MUCILAGE EXTRACTION FROM *OPUNTIA* SPP FOR PRODUCTION OF BIOFILMS

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ABSTRACT: Crops like *Opuntia* spp grows essentially in the tropical and subtropical regions, under arid climate conditions, preventing soil degradation and being helpful in the control of desertification processes. The fruits are widely used for human consumption and the cladodes are essentially used as feed. The cladodes have in its composition mucilage, a complex polymeric carbohydrate structure with the ability to retard water loss and being able to form viscous or gelatinous colloids. Its gelling, thickening and emulsifying properties turn cactus mucilage useful to be applied in different areas such as cosmetics, pharmaceutical, and some other industries. In the food industry, it could find application in food packaging as edible films and coatings because of its elastic flow characteristic and its ability in forming a molecular network. Therefore, the aim of this work is to develop an efficient and sustainable method to extract the mucilage from the cladodes with characteristics suitable to be used as a biofilm material. The methodologies tested comprised the same general steps: 1) Removal of the outer layers from cladodes; 2) Washing and cutting step; 3) Mix with a solvent (water or ethanol); 4) Pressing or centrifugation; 5) Precipitation with ethanol; 6) Drying step to obtain mucilage powder (drying and lyophilization). Based on preliminary results, a yield (in dry basis) of 0.72 and 15.29%, was obtained for pulp and peel, respectively. A critical assessment of the methods tested indicate that there are still options for improvement (e.g. use of different solvents and solvents mixtures) and that higher yield can be obtained.
Keywords: *Opuntia* spp., biopolymers, biobased products, biobased economy, desertification

1 INTRODUCTION

As the world is facing several problems with the increase of the desertification processes as well as the decline of water resources, and considering that approximately 50% of the world area is considered arid, semi-arid it is mandatory to find alternative crops able to grow in those conditions [1,2]. Species like *Opuntia* spp, are one of those that have been identified as capable to grow in those areas and promote the rehabilitation of dry lands, providing improvement of soil properties and the prevention of soil erosion [1]. This crop due to its photosynthetic mechanism, the Crassulacean Acid Metabolism (CAM), have a high-water use efficiency and a high capacity of drought tolerance also showing great potential as feedstock for bioenergy production [1].

Opuntia spp is a perennial crop belonging to the *Cactaceae* family and are known almost 300 species [3, 4]. The crop is native from Mexico and is well established around the world, especially in South America and Mediterranean areas [5]. The fruits are widely used for human consumption and its cactus, constituted by cladodes, are used essentially as feed consumption [6–8].

Cladodes have in its constitution an interesting compound, a complex polysaccharide of high molecular weight, named mucilage [9]. The mucilage allows the plant to retain a large amount of water acting as a water barrier to the plant [10], and having the capacity to form viscous or gelatinous colloids [9]. Due to its gelling, thickening and emulsifying properties, the mucilage could find application in different areas such as cosmetics, pharmaceutical and some other industries [9].

In the food industry, cactus mucilage seems to have a promising application in food packaging as edible films and coatings due to its elastic flow characteristic and its ability to form a molecular network [11].

Bioplastics are becoming a very important alternative to plastics due to its renewable and biodegradable character and also to accommodate the increased awareness of consumers to find safe, healthy and stable foods [11,12]. As an alternative to plastics, some biopolymers have been studied like polysaccharides, proteins, and lipids obtained from different sources like alginate, chitosan, agar, gelatin, kefiran and *Opuntia* nopal mucilage [13,14].

The ability to form edible films from *Opuntia* nopal mucilage has been studied and films seems to be easily formed, however the bioplastic demonstrate poor mechanical properties [15]. Several additives to improve cactus mucilage films resistance [15-17] have been studied and also the use of different plasticizers [11]. Some studies also showed that *Opuntia ficus-indica* mucilage is effective as a coating material for extending shelf-life in fresh strawberries [18], kiwifruit slices [19] and fig fruit [10].

Indeed, cactus mucilage may have interesting potential for application in food industry due to its film-forming capacity, biodegradability, and safety, but still, more studies are needed in order to improve the knowledge towards its application as a bioplastic and as an alternative to plastics. In this work, the cladode is characterized, and the mucilage extraction process is studied in order to develop a more efficient and sustainable method of mucilage extraction from the cladodes with characteristics suitable to be used as a biofilm material.

The MediOpuntia project aims to promote planting cactus on large scale in marginal lands of Mediterranean countries with minimum pressure on available water resources. The target is to develop an integrated management package of inventive low-cost on-farm practices for producing cactus as well as adding value to the final product by introducing innovative manufacturing techniques to produce functional food from cactus seeds, stems and fruits. In the framework of the MediOpuntia project the aim of this work is to develop an efficient and sustainable method to extract the mucilage from the cladodes with characteristics suitable to be used as a biofilm material.

