Antioxidant potential and antimicrobial activity of chitosan–inulin conjugates obtained through the Maillard reaction

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Abstract Chitosan (1%) was glycosylated with inulin (0.5, 1, and 2%) via the Maillard reaction at various initial pH values (5, 5.5, and 6). Higher pHs led to a greater pH drop and increase in the intermediate products and browning intensity (BI). The chitosan–inulin conjugates were then classified into three levels of low, medium, and high BI through *K-means clustering* in order to investigate the effect of BI development on the antioxidant and antimicrobial attributes of the conjugates. Covalent linkage between chitosan and inulin was confirmed by Fourier transform infrared spectroscopy. High BI chitosan–inulin conjugate had significantly higher antioxidant property compared to chitosan and other conjugate fractions. In addition, the conjugates obtained at low pH values mainly presented greater antimicrobial activities than those prepared at high pHs. It can be concluded that chitosan–inulin Maillard-born conjugates can be used as novel antioxidant and antimicrobial prebiotic-based ingredients for food applications.

Keywords Chitosan · Inulin · Antioxidant · Antimicrobial · Maillard reaction

Introduction

The covalent coupling of carbonyl groups of reducing sugars to amino groups of nitrogenous compounds (e.g., amino acids, peptides, proteins, and chitosan) during food thermal processing and storage is mainly referred to the Maillard reaction. It has an important role in the food processes such as in coffee roasting, bread baking, and chocolate making (Nooshkam et al., 2018). The Maillard reaction has gained vast interest in different industries as a safe and promising alternative method to modify the functionality of many biological compounds (Pirestani et al., 2017). In food science, it has been broadly applied to ameliorate the technological functionality (e.g., solubility, thermal stability, foaming capacity, and emulsifying activity) of various proteins (Hamdani et al., 2018).

It has been reported that the Maillard reaction under controlled conditions can be employed as safe procedure to amend the antioxidative and sensory features of food products (de Oliveira et al., 2016). The reaction forms Amadori rearrangement products (ARPs) and melanoidins with metal chelating and oxygen radicals sequestering effects (Wang et al., 2013). It is worth to note that melanoidins have also antimicrobial, antihypertensive, and prebiotic properties (Wang et al., 2011). The antioxidant activity of the Maillard reaction products (MRPs) has been ascribed to their metal chelation, hydrogen peroxide degradation, radical chain breaking, and oxygen radicals scavenging features (Nooshkam and Madadlou, 2016a). Moreover, antimicrobial ability of melanoidins has been mainly attributed to their metal binding capacity (Rufián-Henares and Morales, 2008).

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As mentioned, the Maillard reaction can lead to bioactive conjugates with improved functional properties. One challenge in applying Maillard conjugation in food industry is the saccharide selection. It is important to use saccharides that are well accepted by consumers and confer technological and health benefits. In this study, chitosan (as an amino group source) and inulin, as natural bioactive compounds, were used to synthesize novel antioxidant and antimicrobial chitosan–inulin Maillard-born conjugates.

Chitosan, *N*-deacetylated basic chitin derivative, has several unique features such as metal ions sequestration, film formation, and antimicrobial, antioxidant, and antibrowning effects. These characteristics make chitosan an interesting bioactive compound to be used in food and medicinal industries. Its antimicrobial property is depend on various factors including chitosan type (molecular weight and deacetylation), temperature, pH, and food ingredients (Devlieghere et al., 2004; Kumar, 2000). Chitosan is also found in an abundant level in seafood industry as a by-product. The Maillard reaction has been applied to improve the antioxidant and antimicrobial properties of chitosan as well as its solubility (Rao et al., 2011; Ying et al., 2011; Zhang et al., 2015).

Inulin is a polydisperse oligomer with 10–65 fructose units (usually 12–15) that can be applied as a dietary fiber, prebiotic sugar, fat/sugar substitute, thickener, and gelling agent in reduced calorie food products (Lopes et al., 2015). The small intestinal enzymes (e.g., maltase, sucrase, and α -amylase) in digestive tract of mammals cannot degrade inulin, but it selectively underwent hydrolysis by the β -fructosidase enzyme produced by health-improving bacteria *Bifidobacteria* to form low molecular weight compounds such as organic acids (Schaller-Povolny and Smith, 1999). The presence of fructose, glucose, and fructans produced by the natural enzymatic or hydrolysis reactions show that inulin contains approximately 4.1% reducing sugar, thereby contributing to the Maillard reaction (He, 2015).

