Emulsifying and Antioxidant Properties of a Shrimp (*Pandalus borealis*) Hydrolysate Conjugated With Xylose or Dextran Through the Maillard Reaction by Dry-Heating in Mild Conditions

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Abstract

Maillard Reaction (MR) was performed in 'dry' conditions at 50 °C for 48 h between native shrimp hydrolysate (HN) and xylose or dextran. Resulting emulsifying and antioxidant properties were assessed. HN was compared with a native sodium caseinate (CN) and its glycoconjugates, obtained in the same conditions. Compared to dextran, xylose resulted in significant browning after MR with HN or CN, but HN-xylose conjugates showed the most functional modifications. Increasing xylose/HN ratios led to significant molecular rearrangements in the peptide populations and intermediate aromatic compounds of the MR detected at 220 and 294 nm, respectively. Consequently, HN-xylose conjugates had higher reducing power than HN. Conversely, at 0.5% (w/w), a sharp increase in consistency index and apparent viscosity of emulsions after a 4-h ageing period was obtained with the lowest xylose/HN ratio. These results confirm that MR is a promising process for the production of new natural food ingredients from marine-derived protein sources.

Keywords: antioxidant properties, emulsifying properties, hydrolysate, maillard reaction, shrimp, xylose

Abbreviations: CN: native sodium caseinate; HN: native shrimp hydrolysate; MRPs: Maillard Reaction Products; HX0.6 and HD: MPRs obtained with HN-xylose and HN-dextran ratios = 0.6, respectively; HX1.2: MPRs obtained with HN-xylose ratio = 1.2; CX and CD: MPRs obtained with CN-xylose and CN-dextran ratios = 0.6, respectively ; MW: Molecular Weight

1. Introduction

Consumers often prefer natural ingredients, additives or preservatives as opposed to chemical ones in foodstuffs. Such preferences make the use of fish by-products for the production of innovative molecules a challenge for biotechnology and food industries, as they could offer just such alternatives (Guérard et al., 2010; Gómez-Guillén, Giménez, López-Caballero, & Montero, 2011; He, Franco, & Zhang, 2013; Liu, Ru, & Ding, 2012). Raw materials generally come from by-products such as fish skin, residual muscles on fish carcasses, seafood cooking water or crustacean residues. Although most of these by-products do not have desirable functional properties, enzymatic hydrolysis and/or fractionation using membrane technologies are widely used to improve them (Bourseau et al., 2009). These processes can be coupled to the Maillard Reaction (MR) using either liquid or dry conditions. Whatever the conditions used, MR is a natural process to improve the techno-functional properties such as gelling, foaming and emulsifying (Evans, Ratcliffe, & Williams, 2013; Xue, Li, Zhu, Wang, & Pan, 2013). In parallel with techno-functional improvements, Maillard Reaction Products (MRPs) can have biological activities such as in vitro antioxidant properties (Guérard & Sumaya-Martinez, 2003; Hwang, Kim, Woo, Lee, & Jeong, 2011). Although these properties are now well characterized in simple systems,

such as a reducing sugar with an amino acid residue, MR involving marine by-product hydrolysates still requires detailed study.

The advanced stage of the MR may be responsible for undesirable compounds, such as those with cytotoxic and carcinogenic activities (Cheriot, Billaud, Pöchtrager, Wagner, & Nicolas, 2009; Hongsprabhas, Kerdchouay, & Sakulsom, 2011), and a loss of techno-functional properties, demonstrated by a decrease in the emulsifying properties of glycoconjugates (Lan et al., 2010; Wang & Ismail, 2012). Extreme experimental conditions during MR will result in cross-linking and polymerization during advanced and final stages. The polymerization process increases the size of glycoconjugates, which can affect their diffusion to the oil-water interface and slow the reorientation process once they reach it (Corzo-Martínez, Carrera Sánchez, Moreno, Rodríguez Patino, & Villamiel, 2012). Consequently, the regulation and combinations of parameters such as heating time, temperature, pH, buffering conditions, water activity, and the nature, concentration and ratio of the reactants allow the control of MR pathways (Ames, 1992). Nevertheless, relationships among factors during the course of MR still need to be examined. In the case of functional properties, no correlation has been found between MRPs and the molecular weight (MW) of the reactant saccharides (Li et al., 2013).

In this context, we propose to study the functional properties of a shrimp by-product using xylose as high reactive sugar, which make it possible to apply mild MR conditions with temperatures below 60 °C (the threshold of protein denaturation). We have previously showed that pentoses are more reactive than hexoses due to the higher proportion of their acyclic form, which ultimately condition the sugar reactivity in MR (Laroque et al., 2008). Polysaccharides can be also used to produce MRPs (Evans et al., 2013). Among food polysaccharides used in MR, dextran has attracted increased interest in recent years (Alvarez, García, Rendueles, & Díaz, 2012; Evans et al., 2013; Lesmes & McClements, 2012; Li et al., 2013). It was thus demonstrated that i) protein-dextran conjugates obtained with high MW dextran are more effective for improving the emulsifying properties of protein source (Wong, Day, & Augustin, 2011) and ii) increasing MW of dextran slows down the rate of glycation through MR (Jiménez-Castaño, Villamiel, & López-Fandiño, 2007; Spotti et al., 2013). For this latter reason, MR with dextran is performed in dry conditions in order to ensure low water activity (Kato, 2002).

