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Assessing the effect of Maillard reaction with dextran on the techno-functional properties of collagen-based peptides obtained from bovine hides

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Abstract

The recovery of food processing co-products, in the meat sector, has become a hot topic. Based on previous studies, the enzymatic hydrolysis of bovine hides was proposed as a suitable and efficient recovery methodology to produce protein hydrolysates to be used in the food industry. It was found, however, that maximizing recovery yield lead to hydrolysates presenting very poor functional properties. Maillard reaction has been shown to modify the techno-functional properties of proteins without adding chemical agents. The glycation reaction occurred successfully as proved from the analysis of the free amino groups and the size exclusion chromatography (SEC). However, the glycated hydrolysates did not show an improvement in any of the techno-functional properties here assayed: foaming, gelling and emulsifying capacity. This lack of improvement was attributed to the low molecular weight of the peptides (less than 6.5 kDa in average, being the 60% of them lower than 3 kDa) required for recovering proteins from hides in high yields (>85%). When compared to non-hydrolysed collagen, the number of free amino groups per molecule in the hydrolysate is much lower, meaning that interactions between protein-protein and protein-matrix interactions are less evident.
**Keywords**

meat by-product; Maillard reaction; collagen peptides; gelling; solubility.

1. Introduction

Bovine hides, produced during the slaughter process, account for around 7% of the animal weight (Mullen, Álvarez, Pojić, Hadnadev, & Papageorgiou, 2015), and are used as a raw material for leather production. However, some pieces are not suitable for this industry (too small, damaged, etc.) and consequently they are discarded, imposing an extra cost for the producer. Hides are mainly composed by collagen, which is widely used in food industry as gelling agent, thickener or binder after being transformed into gelatine (Toldrá, Aristoy, Mora, & Reig, 2012). Collagen can also be used as protein supplement once hydrolysed into peptides and free amino acids (Ferraro, Anton, & Santé-Lhoutellier, 2016). Precisely, because of the many uses of collagen and the need of the meat industry to re-valorise the large amounts of processing co-products generated, innovative strategies have been adopted, as for example the use of Alcalase hydrolysis to recover collagen peptides from hides (Anzani et al., 2019). In this previous work the processing conditions were optimized to maximize the recovery yield of hides’ proteins in the form of peptides by means of Alcalase; which was selected based on its ability to hydrolyse the collagen present in this material (Anzani, Prandi, Buhler, et al., 2017; Anzani, Prandi, Tedeschi, et al., 2017). However, when aimed for high recovery yields, the final hydrolysate presents a very high degree of hydrolysis meaning that it is mainly composed of free amino acids and very short peptides (Anzani et al., 2019); which usually don’t exhibit good techno-functional properties (apart of high solubility); as it was also reported by Liu, Kong, Xiong, and Xia (2010) for plasma peptides obtained with Alcalase. In present work, this fact was further confirmed since the techno-functional properties of hides’ hydrolysate (solubility, emulsifying ability, foam capacity and water and
oil holding capacity) were characterised and the results were very poor when compared to intact collagen or gelatine. This loss of functionality will negatively impact the range of application of the hydrolysates as food ingredient.

A possible way to enhance the hydrolysate techno-functional properties is by means of controlled glycation following Maillard’s reaction; which is a procedure that involves food grade reagents and can be safely used for food industries with minimal changes to colour and flavour when carried out under controlled reaction conditions (time, temperature and pH) hence preventing progression to more advanced stages (Sanmartín, Arboleya, Villamiel, & Moreno, 2009; de Oliveira, Coimbra, de Oliveira, Zuniga, & Rojas, 2016). Many studies have reported that glycation can improve the emulsifying and foaming properties, water-holding capacity, and thermal stability of proteins (Chen et al., 2019; Doost, Nasrabadi, Wu, A’yun, & Van der Meeren, 2019). The glycation of proteins is conducted to produce more amphipathic compounds by adding hydrophilic groups in forms of carbohydrates, generating novel glycoproteins (Álvarez, García, Rendueles, & Díaz, 2012). These glycation is accomplished by the covalent attachment of carbohydrates to the protein free amino groups, especially those in lysine (de Oliveira et al., 2016). In this work dextran of 10 kDa has been selected because it has been reported that it can strongly enhance the stability of foams and act as thickening and gelling agents after conjugation (Liu, Zhao, Zhao, Ren, & Yang, 2012); which are the desired properties for collagen based ingredients (Gómez-Guillén, Giménez, López-Caballero, & Montero, 2011). Additionally, gelling and emulsifying properties are improved proportionally to the size of the sugar employed (de Oliveira, Coimbra, de Oliveira, Zuñiga, & Rojas, 2016).

