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Effect of microalgae addition on active biodegradable starch film

Mariel Carissimi, Simone Hickmann Flôres, Rosane Rech*

Institute of Food Science and Technology, Federal University of Rio Grande do Sul (UFRGS), Av. Bento Gonçalves, 9500, Agronomia, 91501-970 Porto Alegre, Rio Grande do Sul, Brazil

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ABSTRACT

Despite its great importance, the traditional packaging is produced from non-renewable and non-biodegradable sources, causing several environmental problems. Therefore, biodegradable packaging has been attracting attention and interest from researchers and industries worldwide. This work developed biodegradable films with antioxidant properties, using cassava starch combined with biomass or biomass extract of microalgae *Heterochlorella luteoviridis* and *Dunaliella tertiolecta*. The films were characterized by their physicochemical, mechanical, barrier, optical and antioxidant properties. The film with the best characteristics was used as salmon packaging aiming protection against lipid oxidation. In general, the addition of biomass increased films' solubility, biodegradability and opacity, while the films with the addition of biomass extract showed the lowest values of these parameters. The addition of biomass increased films' elongation, rupture, and reduced the tensile strength and Young's modulus. Conversely, the addition of microalgae extract caused the inverse effect on these properties. All films with microalgae showed higher antioxidant activity, evaluated by peroxide index, compared to the control ones. The film containing 2.0% of *H. luteoviridis* extract presented the lowest water vapor permeability and good mechanical characteristics, and was applied in salmon packaging. The selected packaging decreased fish moisture and delayed lipid oxidation evaluated by TBARS index. The results showed incorporation of microalgae biomass resulted in biodegradable and highly soluble films, making it difficult to apply in moist foods. The films containing microalgae extracts were suitable to be used in foods with high water content.

1. Introduction

Packaging plays an important role in food preservation as it acts as a barrier against physical impacts, prevents contamination, increases shelf life, and contains important information about packaged food. However, most of the packaging used today is derived from non-renewable sources, such as petroleum. Various polymers have been used as packaging materials for many years because of their economic and technological advantages. These synthetic polymers are hydrophobic, do not undergo the action of microorganisms and take many years to decompose, thus generating a large volume of solid waste, causing serious environmental problems [1,2].

Population and economic growth in most Western countries have resulted in a large increase in waste production. In Brazil, approximately 218 thousand tons of urban solid waste is collected per day, of which only 60% are destined for landfills or recycling [3]. In this context, several countries are recognizing the need to reduce the amount of wasted and discarded plastics, aiming to recycle this material through initiatives that combine practicality and economy.

The food industry is the largest packaging user. So, several studies are being carried out to meet the growing demand related to sustainability and environmental safety of the food industry. These studies involve the development of new materials to produce packaging that degrade faster. The use of vegetable raw materials to produce biodegradable packaging is a favorable alternative. Biopolymers as polysaccharides, proteins, and lipids are highly biodegradable and decompose easily into inorganic by-products like carbon dioxide (CO_2) and water (H_2O). They can be used in combination or separately [1,2]. Starch stands out as being biodegradable, renewable, low costly and widely available worldwide.

Packaging made from biodegradable macromolecules, also called films, are leveraging new research to create packaging that can act as a barrier against the exchange of gases and moisture between food and the environment, or as an antioxidative protection. Usually, food packages are inert barriers, without any interaction with the food, presenting only product protection. However, research studies are focusing on the development of active biodegradable films: a system where an interaction between packaging and food takes place. Such

* Corresponding author.
E-mail address: rrech@ufrgs.br (R. Rech).

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interaction may occur due to the addition of different compounds that delay or decrease the oxidation process in packaged products, such as antioxidants or natural dyes, which retard oxidation due to the light barrier [4,5].

Some species of microalgae can produce compounds with high added value, such as pigments, essential fatty acids, vitamins, and minerals. Due to the presence of biologically active compounds with antioxidant, anti-inflammatory and coloring effects in its biomass, microalgae is already being market as a food supplement, in addition to being used to increase the nutritional content of conventional foods [6]. The species *Heterochlorella luteoviridis* and *Dunaliella tertiolecta* have a good concentration of carotenoids, such as lutein, β-carotene and zeaxanthin, and a considerable content of essential fatty acids, ω3 and ω6 [7,8].

This work studied the effects of microalgae biomass or extract on biodegradable films using cassava starch as a polymeric matrix aiming the development of an active and functional film. The films were characterized by their physicochemical, mechanical and optical characteristics, as well as solubility and biodegradability. Additionally, the protective effect on lipid oxidation was assessed using an accelerated oxidation test. Finally, the film with the best characteristics was evaluated as salmon packaging.

2. Materials and methods

2.1. Microalgae and culture

This study used the microalgae *Heterochlorella luteoviridis* BE002, and *Dunaliella tertiolecta* BE003, from Bioprocess Engineering in Microalgae Laboratory of Federal University of Rio Grande do Sul (Porto Alegre, Brazil). The cells were cultured in photobioreactors as described previously [9,10]. At the end of the culture, the contents of the photobioreactors were centrifuged (10,000 ×*g* for 10 min), the culture medium was discarded, and the biomass was freeze-dried and stored (−18 °C) for subsequent analysis and utilization. A detailed composition of *H. luteoviridis* and *D. tertiolecta* biomasses can be found at Diprat et al. [8].