In the present work, the influence of xylose as pentose, and dextran as polysaccharide in the MR was investigated on a shrimp hydrolysate. The emulsifying and antioxidant properties of this marine by-product were investigated and compared with those of native sodium caseinate and its glycoconjugates obtained under the same MR conditions. As described above, in order to allow glycation between protein sources and dextran and to minimize the production of undesirable compounds, the present MR was conducted using drying conditions at 50°C. The impact of MR was assessed on the basis of the decrease in free amino groups, and MW distribution profiles assessed through chromatography.

2. Materials and Methods

2.1 Materials

Native shrimp (*Pandalus borealis*) hydrolysate was prepared from by-products of northern shrimp and supplied by Diana Naturals (Antrain, France). This hydrolysate has a 55.2% degree of hydrolysis, as measured by OPA (ortho-phthaldialdehyde) assay, and it contained: moisture 3%, ash 14.7% and protein 82.2%. Native sodium caseinate was supplied by Armor Proteines (France). Xylose and dextran (270 KDa) used for the MR, were purchased from Sigma–Aldrich Co. (St Louis, MO, USA). All solutions and dilutions were made using Milli-Q water (Millipore, Molsheim, France).

2.2 Maillard-Induced Glycosylation

The first step consisted of mixing xylose (X) and dextran (D) separately with either native shrimp hydrolysate (HN) or native sodium caseinate (CN), at the following weight ratios: X/HN = 0.6 and 1.2 (these samples were named HX0.6 and HX1.2, respectively); D/HN, X/CN and D/CN = 0.6 (these samples were named HD, CX and CD, respectively). The second step consisted of putting each mixture in distilled water and lyophilizing it (Bioblock Scientific CHRIST® alpha 1-4LD plus; –48 °C; 1.25 mbars; 96 h). The powders obtained were incubated for 48 h at 50°C (Incubator BE400, Memmert) in a hermetic desiccator (Desiccator PP/PC D250mm, Fisher Scientific) containing a saturated NaCl solution with activity of water (a_w) = 0.75. Temperature and relative humidity were monitored during the whole process using a thermohygrometer (Testo 175T1, Testo). Temperature remained constant (50+/0.1 °C) and relative humidity was stabilized at 75 % ± 1.5% from t + 15 h. At the end of incubation, the resulting powder was lyophilized a second time, using the same protocol as described previously. At the end of the process, the a_w of the glycoconjugates was measured using a water activity meter (FA-st/1, GBX, Scientific Instruments) and was found to be 0.242 ± 0.011.

2.3 Determination of Free Amino Group Content

All absorbance (A) measurements were performed using a POLARstar Omega spectrophotometer (BMG Labtech, France).

Available amino groups in the glycoconjugates were quantified by OPA (ortho-phthaldialdehyde, Sigma–Aldrich Co., St Louis, MO, USA) assay as described in (Laroque et al., 2008). Firstly, the following absorbance calibration curves were obtained: $A_{340leu} = 0.22$ [leu] ($r^2 = 0.99$; 0 to 0.66 mg.mL^{-1}), $A_{340HN} = 0.65$ [HN] ($r^2 = 0.97$; HN from 0 to 3 mg.mL^{-1}) and $A_{340CN} = 0.13$[CN] ($r^2 = 0.99$; CN from 0 to 3 mg.mL^{-1}). Then, from these curves, [HN] (mg.mL^{-1}) = 0.33*[leu] (mg.mL^{-1}), and [CN] (mg.mL^{-1}) = 1.61 * [leu] (mg.mL^{-1}) were inferred. These concentrations allowed the conversion of absorbance at 340 nm, obtained with leucine, into concentrations of free amino groups in mg.mL^{-1} HN, CN, or glycoconjugates. Three assays were made for the different protein sources and their glycoconjugates. Data were finally expressed as concentrations (%) relative to initial HN or CN weight.

2.4 Solubility Measurements

For solubility evaluation, the pH of HN and CN and their glycoconjugates, dissolved in distilled water (stock solution of 0.5 mg.mL^{-1}), was adjusted to 3, 4, 5, 6, 7, 8 and 9 using HCl or NaOH 0.5 N. The method used was adapted from (Alvarez et al., 2012; Mu, Tan, Chen, & Xue, 2009). After pH adjustment, a final concentration of 0.1 mg.mL^{-1} was obtained. Absorbance at 220 nm was then measured for the solution and for the supernatant after centrifugation at 3000 *g* for 15 min at 4 °C (Hermle Z233MK-2, Germany). Solubility (%) was then determined in triplicate as ($A_{supernatant}/A_{solution}$)×100.