Therefore, the first objective of this research was to examine the techno-functional properties (solubility, emulsifying ability, foam capacity and water and oil holding capacity) of a high DH (18%) hydrolysate from bovine hides. Second objective was, after assessing their poor
performance, to improve the hydrolysate techno-functional properties by glycation with dextran (10 kDa), aiming to improve their range of application. Finally, commercial gelatines and its Alcalase hydrolysates, used as a high collagen content control, were also analysed after glycation.

2. Material and methods

2.1 Enzymatic hydrolysis of bovine hides and the commercial gelatine powders

Samples consisting of pieces of bovine skin were provided by Inalca Industria Alimentare Carni SpA (Castelvetro, Modena, Italy) and stored at −20 °C. The hydrolysis process, using Alcalase 2.59 U/g, was carried out according to optimised conditions from a previous study (Anzani et al., 2019) using two sample:buffer (v/w) ratios 1:2 and 1:3 (Supplementary 0). As a result two hydrolysates with DH equal to 17.3 and 19.2%, with an extraction yield of 85% and 82%; and protein content of 80% and 85% were obtained respectively for hydromodule 1:2 and 1:3. The enzymatic hydrolysis reaction was performed on the commercial gelatines’ powders (bovine and porcine purchase from Sigma-Aldrich Co. Wicklow, Ireland) following the same protocol; however, in order to prevent its gelation, the sample:buffer solution ratio was increased to 1:4 (v/w). Finally, all the samples were freeze dried (Cuddon FD80, New Zealand) to obtain a powder, and preserved until use in refrigeration.

2.2 Preparation of peptide-dextran glycated samples

Dextran, with an average molecular size of 10 kDa, was supplied by Sigma-Aldrich (Co. Wicklow, Ireland). Freeze dried peptides (hides’ hydrolysates and hydrolysed gelatine powders) were dissolved in distilled water; dextran was added to a final proportion of 1:3 peptides/dextran (w/w) and stirred. This proportion was based on bibliography (Jung, Choi, Kim, & Moon, 2006), where 10 kDa dextran was employed. The peptides/dextran mixture
was then freeze dried to obtain a homogeneous dry powder; then the samples were placed
into hermetic vessels and dry-heated at 75 ºC for 3 hours in an oven (Gallenkamp, Germnay)
(Álvarez et al., 2012). After reaction, samples were removed and kept sealed under
refrigeration (4 ºC) until used for analysis. This process was carried out in duplicate.

2.3 Hydrolysates and glyco-peptides characterisation

2.3.1 Protein content determination

Protein content in all samples was determined using a LECO FP628 (LECO Corp., MI, USA)
Protein Analyser, based on the Dumas method according to the AOAC method (1996). A
conversion factor of nitrogen/protein of 5.56 was used.

2.3.2 Ultra-filtration fractionation of hydrolysates (UF)

In order to confirm the amount of peptides shorter than 3 kDa in the hydrolysates, these
samples were fractionated by using Amicon Ultra devices (Merk Millipore, Billerica, MA,
U.S.A.) equipped with a 3 kDa molecular weight cut-off (MWCO) membrane following
manufactures instructions: 10 mL of each sample were loaded, the volume of the retentate
was kept constant by adding ultrapure water until 10 mL were obtained in the permeate. The
protein amount of the retentate and permeate was determined.

