LWT - Food Science and Technology 75 (2017) 440–446

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Use of soluble chitosans in Maillard reaction products with β-lactoglobulin. Emulsifying and antioxidant properties

Marian Mengíbar [a, 1], Beatriz Miralles [b, 1], Ángeles Heras [a, *]

[a] Instituto de Estudios Biofuncionales/ Dpto, Química Física II, Facultad de Farmacia, Paseo Juan XXIII, nº1, Universidad Complutense de Madrid, 28040 Madrid, Spain
[b] Instituto de Investigación en Ciencias de la Alimentación, CIAL (CSIC-UAM), C/ Nicolás Cabrera nº 9, Campus de la Universidad Autónoma de Madrid, 28049 Madrid, Spain

ARTICLE INFO

Article history:
Received 29 February 2016
Received in revised form
9 September 2016
Accepted 10 September 2016

Keywords:
Chitosan
Conjugate
Emulsifying activity index
DPPH radical scavenging
Reducing power ability

ABSTRACT

The reaction of commercially available soluble chitosans of equal acetylation degree (11–12%) and differing in their molecular weight (56–1.3 kDa) and β-lactoglobulin by Maillard reaction has been conducted in order to evaluate the resulting products and their functional properties. The characterization of the reaction products was performed by measurement of z-potential interactions, solubility, size exclusion chromatography, electrophoretic pattern, and absorbance and fluorescence spectroscopic compounds formation. Higher molecular weight chitosans (39 and 56 kDa) showed a slow progression and a gelled insoluble material appeared. In filtered products, emulsifying properties of the conjugates were improved with regard to those of the unreacted protein. Conjugates formed with the enzymatically depolymerized chitosan (1.3 kDa) displayed a sharp formation of advanced and final products of Maillard reaction. Products with all three chitosans gave rise to antioxidant activity superior to the protein after 2 days of reaction (2 and 3 times in ferric reducing power for 39 and 56 kDa chitosans, and 7 times for the 1.3 kDa chitosan, respectively), and the values correlated well with the spectroscopic and fluorescent compounds from the Maillard reaction. The results are useful with a view to selecting chitosans for development of new ingredients with tailored functional properties.

1. Introduction

Since proteins and polysaccharides are used in a wide variety of technological applications, the study of their conjugation for functional improvement in conditions industrially feasible results attractive. The Maillard reaction produces browning of compounds due to the initial condensation between a non-protonated amino group of aminoacids, peptides or proteins and a carbonyl group, usually a reducing sugar residue, to form a Schiff base. The reaction continues with cyclization to form the corresponding *N*-glycosylamine which undergoes an irreversible rearrangement to give the Amadori product. The process continues with a complex mechanism where the Amadori product is degraded and the advanced products that are formed are referred to as advanced glycation end products (AGEs) and brown pigments (Oliver, Melton, & Stanley, 2006). This spontaneous reaction was proposed as an effective and alternative method to chemical reactions involving cross-linking agents (Darewicz & Dziuba, 2001).

The Maillard reaction between dietary proteins and polysaccharides or sugars has been reported in a large number of publications where solubility, emulsifying activity, foaming capacity, and antimicrobial activity have been assessed in relation to the polysaccharide structure, the reaction time or the overall net charge (Liu, Kong, Han, Sun, & Li, 2014,; Wang & Zhong, 2014; Wang, Bao, & Chen, 2013; Yang et al., 2015). The versatile physico-chemical characteristics of chitosan due to the variable amine group proportion in the chain (degree of acetylation, DA) and the chain length make suitable the modification of the polymer to get distinctive technological and biological functions.

Enhancing the Maillard reaction for an intended use requires an insight into the structure, including the size and charge, of both the protein and carbohydrate moiety starting material (Evans, Ratcliffe, & Williams, 2013). The number of cationized groups in chitosan plays a key role in inhibiting the coalescence of oil droplets to produce a stable emulsion (Song, Babiker, Usui, Saito, & Kato, 2002).

