Biodegradable Agar extracted from *Gracilaria Vermiculophylla*: Film Properties and Application to Edible Coating

A.M.M. Sousa\(^1,a\), A.M. Sereno\(^1,b\), L. Hilliou\(^1,2,c\) and M.P. Gonçalves\(^1,d\)

\(^1\)REQUIMTE, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias 4200-465, Porto, Portugal

\(^2\)I3N- Institute for Nanostructures, Nanomodeling and Nanofabrication, University of Minho Campus de Azurém, 4800-058, Guimarães, Portugal

\(^a\)ana.sousa@fe.up.pt, \(^b\)sereno@programalban.org, \(^c\)loic@dep.uminho.pt, \(^d\)pilarg@fe.up.pt

**Keywords:** *Gracilaria vermiculophylla*, Agar, Biodegradable films, Edible coatings, Cherry tomatoes.

**Abstract.** *Gracilaria* is a red algal genus that biosynthesizes a polymer called agar that is extensively used in the food and pharmaceutical industries as gelling and stabilizing agent. In the last years, many studies have focused on gel properties of this biopolymer; but the agar films and coatings still have few studies reported. The edible film and coating have a protective function, preventing moisture, oxygen and flavour transfers between food and surroundings. The objectives of this work are the production of biodegradable agar films from *Gracilaria vermiculophylla*, collected in Ria de Aveiro, Portugal, and the study of the effect of glycerol, an hydrophilic plasticizer, on the properties of the films and on subsequent application in edible coating of fresh fruits and vegetables. The agar extraction was carried out at specific optimum parameters determined in previous work (3.5 h pre-treatment duration, 6% NaOH concentration and 2 h extraction time). Agar films were made using the knife coating technique and compared with commercial agar. The physical properties of films such as hygroscopicity, mechanical resistance (Young’s modulus, tensile strength and elongation), and permeability to water vapour and oxygen were characterized. As expected, the plasticizer addition revealed and increase on elongation and decrease on tensile strength. The films were transparent and optically clear, showing good properties similar to the commercial agar films. The potential application of the agar/glycerol solution to fresh vegetable preservation was tested. Model fruits and vegetables were coated with the biopolymer/plasticizer solution and compared with a control sample in terms of colour, firmness, weight loss and shelf life. Considering on one hand the abundance of the raw algal material which is actually an invasive species, and the properties of the agar films and coatings obtained on the other hand, commercial use of *G.vermiculophylla* from Ria de Aveiro is well justified.

**Introduction**

As seafood consumption has been rising in the last decades, the world can no longer depend only on fishing and so the cultivation of aquatic organisms under controlled conditions, aquaculture, presents itself as a great alternative to this demand [1]. The use of plants like seaweeds in aquaculture can produce sustainable and cost-effective operations that reduce the environmental impacts of effluents resultant of the aquaculture mechanism [2]. Since the intensive use of this photoautotrophic organisms as biofilters leads to large amounts of seaweeds waste, the objective of this work was to add value to the *G. vermiculophylla* harvested in Ria de Aveiro after it’s use in aquaculture systems.

Biodegradable polymers have been developed recently in response to public concern over the growing environmental problem associated with the use of synthetic polymers leading to plastic waste. The search for low-cost, environmentally friendly materials has led to the development of different biodegradable plastics incorporating natural polymers [3]. Red marine seaweeds like *G. vermiculophylla* are the source of agar, a biopolymer with a simple extraction process and of practical importance in pharmaceutical and biotechnological industries where it is used as gelling, stabilizing...
and encapsulating agent [4]. In this work, it was proposed to study the application of agar to the production of biodegradable films and coating. The protective function of an edible film or coating is to prevent the transfer of moisture, oxygen, flavour and/or oil content between food and the surrounding medium and/or between different compartments in a heterogeneous food [5].

Films of agar extracted from *G. vermiculophylla* using optimum conditions (determined in a previous work [6]) and commercial agar were made using the knife coating technique [4]. Plasticizer was added to the biofilms with the intent to increase their flexibility and oxygen permeability [7]. The functional properties (hygroscopicity, mechanical resistance, and permeability to water vapour and oxygen) of the films as well as an application to fruit preservation were tested.

