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Proceeding Paper

Edible Films with Protein and Bioactive Compounds from *Arthrospira* sp. †

Valter F. R. Martins, Fátima Poças, Manuela Pintado, Rui M. S. C. Morais and Alcina M. M. B. Morais *

CBQF—Centro de Biotecnologia e Química Fina—Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal; s-vfrmartins@ucp.pt (V.F.R.M.); fpocas@ucp.pt (F.P.); mpintado@ucp.pt (M.P.); rcmorais@ucp.pt (R.M.S.C.M.)

* Correspondence: abmorais@ucp.pt; Tel.: +351-225580050

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Abstract: *Arthrospira* sp. is an alternative source of protein in the food chain, but it may also be considered a source of phenolic compounds with interesting properties, such as antioxidant and antimicrobial properties. In active packaging, these two properties are essential. In the present work, two extracts were produced: one extract rich in protein and another in bioactives. These two extracts were used in the production of an edible film composed of alginate (2%) + protein extract (0.5%) + bioactive extract (0.25%) with high antioxidant activity: ABTS of 1537.50 ± 191.87 and DPPH of 190.75 ± 15.53 µM TE/mg film. All the edible films produced had good physical properties, such as 100% solubility in water and ethanolic solutions. The films with alginate and protein-rich extract and or without bioactive-rich extract presented lower water vapor permeability—12.28 ± 3.01 $g \cdot mm^{-2} \cdot day^{-1} \cdot kPa^{-1}$ and 14.39 ± 3.64 $g \cdot mm \cdot m^{-2} \cdot day^{-1} \cdot kPa^{-1}$, respectively—than the alginate film. In addition, the film with alginate- and protein-rich extract presented an acceptable color.

Keywords: microalgae; *Arthrospira* sp.; edible film; protein; bioactive compounds; total phenolic content; antioxidant activity

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1. Introduction

In trying to mitigate environmental hazards caused by non-biodegradable food packaging waste, active and edible films from natural sources have emerged. These films have barrier properties and intend to extend the shelf life of food products. For this reason, other natural compounds with antioxidant and sometimes antimicrobial properties may be added to natural edible film, turning it into a functional preservative for food safety and security for consumers [1,2]. Another environmental concern is the extraction of bioactive compounds to integrate bioactive properties into the edible films. This extraction should be performed using green techniques, such as those assisted by ultrasound and using ambience-friendly solvents, such as water or hydroethanolic solutions [3].

Arthrospira sp., well known as spirulina, is a good ingredient for producing edible films, as it does not compete with arable lands, like other sources, and it is also considered a "functional food" [4]. It is a microalga rich in natural nutrients; it consists of 60–70% protein, including essential amino acids, vitamins (B-complex and vitamin E), minerals (Fe and Ca), and fatty acids (γ-linolenic acid). Thirunavukkarasu and Shankat [5] determined the protein content of pectin-based edible films incorporated with spirulina powder (5%, 10%, and 15%). The films incorporated with this microalga, especially at 15%, showed a significantly higher protein content, i.e., 56.47 g/100 g, than the control (20% pectin).

Kontogianni et al. [6] made an edible film with whey protein containing spirulina for cheese preservation. Balti et al. [7] also developed a film formulation using spider crab (*Maja crispata*) chitosan incorporated with spirulina extract. Nakamoto et al. [8] provided a general overview of the spirulina used in packaging, highlighting its biodegradability, pH monitoring, and phycocyanin bioactivity.

Adding protein to edible films may enhance some of their properties, such as the mechanical resistance of, e.g., protein-kefiran films and alginate films with whey protein; the thickness of, e.g., films with pea protein; and the oxygen barrier, therefore increasing the protection of foods and food ingredients against oxidation, e.g., corn zein, gluten, wheat protein, and whey protein films [9].

A pitfall of using spirulina biomass as a food ingredient is that it normally changes the taste and, therefore, is not well accepted by consumers [4]. Tackling this issue, the use of extract reduces the intensity of the unacceptable flavor because it is used in lower concentrations.