* Corresponding author.
E-mail address: aheras@farm.ucm.es (Á. Heras).
[1] Both authors contributed equally to this paper.

http://dx.doi.org/10.1016/j.lwt.2016.09.016
0023-6438/

Satisfactory results have been achieved with high (Laplante, Turgeon, & Paquin, 2005) and medium molecular weight chitosans (400–600 kDa), (Li & Xia, 2011; Zinoviadou, Scholten, Moschakis, & Biliaderis, 2012). Also, the exposure of electrostatic binding sites of low acetylation degree (below 10%) chitosan contributes to the disruption of cell membranes in antimicrobial Maillard conjugates (Liang, Yuan, Liu, Wang, & Gao, 2014).

On the other hand, the formation of beneficial compounds through the Maillard reaction is currently gaining a lot of attention (Pastoriza & Rufián-Henares, 2014). Among others, *in vitro* studies have demonstrated that these conjugation products may offer substantial health–promoting activity due to scavenging of reactive oxygen species, radical chain-breaking activity and decomposing hydrogen peroxide and metal chelation (Chawla, Chander, & Sharma, 2009; Gu et al., 2010). Chitosan presents ferrous ion-chelating potency (Xing et al., 2005) and it is documented that it minimizes lipid oxidation itself (Li, Shi, Jin, Ding, & Du, 2013) or modified with glucose (Kanatt, Chander, & Sharma, 2008).

The goal of this study has been to carry out Maillard reaction with soluble chitosans of varying molecular weights and β-lactoglobulin (β-lac) to highlight the importance of the selection of the polymer with regard to its physico-chemical features, in particular the polymerization degree, on the intended use. To this aim, the emulsifying and antioxidant properties have been selected.

2. Materials and methods

2.1. Samples

β-lac (~80% PAGE) from milk was purchased from Sigma Chemicals (St Louis, MO, USA). Chitosan A (DA 12%, M_w 39 kDa) and Chitosan B (DA 11%, Mw 56 kDa) were provided by Laboratorios Beslan S.L (Madrid, Spain) and Productos Químicos Gonmisol S.L (Barcelona, Spain), respectively. Chitosan C (DA 12%, Mw 1.3 kDa) was prepared from chitosan A by enzymatic depolymerization using chitosanase from *Streptomyces griseus* (EC 3.2.1.132) (Sigma-Aldrich, St. Louis, MO, USA) by dissolving in 0.2 mol/L acetic/acetate buffer at 5 mg/mL (pH 5.7). One milliliter of enzyme (3.48×10^{-3} mg/mL) was employed per 100 mL of substrate to start the reaction at 37 °C in an orbital Lab Therm LT-Xshaker (Thermo Fisher Scientific Inc, Massachusetts, USA) at 100 rpm for 4 days. Ultrafiltration with cellulose acetate membrane disks of 30, 10 and 5 kDa cut-off (Millipore Corporation, Massachusetts, USA) was carried out to obtain the corresponding fraction of chitosan C and to separate chitosanase. The DA was determined by first derivative UV-spectrophotometric method (Muzzarelli & Rocchetti, 1985) using a spectrophotometer Specord 205 (Analtykjena, Jena, Germany) and the weight average molecular weight (M_w) by size exclusion chromatography (SEC-HPLC). The *Mw* of the different fractions were obtained from the SEC profiles by extrapolation in a calibration curve using different known *Mw* chitosans as standards (Mengíbar, Mateos-Aparicio, Miralles, Heras, 2013).