As seen in the literature and to the best of our knowledge, there is no report dealing with chitosan and inulin conjugation via the Maillard reaction. The aim of the present study was therefore to glycate chitosan with inulin to create a novel functional prebiotic-based chitosan–inulin Maillard-born conjugate with antioxidant and antimicrobial properties.

Materials and methods

Materials

Chitosan (medium molecular weight), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and triphenyltetrazolium chloride were purchased from Sigma-Aldrich Co. (St. Louis, MO).

Inulin (Orafti® GR) with $\sim 92\%$ inulin content and ≥ 10 degree of polymerization was acquired from Beneo Co. (BENEIO GmbH, Mannheim, Germany). According to the producer specification, Orafti® GR also contains other sugars such as glucose, fructose, and sucrose. All other reagents with analytical grade were purchased from Merck Co. (Darmstadt, Germany).

Preparation of chitosan–inulin conjugates

The Maillard conjugates were synthesized according to the procedure of Jiang et al. (2012) with some modification. Chitosan solution (1% w/v) was prepared by dissolving in 1% v/v glacial acetic acid solution (pH 3.6) and stirred at 500 rpm for 1 h at room temperature. The solution was then stored at 4 °C for hydration. Afterwards, inulin was added to the solution at three chitosan to inulin ratios of 1:0.5, 1:1, and 1:2. The mixed solution was stirred for 30 min and then pH was adjusted to 5, 5.5, and 6 by 4 M NaOH. Next, the chitosan–inulin solutions were autoclaved for 15 min. After heating, the conjugated solutions were rapidly cooled in an ice–water bath for further analyses.

pH measurement

The pH of the non-heated and heated solutions was recorded by means of a pH meter (Consort C831, Belgium) at ambient temperature.

Intermediate and final products of the Maillard reaction

The absorbance of the glyated solutions was measured at 294 nm (as an index of intermediate products) and 420 nm (as an index of browning intensity or final products) using a UV–Visible spectrophotometer (WPA-S2000-UV/Vis, UK) (Nooshkam and Madadlou, 2016b).

Conjugate chemistry

Fourier transform infrared (FTIR) analysis was employed to study the alterations in functional groups of chitosan upon conjugation. FTIR spectra of inulin, chitosan, and the conjugate were recorded at 400 to 4000 cm^{-1} by means of a FTIR spectrometer. Freeze-dried samples were mixed with potassium bromide, compressed to form tablets, and then scanned.

Antioxidant activity

Reducing power

Reducing power of the samples was evaluated based on the method of Li et al. (2013) with slight changes. One mL of

the samples (10 mg mL^{-1} based on chitosan content) was charged with 1.0 mL of distilled water and 1.0 mL of 1% w/v potassium ferricyanide ($\text{K}_3[\text{Fe}(\text{CN})_6]$). The mixed solution was incubated in water bath at 50°C for 20 min and then mixed with 2.5 mL of 10% trichloroacetic acid. Afterwards, the obtained mixture was centrifuged for 5 min at 1500 rpm. Next, 2.0 mL of distilled water and 1.0 mL of ferric chloride (0.1% w/v) were mixed with 2.0 mL of the supernatant and after storing at room temperature for 10 min, the absorbance of the mixture was read at 700 nm. Higher absorbance value denotes higher reducing power. Butylated hydroxyl toluene (BHT) with a concentration of 15 mg mL^{-1} in 96% ethanol was used as a standard antioxidant for comparison.

DPPH-radical scavenging (DPPH-RS) activity

The method reported by Bai et al. (2017) was applied with minor alteration to investigate the DPPH-RS activity of samples. One mL of 0.2 mM ethanolic DPPH solution was added and mixed with 2.0 mL of the samples (10 mg mL^{-1} based on chitosan content). After vigorous shaking, the mixture was kept at room temperature in a dark place for 30 min and its absorbance was then recorded at 517 nm against ethanol as blank. The samples were replaced with distilled water to prepare control according to the same procedure. Finally, the following formula was used to calculate the DPPH-RS activity of the samples:

$$\text{DPPH-RS activity}(\%) = \left(1 - \frac{A_s}{A_c}\right) \times 100$$

where A_s represents the absorbance of samples and A_c denotes the absorbance of control.