The bioactive compounds of spirulina are associated with therapeutic properties [10,11]. The edible films made with bioactive-rich extract and protein-rich extract not only increase the shelf life of food products but also have increased nutritional quality due to the addition of protein from an alternative source, such as the spirulina microalga.

2. Methodology

2.1. Microalga Biomass

Arthospira sp. biomass was kindly donated frozen by A4F—Algae for Future, Portugal. It was stored at −20 °C until it was necessary for the assays of extraction of bioactive compounds and protein, when it was previously frozen-dried.

2.2. Extraction of Bioactive Compounds from Dried Arthrospira sp.

The solvent extraction was performed with hydroalcoholic solution (water/ethanol 1:9 *v*/*v*). *Arthrospira* sp. powder (1 g) was placed in 30 mL solution at 50 °C and 120 rpm (Orbital Shaker, MaxQ 6000, Thermo Scientific, Waltham, MA, USA) for 120 min (repeated twice). The mixture was homogenized with an ultrasound probe (Sonics, Vibra cell, Newtown, CT, USA), with 20 kHz pulses of 30 s for 10 min. After that, the solution was filtered, and the ethanol was evaporated (Rotary Evaporator Buchi R-210, Buchi Labortechnik AG, Flawil, Switzerland) and lyophilized to obtain a dried extract [3].

2.3. Bioactive Determination of the Extracts

2.3.1. Total Phenolic Content (TPC)

The TPC was determined using the Folin–Ciocalteu method, following the procedure described by Martins et al. [3]. Absorbance was measured at 765 nm using a Synergy H1 microplate reader (Biotek, Winooski, VT, USA) in a 96-well microplate (Sarstedt, Numbrecht, Germany). Gallic acid was used as a standard for the calibration curve, and the results were expressed as milligrams of gallic acid equivalents per milligram of extract dry weight (mg GAE/mg DW). Three independent analyses were conducted for each of the triplicates.

2.3.2. Antioxidant Activity (AA)

The AA of the extract solutions mentioned above (20 mg/mL) was determined using three different assays: ABTS, DPPH, and ORAC.

The ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid)) assay, DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, and ORAC assay were carried out following the procedure described by Martins et al. [3]. Trolox was used as the standard for the calibration curve, and the results were expressed as μmol of Trolox equivalent/milligrams of

extract dry weight (µmol TE/100 mg DW). Three independent analyses were conducted for each of the triplicates.

2.4. Extraction of Protein from Dried Arthrospira sp.

Cunha et al.'s [12] methodology was used to obtain a protein-rich extract from freeze-dried *Arthrospira* sp. This began with acid hydrolysis using acetic acid (2% at 50 °C, ratio 1:3 w/v and 125 rpm for 1 h), followed by hydrolysis with cellulase (5% at 50 °C and pH 7.5, ratio 1:10 w/v and 125 rpm for 2 h). This step was repeated with protease (3.9% at 40 °C and pH 7.5 with 125 rpm for 2 h). Finally, enzyme inactivation was performed (10 min at 90 °C), followed by centrifugation (20 min at 5000 g), and the supernatant was freeze-dried; it contained the bioactive extract rich in protein hydrolysates.

2.5. Edible Film Production: "Casting" Method

Films were elaborated using Martins et al.'s [13] methodology with some modifications. Three types of films were prepared: a control with alginate (2%), another with alginate (2%) and protein extract (0.5%), and another with alginate (2%), protein extract (0.5%), and bioactive-rich extract (0.25% w/v). Sodium alginate 2% (w/v) (Sigma, Aldrich Chemie Gmbh. Steinheim, Germany) was dissolved in hot, distilled water, and then, 0.5% of the protein extract (Section 2.3) was added until total dissolution, and it was left under stirring for 1 h. After that, 0.6% (v/v) diacetyllauroyl glycerol (Tokyo Chemical Industry, Toshima, Kita-ku, Tokyo, Japan) was added to the alginate and protein solution, and the mixture was stirred for 1 h at room temperature (25 °C). The film solution with protein extract and enriched with bioactive extract from *Arthrospira* sp. was obtained by adding the bioactive-rich extract (0.25% w/v) to the solution under stirring until complete dissolution. To obtain the film, 20 mL of the solution was poured into a 10 cm diameter plastic Petri dish and left to dry at 40 °C for 18 h in an oven (Memmert GmbH, Schwabach, Germany).

