to the variation between the colour parameter of film and that of the white standard plate used as background.

2.4. Transparency

The absorbance of films (600 nm; spectrophotometer UNICAM Uv/Vis UV2; ATI-UNICAM, Cambridge, United Kingdom) and film thickness (mm; in the same location of absorbance readings) were measured ($n = 5$ for each film) in order to address film transparency using the following equation:

$$\text{Transparency} = \frac{A_{600}}{x}$$

where A_{600} is the absorbance of films and x is the film thickness (mm) (Shiku, Hamaguchi, & Tanaka, 2003). Films less transparent show higher transparency values.

2.5. Opacity

Opacity was measured accordingly to the Hunterlab method (Anonymous, 2008) using the same equipment of colour measurement. The opacity (%) of films was calculated with reflectance measurements of each film ($n = 6$) with standard black and white backing plates, accordingly to the following equation:

$$\text{Opacity} = \frac{Y_{\text{black backing}}}{Y_{\text{white backing}}} \times 100$$

where Y is the CIE tristimulus value of the film with the black ($Y_{\text{black backing}}$) or white ($Y_{\text{white backing}}$) backing plates.

Please cite this article in press as: Teixeira, B., et al., Characterization of fish protein films incorporated with essential oils of clove, garlic and origanum: Physical, antioxidant and antibacterial properties, *LWT - Food Science and Technology* (2014), http://dx.doi.org/10.1016/j.lwt.2014.04.024