Antimicrobial activity

The hereunder antimicrobial assays were used to investigate in vitro antimicrobial effect of the conjugated samples towards pathogenic bacteria strains (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Bacillus subtilis* ATCC 23857) and fungal strains (*Candida albicans* PTCC 5027 and *Aspergillus niger* ATCC 23857).

Minimum inhibitory concentration (MIC)

The quantitative broth microdilution-based test in sterilized 96-well plate was employed to determine MIC of the conjugates. In brief, 20 μL of the conjugated and chitosan solutions (10, 5, and 2.5 mg mL^{-1} based on the chitosan content) was poured to the wells containing MHB (for bacteria) and SDB (for fungi) media. Afterwards, 20 μL of the microbial suspension (equivalent to 0.5 McFarland)

was added and the plates were incubated for 24 h at 37°C (for bacteria strains) or 72 h at 27°C (for fungi strains). Next, 20 μL of triphenyltetrazolium chloride solution (5% w/v) was added followed by re-incubation at abovementioned conditions. The presence of a dark red or purple color in the wells after 30 min incubation for bacteria and 24 h for fungi indicates the microbial growth. In this regards, the first sample concentration, in which the color did not form, was reported as MIC of the samples.

Minimum bactericidal (MBC)/fungicidal (MFC) concentration

MBC and MFC were determined via culturing all opacity-free wells on the MHA and SDA media, separately. The media were then incubated at 24 and 72 h for bacteria and fungi, respectively. The conjugated/chitosan concentration suppressing bacteria/fungi growth was reported as MBC and MFC.

Statistical analysis

SPSS software (version 21) was used for data analysis and Duncan's test at $p < 0.05$ was used for determining the differences between means. All experiments were done triplicate.

Results and discussion

pH changes

It has been proposed that pH decrement during the Maillard reaction is likely due to the generation of acidic substances like formic and acetic acids in the intermediate stage and the amino groups consumption in the early stage of the non-enzymatic glycosylation (Jiang and Brodtkorb, 2012). The alterations in the pH of chitosan–inulin conjugates as a function of initial pH and chitosan: inulin ratio are presented in Table 1. High initial pH values (5.5 and 6.0) resulted in a marked decrease in the pH of the conjugated systems, probably due to the creation of acidic compounds. However, the Maillard reaction at pH 5.0 did not lower the pH value of chitosan–inulin mixture and different chitosan to inulin ratios had no obvious effects on the pH changes during conjugation process.

The unchanged pH at acidic conditions, especially at pH 5.0 could be due to the more positively charged amino groups ($-\text{NH}_3^+$) in chitosan compared to pHs 5.5 and 6.0. It was reported that the rate of the Maillard reaction is low at acidic pHs because of the protonation of amino groups and rising pH up to approximately 10 leads to a manifest increment in the reaction rate (O'Brien et al., 1989).

Table 1 pH changes, intermediate products ($\text{Abs}_{294 \text{ nm}}$), and browning intensity ($\text{Abs}_{420 \text{ nm}}$) of chitosan–inulin Maillard-derived conjugates at various pH and chitosan:inulin ratios