2.6. Edible Film Characterization

2.6.1. Determination of the Physical Properties of the Edible Film

- Thickness

A digital thickness gauge (Adamel Lhomargy, Ivry-sur-Seine, France) was used to measure the film thickness. Three replicates were prepared for each sample, and five thickness measurements (at random positions) were taken for each replicate [14].

- Color

The color of each film was analyzed with a colorimeter Chroma Meter CR 400 (Konica Minolta Sensing, Osaka, Japan) calibrated with a white standard color plate. Color measurements were denoted in the system as L^*, a^*, and b^* [15]. Five measurements were performed for each film. Hue and chroma were determined using the following equations:

$$\text{Hue} = \arctan(b^*/a^*) \quad (1)$$

$$\text{Chroma} = \sqrt{a*^2 + b*^2} \quad (2)$$

- Water vapor permeability

The water vapor transmission rate (WVTR) and the water vapor permeability (WVP) of the films were determined gravimetrically at 23 ± 3 °C and 50% relative humidity (RH) following the standard ASTM E-96 [16]. Briefly, this technique consisted of placing dried calcium carbonate in a capsule and closing the capsules with the film. The capsule was weighed twice a day. Three replicates were performed for each film. The WVTR

($g \cdot m^{-2} \cdot day^{-1}$) and WVP ($g \cdot mm \cdot m^{-2} \cdot day^{-1} \cdot kPa^{-1}$) were determined through linear regression and application of the following formulae:

$$\text{WVTR} = \text{weight of water that passed through the film}/(\text{area} \times \text{time}) \quad (3)$$

$$\text{WVP} = \text{WVTR} \times \text{thickness of the film}/(\text{water vapor saturation pressure} \times \Delta\text{RH}) \quad (4)$$

- Solubility

The determination of the film solubility was performed in triplicate following the regulation EU N°10/2011 [17]. Briefly, five solutions were prepared to test the film migration (water, water to acetic acid 3%, and water to ethanol 1:9, 2:8, and 5:5 *v*/*v*). The films were maintained in the solution for 24 h, and the solubility was determined using the following equation:

$$\text{Solubility (\%)} = 100 - \text{film weight after 24 h immersion}/\text{film initial weight} \times 100 \quad (5)$$

2.6.2. Edible Film Antioxidant Activity (ABTS, DPPH)

The antioxidant activity of the films was determined by the 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assays, according to Lopes et al. [18]. Briefly, the ABTS solution concentration was adjusted with water to an initial absorbance of 0.700 ± 0.020 at 734 nm (Synergy H1, Biotek, Winooski, VT, USA). The DPPH working solution (90 µM) was prepared with methanol so that the absorbance reached 0.600 ± 0.100 at 515 nm. Each film was cut into 1 mg pieces, which were placed into test tubes, and the ABTS or DPPH solution (2–8 mL) was pipetted into each tube and protected from light exposition and at room temperature (25 °C). The tubes with ABTS and DPPH were left to react for 6 min and 30 min, respectively, and then, the absorbance was measured at 734 nm (ABTS) and 515 nm (DPPH). The results were expressed as µM Trolox equivalents per mg of film (TE µM/mg film). All analyses were performed in quadruplicate.

2.7. Statistical Analysis

The results were expressed as the mean ± standard deviation of three independent replicates (n = 3). Shapiro–Wilk (for normality) and Levene (for homogeneity of variance) tests were performed on the residuals of the fitted model. All data demonstrated normal distribution and were statistically compared (control vs. different formulations) using one-way ANOVA followed by Tukey's test ($p \leq 0.05$) in the case of homogeneous variance or followed by Dunnett's C test ($p \leq 0.05$) in the case of heterogeneous variance. SPSS Base 23.0 for Windows (SPSS Inc., New York, NY, USA) was used for the statistical analysis.

