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# Development and characterization of hybrid corn starch-microalgae films: Effect of ultrasound pre-treatment on structural, barrier and mechanical performance

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ABSTRACT

This work is aimed at evaluating the ability of the microalgae to partially substitute biodegradable materials with improved physicochemical properties. To this end, starch films containing the microalga *Nannochloropsis gaditana sp.* (*N. gaditana*) have been developed and characterized. Initially, different ultrasound treatments were evaluated for microalgae cell wall disruption. Although surfactant-aided sonication was the most efficient disruption method, the presence of the surfactant hampered continuous film formation. Subsequently, the ability of intact and ultrasound-treated *N. gaditana* cells to improve barrier properties of starch films was evaluated. Combined small and wide angle X-ray scattering experiments (SAXS/WAXS, respectively) evidenced slight nanostructural and crystallinity changes induced by the presence of *N. gaditana* cells. Incorporation of intact or ultrasound-treated microalgae into starch led to more hydrophobic films, with enhanced barrier properties for most of the formulations. However, the films containing the microalgae treated with the greatest ultrasound intensity, did not show any barrier improvement due to the plasticization promoted by the cell components (probably lipids) released during the ultrasound treatment, as suggested by SAXS/WAXS and the mechanical properties.

## 1. Introduction

Research is currently focused on finding promising applications of microalgae components in different areas, such as for biodiesel production from the fatty acids present in the biomass, development of functional foods enriched with healthy bioactive molecules or for improving the nutritional content in animal feed [1–3]. However, another potential market for microalgae products, which has been scarcely explored, is the development of biodegradable packaging materials for food applications. Current consumer trends require materials that are inexpensive, versatile, and convenient in making plastics. A primary limitation of conventional bio-based plastics is that the biomass resources compete with food and food applications, and these agro-crops consume large amounts of petroleum products in their life cycle. In addition, these terrestrial crops require large amounts of fertile land, irrigation water, and fertilizers and take time to grow in between harvest to produce the quantities of biomass required to replace conventional plastic feedstock markets. Microalgae, as an alternative biomass, can serve as an excellent feedstock for plastic production owing to its many advantages, such as high yield and the ability to grow in a wide range of environments. These small aquatic organisms present high protein contents (for instance, Spirulina contains 46%–63% of protein in dry weight), which together with their small size makes microalgae suitable for plastic conversion without the need of any pre-treatment process, more cost-effective scalable production and reducing waste in production [4]. However, there are only few studies reporting on the use of microalgae in packaging materials. Composite materials incorporating *Chlorella* in synthetic resins, such as polyethylene (PE) or polyvinyl chloride (PVC) have been studied aimed at making eco-friendly building materials with $CO_2$ fixation properties [5–6].

*Abbreviations:* SAXS, small angle X-ray scattering; WAXS, wide angle X-ray scattering; US, ultrasound

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Similarly, thermoplastic starch films containing diverse microalgae for "bio$CO_2$" fixation were produced, having mechanical properties comparable to neat polyurethane or polyethylene films [7]. Several studies have focused on blending polymers with residual microalgae biomass (RMB), having the added value of lower production costs and smart use of resources and waste streams. For instance, extrusion and injection moulding processes, commonly used in the plastic industry, have been evaluated for the development of hybrid plastic-based composites containing RMB, showing that it is feasible to partially substitute the synthetic polymer with up to 20% RMB, providing materials with reasonably good mechanical properties [8].

Surprisingly, little information has been reported about the potential use of microalgae in the development of biodegradable and renewable materials [9] or even in the development of edible films and coatings. The present work has been carried out with *Nannocloropsis gaditana*, a microalga with a flexible cell membrane and hard cell wall containing considerable amounts of polyunsaturated fatty acid oils, antioxidants and pigments which could provide functional properties to the packaging materials and edible coatings [10]. Therefore, in this work, *N. gaditana* has been incorporated within a cost-effective biodegradable material (starch) by a solvent-casting methodology.

This proof-of-concept study is aimed at providing alternative biomass resources to develop biodegradable hybrid starch-based films with improved barrier properties. To this end, different methodologies were first evaluated to disrupt the cell walls in order to favor their dispersion within the biopolymer matrix and, the efficiency of the disruption step was qualitatively evaluated. Subsequently, corn starch-*N. gaditana* films were developed and characterized. Corn starch was chosen as biopolymer matrix as it combines several advantages such as low price, wide availability, high purity, non-toxicity, biodegradability and environmental compatibility [11].

## 2. Materials and methods

### 2.1. Materials

Corn starch (28% amylose) and *Nannocloropsis gaditana* were kindly supplied by Roquette (Roquette Laisa España, Benifaió, Spain) and Buggypower S.L. (Murcia, Spain), respectively. Triton™ X-100, glycerol and phosphate buffered saline (PBS) were purchased from Sigma Aldrich (Madrid, Spain) and Panreac Quimica, S.A. (Castellar Del Vallés, Barcelona, Spain), respectively. All products were used as received without further purification.