2.5 Determination of Reducing Power

The reducing power of MRPs samples was determined according to the method proposed by (Oyaizu, 1986). Following this procedure, 250 µL MRPs samples ranging from 0.1 to 20 mg.mL^{-1} were mixed with 250 µL 0.2 M sodium phosphate buffer (pH 6.6) and 250 µL 1% potassium ferricyanide ($K_3Fe(CN)_6$). The reaction mixtures were incubated in a water bath at 50°C for 20 min, followed by addition of 250 µL 10% trichloroacetic acid after cooling at room temperature. The mixtures were vortexed thoroughly, then centrifuged at 5000 *g* for 15 min at 4 °C. Then, 500 µL distilled water and 200 µL 0.1% FeCl$_3$ were added to 500 µL of supernatant. The absorbance was measured in triplicate at 700 nm and reducing power results were expressed using absorbance units.

2.6 Molecular Weight Measurements

The MW distribution profiles of the samples were assessed by fast protein size-exclusion liquid chromatography (SEC-FPLC) using a Superdex^{TM} Peptide 10/300 GL column (GE Healthcare; fractionation range: 7000-100 Da) and a Superdex^{TM} 200 10/300GL column (GE Healthcare, fractionation range: 600-10 kDa). Chromatographic system and conditions of injection, as well as calibration of the columns were presented in details in Decourcelle, Sabourin, Dauer, and Guérard (2010). As described by authors, for the calibration of the two columns, standards of decreasing MW were used. For the first column, standards lead to logMW= − 0.12 Rt + 6.3, and for the second one, logMW= −0.107 Rt + 9.02 (Rt is the retention time, expressed in minutes). For each chromatogram extracted at 220 nm and 294 nm, peptides or MRPs, respectively, were sorted into five fractions of decreasing MW. The relative proportions of compounds were deduced from total areas under the curve and expressed as percentages (Table 1).

2.7 Preparation of Emulsions

Preparations of stock solutions of CN, HN or glycoconjugates and emulsions were adapted from our previous work (Decourcelle et al., 2010). The pH was adjusted to 7 with 0.1N NaOH or 0.1N HCl solution (Sigma–Aldrich Co.; St Louis, MO, USA). Two emulsions with a 50/50 (w/w) oil/water ratio were prepared.

2.8 Assessment of the Oil Phase Volume Fraction

Emulsion samples were placed in two 20 mL graduated glass vessels (Duran glass, 17×13×207mm, Fischer Scientific). The oil phase volume fraction (ϕ_f) was determined after a 4-h ageing period, after which no creaming was observed. Then, the volume of the upper cream layer (V_d in mL) was determined from the visible boundary between the continuous phase and the upper cream layer (Chanamai & McClements, 2000; Chanamai & McClements, 2000; Moates, Watson, & Robins, 2001). ϕ_f was then inferred: $\phi_f = V_d / (V_d + V_c)$, where V_c is the volume of continuous phase of emulsions in mL (Rojas, 2007).

A blue dye (E131 at 1.10^{-3} % w/w) was added to the emulsions to facilitate observation of the boundary; we had already verified that this dye had no effect on emulsifying properties (results not shown).

2.9 Rheological Analysis

After the 4-h ageing period, emulsions were collected from the middle of the cream layer and characterized using a speed-controlled rotational VT550® Haake™ viscometer with a double-gap Couette type geometry (NV sensor system (cup and rotor), Thermofisher). The two samples to be tested were put into the cup device, and the rotor was then lowered carefully into the emulsion in order to avoid it losing its structure during loading. All measurements were performed at room temperature. Rheological characterization of emulsions was performed by carrying out simple shear steady-state experiments. The flow curve, which is the shear stress as a function of shear rate, was obtained for gradually increasing shear rates ranging from 0 to 500 s^{-1} over a period of 120 s. The consistency index (K in Pa.s^n) and power law index (n) were obtained by fitting data to Ostwald's model ($\tau = KD^n$, where τ is the stress in Pa, and D is the shear rate in s^{-1}). The apparent viscosity (η in Pa.s) was determined at 13.5 s^{-1}. Data were treated with Rheowin Pro® v.4.30.0016 software (ThermoHaake™).

2.10 Statistical Analysis

ANOVA with Duncan's test was performed using Statgraphics™ Plus 4.0 software, with a confidence level of 5%.

3. Results and Discussion

3.1 Solubility

The solubilities of native protein sources (CN and HN) and their glycoconjugates in the pH range 3-9 are shown in Figure 1. As expected, CN showed a minimum of solubility close to pH 4.5. The results revealed that HN and its glycoconjugates (HD, HX0.6 and HX1.2) were highly soluble over a wide range of pH, whatever the type of sugar used for the MR; this is due to enzymatic hydrolysis, which leads to small peptides and therefore small glycoconjugates with MW < 7 kDa (Table 1).
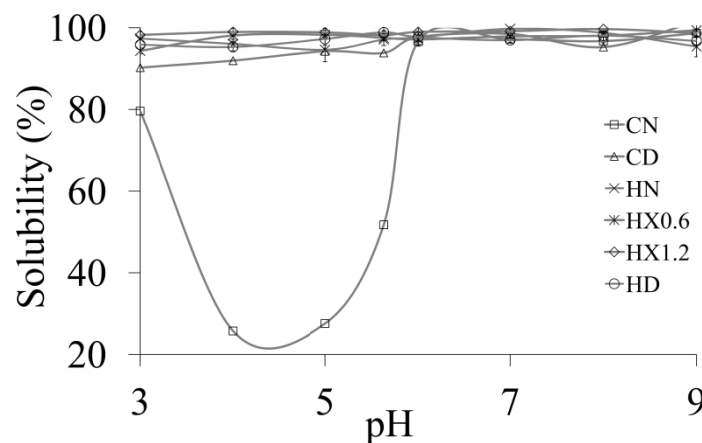