3. Results and Discussion

3.1. Yield of Extraction of Bioactive Compounds and Protein from Freeze-Dried Arthrospira sp.

The yield of the extraction of the bioactive compounds was 12.99 ± 0.90%, and for protein, it was 46.07 ± 4.22%. Martins et al. [3] used the same methodology to extract bioactives from olive pomace and obtained a yield of extraction of 10.7 ± 0.40%. To extract protein, Cunha et al. [12] optimized the extraction of protein from *Chlorella vulgaris* using a Box–Behnken factorial design and obtained a yield of extraction of 61 ± 0.5%. That extract contained 45 ± 1.7% of protein, corresponding to 52.37 ± 0.36% of the protein in the alga biomass. Cunha et al. [19] repeated this procedure with *Nannochloropsis oceanica* microalga and obtained 67 ± 0.7% yield of extraction with an extract containing 63 ± 0.7% protein, corresponding to 31% of the alga biomass.

3.2. Antioxidant Activity of Bioactive Rich Extracts

The antioxidant activity assays revealed high ORAC, ABTS, and DPPH values (Table 1).

Guler et al. [20] determined the antioxidant activity of an aqueous extract from *Arthrospira platensis* and found 40.65 ± 0.21% and 81.93 ± 0.61% for DPPH and ABTS, respectively. Mondal et al. [1] obtained an extract from *Dunaliella tertiolecta* using ethanol and ultrasounds, and it presented 40 ± 1.41% of DPPH radical scavenging activity and a total phenolic content of 515.72 ± 4.67 μg g^{-1}.

Table 1. Total phenolic content and antioxidant activity (ABTS, DPPH, and ORAC) of bioactive extracts.

TPC (mg GAE/100 mg DW)	ABTS	DPPH (μmol TE/100 mg DW)	ORAC
0.973 ± 0.061	2.846 ± 0.452	2.284 ± 0.064	18.378 ± 1.004

3.3. Edible Films Produced

Using the "casting" method, alginate alone and with both extracts (bioactive and protein) showed a good film-forming ability. In Figure 1, it is possible to observe the three types of edible films in the study. The main difference was the color (Section 3.4.1).

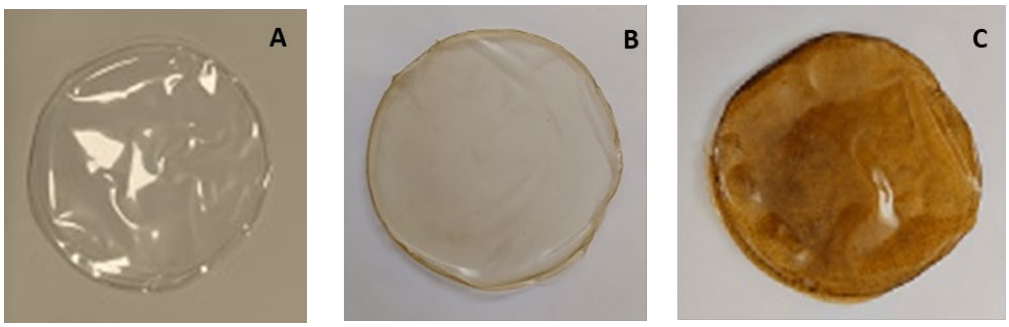

Figure 1. Edible films photos: (**A**) alginate 2%; (**B**) alginate 2% + protein-rich extract 0.5%; (**C**) alginate 2% + protein-rich extract 0.5% + bioactive-rich extract 0.25%.

3.4. Edible Films Characterization

3.4.1. Physical Properties

- Thickness

The thickness of the film with alginate, protein extract, and bioactive extract (0.076 ±0.006 mm) was greater than the thickness of the alginate film (0.058 ± 0.002 mm) and that of the film with alginate and protein extract (0.063 ± 0.005 mm).

