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PHYSICOCHEMICAL PROPERTIES OF ALGINATE FILMS ADDED WITH *Chlorella vulgaris* BIOMASS

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ABSTRACT

Microalgae are versatile microorganisms widely distributed in aquatic and terrestrial environments. They are rich sources of high-added-value biomolecules such as lipids, proteins, carbohydrates, pigments, vitamins, and sterols with known nutritional and health benefits. These can be used as functional food and feed ingredients, cosmetics, pharmaceuticals, agricultural products, and biofuels. The association of microalgal biomass with biopolymers to develop bio-based materials, like alginate films and coatings, represents a potential use for microalgae that has been scarcely explored. Microalgal biomass and its extracts are promising as reinforcement fillers or additives for biopolymeric materials, as they can add new functionalities such as antimicrobial and antioxidant activity. *Chlorella vulgaris* is a green microalga that can grow under autotrophic, heterotrophic, and mixotrophic conditions producing biomass and bioactive compounds for several applications. This work aimed to characterize alginate films added with *C. vulgaris* biomass. The films showed a continuous polymer matrix and the characteristic green color of the microalgae with lowered light transmittance. The biomass cell disruption process was crucial to promote intermolecular interaction between polymer chains and microalgae components, enhancing film mechanical and water barrier properties. Alginate films added with *C. vulgaris* biomass show good perspectives for application as active food packaging or in agriculture.

Keywords: Biopolymers, Active packaging, Alginate, Microalgae.

1 INTRODUCTION

Recent studies have focused on the biotechnological potential of microalgae, which are considered strategic resources as they do not require large areas like arable crops, avoiding competition with land use. The immense biodiversity and variability in the composition of microalgal biomass, genetic improvement, and large-scale cultivation technologies have enabled the commercial use of various microalgae species.[1] Besides, studies have demonstrated the viability of cultivating microalgae in agro-industrial effluents, promoting nutrient recovery and pollutant removal, and being integrated as a biorefinery.[2] The cultivation of microalgae can be explored for biomass production to be added to several products and also for extracting bioactive compounds. Proteins, lipids, carbohydrates, pigments, vitamins, sterols, biopolymers, and other biomolecules derived from microalgae can be used in various industrial segments, such as food, feed, bioenergy, pharmaceutical, cosmetics, and agriculture.[2,3]

Chlorella vulgaris is a green microalga belonging to the *Chlorellaceae* family. Its cells are spherical or ellipsoid measuring 2-10 µm. It can grow under autotrophic, heterotrophic, and mixotrophic conditions, and the growth and nutritional composition are influenced by environmental conditions such as the composition of the culture medium, pH, salinity, temperature, and light intensity/period.[4] *C. vulgaris* contains 42-58% protein, 5-40% lipids, 12-55% carbohydrates, vitamins (B complex, thiamine, C, D, E, and K), and minerals such as iron, potassium, calcium, phosphorus, and magnesium. It is rich in carotenoids (β-carotene, lutein, astaxanthin, canthaxanthin) and chlorophylls (a and b) whose concentration depends on the growing conditions.[5] Nonetheless, the cell wall integrity may significantly limit nutrient availability, since the structures of many microalgae species are covered with resistant multiple layers which limit the release of cellular constituents. The cell wall represents a natural barrier that results in the low bioavailability of intracellular molecules. Chemical, physical, and biological methods are employed, sometimes in combination, but an optimal method has not been established.[6] Physical methods have been used for cell wall disruption, as they potentially avoid chemical contamination and preserve most of the functionality of the intracellular biomolecules.[7]

Another potential market for the application of microalgal biomass, yet to be explored, is the development of renewable materials for food, pharmaceutical, and agricultural applications. Microalgal biomass as a raw material, additive, or reinforcement for biodegradable films can add functionalities to the materials, as it is a source of biopolymers and bioactive molecules.[8,9] Alginate is an anionic polysaccharide extracted from several species of brown algae. Due to its ability to irreversibly crosslink with divalent ions, such as calcium, alginate forms strong films with good mechanical and barrier properties.[10] Packaging usually acts as an inert barrier to gas and moisture exchanges between product and environment, however, many studies have been focusing on the development of active films, for example, by the addition of different compounds, such as antioxidants or natural pigments, that delay or decrease the oxidation process of the product. This work aims to evaluate the effect *C. vulgaris* biomass on the properties of alginate films intending food and agricultural applications.

2 MATERIAL & METHODS

Medium-viscosity sodium alginate (Sigma Aldrich, USA), calcium chloride dihydrate ($CaCl_2.2H_2O$) (Synth, Brazil), and glycerol P.A. (Synth, Brazil). All other reagents were of analytical grade. *C. vulgaris,* from the Freshwater Microalgae Culture Collection

(CCMA/UFSCar), was maintained in BG 11 medium in 500 mL Erlenmeyer flasks under stirring and with 12-hour photoperiod (light/dark) until the cell concentration reached approximately 1 g L^{-1}.