2.3.3 Amino acid profile

Amino acid profile was analysed following the method reported by Hill (1965). Hide’s
hydrolysates were hydrolysed in 6 mol/L HCl at 110 ºC for 23 hours and the resulting
hydrolysates were quantified using a Jeol JLC-500/V amino acid analyser (Jeol (UK) Ltd.,
Garden city, Herts, UK) fitted with a Jeol Na+ high performance cation exchange column.
Norleucine was employed as internal standard.
2.3.4 Determination of free amino groups (FAG)

To calculate the FAG as meq of leucine/g of sample (meq/g) a method with slight modifications was followed (Anzani, Prandi, Tedeschi, et al., 2017). The OPA/NAC (o-Phtalaldehyde/N-acetyl-cysteine) reagent (100 mL) was prepared by combining 10 mL of 50 mM OPA (in methanol) and 10 mL of NAC 50 mM (in methanol), 5 mL of 200 g/L SDS, and 75 mL of borate buffer (0.1 mol/L, pH 9.5). The OPA assay was carried out by the addition of 20 µL of sample (diluted in water) to 2.4 mL of OPA/NAC reagent. The absorbance of this solution was measured at 340 nm with JASCO B-530 UV–Vis-spectrophotometer (JASCO, Oklahoma City, OK, U.S.A.). A standard curve was prepared using l-isoleucine (0–2 mg/mL).

2.3.5 Size exclusion chromatography (SEC)

Phosphate buffer (pH 7.0, 0.15 mol/L) was used as mobile phase with a flow of 0.35 mL/min in a Waters HPLC (2795 Separation Module) system coupled to an AdvanceBio SEC 130A 2.7 µm 4.6x300 mm column attached to an AdvancedBio SEC130A 2.7 µm 4.6x50 mm Guard. The result was monitored at 214 nm in a Photodiode Array Detector (Waters 2996) and the retention time of each peak was evaluated using the Empower Pro 2 software (Waters Corporation). A calibration curve was made using blue dextran (2000 kDa), albumin (66 kDa), carbonic anhydrase (29 kDa), lysozyme (14.3 kDa) and Vitamine B12 (1.3 kDa).

2.4 Analysis of techno-functional properties

2.4.1 Solubility

Exactly 0.5 g of the sample was dissolved in 10 mL of distilled water and pH adjusted between 3 and 8 by addition of 1 mol/L NaOH or HCl. The solution was centrifuged at 2400g for 30 min (Lynx6000, Thermo Fisher Scientific, Hempstead, UK) (Penteado, Lajolo, &
The amount of soluble peptides before and after centrifugation was determined by the Nanodrop ND1000 system (Thermo Fisher Scientific, Wilmington, DE, U.S.A.). Solubility was calculated as follows:

\[
\% S = \left( \frac{P_d}{P_t} \right) \times 100 \tag{Equation 1}
\]

Where \( P_d \) is the amount of soluble protein (g) and \( P_t \) the amount (g) the total protein used in the assay.

### 2.4.2 Water- and oil-holding capacities

The methods of Vioque, Sánchez-Vioque, Clemente, Pedroche, and Millán (2000) were used with modifications. Ten g of distilled water or rapeseed oil were mixed with 0.5 g of sample, held for 30 min, stirred twice gently, and centrifuged at 2,700g for 30 min (Lynx6000). The volume of the supernatant was weighed again. The water- (WHC) or oil-holding capacities (OHC) were expressed as following:

\[
\text{WHC/OHC} = \frac{(\text{initial weight} - \text{weight supernatant})}{\text{(grams protein employed)}}
\]

### 2.4.3 Lowest gelation concentration value (LGC)

The method of Coffmann and Garcia (1977) was followed with slight modifications. Aqueous solutions from 20 to 120 g/L of freeze-dried samples were adjusted to pH=6 using 1 mol/L NaOH or HCl; the solutions were heated in a thermostatic bath at 85 °C for 30 min. After heat-induced gelation, samples were cooled and stored at 4 °C for 24h. The lowest gelation concentration (LGC) is when the test tube is inverted, and the gel does not slip down.
2.4.4 Foam capacity and stability