Figure 1. Solubility of native sodium caseinate (CN), native shrimp hydrolysate (HN) as well as their glycoconjugates obtained with xylose (HX0.6; HX1.2) or dextran (HD; CD)

Table 1. a) Molecular weight distribution of native shrimp hydrolysate before (HN) and after glycation with xylose (HX0.6; HX1.2). b) MW distribution of native sodium caseinate and native shrimp hydrolysate before (CN; HN) and after glycation with dextran (CD; HD)

a)	λ (nm)	> 7 kDa	7 < kDa < 3	3 < kDa < 1	1 < kDa < 0.5	< 0,5 kDa	AUC_{MR}/AUC_{Ref}
HN	220	0.2	4.4	13.4	13.5	68.5	Reference
	294	0.1	2.4	8.7	8.1	80.7	
HX0.6	220	5.6	20.6	25.1	11.9	36.8	2.7
	294	5.5	18.9	21.3	11.5	42.8	11.5
HX1.2	220	3.6	16.4	23.9	13.9	42.2	3.6
	294	2.6	13	19.7	12	52.7	27.4
b)	λ (nm)	> 400 kDa	400 < kDa < 200	200 < kDa <100	100 < kDa < 50	< 50 kDa	AUC_{MR}/AUC_{Ref}
CN	220	21.5	3.8	9.1	39.2	26.4	Reference
	294	37.7	5.3	42.3	12.6	2.1	
CD	220	26.1	6.5	17.6	31.9	17.9	1
	294	41.6	6.3	18.4	23.2	10.5	1.1
HN	220	0.3	nd	0.2	0.2	99.3	Reference
	294	nd	nd	nd	nd	100	
HD	220	0.2	nd	nd	0.1	99.7	1.1
	294	0.8	nd	0.3	nd	98.9	1.3

For each chromatogram extracted at 220 or 294 nm, native protein sources or MRPs, respectively, were presented into 5 fractions of decreasing MW. The relative areas (%) of each fraction are given as their percentage of the total area. Data presented on a) were obtained using a Superdex™ peptide HR 10/300GL column and those shown on b) were obtained using a Superdex™ 200 10/300 GL column, (nd: not detected; AUC_{MR}/AUC_{Ref}: ratio of areas under curve between MRPs and native protein sources; Ref: Reference).

The high solubility of HN and its glycoconjugates suggests that these glycoconjugates have a hydrophilic character (Klompong, Benjakul, Kantachote, & Shahidi, 2007). Figure 1 also shows that the solubility of CD increased (~90%) around pH at the isoelectric point in comparison to CN. This was likely due the modification in protein charges after MR (Corzo-Martínez et al., 2012). Similar results were shown for whey protein isolate glycated with dextran (Wang & Ismail, 2012), although glycation was limited between the two reactants. Our results on the extent of the present MR are discussed below.

The glycoconjugate CX obtained between xylose and native sodium caseinate is not shown on Figure 1. Indeed, while initial mixtures (Figure 2a and 2c) and HX0.6 (Figure 2b) resulted in fully soluble solutions, Figure 2d shows that solution of CX resulted in two phases, revealing a deposit of light brown matter and an upper aqueous phase. Therefore, it was not possible to study CX functionalities throughout the study.