### 2.2. Microalgae cell wall disruption

The starting material consisted of *N. gaditana* dispersed in water at a concentration of 0.4 g of dry weight $L^{-1}$. Cell wall disruption was carried out using an UP-400S ultrasound equipment (Hielcher GmbH, Germany) providing a maximum power of 400 W at a constant frequency of 24 kHz. The sonication time was varied within 0–30 min, while two different powers of 200 and 400 W were applied, maintaining the solution in an ice bath. The experiments were carried out at room temperature. Finally, another batch containing 0.4 g of dry weight/L and 0.3% of Triton™ X-100 was also prepared following the same disruption processes described above. Table 1 summarizes the disruption techniques used for *N. gaditana* and the corresponding sample codes.

### 2.3. Optical microscopy

In order to evaluate the impact of the different disruption methodologies on the microalgae cell wall structure, *N. gaditana* samples were prepared at 0.4 g $L^{-1}$. Microalgae samples and glycerol were mixed in 1:1 ratio to restrict the cell movement during the observation. Furthermore, *N. gaditana* samples were stained with a 0.01% Calcofluor White (Sigma-Aldrich, Inc. Madrid, Spain) solution for 5 min, as this dye can strongly bind to cellulose and β-linked glucans. The cells were then centrifuged at 3000*g* for 1 min and washed twice with PBS. Digital images were taken using an Eclipse 90i Nikon microscope (Nikon corporation, Japan) equipped with 5-megapixels cooled digital color microphotography camera Nikon Digital Sight DS-5Mc. Acquired images were analyzed and processed by using Nis-Elements Br 3.2 Software (Nikon corporation, Japan).

**Table 1**
Nomenclature and disruption conditions used for *Nannochloropsis gaditana*.

| Sample | g Triton™ X-100/100 g sample | Technique | Time (min) | Power (W) |
|---|---|---|---|---|
| S | | – | – | – |
| S/M | | – | – | – |
| S/M-US-5-200 | | US | 5 | 200 |
| S/M-US-5-200-T | 0.3 | | | |
| S/M-US-30-200 | | | 30 | |
| S/M-US-30-200-T | 0.3 | | | |
| S/M-US-5-400 | | | 5 | 400 |
| S/M-US-5-400-T | 0.3 | | | |

### 2.4. Development and characterization of microalgae-containing starch films

Eight different formulations based on starch, glycerol and microalgae were prepared. Corn starch was dispersed in water to obtain 2% (w/w) polysaccharide dispersions. These were subjected to gentle stirring at 90 °C for 30 min to induce starch gelatinization. Afterwards, the plasticizer was added in a starch:glycerol ratio of 1:0.25 and the dispersions were homogenized (13,500 rpm,1 min) at room temperature, using an homogenizer (D9, MICCRA GmbH, Müllheim, Deutschland). In the case of the dispersions containing *N. gaditana* (0.4 g weight $L^{-1}$), the microalgae were first treated (as described in Table 1) in deionized water and, subsequently, starch was incorporated into the mixture prior to the gelatinization step. Homogenized film forming dispersions, containing 15 mg of total solids per $cm^2$, were spread evenly over a Teflon casting plate resting on a levelled surface. Films were formed by drying for 48 h at 45% RH and 30 °C. These conditions were selected after previous experiments to ensure that homogenous dry films could be peeled intact from the casting surface.

Samples were equilibrated for one week at 0% relative humidity and 25 °C prior to barrier, mechanical, contact angle and SAXS/WAXS analyses. Film thickness was measured with a Palmer digital micrometre (Vidrafoc, Spain) to the nearest 0.0025 mm at five random positions.

#### 2.4.1. Scanning electron microscopy (SEM)

Microstructural analysis of the films was carried out using a Scanning Electron Microscope (Hitachi S-4800). Three different samples of each film were cryo-fractured after immersion in liquid nitrogen and randomly broken to investigate the cross-section of the samples. Samples were fixed on M4 Aluminium Specimen Mount, gold–palladium coated and observed using an accelerating voltage of 10 kV and a working distance of 12 mm.

#### 2.4.2. Water vapor permeability

Water vapor permeability (WVP) was measured, in triplicate, according to the ASTM E96/E96M-10 a gravimetric method [12]. Deionized water was placed inside the Payne permeability cups (Elcometer SPRL, Hermelle/s Argenteau, Belgium) to expose the film to 100% RH on one side (the exposed area was $9.6 \times 10^{-4}$ $m^2$). Once the films were secured, each cup was placed in an equilibrated relative humidity cabinet at 0% RH and 25 °C. The cups were weighed periodically ( ± 0.0001 g). Cups with aluminium films were used as control samples to estimate solvent loss through the sealing. Water vapor permeation