Kontogianni et al. [4] produced a film with whey protein and *Arthrospira* sp. biomass and obtained a thickness between 0.188 mm and 0.250 mm for the control (0%) and 4% biomass spirulina, respectively. Erfani et al. [21] found that thickness affects other parameters, such as solubility and water vapor permeability. They observed that a film with polyvinyl alcohol (PVA) (2%), AgCl nanoparticles, and spirulina alga powder presented a thickness that increased when the concentration of nanoparticles and spirulina increased in the formulation.

- Color

The color parameters (Table 2) were different when the extracts (rich in bioactives or protein) were added to the alginate. Alginate (2%) + protein extract (0.5%) had the highest hue value.

Balti et al. [7] made a film with chitosan and a polyphenol-rich extract from *Arthrospira* and obtained values L^* (lightness) between 88.60 ± 2.20 to 65.53 ± 1.20 when the concen-

tration of this extract increased from 0 to 20%. The values of a^* increased from 1.11 ± 0.04 to 3.82 ± 2.65, and b^* values increased from 16.80 ± 2.51 to 38.87 ± 2.64. These values are higher than the results of the present study when using *Arthrospira* sp. extracts.

Table 2. Edible films color parameters.

Film	L^*	a^*	b^*	Hue	Chroma
Alginate (2%)	51.79 ± 3.77 ^{a}	2.30 ± 0.15 ^{a}	0.93 ± 0.11 ^{b}	21.85 ± 1.32 ^{c}	2.48 ± 0.18 ^{b}
Alginate (2%) + Protein extract (0.5%)	61.16 ± 9.54 ^{a}	1.76 ± 0.24 ^{b}	6.80 ± 0.48 ^{a}	75.5 ± 1.90 ^{a}	7.02 ± 0.49 ^{a}
Alginate (2%) + Protein extract (0.5%) + Bioactive-rich extract (0.25%)	20.66 ± 1.21 ^{b}	2.58 ± 0.32 ^{a}	6.90 ± 0.45 ^{a}	69.46 ± 2.79 ^{b}	7.38 ± 0.42 ^{a}

Different letters in each column mean significant differences ($p < 0.05$).

- Water vapor permeability (WVP)

The WVP of the alginate film (22.08 ± 0.59 g·mm·m^{-2}·day^{-1}·kPa^{-1}) was higher than that of the other films. It was 14.39 ± 3.64 g·mm·m^{-2}·day^{-1}·kPa^{-1} for alginate + protein extract and 12.28 ± 3.01 g·mm·m^{-2}·day^{-1}·kPa^{-1} for alginate + protein extract + bioactive extract.

Fabra et al. [22] added green tea and grape seed extracts to alginate films, and they also observed that the incorporation of both extracts enhanced the barrier properties of the films, decreasing WVP. This may be attributed to the polyphenols present in the extracts. Balti et al. [7] made a film with chitosan and a polyphenol-rich extract from *Arthrospira*. When increasing the concentration of the extract from 0 to 20%, the values of WVP decreased from 524.2 ± 11 to 378.6 ± 23 (g·mm·m^{-2}·day^{-1}·kPa^{-1}). Erfani et al. [21], with their film formulation of PVA (2%), AgCl nanoparticles, and spirulina alga powder, observed that the increase in spirulina concentration caused the increase in WVP. The present study does not confirm these observations since adding the extracts to alginate decreased WVP.

- Solubility

The three types of edible films produced were completely soluble in water and hydroalcoholic solutions (at 10, 20, and 50% EtOH). However, they were not soluble in acetic acid (3%). In addition, there were significant differences between the alginate (2%) film and the alginate (2%) + protein extract (0.5%) (Table 3). The assays used were intended to mimic food stuffs with the different characteristics of water and ethanol contents, with EtOH 10 and 20% simulating hydrophilic foods, EtOH 50% simulating hydrophobic foods, and acetic acid 3% simulating foods with pH below 4.5 [17]. The results of the assays on the solubility in acid acetic show that the films produced practically will not be soluble in contact with acidic foods, while the same films would be completely soluble in contact with both hydrophilic and hydrophobic foods.

Table 3. Edible films' solubility.