2.2. Microalgae extract

The extract was obtained by alcoholic extraction with absolute ethanol. The dry biomass was hydrated with distilled water (24 h, 4 °C, dark) and macerated with the addition of absolute ethanol and centrifuged (3000 ×*g* for 10 min). The extract was separated, and the precipitate was returned to the maceration step following the same procedure. The process was repeated until a white biomass was obtained. The solvent was separated in a rotary evaporator (30 °C), and the remaining extract was freeze-dried.

2.3. Film preparation

The films were prepared and analyzed at the Bioactive Compound Laboratory of the Institute of Food Science and Technology (ICTA). Commercial cassava starch (Yoki), glycerol plasticizer (Synth) and water were used for the formulation of the films. The microalgae applied to the films were cultured as described above.

All the films were prepared by the casting method, as described in the following paragraphs. After some preliminary tests, the contents of starch and plasticizer were fixed, and tests were performed to determine the microalgae biomass ones. The drying time and temperature were determined with previous tests, using different time/temperature combinations until reaching an ideal binomial.

The biomass films were prepared from a film-forming solution of 4% cassava starch (4 g starch in 100 g solution) maintained in a water bath with mechanical stirring for 15 min at 80 °C until complete gelatinization. The solution was then cooled to 40 °C, and the plasticizer (glycerol, 1% w/w film-forming solution) and dry biomass were added maintaining the stirring for another 30 min. The microalgae concentrations (mass fraction) were 0% (control CB) and 0.5%, 1.0%, and 2.0% (w/w) of the microalgae *H. luteoviridis* (BH) or *D. tertiolecta* (BD) biomass.

For the films with biomass extracts of *H. luteoviridis* or *D. tertiolecta,* a film-forming solution was also prepared with a suspension of 4% of cassava starch as described for the biomass films. After cooling the solution to 40 °C, the plasticizer (glycerol, 1% w/w film-forming solution) and polysorbate 80 (Tween 80, 0.1% w/w film-forming solution) were added (EC control) and along with 0.12%, 0.25% and 0.5% (w/w) of the microalgae *H. luteoviridis* (EH) or *D. tertiolecta* (ED) extract, maintaining the stirring for a further 30 min. Preliminary tests showed the need of using an emulsifier (Tween 80) to help the homogenization of the film-forming solutions using the extracts.

The extract amounts of 0.12%, 0.25%, and 0.5% were determined using Eq. (1). The extract yield of both microalgae that was 25%.

$$Extract\ (\%) = \frac{Biomass\ (\%) \times Extract\ yield\ (\%)}{100} \quad (1)$$

Each formulation was evenly poured into acrylic plates using 0.25 g/cm^2 and dried using forced air circulation drier (DeLeo, B5AFD, Brazil) at 35 °C for 16 h.

After drying, the films were kept in a desiccator with controlled relative humidity (58%, 25 °C) for 48 h. The films were then removed from the plates and prepared for the characterization analyzes through mechanical, physicochemical, barrier and optical properties, as well as the evaluation of the protection against lipid oxidation and biodegradability.

2.4. Film characterization: mechanical and physicochemical analysis

The mechanical properties of tensile strength, elongation at break and Young's modulus were determined using a texturometer TA-XT2 (Stable Micro Systems, UK), according to the ASTM standard method D882-12, at 25 °C, initial claws separation of 50 mm, and probe velocity of 0.8 mm s^{-1} [11]. The films were cut into 10 test pieces (80 mm × 10 mm) conditioned for two days at room temperature and 58% relative humidity before measurement.

The films' thickness was measured by a digital micrometer (model IP40, Digimess, Brazil) with a scale from 0 mm to 25 mm and an accuracy of 0.001 mm. Results represent the mean of 5 measurements randomly performed over each evaluated sample.

The moisture content was determined by the gravimetric method according to AOAC [12]. The water solubility analysis was performed sequentially after the moisture analysis, due to the need of a free-moisture sample, according to the methodology described by Colla et al. [13] using a reduced amount of distilled water in the solubilization step: after weighing, the films samples from the moisture analysis were reconditioned in metallic containers with 30 mL of distilled water and put under slow agitation for 24 h at 25 °C (model NT145, Nova Técnica, Brazil). After this time, the water content in the vessel was filtered using a previously weighted filter and dried (105 °C for 24 h). The mass difference was used to calculate the solubility. The analysis was performed in triplicate.

The evaluation of the water vapor permeability (WVP) of films was based on ASTM standard E96 [14] and the procedure described by Talja et al. [15], however using different size of permeation cells and a smaller amount of $CaCl_2$: the films were fixed onto aluminum permeation cells (inner diameter: 63 mm; height: 25 mm) containing 1,5 g of granular anhydrous $CaCl_2$. The temperature was maintained at 25 °C to achieve a relative humidity gradient of 0 to 75%. The progress of mass gain was followed gravimetrically at specified times (1 h, 2 h, 12 h, and 24 h).