pH	Chitosan:Inulin ratio	pH changes	Intermediate products ($\text{Abs}_{294 \text{ nm}}$)	Browning intensity ($\text{Abs}_{420 \text{ nm}}$)
		$5.01 \pm 0.01^{\text{a1}}$		
5.0	1:0.5	$4.99 \pm 0.00^{\text{a}}$	$0.584 \pm 0.036^{\text{d}}$	$0.090 \pm 0.017^{\text{c}}$
5.0	1:1	$4.99 \pm 0.00^{\text{a}}$	$0.603 \pm 0.057^{\text{d}}$	$0.110 \pm 0.016^{\text{bc}}$
5.0	1:2	$5.00 \pm 0.01^{\text{a}}$	$0.585 \pm 0.015^{\text{d}}$	$0.125 \pm 0.007^{\text{bc}}$
		$5.51 \pm 0.01^{\text{a1}}$		
5.5	1:0.5	$5.48 \pm 0.01^{\text{b}}$	$0.674 \pm 0.023^{\text{cd}}$	$0.098 \pm 0.009^{\text{bc}}$
5.5	1:1	$5.48 \pm 0.01^{\text{b}}$	$0.749 \pm 0.003^{\text{bc}}$	$0.104 \pm 0.003^{\text{bc}}$
5.5	1:2	$5.48 \pm 0.02^{\text{ab}}$	$0.594 \pm 0.019^{\text{d}}$	$0.113 \pm 0.006^{\text{bc}}$
		$6.01 \pm 0.01^{\text{a1}}$		
6.0	1:0.5	$5.87 \pm 0.01^{\text{b}}$	$0.760 \pm 0.013^{\text{bc}}$	$0.186 \pm 0.009^{\text{a}}$
6.0	1:1	$5.89 \pm 0.01^{\text{b}}$	$0.825 \pm 0.002^{\text{b}}$	$0.130 \pm 0.001^{\text{b}}$
6.0	1:2	$5.88 \pm 0.03^{\text{b}}$	$0.932 \pm 0.021^{\text{a}}$	$0.134 \pm 0.004^{\text{b}}$

Values with different superscripts for each parameter differ significantly ($P < 0.05$)

¹Initial pH value

Preliminary experiments showed the chitosan solution experienced intense turbidity at pHs more than 6.5, which was attributed to the chitosan insolubility at neutral and basic conditions (Gullón et al., 2016). Therefore, the experiments were performed at lower pH values. The pH reduction during the Maillard reaction between reducing sugars and amino groups has been reported in the literature (Jiang et al., 2013).

UV absorbance and browning intensity (BI)

The degradation of early MRPs (e.g., ARPs) into intermediate components is a pH-dependent pathway; hydroxymethylfurfural or furfural is synthesized at pHs ≤ 7.0 , which was employed in the present study, while basic pH values lead to the generation of reductones and fission components such as diacetyl, acetol, and pyruvaldehyde (Nooshkam and Madadlou, 2016b). The intermediate compounds have maximum absorbance at 294 nm in conjugation with yellowish color and reducing power (Nooshkam and Madadlou, 2016a).

The effects of the initial pH and chitosan to inulin ratios on $\text{Abs}_{294 \text{ nm}}$ of the conjugates were significant ($p < 0.05$). At pH 6.0, the intermediate products increased significantly as the level of inulin increased (Table 1). It appeared the formation of intermediate products initially increased and then declined as inulin content increased at pHs 5.0 and 5.5, however; no significant differences were observed between Abs_{294} of the conjugates formed at pH 5.0. It is hypothesized that higher UV absorbance of the chitosan–inulin conjugates at pH 6.0 is likely due to the greater reaction rate under elevated pH values (more deprotonated amino groups), which subsequently will

provide higher conversion yields of the early MRPs to the intermediate ones. The results are in line with pH changes in the previous section (Table 1). In accordance with the findings of the present communication, it was reported that glycation of sugars and protein/peptides results in a significant increase in the synthesis of intermediate Maillard components (Zhang et al., 2015).

The intermediate compounds undergo condensation process to fabricate brownish and nitrogen-bearing polymers named melanoidins with the highest absorbance at 420 nm, which is frequently used as an index of the Maillard reaction progression (Wang et al., 2013). BI increased gradually with rising inulin levels at pH values of 5.0 and 5.5. The highest BI observed at the 1:0.5 chitosan to inulin ratio at pH 6.0 and the BI was then significantly decreased as the content of inulin increased. It might be explained that the high initial pHs (pH 6.0) can manifestly increase the intermolecular mobility of chitosan amino groups (via increasing deprotonated NH_3 contents) and inulin carbonyl groups, thereby accelerating the conjugation rate in comparison to the lower pH values, which is supported by greater changes in pHs and the intermediate compounds (Table 1). However, the decreased BI with rising inulin content at pH 6.0 could be ascribed to the fact that high molecular weight conjugates can be formed at high inulin levels, which can lower the conversion yield of the intermediate compounds to browning ones through increasing the solution viscosity and in turn lowering molecular motion (limiting the contact area between carbonyl and amino groups). Although, chitosan has poor solubility in alkali and neutral conditions (Gullón et al., 2016), Huang et al. (2007) reported that chitosan conjugation with xylose at pH 9.0 resulted in a marked increase

in the BI compared to lower pH values (5, 6, and 7). Brown color development upon heating the mixture of chitosan and reducing sugars was reported in many researches (Chung et al., 2005; Ying et al., 2011; Gullón et al., 2016).