**Film preparation.** Solutions of agar (commercial Sigma Aldrich and *Gracilaria* extracted) 1% (w/w) were prepared by dissolving the biopolymer in distilled water at 95°C for at least 30 minutes under stirring. Then, glycerol was added to the solution at a concentration of 15% of the total dry basis. The film forming solution was kept at the same temperature and stirred for 10 minutes after which the solutions were cooled down to 60°C and then spread over an acrylic plate, using an automatic knife film applicator (Sheen, model 1132N, UK) with application speed of 300 mm/s. The films were allowed to dry for 2-3 hours, under air convection at room temperature. Using this knife coating technique homogeneous films with uniform thickness of (7±1) µm (measured with an Absolute Digimatic Indicator model ID-F150 from Mitutoyo Co., Japan) were obtained (Fig. 1).

![Fig. 1: Agar film obtained with the optimized extract from *Gracilaria.*](image)

**Film properties**

**Films hygroscopic properties.** Water sorption isotherms were obtained by the gravimetric method [4]. Film samples with 25 mm x 25 mm dimensions, previously dried in a vacuum oven at 60°C for 24h, were placed in flasks with different controlled relative humidities, and stored until they reached a constant weight. The experiment was carried out at 25°C. The film’s water sorption isotherm is defined as the relation between the equilibrium water activity and the corresponding moisture content (dry basis) of the sample at a given temperature. The Guggenheim-Anderson-de Boer (GAB) [8] model (Eq.1) was used to represent experimental sorption data,

$$X_e = \frac{CkX_0a_w}{[(1-ka_w)(1-ka_w+Ca_w)]},$$

(Eq.1)

where $X_e$ is the equilibrium moisture content at the water activity $a_w$, $X_0$ is the monolayer moisture content and represents the water content corresponding to saturation of all primary adsorption sites by one water molecule, $C$ is the Guggenheim constant and represents the energy difference between the water molecules attached to primary sorption sites and those absorbed to successive sorption layers, and $k$ is the corrective constant taking into account properties of multilayer molecules with respect to the bulk liquid. Both agar/glycerol films showed similar hygroscopic behaviour as shown in Fig. 2. The values of $k$ (<1) and the correlation coefficient ($r$>0.98) show that GAB equation gives a good fit to experimental values (Table 1). The GAB parameters $k$ and $X_0$ were identical for both agar/glycerol biofilms but the energy constant $C$ was slightly higher for commercial agar films probably due to chemical differences between the to agar types studied. In spite of glycerol addition, agar biofilms sorbed less water for the same $a_w$ when compared with other biopolymers previously studied (“Sargaço”, commercial alginate and κ,ι-carrageenan) [4]. This less hydrophilic nature constitutes an advantage when it comes to food packaging.

---

Advanced Materials Forum V
Fig. 2: Experimental data for moisture content isotherms of agar/glycerol films and the respective fitted GAB curves.

Table 1: GAB parameters (C, k and X₀) and respective correlation coefficient (r²) for agar/glycerol films.

<table>
<thead>
<tr>
<th>Film composition</th>
<th>C</th>
<th>k</th>
<th>X₀ (g of H₂O/g of solids)</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gracilaria agar + 15% Glycerol</td>
<td>2.264</td>
<td>0.927</td>
<td>0.084</td>
<td>0.997</td>
</tr>
<tr>
<td>Commercial agar + 15% Glycerol</td>
<td>6.649</td>
<td>0.949</td>
<td>0.073</td>
<td>0.996</td>
</tr>
</tbody>
</table>

**Water vapour permeability (WVP).** WVP tests were conducted using ASTM method 96 [9]. Commercial and *Gracilaria* extracted agar films have shown similar WVP, respectively (3.32±0.37) x10⁻¹¹ and (3.44±0.09) x10⁻¹¹ g.m⁻¹.s⁻¹.Pa⁻¹. In spite of plasticizer addition, both agar/glycerol films exhibited lower WVP than other biopolymers previously studied [4].