Blank alginate films were prepared by casting in a two-stage procedure.[11] Glycerol (0.6 g/g alginate) and alginate (1.5 % w/v) were added to distilled water (370 mL) under constant mechanical stirring (1000 rpm) for 1 h. As a pre-crosslinking stage, 30 mL $CaCl_2.2H_2O$ 0.8 % w/v was slowly added to the solution. The film-forming solution was poured into polypropylene Petri dishes (d=14 cm) and dried at 40ºC for 20h in a circulation oven. Films were crosslinked by immersion in 50 mL of $CaCl_2.2H_2O$ (5% w/v) and glycerol (5% w/v) for 20 minutes and then dried at 25°C for 3h. To prepare alginate films with microalgal biomass, *C.vulgaris* suspension was centrifugated at 1844 g for 20 min and resuspended in distilled water. Half of the suspension volume was submitted to cell disruption (55ºC, 15 minutes, 1600 rpm, with glass beads in a cell disruptor TE-099 - TECNAL). The biomass (0.1 g dry mass/100 mL) was added to the film-forming solution before pre-crosslinking and followed the same procedure described for blank alginate films. The films were designated as ALG (blank alginate), ALG/B (with non-disrupted biomass), and ALG/DB (with disrupted biomass).

Films were characterized according to the following: thickness (δ, digital micrometer), morphology (scanning electron microscopy, SEM), moisture content (W, 105°C/24h), water solubility (S, immersion in water for 24h followed by drying at 105°C/24h), mechanical properties (TS and %E)[12], water vapor permeability (WVP)[13], swelling degree (SD, in water 1h at 25°C, gravimetric), and light transmittance (UV-vis spectrophotometry), and opacity (O = -log T_{600}/δ). Analysis of variance (ANOVA) and the Scott-Knott test at a significance level of 5% ($p<0.05$) was performed to detect significant differences between averages using the software R Studio 3.2.4.

3 RESULTS & DISCUSSION

The visual aspect and the surface and cross-section morphology of the films are shown in Figure 1 A, all films exhibit continuous, homogeneous, and flexible matrices. ALG films were visibly transparent, while films with microalgae biomass (ALG/B and ALG/DB) showed a characteristic green color of *C.vulgaris*. The physical and functional properties of materials are influenced by surface morphology and microstructural organization of the film components. SEM micrographs indicate that all films had a continuous surface with no holes or cracks. Blank ALG films showed a smooth appearance, consistent with the formation of a compact arrangement of polymer chains, whereas ALG/B showed a coarse aspect and small precipitates in the film surface suggesting that the polymer chain packing was changed by microalgae biomass. The same was observed by Deshmukh et al.[14] for chitosan films with defatted *Chlorella* biomass. The surface of ALG/DB was smoother compared to ALG/B, indicating a positive effect of the cell disruption pre-treatment. Fabra et al.[8] also reported changes in the morphology of starch films added with microalgal biomass pretreated with ultrasound. The authors attributed this effect to a greater plasticization of the starch matrix promoted by the components extracted from the microalgae through the ultrasound treatment.

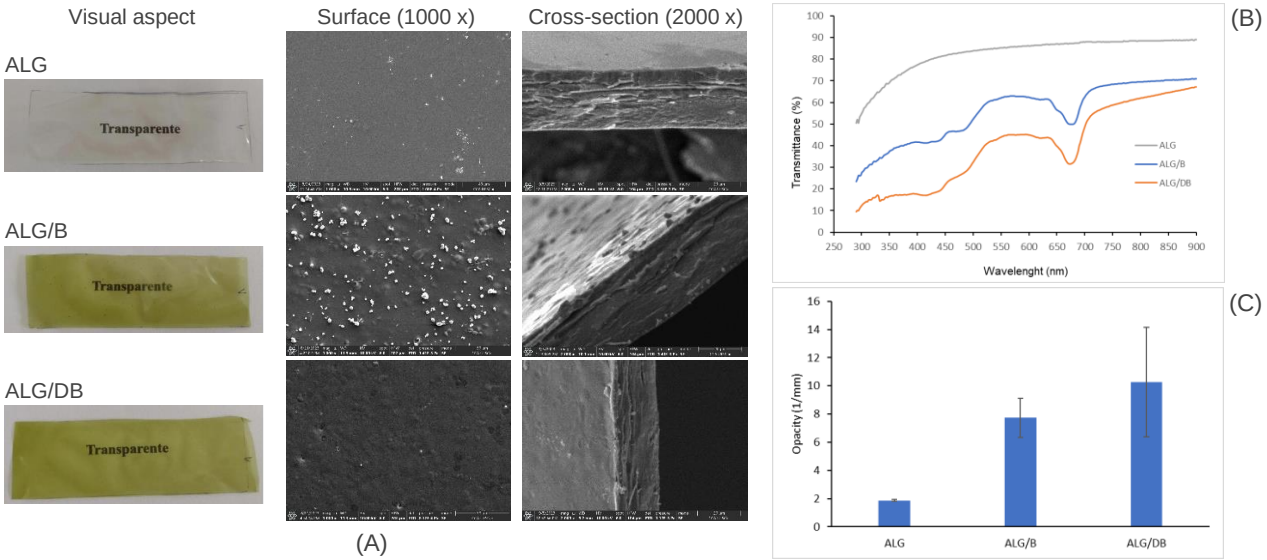

Figure 1 Visual aspect and SEM micrographs (A), UV-vis transmittance spectra (B), and opacity (C) of blank alginate films (ALG), alginate films with *C. vulgaris* biomass (ALG/B), and alginate films with disrupted *C. vulgaris* biomass (ALG/B).