K-means clustering

Classification of bioactive Maillard-type conjugates could help in the prediction of technological functionality and biological properties as the first stage in their application as food preservatives. This object can be obtained by clustering analysis as a part of pattern recognition theory. In this method, a partial membership can be shared by two or more classes; whilst, the membership of a product in the traditional clustering methods is exclusive for only a class.

Clustering methods are employed to find suitable grouping of samples in a known data set. *K*-means clustering technique classifies a set of data into disjoint clusters with *K*-numbers through searching the inherent patterns in the set. The technique is completed according to the following steps: (1) defining random *k* centroids for each clusters, (2) associating each samples' data to the closest centroid, (3) formation of an early clustering via completing the first step, (4) estimating new *k* centroids through measuring the data average in each cluster, (5) re-allocating the sample data to the closest new centroids, and (6) generating a loop with repeatedly changing cluster centroid until *k* centroid is not more changed. In this way, samples having particular characteristics can be classified into *k* clusters.

It is also worthwhile to mention that *K*-means clustering has some properties such as (1) working better in the process of many data sets, (2) working different on numerical values and expression, and (3) completion mainly at most favorable point (Joon et al., 2019).

In the present study, 9 treatments were individually clustered into 3 various groups with 3 BI levels, i.e. low, medium, and high BIs. The highest distance to the cluster mean was considered for the first (low BI) and third (high BI) clusters, whereas the intermediate pH value with the highest BI was taken into account for choosing the second cluster (medium BI). The hereunder treatments were finally selected to be evaluated for antioxidant, antimicrobial, and structural properties.

Low BI: pH 5.0, 1:0.5 chitosan:inulin ratio.

Medium BI: pH 5.5, 1:2 chitosan:inulin ratio.

High BI: pH 6.0, 1:0.5 chitosan:inulin ratio.

FTIR spectra of chitosan–inulin conjugates

Figure 1 shows the FTIR spectra of chitosan, inulin, and chitosan–inulin conjugates with different BIs. The peaks at

around 3442 cm^{-1} and 2873 cm^{-1} in chitosan spectrum ascribed to $-\text{OH}$, $-\text{NH}$, and $-\text{CH}$ stretching vibration, and the inter- and intra-molecular hydrogen bonds (Zhang et al., 2015). Other characteristic absorption bands were also observed in chitosan spectrum: characteristic amide I band at 1661 cm^{-1} associated to $\text{C}=\text{O}$ vibration of the $-\text{CONH}_2$ groups in acetylated units; band at 1597 cm^{-1} assigned to the amide III ($-\text{NH}_3^+$ groups); band at 1160 cm^{-1} ascribed to $\text{C}-\text{O}-\text{C}$ symmetric stretching; and the bands at 1079 cm^{-1} and 1029 cm^{-1} attributed to the $\text{C}-\text{O}$ stretching vibration. The two strong peaks at around 1647 cm^{-1} and 1421 cm^{-1} in inulin spectrum are mainly due to the carboxylic acid asymmetric and symmetric stretching vibrations (Espinosa-Andrews et al., 2010).

The attachment of inulin to chitosan through the Maillard reaction led to some remarkable changes in the chitosan absorption bands. The peak at 1661 cm^{-1} decreased and shifted to 1582 cm^{-1} (in low BI spectrum), 1580 cm^{-1} (in high BI spectrum), and 1576 cm^{-1} (in medium BI spectrum), suggesting the creation of Schiff base ($\text{C}=\text{N}$ double bond) between the chitosan amine group and inulin carbonyl group (Gullón et al., 2016). In addition, the new bands at 1411 cm^{-1} in high BI spectrum and 1412 cm^{-1} in low and medium BIs spectra confirmed the incorporation of inulin into the chitosan molecule through the Maillard reaction (Gullón et al., 2016; Zhang et al., 2015). Furthermore, the peaks at 1600 cm^{-1} and 1421 cm^{-1} (inulin carbonyl region) and 1597 cm^{-1} (NH_3 groups of chitosan) disappeared after the reaction, mainly due to the Maillard glycation between the sugars.