**Oxygen permeability (OP).** Oxygen transmission rate tests were performed using a static method, simulating the real package situation, as described in [7]. Film discs were cut with a 85 mm diameter and conditioned for 7 days at room temperature in a desiccator under controlled relative humidity, after which they were attached over and sealed in a circular opening of a diffusion cell. After purging the cell with helium until there was no detectable amount of oxygen, the test was initiated. At given time intervals, the oxygen concentration was measured using a CheckMate II gas analyzer (PBI, Dansensor Denmark). The oxygen permeability (OP) obtained for commercial and Gracilaria extracted agar/glycerol films were, (1.39±0.27) x10⁻¹⁰ and (1.19±0.39) x10⁻¹⁰ ml.m.m⁻².s⁻¹.Pa⁻¹. The higher OP values obtained for agar films when compared with the synthetic polymers used in food packaging are due to the hydrophilic nature of the film. Once again the two biofilms revealed similar properties.

**Mechanical Properties.** Tensile tests were performed in a TAXT2 (Stable Micro Systems) texture analyzer equipped with tensile tests attachments as described in [4]. Films were cut into 25 mm x 100 mm strips and tested after storage for 7 days at room temperature in a desiccator with controlled relative humidity (53%). Again both agar/glycerol films revealed similar properties, showing high stress at break, (50.3±12.3) and (49.9±12.8) MPa, and high strain at break, (2.55±1.12) and (2.06±0.74) % respectively for *Gracilaria* and commercial agar (Table 2). Comparing with biofilms made from “Sargaço”, they are less plastic since a smaller strain at break and a larger stress at break are observed. They are also harder as a larger Young modulus was measured [4].
properties measured agree with film’s hygroscopic behaviour since films with a higher moisture content (“Sargaço”) show a more plasticizing effect. Commercial agar/glycerol films have better mechanical characteristics for food packaging, as they are stronger (higher Young modulus) in spite of being less plastic (lower strain at break).

Table 2: Mechanical properties of agar/glycerol films.

<table>
<thead>
<tr>
<th></th>
<th><em>Gracilaria</em> agar + 15% Glycerol</th>
<th>Commercial agar + 15% Glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young Modulus (MPa)</td>
<td>25.3 ± 5.1</td>
<td>31.7 ± 5.8</td>
</tr>
<tr>
<td>Strain at break (%)</td>
<td>2.55 ± 1.12</td>
<td>2.06 ± 0.74</td>
</tr>
<tr>
<td>Stress at break (MPa)</td>
<td>50.3 ± 12.3</td>
<td>49.9 ± 12.8</td>
</tr>
</tbody>
</table>

Application to fruit preservation

**Coating preparation.** Cherry tomatoes were harvested and purchased in January 2009. Fruits of homogeneous colour, size and without injuries were selected, washed and air-dried at room temperature. The edible coating formulations were similar to those used for film preparation. Coating was applied by dipping the tomatoes into the solution for about 1 minute. It was observed that for the commercial agar solution, its adherence to the fruit surface was very low at any temperature. Therefore the coating study was made with *Gracilaria* extracted agar only, which, adhered well to the fruit surface at a minimum temperature of 45 °C. All *Gracilaria* agar coatings were compared with a control sample, coated with water at 45°C. After dipping, fruits were air dried with a help of a fan and stored at (30±2)°C and 58% relative humidity. Each day, 4 samples were tested in terms of firmness, weight loss and colour variation.

**Weight loss.** Weight of individual lots was recorded following treatment (day 0) and everyday until the end of the experiments (day 7). Cumulative weight loss was expressed as percent of original weight. Results showed that weight loss increased progressively with storage time and that the agar coating slightly reduced the weight loss of cherry tomatoes during storage when compared with the control, though the difference vanished after 6-7 days, as seen in Fig. 3. This may be due to the delaying of moisture migration from the fruit into the environment, thus reducing weight loss during the storage.