At 12 mg.mL^{-1}, differences in color appeared between HX0.6 (Figure 2b) and CX (Figure 2d) revealing a lighter browning in CX, likely due the lower content of free amino groups in caseinate compared with shrimp hydrolysate. Nevertheless, it can be inferred from Figure 2d that browning occurred significantly in CX, likely due to the presence of advanced-stage MRPs. This high reactivity of the caseinate may conjointly be explained by the concentration of reactants resulting in dry conditions and by the preponderance of the open conformation of caseinate, which hence reacted rapidly with xylose. Then, although mild conditions were used in the present MR, changes in caseinate conformation and cross-linking among the two reactants might largely occur (Corzo-Martínez et al., 2012; Oliver, Melton, & Stanley, 2006). This result requires further investigation.
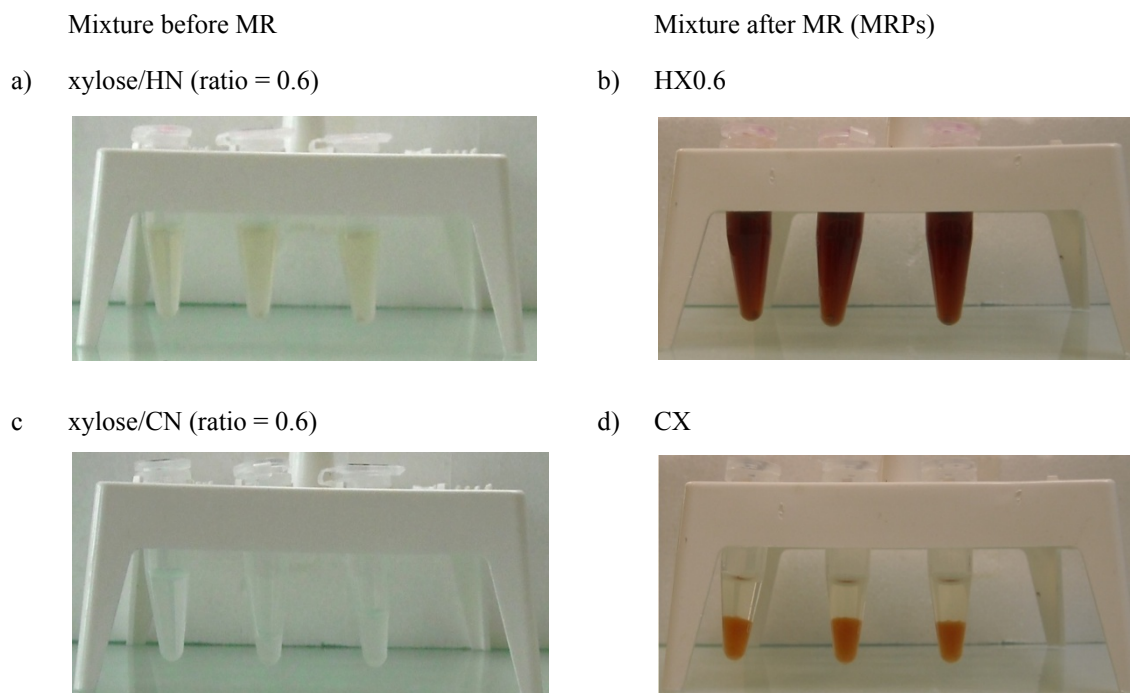
Mixture before MR   Mixture after MR (MRPs)

a)   xylose/HN (ratio = 0.6)   b)   HX0.6

c   xylose/CN (ratio = 0.6)   d)   CX

Figure 2. Images of 12 mg.mL^{-1} solutions containing HN-xylose or CN-xylose mixture before and after MR (HX0.6 and CX: MPRs obtained with HN-xylose and CN-xylose ratios = 0.6, respectively)

3.2 Molecular Weight Size Distribution of MR Products

Size-exclusion chromatography (Figure 3) was used to investigate MW distribution of protein sources before and after the MR. Rearrangements obtained after MR are shown in Table 1 through the appearance of compounds whose chromatographic profiles had increasing areas under the curve (AUC).

When considering HN-xylose conjugates (HX0.6 and HX1.2), the MR lead to significant molecular modifications in the protein hydrolysate detected at 220 nm, and in MRPs detected at 294 nm, as shown in Figure 3a and 3b. In addition, the AUCs, calculated from the elution profiles of native and glycated mixtures at 220 nm (Table 1a), were increased by factors of 2.7 and 3.6 in HX0.6 and HX1.2 samples, respectively. This means that MR led to a wide range of new compounds and to the apparition of new amide bonds (Figure 3a). As shown in Table 1a and Figure 3b at 294 nm, the AUC of glycoconjugates obtained with xylose in HX0.6 and HX1.2 were higher than that of HN by factors of ~11.5 and ~27.4, respectively. This reflects the production of aromatic intermediates of the MR and is in accordance with results obtained using high reactive sugars such as pentoses (Laroque et al., 2008).

Figure 3c and 3d show that glycoconjugates with dextran exhibited chromatographic patterns quite similar to those with native protein sources (CN and HN). Particularly, comparisons of CD and HD conjugates with their native protein sources revealed 1.1-fold higher and 1.3-fold higher AUC results at 294 nm, respectively (Table 1b). This is likely due to the lower content of reducing functions of dextran coupled to the low temperature used in the course of the MR.