Antioxidant properties

Reducing power

MRPs have the ability to reduce the ferric chloride (Fe^{3+})-ferricyanide complex to Fe^{2+} , which the concentration of the latter can be measured at 700 nm (Vhangani and Van Wyk, 2013). The reducing power of low, medium, and high BI fraction of chitosan–inulin conjugates is presented in Table 2. High BI conjugates rendered significantly higher reducing power than other pairs and chitosan solutions, indicating a positive correlation between BI and antioxidant potential. The improved reducing power of chitosan upon glycation could be attributed to by the reducing effect of hydroxyl and pyrrole groups of the conjugates (Bai et al., 2017). In agreement with our results, Zhang et al. (2015) stated that the increased reducing power of chitosan after conjugation with fructose is due to the hydrogen donating activity of the compounds formed at intermediate (reductones) and final (melanoidins) stages of the Maillard reaction. In addition, the authors claimed that the more content of hydroxyl groups was exposed in chitosan

Fig. 1 FTIR spectra of chitosan, inulin, and chitosan–inulin conjugates with low, medium, and high browning intensity (BI)

molecule via destroying intermolecular hydrogen bonds upon conjugation.

DPPH-RS activity

DPPH[•] is able to react directly with antioxidative compounds. In this way, the potent antioxidant can scavenge DPPH[•] via hydrogen donation concomitant with purple color transformation into yellow one (Rao et al., 2011). DPPH-RS activity increased significantly ($p < 0.05$) as a

function of BI (Table 2). It is noteworthy that high BI conjugate presented significantly greater scavenging effects towards DPPH[•] compared to low and medium BI conjugates and chitosan solutions. These results are congruent with those of Ying et al. (2011), who reported that the Maillard conjugation of chitosan with reducing sugars resulted in an increase in the DPPH-RS activity of the obtained MRPs. Furthermore, Benjakul et al. (2005) demonstrated that radical suppressing potential was well correlated with BI and the progress of intermediate

Table 2 Antioxidant and antimicrobial properties of low, medium, and high BI (browning intensity) fractions of chitosan–inulin Maillard reaction products (MRPs)

Biological assays	Chitosan–inulin MRPs		Chitosan solution (10 mg mL ⁻¹)			Standard BHT (15 mg mL ⁻¹)
	Low BI	Medium BI	High BI	pH 5.0	pH 5.5	pH 6.0
Antioxidant activity (10 mg mL ⁻¹ based on chitosan content)						
<i>Reducing power (AU)</i>	0.119 ± 0.008 ^b	0.131 ± 0.019 ^b	0.192 ± 0.003 ^a	0.117 ± 0.015 ^b	0.106 ± 0.011 ^b	0.128 ± 0.008 ^b
<i>DPPH-RS activity (%)</i>	15.171 ± 3.324 ^{bc}	20.406 ± 0.755 ^b	60.256 ± 3.022 ^a	9.508 ± 2.266 ^c	10.791 ± 0.755 ^c	11.752 ± 3.626 ^c
Antimicrobial activity						
<i>Minimum inhibitory concentration (MIC) (mg mL⁻¹)</i>						
<i>B. subtilis</i>	2.5	2.5	2.5	2.5	2.5	2.5
<i>E. coli</i>	2.5	2.5	5	2.5	5	5
<i>P. aeruginosa</i>	2.5	2.5	2.5	2.5	2.5	2.5
<i>S. aureus</i>	2.5	2.5	5	2.5	2.5	5
<i>A. niger</i>	2.5	2.5	2.5	2.5	2.5	5
<i>C. albicans</i>	2.5	2.5	5	2.5	5	5
<i>Minimum bactericidal concentration (MBC) (mg mL⁻¹)</i>						
<i>B. subtilis</i>	5	5	5	5	5	5
<i>E. coli</i>	5	5	10	5	10	10
<i>P. aeruginosa</i>	5	5	5	5	5	5
<i>S. aureus</i>	5	5	10	5	5	10
<i>Minimum fungicidal concentration (MFC) (mg mL⁻¹)</i>						
<i>A. niger</i>	5	5	5	5	5	10
<i>C. albicans</i>	5	5	10	5	10	10

