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Synthesis, optimization and structural characterization of a chitosan–glucose derivative obtained by the Maillard reaction

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ABSTRACT

Chitosan (Chit) was submitted to the Maillard reaction (MR) by co-heating a solution with glucose (Glc). Different reaction conditions as temperature (40, 60 and 80 °C), Glc concentration (0.5%, 1%, and 2%, w/v), and reaction time (72, 52 and 24 h) were evaluated. Assessment of the reaction extent was monitored by measuring changes in UV absorbance, browning and fluorescence. Under the best conditions, 2% (w/v) of Chit, 2% (w/v) of Glc at 60 °C and 32 h of reaction time, a chitosan–glucose (Chit–Glc) derivative was purified and submitted to structural characterization to confirm its formation. Analysis of its molecular weight (MW) and the degree of substitution (DS) was carried out by HPLC–Size Exclusion Chromatography (SEC) and a colloid titration method, respectively. FT-IR and ^{1}H NMR were also used to analyze the functional groups and evaluate the introduction of Glc into the Chit molecule. According to our objectives, the results obtained in this work allowed to better understand the key parameters influencing the MR with Chit as well as to confirm the successful introduction of Glc into the Chit molecule obtaining a Chit–Glc derivative with a DS of 64.76 ± 4.40% and a MW of 210.37 kDa.

1. Introduction

In recent years, chitosan (Chit) has received much attention from the scientific community. As it is well known, Chit is a deacetylated derivative of chitin mainly extracted from the exoskeleton of crustaceans, fungi and insects and it is an abundant byproduct of the seafood industry (Rao, Chawla, Chander, & Sharma, 2011). Chit is a biopolymer possessing different interesting bioactive properties such as antimicrobial, anti-inflammatory as well as technological properties such as fat binding capacities or texture enhancer, among others, which make Chit an interesting compound to be used in different fields such as in the food industry, pharmacy, dentistry, medicine and environment field (Dutta, Dutta, & Tripathi, 2004).

However, despite this broad spectrum of properties, Chit possesses certain limitations, as its poor solubility at neutral or basic pH (Yang, Chou, & Li, 2002). Drawbacks as well as functional characteristics of Chit depend on the degree of deacetylation (DD), the distribution of acetyl groups along the main chain, the molecular weight (MW) and the nature of the acid used for protonation (Pillai, Paul, & Sharma, 2009).

For this reason, numerous research works have been focusing their objectives in obtaining Chit derivatives with different or improved properties than native Chit.

Among all the followed strategies used for Chit modification, one of the most used is the substitution of the amino groups into the molecule with specific residues, which can confer to Chit the desired characteristics. Thus, for example, for improving solubility, the most common strategy involves introducing a hydrophilic functional group into the Chit molecule. Carbohydrates, especially, mono- and disaccharides have been extensively used to carry out this modification, since are simple and cost-effective molecules. Carbohydrate introduction can be performed by mean different chemical reactions, including reductive N-alkylation (Chung, Kuo, & Chen, 2005; Chung, Tsai, & Li, 2006), amide formation (Il'ina and Varlamov, 2007; Ruiz-Matute et al., 2013), quaternary reaction (Ignatova, Manolova, & Rasgkov, 2007; Verheul et al., 2008), carboxymethyl reaction (Mourya, Inamdar, & Tiwari, 2010; Song,

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Zhang, Gao, & Ding, 2010), acylation reaction (Shelma & Sharma, 2010) and Maillard reaction (Chung et al., 2006; García-Bermejo et al., 2012; Luo et al., 2013).

The Maillard reaction (MR), also named as nonenzymatic browning, is a chemical reaction involving the condensation between an amino group of amino acids, proteins or any nitrogenous compound and a carbonyl group of reducing sugars, aldehydes or ketones (Hodge, 1953) and is one of the main reactions taking place in processed foods. Thus, the presence of free amino groups makes Chit a candidate to react with the carbonyl group of reducing sugars, and allows it to participate in the MR. It has also been well reported that MR products contribute to flavor formation, antioxidant and antimicrobial effects and to the improvement of functional properties (Chevalier, Chobert, Genot, & Haertle, 2001). The formation of these compounds can be influenced by many factors, including reactive concentration, temperature, time of heating, initial pH and the type of reducing sugar used (Chung et al., 2006).

Different water-soluble chitosans, mainly derived from Chit and mono- or/and disaccharides have been produced, and their rheological, antioxidant and antimicrobial properties have been demonstrated (Kosaraju, Weerakkody, & Augustin, 2010; Wu et al., 2014; Ying, Xiong, Wang, Sun, & Liu, 2011). The results have shown that the obtaining Chit derivatives by the MR is quite promising, especially for the food industry due to their improved technological properties. However, from our point of view, the information reported in these studies is not enough, being in most cases, incomplete. In many publications, MW of Chit has not been reported or the structural characterization confirming the obtained derivative was not included. This implies that a relation between structure and properties cannot be established and it is well known that if the MW and the DD of Chit (or their derivatives) change, their biological and functional properties also change. Moreover, the assessment of the parameters affecting the reaction has been deeply evaluated.