The relative proportions of compounds classified according to their MW obtained at 220 nm and 294 nm (Table 1a) suggest an increase of MRPs derived from xylose, but it was also noticed that AUC measured after MR with dextran was slightly higher (Table 1b).
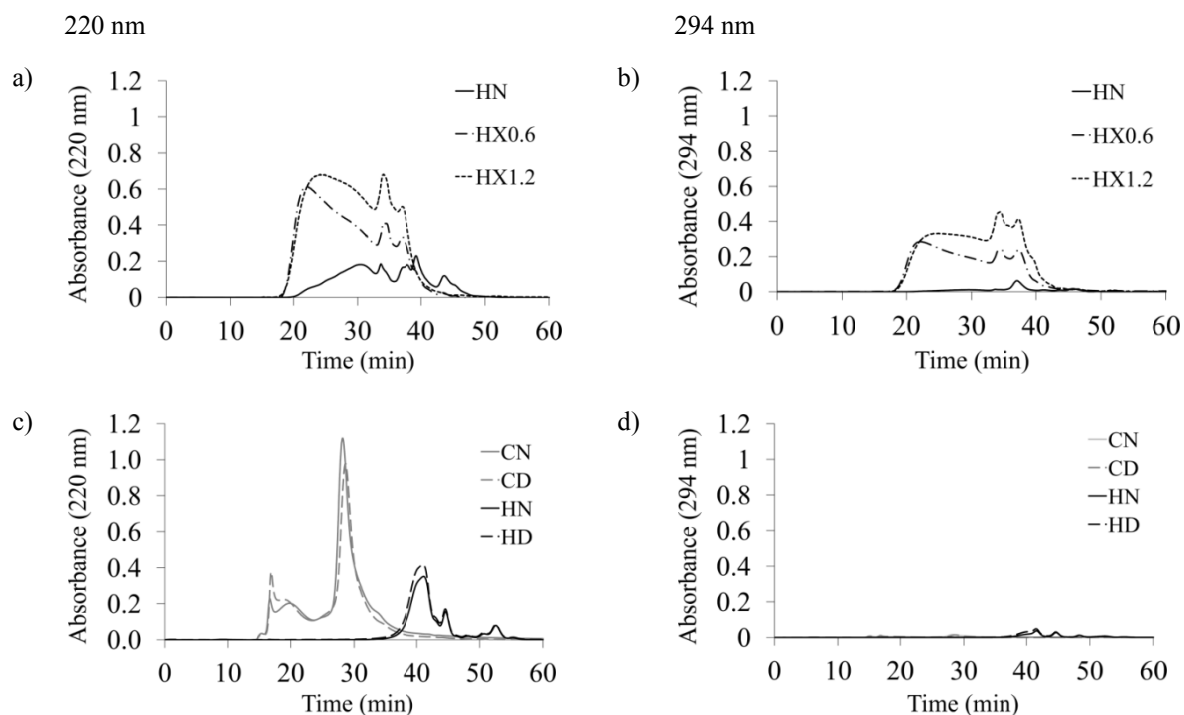
220 nm

294 nm

Figure 3. Chromatograms at 220 nm and 294 nm for shrimp hydrolysate and sodium caseinate (HN) and CN) before and after glycation with xylose (HX0.6; HX1.2) or dextran (HD; CD). Spectra a) and b) were obtained with a Superdex™ peptide HR 10/300GL column, whereas spectra c) and d) with a Superdex™ 200 10/300 GL column

3.3 Free Amino Groups

Table 2 indicates the amounts of free amino groups (mg.mL^{-1}) and their relative percentages (%) in the glycoconjugates and native protein sources.

Table 2. Results of OPA assay using to quantify amounts of free amino groups converted to mg.mL^{-1} proteins and to their relative content

	Amount of free amino groups (mg.mL^{-1} proteins)	SD	Relative percentage of free amino groups (%)
Without MR			
CN	151.5	1	100
HN	148.5	0.1	100
After MR			
CN	144.7	1.7	95.8
CD	135.7	1.3	89.6
HN	144.7	1.7	97.4
HX0.6	129.1	1.2	87
HX1.2	83	0.5	55.9
HD	144.9	0.5	97.5

The loss of available NH$_2$ primary groups allowed the evaluation of the reactivity between protein sources and sugar. It should, however, be mentioned that the MR can also occur to a lesser extent with other amino residues of proteins (Li et al., 2013; Wang & Ismail, 2012). Table 2 shows that xylose significantly decreased the quantity of free amino groups compared with HN. Furthermore, increasing xylose/HN ratio led to a greater decrease in

free amino groups. This decrease is in accordance with the results of size exclusion chromatography, showed by the linear relationship on Figure 4 and allows us to deduce the lowest concentration of xylose for which conditions would become limiting for the MR. In addition, the smallest decrease of free amino group concentration appeared after MR with HD and CD (Table 2). The weakest reactivity of dextran is likely related to its low contents of reducing functions and may also be related to steric hindrance effects (Jiménez-Castaño et al., 2007; Kato, 2002; Spotti et al., 2013).
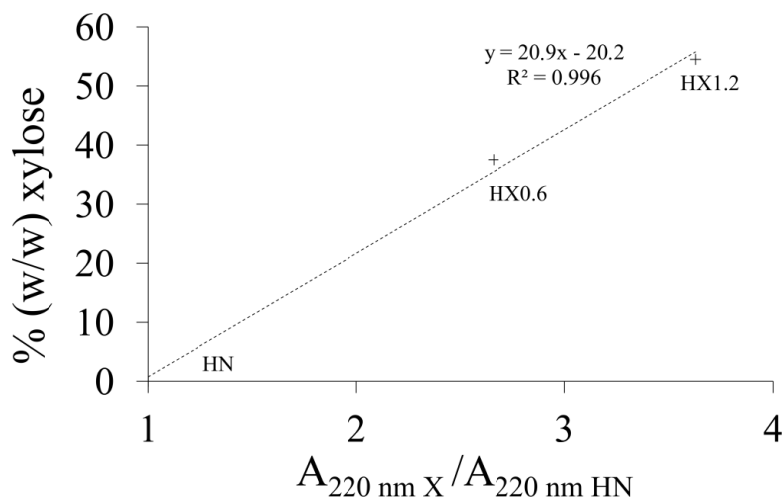
Figure 4. Relationship between xylose concentration in initial mixture (before MR) and reactivity of MR evaluated by the ratio $A_{220nmX}/A_{220nmHN}$ (X: HX0.6 or HX1.2)