![Fig. 3: Effect of *Gracilaria* extracted agar/glycerol coating on weight loss of cherry tomatoes stored at (30±2) °C and 58% relative humidity.](image-url)
Firmness. Texture analysis was performed using a TA.XT2 Stable MicroSystems texture analyzer (Surrey, England). The system was equipped with a load cell of 49N and a cylindrical probe of 2 mm diameter, moving at 0.1 mm/s until 10% of sample deformation. Firmness was measured as the maximum penetration force (N) reached during tissue breakage. For both control and coated fruits, maximum force at break decreased as a function of storage time (Fig. 4). During the first half period of the test the effect of the coating was clear (coated samples showed higher firmness) but for the final half treatment days both control and coated samples agree with weight loss because texture loss and change in appearance are related to metabolic changes and weight losses.

![Firmness graph](image1)

**Fig. 4:** Effect of *Gracilaria* extracted agar/glycerol coating on firmness of cherry tomatoes stored at (30±2) °C and 58% relative humidity.

Optical properties. Surface colour was measured with a Minolta colorimeter CR300 series (Tokyo, Japan) using the CIELab colour parameters, lightness (L) and chromaticity parameters a (red – green) and b (yellow – blue) were measured. Results showed that lightness values (L) were significantly higher for the agar coated tomatoes (Fig. 5). The coated fruits showed an increasing trend of parameter L, especially between day 1 and day 3, whereas the control samples L value decreased during the second half of the test period. Regarding the chromaticity parameters, results were not conclusive for both samples.

![Optical properties graph](image2)

**Fig. 5:** Effect of *Gracilaria* extracted agar/glycerol coating on lightness (L) of cherry tomatoes stored at (30±2) °C and 58% relative humidity.
Conclusions

The agar/glycerol films (commercial and Gracilaria extracted) showed similar properties. Considering that one of the main functions of food packaging is to reduce the moisture content transferred between food and surroundings, the less hygroscopic nature of agar compared with other biopolymers (“Sargaço”, κ-t-carrageenan and commercial alginate) constitutes a great advantage of agar biofilms for this application. The higher tensile strength and good deformability (high strain at break) are also good parameters for packaging applications. The oxygen permeability was identical for both agar/glycerol films and higher when compared with the synthetic polymers used in food packaging. Agar/glycerol films were transparent and optically clear. The amount of glycerol added led to good results not compromising the potential applications for food packaging.

Regarding the coating application tests, results showed that coatings made with Gracilaria extracted agar/glycerol solutions were effective in extending cherry tomatoes shelf life in terms of weight loss and firmness although during the second half of the test period this difference tended to vanish. This fact may be due to the use of drastic storage conditions for performing the test.

Visual inspection of the fruits revealed that the control fruits lost their gloss whereas the coated fruits kept a light gloss up to the end of the test. This result is supported by the colour parameter L which indicates higher lightness of coated samples throughout the test. The commercial agar/glycerol formulation used wasn’t able to ensure its adherence to the fruit surface.

The results obtained show that agar extracted from G.vermiculophylla constitutes a good and cheap alternative to commercial agar regarding the food packaging application and coating.

Agar is well known in the food industry due to its excellent gelling properties but very few studies were made with the intent to study its application as edible film or coating. The main objectives of this work were clearly achieved: the potential use to algal waste that is produced by aquaculture processes was given and new possibilities for agar applications that go beyond his gelling properties were shown. For future work, an improvement of agar characteristics for films and edible coatings will be sought, namely by studying different formulations, possibly with different plasticizers.

References

[7] F.D.S. Larotonda in: Biodegradable films and coatings obtained from carrageenan from Mastocarpus stellatus and starch from Quercus suber , thesis presented to the Faculty of Engineering of University of Porto, chapters 5 and 6 (2007).
Biodegradable Agar Extracted from *Gracilaria Vermiculophylla*: Film Properties and Application to Edible Coating

10.4028/www.scientific.net/MSF.636-637.739

**DOI References**