A sample solution of 10 g/L was homogenised in an Ultraturrax (Ultraturrax T25, Janke & Kunkel IKA-Labor technick, Staufen im Breisgau, Germany) at 10000 rpm for 5 min. The percentage of increase in foam volume was recorded as foam capacity. The change in the foam volume after 0, 15, 30, 45 and 60 min of standing at room temperature was recorded as foam stability (Lawhon, Cater, & Mattil, 1972).

\[
\text{Foam capacity} (\%) = \frac{(\text{foam volume})}{(\text{liquid} + \text{foam volume})} \quad \text{Equation 3}
\]

2.4.5 Emulsifying capacity

Sample solutions ranging between 2 and 10 g/L were added with 13 mL of rapeseed oil. After stirring the samples for 2 min at 15000 rpm with a homogenizer (omni-prep Homogenizer, OMNI Inc, Kennesaw, Georgia, U.S.A) the samples were centrifuged at 1200 × g for 10 min (Lynx6000) (Inklaar & Fortuin, 1969). The percentage of emulsification was calculated as:

\[
\%E = \frac{(Ve/Va)}{\times 100} \quad \text{Equation 4}
\]

where Ve is the volume of the emulsified oil (mL) and Va the volume of the added oil (mL).

2.5 Statistical analysis

Differences between controls and treatments were analysed by one-way analysis of variance (ANOVA). When analysis of variance revealed a significant effect (p < 0.05), means were compared by Tukey test at 5% significance level, using the software IBM SPSS Statistics 24 software (Armonk, NY, U.S.A).
3 Results and discussion

3.1 Molecular weight and amino acid profile of hydrolysates

It was found that the weight distribution, after ultrafiltration, of hydrolysates 1:2 was composed by a 61% of peptides larger than 3 kDa and 39% shorter than 3 kDa, with these values being 64% and 36%, respectively, for hydrolysates 1:3 (Supplementary 1). SEC analysis revealed that both hydrolysates have an average molecular weight around 6.5 kDa (Figure 1). The amino acid profile (Supplementary 2) showed that there is no significant difference (p<0.05) between the two hydrolysates. Glycine and proline were the most abundant amino acids; which corresponds to collagen profile, the main constituent of hides.

3.2 Hydrolysates’ functional properties

The protein content of the freeze dried hydrolysates was of 79.8±0.3 and 85.1±0.2 g/100g, for 1:3 and 1:2 ratios respectively. Such powders were employed to analyse the functional properties, as summarized in Table 1 and Figure 2. No significant differences (p>0.05), regarding the functional properties, were found between both samples, with the exception of foam capacity which was higher for hydrolysate 1:3 (p<0.05); as shown in Figure 3. The reduced efficiency of small peptides as emulsifiers was related to the charge repulsion which prevented the peptides agglomerating to produce a layer around fat globules. Foam stability is also linked to the molecular properties, as small peptides do not have the strength needed to maintain stable foams (Karami & Akbari-adergani, 2019). Solubility was found to be 100% in the range of pH analysed; which is a positive characteristic that may play an important role in using hide hydrolysates as food ingredients in liquid formats or for easy incorporation into food matrices. Precisely, due to its high solubility, these peptides have no WHC since, under experimental conditions; a solution is formed rather than gel. Short peptides are unable to
create a hydrophilic matrix that can cause water retention. OHC of hydrolysates (Table 1) when compared to other proteins extracted from meat co-products performed poorly (Alvarez, Drummond, & Mullen, 2018). Finally, the most relevant functionality for collagen derived products, gel formation, was completely lost due to the hydrolysis. Short peptide chains are not able to generate enough intermolecular bonds to establish a 3-dimensional protein structure; therefore, water cannot be entrapped and retained to form a gel.

Since no main differences were found in the functional properties, size distribution or amino acid profile between both hydrolysates, the one generated at 1:2 ratio was selected for further research, given the fact that water consumption is minimised.

