Valorization of olive leaves extract in Natural Deep Eutectic Solvents for the development of bioactive chitosan films and hydrogels

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Introduction:

Chitosan is a copolymer of N-acetyl-D-glucosamine and D-glucosamine, produced by partial deacetylation of chitin from the exoskeletons of crustacean shellfish, such as shrimps and crabs. It is non-toxic, biodegradable and biocompatible with the human digestive system. The cationic nature of chitosan leads, under acidic conditions, to the development of various forms, such as nano/micro-particles, emulsions, fibers, hydrogels, films and membranes [1]. Hydrogels are 3D hydrophilic polymeric networks that do not dissolve but can swell, in water. In the last few years, there is increasing interest in these materials thanks to their solid and liquid-like properties, high biocompatibility, easy preparation, and versatile applications.

The Maillard reaction is usually known as a spontaneous and non- enzymatic browning reaction between the amino group of amino acids and the carbonyl group of reducing sugars which is promoted by heating. Chitosan contains amino groups that can react with the carbonyl groups of reducing sugars to generate Maillard reaction products (MRPs) such as Schiff bases and Amadori or Heyns rearrangement products. Some of the Maillard reaction products (MRPs) produced by chitosan improve the antioxidant, antimicrobial and emulsifying properties of the biopolymer.

Deep Eutectic Solvents (DES) are mixtures of two or more components, a hydrogen bond acceptor and a hydrogen bond donor, with a low-temperature eutectic point. When the components of the DES are naturally occurring compounds, the solvents are characterized as Natural Deep Eutectic Solvents (NADES) [2-5]. The components of the NADES can be task-specifically selected so as to fit to the desired application.

In this context, we decided to investigate the film and hydrogel formation capacity of chitosan dissolved in NADES and NADES extracts. The NADES of choice was Glucose/Lactic Acid/Water, which contains a monosaccharide able to react with chitosan via the Maillard reaction. This NADES is also an excellent solvent to efficiently extract polyphenols from olive leaves biomass. Thus, we set out to prepare chitosan films and hydrogels using the "as obtained" NADES-olive leaves extract as the dissolution, crosslinking and gelating agent.

Material and methods:

The heating and stirring method using NADES as extraction solvent was applied in order to obtain NADES-Olive Leaves Extract (NADES-OLE).

The as-obtained extract, rich in phenolic antioxidants, was used to prepare chitosan films and hydrogels. The components of the NADES were selected on the basis of their safety regarding pharmaceutic and food applications as well as on the basis that, when dissolved in water, the NADES results to an acidic solution (pH 1.62), which is an indispensable requirement for chitosan dissolution. The casting method was employed for the preparation of the chitosan films. The formation of chitosan hydrogels is a two-step process involving polymer dissolution and physical and chemical cross-linking

FT-IR spectroscopy was used to decipher the structure of the biopolymer after the formation of films and hydrogels. The bioactivity of the obtained chitosan films and hydrogels was evaluated regarding their ability to scavenge the stable free radical DPPH and their ability to inhibit lipid peroxidation induced by the thermal free radical initiator AAPH.

Results and discussion:

- Characteristic absorption bands of chitosan observed at around 1649 and 1584 cm^{-1} were assigned to amide I and primary amino groups, respectively. After the reaction with the glucose component of the NADES, a new absorption band at 1572 cm^{-1} appeared which can bea ttributed to the C=N stretching of the produced Schiff base.
- Swelling ratio of the chitosan-based Hydrogels-NADES-OLE in aqueous solution increased rapidly in the first 5 min reaching 559%. The water retention ratio remained over 70%, after keeping the swollen sample in phosphate buffer solution at pH=5.5 for 3 hours.
- The antioxidant activity of chitosan was higher after the Maillard reaction.

Conclusions:

- Olive leaf (OLE) biowaste was extracted using the Glucose/Lactic Acid/Water NADES
- The NADES and the NADES-OLE acted as efficient cross-linking agents and plasticizers for the production of the chitosan films and hydrogels
- FT-IR confirmed the formation of Schiff base between glucose and chitosan
- The obtained films and hydrogels have different biological and mechanical properties to later be used in different applications, especially in those concerning the food industry.

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