In this work, Chit was characterized and submitted to the MR using the monosaccharide Glc. The factors affecting the reaction, including sugar concentration, temperature and reaction time were evaluated. Measurements of absorbance and fluorescence were used to determine the extent of the reaction and for determining the best condition of reaction. Furthermore, a complete characterization using ^{1}H NMR and FT-IR was also carried out to confirm the successful formation of the derivative. The DD and the MW were also determined. As it was mentioned before, several studies have been developed on the obtaining of chitosan derivatives by MR. However, to date, there is no study, to our knowledge, that include not only a study of the different conditions influencing the MR, but also a complete characterization of Chit and its corresponding derivative, which, can be in the future be related to their functional properties.

2. Materials and methods

2.1. Chemicals

Chitosan (Chit) powder with low MW, acetic acid and D-glucose (Glc) were purchased from Sigma–Aldrich Co. (Steinheim, Germany). Potassium polyvinyl sulphate titration solution (PVSK, *N*/400) was acquired from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2. Synthesis of chitosan–glucose (Chit–Glc) complex

The synthesis of chitosan–glucose (Chit–Glc) complex by the MR was carried out according to the method of Chung et al. (2006) slightly modified. Briefly, Chit was dissolved in 1% (v/v) acetic acid to give a final concentration of 2% (w/v) and the pH of the solution was adjusted to 5.5 by the slow addition of 0.1 M aqueous NaOH. Subsequently, 0.5%, 1% and 2% (w/v) of Glc were added under stirring to each Chit solution. Reactions were performed at 40 °C, 60 °C or 80 °C under shaking at 100 rpm during 72 h, 52 h and 24 h, respectively. Samples (3 mL) were withdrawn every 4 h to evaluate the extent of the MR. Reactions were stopped by keeping the solution in an ice bath during almost 10 min. Then, the reaction product was purified by dialysis (cut-off of MW of 3500 Da, Thermo Scientific Inc., USA) against distilled water for 2 days and frozen for subsequent freeze-drying. The freeze-drying process was performed using a Vacuum Freeze Drier (Model FT33, Armefield, UK), under a pressure of 100 mTorr; the temperature in the freezing chamber was −46 °C and the temperature in the sample chamber was 15 °C.

2.3. Analytical determinations

2.3.1. Determination of the extent of the Maillard reaction (MR)

The samples collected along the reaction were submitted for spectrophotometric analyses with the main objective to establish the extent of the MR. Thus, UltraViolet (UV) absorbance and browning of the sample were measured according to Ajandouz, Tchiakpe, Ore, Benajiba, and Puigserver (2001). Appropriate dilutions were made with distilled water and the absorbance was measured at 294 and 420 nm. The equipment used was an UV mini 1240 spectrophotometer (Shimadzu, Tokyo, Japan).

Suitable dilutions of the sample were taken for measurements of fluorescence as described by Morales and Jimenez-Perez (2001). Thus, the fluorescence intensity was measured at an excitation wavelength of 350 nm and an emission wavelength of 420 nm using an automated microplate reader (Fluostar Optima, BMG Labtech, Germany).

2.3.2. Characterization of chitosan (Chit) and chitosan–glucose (Chit–Glc) derivative

2.3.2.1. High Performance Liquid Chromatography–Size Exclusion Chromatography analysis (HPLC-SEC). Both samples, Chit and Chit–Glc derivatives were submitted to HPLC–SEC analysis in order to analyze their chromatographic profile as well as their average MW. The methodology was carried out following that indicated in Ruiz-Matute et al. (2013) slightly modified. Two ultrahydrogel columns (Ultrahaydrogel 250 × Ultrahydrogel 2000) along with an Ultrahydrogel guard column (Waters, MA, USA) were combined and coupled to a RID-10A Shimadzu refractive index detector. Analyses were performed at 30 °C using 0.25 M acetic acid/0.1 M sodium acetate as mobile phase at a flow rate of 0.8 mL min^{-1}. Mobile phase was filtered before use through a HVLP filter with 0.45 μm pore size (Millipore, Ireland). 100 μL of Chit solution were injected into the chromatographic system. Commercial pullulan samples (Waters, MA, USA) of different MW (0.3–800 kDa) were used for the preparation of the calibration curve.

2.3.2.2. Colloid titration method with potassium polyvinyl sulfate (PVSK). The degree of acetylation (DA) of Chit and their derivatives was determined following the method described by Ying et al. (2011) with minor modifications. Briefly, 20 mg of sample were dissolved in 10 mL of acetic acid (0.1 mol/L) and completely dissolved for 1 h at room temperature. Then, the mixture was diluted with 40 mL distilled water. 5 mL of the diluted solution was withdrawn and one drop of 1% (w/v) toluidine blue (Sigma–Aldrich Co., Steinheim, Germany) was added as an indicator. PVSK (*N*/400) was successively added until the titration end point was reached (a pronounced flocculation in the solution could be observed). Since the consumption of PVSK (*N*/400) (*A* mL, Eq. (1)) might correspond to the glucosamine (GlcN) unit in Chit and their derivatives, the total