2.2. Preparation of Maillard reaction products

Freeze-dried Chitosan: β-lac mixtures (weight ratio 2:1), previously dissolved in 0.1 mol/L acetic acid (Chitosan: β-lac 6.6 and 3.3 mg/mL, respectively) and adjusted to pH 6, were incubated for 7 days at 40° C and 79% relative humidity. Samples were taken at 2, 4 and 7 days, dissolved at 1 mg/mL in deionized water, filtered through 11 μm pore-sized cellulose acetate membrane filters, and freeze-dried. The solubility was determined from the weight difference between the samples after both freeze-drying processes. Solutions of samples in water (2.5 mg/mL) were measured by microelectrophoresis using a Malvern Zetasizer Nanoseries Nano ZS (Malvern Instruments, Herrenberg, Germany).

2.3. Chromatographic separation of products

SEC-HPLC was performed in Waters 625 LC System pump with an Ultrahydrogel column (Waters, i.d = 7.8 mm, l = 300 mm) thermostated at 35 °C. Waters 2414 differential refractometer and Evaporative Light Scattering (ELS Waters 2424 Milford, MA, USA) were connected online. A 0.15 mol/L ammonium acetate/0.2 mol/L acetic acid buffer (pH 4.5) was used as eluent. The flow rate was 0.6 mL/min and 20 μL of samples dissolved in the buffer were injected.

2.4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE (Laemmli, 1970) was carried out employing a 150 g/L acrylamide separating gel and a 50 g/L acrylamide stacking gel. Samples (15 μL, 5 mg/mL) were dissolved in Tris–HCl buffer (pH 6.8) containing 10 mg/mL 2-mercaptoethanol. Electrophoresis was conducted for 1 h at a constant voltage of 180 V. Subsequently, the gels were stained with 0.5 mg/mL Coomassie brilliant blue-R250.

2.5. Absorbance and fluorescence measurements of Maillard reaction products

Aqueous solutions of the filtered reaction products (1 mg/mL) were prepared, to follow the formation of intermediate products by measuring the absorbance at 294 nm according to the method proposed by (Lerici, Barbanti, Manzano, & Cherubin, 1990). The formation of AGEs was measured by FAST Index method (Fluorescence of Advanced Maillard products and Soluble Tryptophan) with slight modifications as described previously (Birlouez-Aragon, Leclere, Quedraogo, Birlouez, & Grongnet, 2001). The fluorescence of soluble peptide tryptophan (Trp) and of advanced Maillard products (AMP) were measured at the wavelength λ_{Ex} = 338/λ_{Em} = 410 nm for AMP and λ_{Ex} = 290/λ_{Em} = 340 nm for Trp.

2.6. Emulsifying properties

The emulsifying properties of the filtered reaction products were determined according to the method of Pierce and Kinsella (1978). β-lac, Chitosan or Chitosan: β-lac reaction products were dissolved at 1 mg/mL in 0.1 mol/L acetate buffer (pH 4). Emulsions, consisting of 1 mL sunflower oil and 2 mL of the above solutions, were shaken and then homogenized in an Ultra Turrax instrument (IKA, Staufen, Germany) at 13,000 rpm for 2 min and at 20 °C. To determine the stability of the emulsions, a 50 μL aliquot of the emulsion was taken from the bottom of the container at different time intervals (0, 2, 4, 6, 8 and 10 min) and diluted with 5 mL of a 1 mg/mL SDS solution. The absorbance of the diluted emulsion was then determined at 500 nm.

2.7. Antioxidant properties

The *α,α-Diphenyl-β-picrylhydrazyl* DPPH radical-scavenging activity of Maillard reaction products was assayed by the method proposed by Chen, Tsai, Huang, and Chen (2009) with slight modifications. A total of 250 μL of sample solution in 5 mg/mL acetic acid was mixed with 1 mL of methanolic DPPH solution (100 μmol/L). The mixture was shaken and kept at room temperature in the dark. After 60 min, the absorbance was measured at 517 nm.

The FRAP (ferric reducing antioxidant power) assay was carried out according to the method proposed by Benzie and Strain (1996) and modified by Pulido, Bravo, and Saura-Calixto (2000) using a