Values with different superscripts for each parameter differ significantly ($P < 0.05$)

compounds of porcine plasma protein–sugar conjugates. The greater DPPH-RS activity of the high BI chitosan–inulin conjugates than other counterparts could be mainly ascribed to the formation of melanoidins with outstanding radical trapping effect (Wang et al., 2011). Additionally, Rao et al. (2011) declared that gamma radiation-assisted Maillard reaction increased the chitosan DPPH-RS activity with rising radiation dose, which was in line with development of brown color. This could be due to the chitosan de-polymerization upon gamma radiation, unmasking free amino groups with RS activity (Rao et al., 2006) in conjugation with melanoidins formation.

Antimicrobial activity

The antimicrobial property of the chitosan–inulin conjugates with different BI against some microorganism is shown in Table 2. The MIC and MBC/MFC of the conjugates towards the microorganisms ranged between 2.5–5 and 5–10 mg mL⁻¹, respectively. However, the inhibitory effects varied due to the conjugate and microorganism types. In most cases, low and medium BIs formed at low pH values of 5.0 and 5.5, respectively, had higher inhibitory effects than the high BI conjugate. Similarly, the chitosan solutions with low pHs had mainly higher microbiostatic and microbiocidal effects compared to chitosan with high pH value. This could be mainly attributed to the more protonated amino groups at such low pH values. In this way, low level of chitosan was likely conjugated with inulin and therefore the obtained conjugate had high antimicrobial activity.

It is also worthwhile to note that the antimicrobial effect of MRPs has been mainly attributed to the chelation activity of melanoidins as final products (Rurián-Henares and Morales, 2008a; Wang et al., 2011), while both intermediate and final products are contributed to the antioxidant potential of MRPs (Benjakul et al., 2005; Zhang et al., 2015). The lower antimicrobial capacity of high BI chitosan–inulin conjugate compared to other pairs could be therefore due to the fact that the Maillard reaction in this study is not significantly progressed towards the advanced stage (melanoidin formation). In this context, more unprotonated amino groups (due to high pH value of the system) are likely contributed to the Maillard reaction to produce early Maillard conjugates with high glycation degree and antioxidant potential. However, it seems that the glycation process is mainly performed at the initial stages (based on UV absorbance and A₄₂₀ nm) and is not sufficient to synthesize melanoidins with antimicrobial effect. In accordance with our results, Chung et al. (2011) reported a higher antibacterial activity of chitosan-based conjugates at low pH value of 5.0 compared to those obtained at pH 7.0.

Several antimicrobial mechanisms have been demonstrated for MRPs, especially melanoidins. It was reported that melanoidins, due to their anionic nature, can present bacteriostatic effect at low concentration via iron chelating from the culture medium, and bactericidal effect at high concentration through magnesium chelating from the outer cell membrane and subsequently disrupting the outer and inner membranes (Rufian-Henares and de la Cueva, 2009; Rurián-Henares and Morales, 2008a; 2008b). In addition, Muppalla et al. (2012) claimed that the antimicrobial activity of MRPs can be due to their high surface activity, interference with serine, glucose, and oxygen uptakes, inhibition of enzymes catabolizing carbohydrates, and antioxidant activity. Furthermore, H₂O₂ is produced during the Maillard reaction and its antimicrobial effect has been observed (Hauser et al., 2014).

In conclusion, the chitosan–inulin conjugate with high browning intensity, which was obtained at high pH value, rendered manifestly higher reducing power and DPPH-radical scavenging activity than other conjugates. In addition, the Maillard conjugation mainly boosted the antimicrobial effect of chitosan and this biological activity was more pronounced in conjugates fabricated at lower pH values. This study demonstrated that the Maillard reaction can be used as a facile and promising method to prepare bioactive conjugates with potential antibacterial and antioxidant properties in food and pharmaceutical industries. However, more studies are needed to identify the compounds formed during Maillard reaction between inulin and chitosan. Furthermore, further studies are required to evaluate potential applications of inulin–chitosan conjugates in food systems as preservatives, particularly in beverages and food packaging.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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