3.3 Changes in free amino groups and molecular size

In all cases a significant decrease (p<0.05) in the number of FAG was observed, demonstrating that the glycation reaction took place (Table 2). In the case of hydrolysate 1:2, the decrease was not significant (p=0.06); however, the conjugation took place as confirmed by SEC analysis and a clear trend in the FAG decrease was observed. After hydrolysis, larger amounts of FAG are available for glycation; however, non-hydrolysed commercial gelatine showed a higher percentage decrease than the observed after being hydrolysed, as also observed by Cermeño et al. (2018). The mili-equivalents of FAG per gram of sample, considering an average molecular weight of 50 kDa for bovine commercial sample and 6.5 kDa for the hydrolysates (as observed in SEC analysis), was also calculated (Table 2). After hydrolysing, around 10 times more free amino groups (mainly amino terminal) are available per gram of sample; which is in agreement with the 20% DH observed. Therefore, although in percentage a lower reduction in FAG is observed, in absolute numbers a remarkably higher number of FAG are conjugated when hydrolysates are glycated.
The SEC analysis is further evidence that the glycation reaction occurred since an increment in the molecular weight of peptides was observed. Particularly, Figure 1 shows the example of the hydrolysate 1:2 and the porcine commercial gelatines before and after the treatment.

After the treatment, Figure 1.B, there is a clear shift in the molecular weight, where an average value of 150 kDa was found, compared to the initial 6.5 kDa. A combination of both conjugation and further polymerization reactions, will explain the higher molecular size observed (Van Boekel, 1998). Additionally, the complete disappearance of the peak around 10 minutes demonstrated that all the molecules have gone through the glycation reaction. A similar trend was observed for the commercial gelatine from pork (Figure 1C and D) and bovine (data not shown), where most of the peaks shifted to shorter retention times indicating an increased average MW.

3.4 Functional properties of glycated proteins

Protein content of glycated proteins, both from commercial and hide hydrolysates, was evaluated prior to characterising functional properties, as shown in Supplementary 3.

3.4.1 Solubility

In the case of the hides hydrolysates no differences were observed after the treatment: complete solubility was detected before and after the treatment within the pH range tested.

In the case of the porcine gelatine, the solubility was increased (p<0.05) in all the pH range used (3<pH>8) after glycation, as observed by other research groups on different starting materials (Li, Enomoto, Hayashi, Zhao, & Aoki, 2010; Mu, ZHao, ZHao, Cui, & Liu, 2011; Qi, Yang, & Liao, 2009). Solubility of bovine gelatine was slightly improved (p<0.05) after glycation in the pH range from 3 to 6, except for a remarkable decrease at pH 4 and a slight decrease in alkaline range. A similar trend was observed also by Mulcahy, Mulvihill, and
O'Mahony (2016), on whey protein isolate treated with maltodextrin. After hydrolysis and the following glycation of the commercial gelatin powders, the solubility between pH 3 and 8 was maximum for all the samples assayed. This increase in protein solubility after conjugation might be attributed to an increase in the hydration of the collagen proteins due to the covalent bonding of hydrophilic dextran, and modification of the net charge of the protein, contributing to greater repulsion between the protein molecules (O'Mahony, Drapala, Mulcahy, & Mulvihill, 2017).

### 3.4.2 WHC and OHC

A significant (p<0.05) decrease in OHW and WHC (Table 3) was observed in the commercial gelatines after the hydrolysis process; however, no significant variation was observed between hydrolysates and the hydrolysates after glycation. This behaviour was also observed by Lillard, Clare, and Daubert (2009) in whey protein concentrates glycated with dextran. OHC and WHC of dextran were also evaluated and values were similar to those found in the hydrolysates and the glycated samples, showing that there was no beneficial effect following glycation.

### 3.4.3 Foam capacity and foam stability

Foaming property is an important attribute in food ingredients; for products such as desserts, baked products or ice creams. In literature, examples of improved foam capacity and stability after Maillard reaction have been reported (Fu et al., 2019). Figure 3 illustrates the percentage of foam formation and its stability in both bovine and porcine gelatines.