Film	H_2O	Acetic Acid 3%	EtOH 10%	EtOH 20%	EtOH 50%
Alginate (2%)	100 ± 0.0	16.9 ± 1.6 ^{b}	100 ± 0.0	100 ± 0.0	100 ± 0.0
Alginate (2%) + Protein extract (0.5%)	100 ± 0.0	27.3 ± 6.8 ^{a}	100 ± 0.0	100 ± 0.0	100 ± 0.0
Alginate (2%) + Protein extract (0.5%) + Bioactive-rich extract (0.25%)	100 ± 0.0	20.5 ± 2.3 ^{ab}	100 ± 0.0	100 ± 0.0	100 ± 0.0

Different letters mean significant differences between different edible films ($p < 0.05$).

In an acidic medium, the alginate chains tend to move closer together, forming intermolecular bonds through hydrogen bonds between the COOH groups. These interactions promote the formation of a more compact structure or even a gel, which is insoluble in an acidic aqueous medium [23]. When proteins are incorporated into alginate films, they probably will interfere with the hydrogen bonding between alginate molecules, loosening

the matrix structure and likely enhancing the solubility of the film, but this depends of several factors [24].

Erfani et al. [21], with their film formulation containing PVA (2%), AgCl nanoparticles, and spirulina alga powder, observed that an increase in spirulina concentration increased the film solubility in water, probably due to its hydrophilic nature.

3.4.2. Antioxidant Activity

In general, ABTS and DPPH showed an increase when the extracts were added. The film with alginate (2%), protein extract (0.5%), and bioactive-rich extract (0.25%) presented higher values than the other films (Table 4). The results for ABTS were higher than for DPPH because ABTS determines the activity for hydrophilic and hydrophobic compounds, and DPPH only determines the activity of hydrophobic compounds.

Table 4. Edible films' antioxidant activity.

Edible Films	ABTS (μM TE/mg film)	DPPH (μM TE/mg film)
Alginate (2%)	120.15 ± 6.81 ^{c}	85.97 ± 3.19 ^{b}
Alginate (2%) + Protein extract (0.5%)	922.36 ± 129.06 ^{b}	95.93 ± 15.91 ^{b}
Alginate (2%) + Protein extract (0.5%) + Bioactive-rich extract (0.25%)	1537.50 ± 191.87 ^{a}	190.75 ± 15.53 ^{a}

Different letters mean significant differences between different edible films ($p < 0.05$).

Balti et al. [8] made a film with chitosan and a polyphenol-rich extract from *Arthrospira* and observed that DPPH values increased from 29.76% to 58.77% when the polyphenol-rich extract concentration increased, with the same happening for FRAP: an increase from 3.77 to 11.71 (mg TE/g of film).

4. Conclusions

It is possible to use *Arthrospira* sp. as a source of protein and bioactive compounds to produce edible films, opening the doors for food packaging applications and for possible coating formulations for perishable foods to increase their shelf life. Spirulina protein-based edible films offer promising properties for sustainable food packaging. The antioxidant activity of the films increased when both extracts, i.e., the protein-rich extract and the bioactive-rich extract, were added. The water vapor permeability decreased when the extracts were added, and all the films produced had higher solubility in water and hydroethanolic solutions. This suggests that the films produced are biodegradable, offering an eco-friendly alternative to conventional plastic packaging, and the sustainable production of spirulina adds to their environmental appeal.

Edible films incorporated with spirulina protein- and bioactive-rich extracts may have multiple applications across industries. In the food industry, they could serve as biodegradable packaging for fresh produce, bakery items, and snacks to extend shelf life. Spirulina films might also inspire culinary innovation, with applications in food decoration and haute cuisine. In addition, in healthcare, spirulina films could act as drug delivery systems for, e.g., vitamins, slow-release agents, or wound dressings with antimicrobial and anti-inflammatory properties. The cosmetic industry could also use spirulina films in biodegradable sheet masks or nutrient-rich skin patches.

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project administration, A.M.M.B.M., R.M.S.C.M. and M.P.; funding acquisition, M.P. All authors have read and agreed to the published version of the manuscript.

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