3.4 Reducing Power

The reducing power of HX0.6 and HX1.2 was much improved compared with that of HN (Figure 5) at 5 mg.mL^{-1}. HX0.6 and HX1.2 reducing power was ~13.5 and ~16 times higher than HN, respectively. These results could be related to the MW distribution, as shown by the chromatographic analysis at 294 nm (Figure 3). Indeed, this analysis revealed the presence of high MW MRPs in HX0.6 and HX1.2 conjugates that were able to achieve reducing power (Gu et al., 2010). It may thus be inferred that these two glycoconjugates may act as reducing agents and could function as electron donors (Benjakul et al., 2005). In contrast, CD and HD conjugates did not significantly improve the reducing power of their native protein source despite little changes observed for HD both in AUC$_{MR}$/AUC$_{Ref}$ (Table 1) and in free amino groups (Table 2) in comparison to HN.
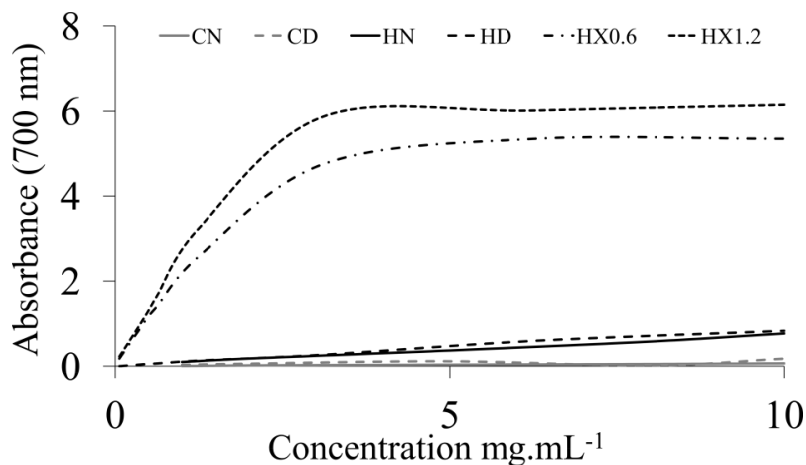

Figure 5. Reducing power of native protein sources (CN and HN) and their glyconconjugates (CD, HD, HX0.6, HX1.2) as a function of their concentration (mg.mL^{-1})

3.5 Oil Phase Volume Fraction

In the emulsions, since the initial oil/water ratio was 50/50, oil phase volume fraction at the beginning of the ageing period (ϕ_i) was 0.5. The destabilization of emulsions during the 4-h ageing led to an increase of the apparent oil volume fraction up to a final value (ϕ_f).

No destabilization phenomenon was observed with CN or CD: ϕ_f =0.5± 0.02~ ϕ_i, likely due to the effective surfactant effect of CN (Flanagan & FitzGerald, 2002) and its ability to cover and to charge the oil/water interface. The comparison between CN and CD also suggests that the inferior glycation with dextran does not modify the emulsifying properties of CN.

HN and its glycoconjugates exhibited similar and significant evolution of the oil volume fraction over time, leading to the same ϕ_f ~0.8 ± 0.015 >> ϕ_i. In these resulting emulsions, two distinguishable layers appeared (not shown): the oil droplets moved up in the emulsions to form a uniform and stable close-packed emulsion in the upper cream layer, and a lower layer appeared that corresponded to the remaining continuous phase (Robins, Watson, & Wilde, 2002). Thus, HX0.6 and HX1.2 did not lead to a better stabilization effect than HN or CN. Nevertheless, it was noted that these cream layers showed different 'apparent thicknesses'. Therefore, differences in the MW of HX-conjugates (Table 1a), but probably also new amphiphilicity and/or flexibility levels of these compounds, may influence the emulsion properties (Klompong et al., 2007). Such new properties were thus hypothesized for HX0.6 and HX1.2 emulsions and were demonstrated, as detailed below, by the rheological analysis.

3.6 Rheology

Shear stress vs. shear strain measurements were additionally used to evaluate the rheological behavior of the resulting emulsions obtained at the end of the 4-h ageing period (Sathivel, Yin, Bechtel, & King, 2009). Figure 6 shows the flow curves of emulsions for CN or HN, with or without glycation. The consistency index (K), power law index (n), coefficient of correlation (r) and apparent viscosity (η) are presented in Table 3.
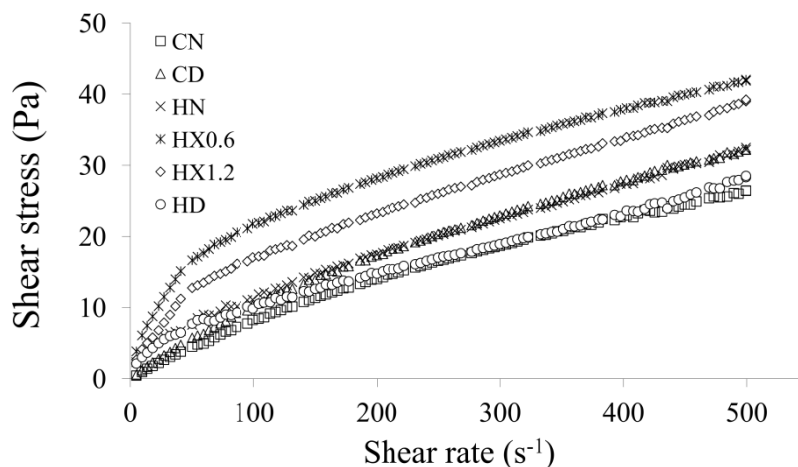