In the case of the bovine gelatine, the foam capacity is not modified after glycation (p>0.05); however, the stability at 45 minutes of analysis was negatively affected (p<0.05). In the case of the porcine gelatines, glycation and the glycation after the hydrolysis also negatively affected the foam capacity and stability (p<0.05) when compared to the control; however,
foam was more stable within each treatment. Similar negative results were obtained for the hide hydrolysates, since no improvement was observed after Maillard reaction (Figure 3b). The same outcome was found out or by Corzo-Martínez, Sánchez, Moreno, Patino, and Villamiel (2012) when β-lactoglobulin was conjugated with galactose.

3.4.4 Emulsifying capacity

Emulsifying properties play an important role in food systems, as they contribute directly to texture and sensory properties of food. There are several examples in literature showing an increase of the emulsion activity and stability after conjugation with dextran (Cheng, Tang, Xu, Wen, & Chen, 2018; Zhang, Yu, Wang, Wang, & Zhang, 2019). The increment depends on the Hydrophilic-Lipophilic Balance (HLB) of the protein-polysaccharide conjugates. In this sense, the saccharides attract water molecules around the oil droplet, while the hydrophobic residues of the protein molecules are attached on the oil droplets inhibiting the oil droplets coalescence (Doost et al., 2019). The commercial gelatines behaved in a different way, as previously noticed in the foaming properties (Figure 6).

In the case of bovine gelatines, the glycation did not significantly affect the emulsifying capacity and stability (p>0.05). By contrast, after the glycation of the porcine commercial gelatines, a dramatic decrease in emulsifying properties was observed. Glycated hydrolysed samples showed no emulsifying capacity. Hydrolysed hides after glycation performed equally poor as emulsifier agents; as glycation had no positive effect. It is likely that the short peptides forming part of the glycoprotein had not enough hydrophobic groups to stabilise the emulsion.

3.4.5 Gelling properties
Commercial gelatines were negatively affected by conjugation after hydrolysis (Supplementary 4). There was no improvement in the hide hydrolysates. Similar decrease in LGC values was reported for purified bovine blood proteins (Álvarez et al., 2012); where it was reported that dextran incorporated to the protein impeded the protein-protein interaction, hence increasing the protein concentration required to form a stable gel.

4. Conclusions

Results obtained in this study suggest that, despite Alcalase provides an excellent mechanism to hydrolyse hides proteins and therefore, recover valuable amino acids and peptides; is not a suitable method to recover peptides that can be used as a techno-functional ingredient by the food industry. Apart from high solubility in a wide pH range (3>pH>8), bovine hides’ hydrolysates showed poor techno-functional properties. Aiming to enhance the techno-functional properties of the hydrolysates, the dry-glycation reaction with dextran 10 kDa was used to generate glyco-peptides. The significant (p<0.05) decrease of the free amino groups and the presence of high molecular weight molecules in the size exclusion chromatography demonstrated that the glycation was successful. However, it was clear that the glycation, using dextran of 10 kDa under these experimental conditions, did not influence the techno-functional properties of the hides’ peptides, probably because of its low molecular weight. The reduced number of functional groups per molecule, compared to intact protein, decreases the number of interactions (hydrophobic, hydrophilic etc) between peptides and other molecules (lipids, water or proteins), which are no sufficient to form stable gels, emulsions or foams. Therefore, in order to obtain a functional hydrolysate a lower DH is recommended, acquiring a compromise between recovery yield and functionality.

The application of peptides recovered from bovine hides conjugated with functional prebiotic carbohydrates in liquid food matrix, will be a possible avenue to add value to this product;
based on its high solubility and the low interaction with other ingredients (water, fat or large 
proteins) present in the formulation.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figure Captions

Figure 1: SEC profiles of native and solution-treated samples: A hydrolysate 1:2, B hydrolysate 1:2 glycated, C porcine commercial gelatine, D porcine commercial gelatine glycated.