2 MATERIALS AND METHODS

Young *Opuntia ficus-indica* cladodes were collected in April 2019 and were provided by Pepe Aromas, Alentejo, Portugal. Cladodes were stored at 4ºC until further analysis. The cladodes were washed, and the spines were removed manually.

The characterization of the cladodes was performed in both outer (peel) and inner (pulp) layers in separated, peel and pulp respectively. Total nitrogen content was determined by the Kjeldahl method [20], and total protein content was calculated using a 6,25 factor, expressed in percentage (%) in fresh weight basis. The total phosphorus expressed in % fresh weight basis [20]. Ash content, expressed in % in fresh weight basis, and moisture, expressed in % in fresh weight basis were evaluated according to [21]. Total fiber content, expressed in % fresh weight basis, was evaluated by the Van Soest method [22]. Colour analysis was measured using a colorimeter CR-410, Konica Minolta, obtaining de L, a, b coordinates and the Hue Angle [23].
Mucilage was extracted from both parts of the cladodes (inner and outer layer) according to the method reported by Espíno-Díaz et al, 2010 [15] with some modifications. Cladodes were cut, homogenized with water in the proportion of 1:1 w/w and boiled for 20 min at 85ºC in a water bath. The mixture was filtered, and the viscous material was washed with 65% ethanol aqueous solution (v/v) for 20h at 4ºC. After precipitation, the samples were washed with 96% ethanol and filtered under vacuum conditions recovering the solid part. The samples were then dried in a vacuum oven at 20ºC. The yield of extraction was expressed in % of mucilage of dry weight. The lipid and reducing sugars content were calculated by default.

3 RESULTS AND DISCUSSION

3.1 Cladode characterization

Cladodes constitution is affected and modified by several factors as maturity stage, environmental conditions, harvest season, post-harvest season, and the type of species [24].
Cladodes pulp and peel were characterized in terms of moisture, ash, total fiber, total protein, lipids and reducing sugars content. The results for chemical characterization obtained for cladodes pulp are represented in **Figure 1** and for cladodes peel in **Figure 2.** The results will be compared simultaneously in the following paragraphs.

Cladodes pulp showed a high content in moisture with 95.39% and peel about 92.22%, similar to the result reported for the whole cladode of *Opuntia ficus-indica*, in fresh weight of 91.9% [25]. High percentages of moisture are reported for younger cladodes which supports the results obtained [25].
Cladodes pulp (0.43%) showed a low content in total fiber and peel showed a slightly higher value of 1.29%. The content in fiber varies according to the plant species and stage of maturity but, in general, the peel contains a large amount of this component which is also verified in the results obtained [26]. The results obtained are lower than the ones reported in previous work for *Opuntia ficus-indica*, with a value of 2.70% [27].
Cladodes are reported to be rich in mineral content, with 1-2% in fresh weight of ash content [2], [28] and the results obtained in this work showed similar values for pulp (1.13%) and peel (1.89%).

The *Opuntia* spp cladodes are reported to have a low content in lipids and a substantial amount of carbohydrates [2], which is verified in this work for both parts of the cladode, with 2.45% and 4.18% for pulp and peel respectively. Regarding protein content, values of 0.60% and 0.41% were obtained for pulp and peel, respectively. The protein content in both parts of the cladodes is similar to the literature, which reports values in a range of 0.5 to 1% of protein for the whole cladode in fresh weight basis [2] [27].

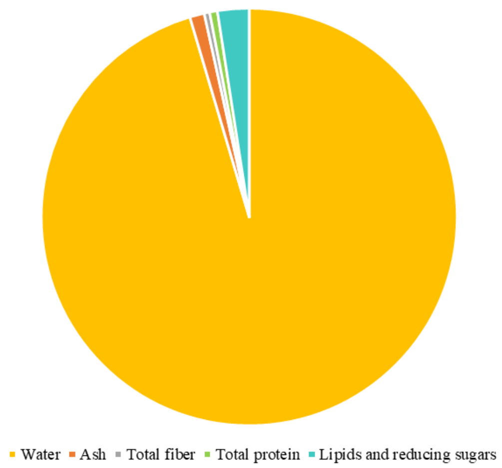

Figure 1: Chemical composition of cladode pulp, in fresh weight basis

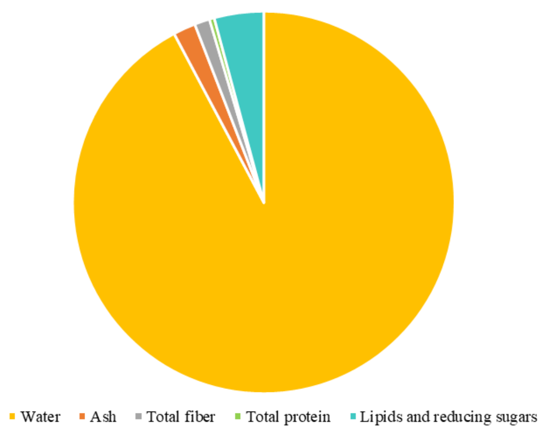

Figure 2: Chemical composition of cladode peel, in fresh weight basis.