Figure 6. Flow curves of emulsions containing native protein sources or glycoconjugates at 0.5 % (w/w) after a 4-h ageing period

MR with xylose led to significant and interesting changes in the rheological properties of emulsions for HX0.6 and HX1.2. Indeed, compared with HN, ~9-fold higher K and ~3-fold higher η were obtained for HX0.6, and ~3-fold higher K and ~1.5-fold higher η were obtained for HX1.2 (Table 3a). Moreover, the results show that increasing the xylose/HN ratio by 2 decreased K by ~3 and η by ~2. This may be caused by excessive sugar attached to the protein source, which restricted the formation of a protein network (Oliver et al., 2006). In contrast, the results in Table 3b show that glycation with dextran had no significant effects on rheological properties of emulsions with either HN or CN, probably because the reactivity of dextran is too low, as demonstrated above. The same results were shown in the literature, with a lower MW for dextran (10 kDa), which did not significantly modify rheological properties of sodium caseinate after MR (Corzo-Martínez, Moreno, Villamiel, & Harte, 2010). Additionally, one can see that many studies have found discrepancies due to the use of dextran with different MW and/or MR conditions (Fechner, Knoth, Scherze, & Muschiolik, 2007; Li et al., 2013; Zhang, Wu, Yang, He, & Wang, 2012; Zhu, Damodaran, & Lucey, 2008).

Table 3. a) Consistency index (K), flow index (n), coefficient of correlation (r) and apparent viscosities (η at 13.5 s^{-1}) obtained with HN and its glycoconjugates or b) with CN and CD

a)	K (Pa.s^n)	n	r	η (Pa.s)
HN	0.54[a]	0.66[a]	0.998	0.27[a]
HX0.6	4.72[b]	0.37[b]	0.998	0.81[b]
HX1.2	1.76[c]	0.50[c]	0.998	0.42[c]
HD	0.57[a]	0.62[a]	0.995	0.27[a]

Superscript letters indicate significant differences ($p < 0.05$).

b)	K (Pa.s^n)	n	r	η (Pa.s)
CN	0.28[a]	0.73[a]	0.998	0.10[a]
CD	0.39[a]	0.72[a]	0.999	0.13[a]

Superscript letters indicate significant differences ($p < 0.05$).

The emulsions with CN and CD exhibited ϕ_f ~0.5 and showed quasi Newtonian behavior (Figure 6). In these emulsions, increasing the shear rate leads to the breakup of droplet interactions, which induces the release of the continuous phase (Binsi, Shamasundar, Dileep, Badii, & Howell, 2009; McClements, 1999; Oliver et al., 2006; Wang, Li, Wang, & Özkan, 2010). In contrast, emulsions with HN and its glycoconjugates showed a large decrease of n, suggesting the existence of an apparent yield stress (Pal, 1999; Princen, 1985) likely due to the greater value of $\phi_f > 0.74$ (Allouche, 2003; Alvarez Solano, 2006; Derkatch et al., 2007; Qi & Tanner, 2012). Indeed, in such emulsions, the internal structures result in particular rheological properties governed by the contact area of droplets and the interdroplet film thickness (Das & Ghosh, 1990; Gladwell, Rahalkar, & Richmond, 1986; Pal, 2006). It can therefore be inferred that HX0.6 has a large effect on interphase and droplet contact, which is greater than that of HX1.2. This could be related to their differences in glycation rate and MW distribution, as shown in Figure 3 (Gerrard, 2002; Lu, Su, & Penfold, 1999). Additionally, results showed that solubility of HN and its conjugates was not correlated with the rheological data of the emulsions, suggesting that interfacial adsorption of HX0.6 might be distinct from that of HN and HX1.2 because of differences in hydrophilic/lipophilic balance (Li et al., 2013), amphiphilicity or flexibility.

We can conclude from these latter results that the modifications of the functional properties of the shrimp hydrolysate were strongly affected by the protein/xylose ratio through the MR, although extreme conditions were avoided. It was thus demonstrated that the reducing power of the shrimp hydrolysate was substantially improved via the MR at the highest content of xylose, but conversely, that the highest rheological properties in the cream layer were observed with the lowest xylose content. This reveals the complexity of using MR to optimize both reducing power and emulsifying properties conjointly. Therefore, the challenge for future research will be to obtain better control over the combination of MR parameters in order to make further improvements in the bi-functionalities of protein sources.

Acknowledgments

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