Figure 2: Solubility of bovine (A) and porcine (B) collagen. Circles represent native collagen, squares collagen glycated; and triangles hydrolysed and glycated collagen. Bars reflecting SD are included. Data within group sharing in common capital letter are not significantly different (p<0.05) Data within same sampling time in common lower-case letter are not significantly different (p<0.05)

Figure 3 Foam capacity and stability of bovine (A) and porcine (B) collagen. Circles represent native collagen, squares collagen glycated; and triangles hydrolysed and glycated collagen. Values reported as mean values (n=2) and standard deviation. Data within group sharing in common capital letter are not significantly different (p<0.05) Data within same sampling time in common lower-case letter are not significantly different (p<0.05)

Figure 4: Emulsifying capacity of bovine (A) and porcine (B) collagen. Circles represent native collagen, squares collagen glycated; and triangles hydrolysed and glycated collagen. Data within group sharing in common capital letter are not significantly different (p<0.05) Data within same sampling time in common lower-case letter are not significantly different (p<0.05)
Table 1: Analysis of the functional properties of hide hydrolysates obtained at both sample/buffer ratios.

| Functional Property                  | Results                                                                 |
|--------------------------------------|-------------------------------------------------------------------------|
| Solubility 3<pH>8                    | Completely soluble                                                      |
| Water holding capacity               | No water was hold since a solution was formed                           |
| Oil holding capacity                 | 0.89 ±0.05 g oil/g protein (hydrolysates 1:2)<sup>a</sup>               |
|                                      | 0.96 ±0.04 g oil/g protein (hydrolysates 1:3)<sup>a</sup>               |
| Gelling properties                   | No gel was obtained                                                     |
| (20-120 g/L of protein)              |                                                                         |
| Emulsifying capacity                 | Less than 10% of added oil                                             |

Data within columns sharing superscript are not significantly different (p<0.05)
Table 2: Free amino groups (FAG) (equivalents of Ile/mol and meq/g) detected in native and glycated proteins.

|                  | (eq/mol) Native | (eq/mol) Glycated | (meq/g) Native | (meq/g) Glycated | Decrease (%) |
|------------------|-----------------|-------------------|----------------|------------------|--------------|
| **Bovine gelatin** | 6.24±0.24A      | 1.60±0.01B        | 0.12±0.01A     | 0.03±0.01B       | 74.31±0.83a  |
| Hydrolysed bovine gelatin | 7.85±0.82A      | 5.00±0.02B        | 1.20±0.12A     | 0.77±0.03B       | 36.30±2.64b  |
| **Porcine gelatine** | 4.37±0.70A      | 0.54±0.01B        | 0.09±0.01A     | 0.01±0.01B       | 87.58±2.87a  |
| Hydrolysed porcine gelatine | 6.05±0.72A      | 4.08±0.39B        | 0.93±0.11A     | 0.63±0.06B       | 32.51±1.36b  |
| **Hydrolysate 1:2** | 8.86±0.73A      | 7.49±0.56A        | 1.36±0.11A     | 1.15±0.08A       | 15.46±1.03d  |

Differences in FAG within same sample before and after glycation sharing in common capital case superscript are not significantly different (p <0.05). Decrease values sharing in common lower-case superscript are not significantly different (p<0.05).
Table 3: WHC and OHC (g of oil or water per gram of sample) values in native and glycated samples. Results expressed as mean and SD

| Sample                         | WHC      | OHC      |
|--------------------------------|----------|----------|
| Bovine gelatine                | 5.54±0.33a | 1.20±0.08b |
| Bovine gelatine glycated       | 1.40±0.03b | 0.77±0.03c |
| Hydrolysed bovine glycated     | 1.22±0.01b | 0.86±0.04c |
| Porcine gelatine               | 5.38±0.53a | 1.54±0.08a |
| Porcine gelatine glycated      | 1.28±0.03b | 0.78±0.03c |
| Hydrolysate 1:2 glycated      | 1.25±0.03b | 0.85±0.06c |
| Hydrolysate 1:2                | ND       | 0.89±0.05c |
| Dextran                        | 1.56±0.03b | 0.53±0.01d |

Data within columns sharing superscript are not significantly different (p<0.05). ND: not detected as sample was too soluble.
Figure 1
Figure 3
- Hides’ peptides with low molecular weight (<6.5 kDa) have poor functionality
- Hides’ peptides were successfully conjugated with dextran of 10 kDa
- The techno-functional properties of hides’ peptides were not modified after glycation