The colour parameters were also evaluated and showed slight differences between peel and pulp.

In pulp (**Figure 3**) the L and b parameters were higher than in the peel (**Figure 4**) showing that the pulp is lighter than the outer layer. The a value is higher in the pulp than in the peel once it is referred to the change of the coordinates between yellow (+) and green (-) [23] and it is verified that the peel has a clear green colour than the pulp.

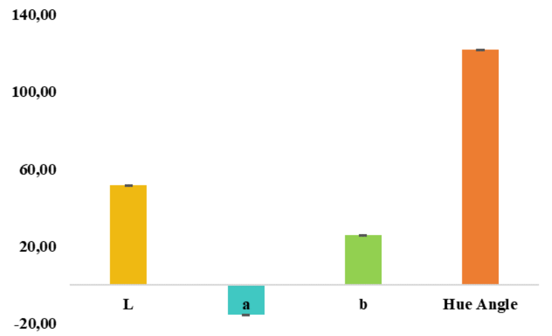

Figure 3: Colour parameters L, a, b, and Hue Angle of cladode pulp.

The Hue Angle is referred to the precise colour of the samples and was obtained similar values between the pulp (121.80º) and peel (123.20º) locating the colour of both parts in the green zone.

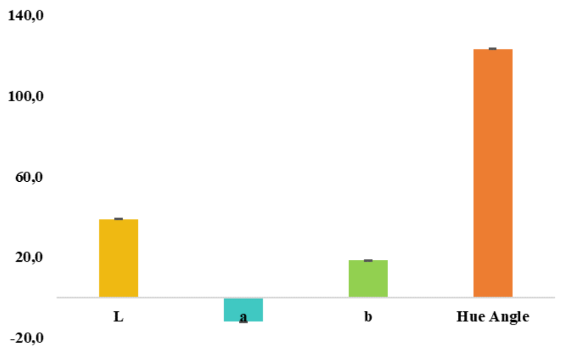

Figure 4: Colour parameter L, a, b and Hue Angle of cladode peel.

3.2 Mucilage extraction

The yield of extraction of mucilage showed different values for pulp and peel. **Figure 5** represents the results obtained for both parts of the cladodes and it is clear the difference between them. A yield of 0.72% was obtained for the pulp and 15.29% for the peel, in dry weight basis.

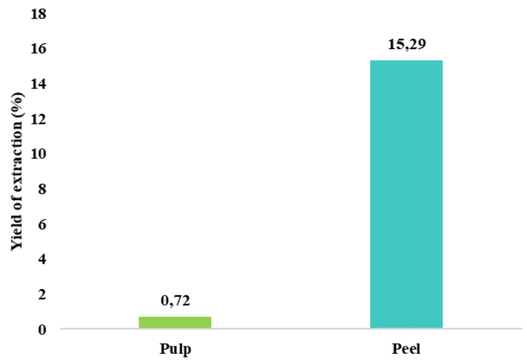

Figure 5: Yield of extraction of cladode mucilage from pulp and peel, expressed in percentage in dry weight basis.

From previous studies by other authors, yields of extraction of 1.5% on dry weight basis were obtained for the whole cladode [29]. In a fresh weight basis, the results obtained correspond of 0.033% for pulp and 1.19% for the peel. The result obtained for the peel is slightly higher than the ones reported by other authors with values of 0.68% for the whole cladodes [15] and lower than 1.5% [25]. The mucilage yield from pulp is lower than the results found in the literature.

The mucilaginous cells are more abundant in the parenchyma (related to the pulp) than the chlorenchyma (related to the peel) so it was expected to obtain a higher mucilage yield for the pulp instead of the peel [25]. It is possible that occurred not only the mucilage extraction but also the extraction of some other components like fibers. The yields of mucilage extraction obtained in this work could be related to the method employed, the conditions used in the method, solvents, and also the characteristics of the cladodes used [29].

4 CONCLUSIONS

Cladodes were characterized in terms of ash, moisture, total fiber, total protein, lipids and reducing sugars content. Both peel and pulp are rich in water, ash, and sugars and their composition is similar to a small difference in total fiber content. Also, the colour parameters L, a, b and the Hue Angle were also accessed, and all the parameters are similar. The mucilage extraction was attempted to develop a more efficient and sustainable method of extraction from the cladodes with characteristics that fit to be used as a biofilm material. Both parts of the cladode, pulp and peel presented a favorable potential for mucilage extraction and shows to be suited to application in the food industry due to properties reported in previous studies. To be applied as a potential packaging material, more studies are needed in order to achieve a higher yield of mucilage extraction from the cladodes.

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