The presence of *C. vulgaris* biomass decreased light transmittance and increased opacity at 600 nm of alginate films (Figure 1 B and C) denoting the ability of microalgal biomass to improve the film's light barrier property and therefore provide protection for light-sensitive food components. UV-blocking property increases its efficiency as food packaging material in limiting UV light-induced lipid oxidation in food systems.[14] In agriculture applications, such as mulching, light transmittance properties affect crop growth, quality, and physiological features. The transmittance at 450 nm has been used to characterize mulching films since blue light has the greatest impact on plant photosynthesis, as it is the most absorbed light by chlorophyll and carotenoids.[15]

Table 1 shows the physicochemical properties of films. Film thickness depends on formation technique, composition, and interaction between film components. The incorporation of non-disrupted microalgae biomass (ALG/B) increased film thickness compared to the control (ALG), However, there was no significant difference when the disruption process was performed (ALG/DB). This can suggest that the release of cell components contributed to a more compact polymer chain arrangement as observed by SEM micrographs. The moisture content of films varied from 15 to 20%, which are typical values of polysaccharide films.[11] ALG/DB exhibited the highest moisture content, probably due to the plasticizing effect of microalgal cell components interacting with the alginate matrix.

Table 1 Thickness, moisture content, solubility, swelling degree, water vapor permeability, tensile strength, and elongation at break of blank alginate films (ALG), alginate films with *C. vulgaris* biomass (ALG/B), and alginate films with disrupted *C. vulgaris* biomass (ALG/B).

Film	δ ** (mm)	W* (%)	S*(g /100g)	SD* (gH_2O/100g)	WVP* (g.mm/ m^2.day.kPa)	TS** (MPa)	E** (%)
ALG	0.069 ± 0.002^b	15.65 ± 1.09^b	6.14 ± 0.27^b	79.25 ± 7.00^a	9.78 ± 0.29^a	31.26 ± 4.47^b	3.06 ± 0.95^b
ALG/B	0.078 ± 0.003^a	17.32 ± 0.46^b	18.10 ± 2.97^a	74.69 ± 6.81^a	9.25 ± 0.45^a	19.36 ± 3.60^c	3.34 ± 1.06^b
ALG/DB	0.064 ± 0.004^b	20.10 ± 0.87^a	8.33 ± 0.73^b	80.69 ± 1.76^a	7.20 ± 0.55^b	47.85 ± 8.16^a	8.48 ± 2.13^a

*average ± SD of three experimental measurements. **Average ± SD of ten experimental measurements. Different letters in the same column indicate significant differences by the Scott-Knott test ($p<0.05$).

The water solubility of ALG/B films was significantly higher compared to the control (ALG) and ALG/DB, indicating less intense intermolecular interactions in the film matrix. This can also explain the lower swelling degree, despite not being significant, observed for ALG/B which could have been affected by mass loss during water immersion. WVP relates to the ability of the material to interact with water molecules. Water vapor permeation is a combined dissolution and diffusion-controlled process governed not only by concentration and chemical structure of film components, but also by chain mobility, which depends on formation kinetics, intermolecular forces, degree of crosslinking, and crystallinity. ALG films exhibited WVP value in agreement with those reported in the literature.[15] Adding disrupted biomass to the film significantly decreased the WVP, confirming a more structured polymer matrix. Lower WVP for food packaging and plastic mulch is desired allowing greater water barrier for the food product and longer irrigation intervals, respectively. Mechanical properties are crucial to predict film functionality and stability, providing information about the magnitude of intermolecular forces within the polymer matrix. Tensile strength (TS) is a measure of force, while elongation is a measure of the film's ability to stretch before breaking. ALG films exhibit typical values for calcium crosslinked alginate films which tend to be strong but with limited flexibility.[15] ALG/B tended to reduce the TS, corroborating the SEM results. On the other hand, films with disrupted microalgal biomass (ALG/DB) exhibited a significant improvement in TS and %E contributing as a mechanical reinforcement in the alginate matrix.

4 CONCLUSION

Alginate films added with *C. vulgaris* biomass showed a continuous polymer matrix and the characteristic green color of the microalgae. Microalgae biomass lowered the UV-vis light transmittance, conferring better UV-blocking capacity to the films. Biomass cell disruption proved essential in promoting intermolecular interaction between polymer chains and microalgae components and enhancing film mechanical and water barrier properties. Alginate films added with *C. vulgaris* biomass show good perspectives for application as active food packaging or in agriculture.

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