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Cecilia Anzani, Carlos Álvarez, Anne Maria Mullen

PII: S0023-6438(19)31142-9

DOI: https://doi.org/10.1016/j.lwt.2019.108800

Reference: YFSTL 108800

To appear in: *LWT - Food Science and Technology*

Received Date: 25 July 2019

Revised Date: 30 October 2019

Accepted Date: 31 October 2019

Please cite this article as: Anzani, C., Álvarez, C., Mullen, A.M., Assessing the effect of Maillard reaction with dextran on the techno-functional properties of collagen-based peptides obtained from bovine hides, *LWT - Food Science and Technology* (2019), doi: https://doi.org/10.1016/j.lwt.2019.108800.

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

3 Cecilia Anzani[1,2], Carlos Álvarez[1], Anne Maria Mullen[1*]
4 1.- Department of Food Quality and Sensory Science, Teagasc Food Research Centre,
5 Ashtown, Dublin 15
6 2.- Department of Food and Drug Science, University of Parma, via Parco Area delle Scienze
7 49/a, 43123 Parma, Italy.
8 *Corresponding author: anne.mullen@teagasc.ie

9

10 **Abstract**

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

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26 **Keywords**

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

28

29 ## 1. Introduction

30 Bovine hides, produced during the slaughter process, account for around 7% of the animal
31 weight (Mullen, Álvarez, Pojić, Hadnadev, & Papageorgiou, 2015), and are used as a raw
32 material for leather production. However, some pieces are not suitable for this industry (too
33 small, damaged, etc.) and consequently they are discarded, imposing an extra cost for the
34 producer. Hides are mainly composed by collagen, which is widely used in food industry as
35 gelling agent, thickener or binder after being transformed into gelatine (Toldrá, Aristoy,
36 Mora, & Reig, 2012). Collagen can also be used as protein supplement once hydrolysed into
37 peptides and free amino acids (Ferraro, Anton, & Santé-Lhoutellier, 2016). Precisely, because
38 of the many uses of collagen and the need of the meat industry to re-valorise the large
39 amounts of processing co-products generated, innovative strategies have been adopted, as for
40 example the use of Alcalase hydrolysis to recover collagen peptides from hides (Anzani et al.,
41 2019). In this previous work the processing conditions were optimized to maximize the
42 recovery yield of hides' proteins in the form of peptides by means of Alcalase; which was
43 selected based on its ability to hydrolyse the collagen present in this material (Anzani, Prandi,
44 Buhler, et al., 2017; Anzani, Prandi, Tedeschi, et al., 2017). However, when aimed for high
45 recovery yields, the final hydrolysate presents a very high degree of hydrolysis meaning that
46 it is mainly composed of free amino acids and very short peptides (Anzani et al., 2019);
47 which usually don't exhibit good techno-functional properties (apart of high solubility); as it
48 was also reported by Liu, Kong, Xiong, and Xia (2010) for plasma peptides obtained with
49 Alcalase. In present work, this fact was further confirmed since the techno-functional
50 properties of hides' hydrolysate (solubility, emulsifying ability, foam capacity and water and

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51 oil holding capacity) were characterised and the results were very poor when compared to
52 intact collagen or gelatine. This loss of functionality will negatively impact the range of
53 application of the hydrolysates as food ingredient.

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

73 Therefore, the first objective of this research was to examine the techno-functional properties
74 (solubility, emulsifying ability, foam capacity and water and oil holding capacity) of a high
75 DH (18%) hydrolysate from bovine hides. Second objective was, after assessing their poor

76 performance, to improve the hydrolysate techno-functional properties by glycation with
77 dextran (10 kDa), aiming to improve their range of application. Finally, commercial gelatines
78 and its Alcalase hydrolysates, used as a high collagen content control, were also analysed
79 after glycation.

80

81 **2. Material and methods**

82 **2.1 Enzymatic hydrolysis of bovine hides and the commercial gelatine powders**

83 Samples consisting of pieces of bovine skin were provided by Inalca Industria Alimentare
84 Carni SpA (Castelvetro, Modena, Italy) and stored at −20 °C. The hydrolysis process, using
85 Alcalase 2.59 U/g, was carried out according to optimised conditions from a previous study
86 (Anzani et al., 2019) using two sample:buffer (v/w) ratios 1:2 and 1:3 (Supplementary 0). As
87 a result two hydrolysates with DH equal to 17.3 and 19.2%, with an extraction yield of 85%
88 and 82%; and protein content of 80% and 85% were obtained respectively for hydromodule
89 1:2 and 1:3. The enzymatic hydrolysis reaction was performed on the commercial gelatines'
90 powders (bovine and porcine purchase from Sigma-Aldrich Co. Wicklow, Ireland) following
91 the same protocol; however, in order to prevent its gelation, the sample:buffer solution ratio
92 was increased to 1:4 (v/w). Finally, all the samples were freeze dried (Cuddon FD80, New
93 Zeland) to obtain a powder, and preserved until use in refrigeration.

94

95 **2.2 Preparation of peptide-dextran glycated samples**

96 Dextran, with an average molecular size of 10 kDa, was supplied by Sigma-Aldrich (Co.
97 Wicklow, Ireland). Freeze dried peptides (hides' hydrolysates and hydrolysed gelatine
98 powders) were dissolved in distilled water; dextran was added to a final proportion of 1:3
99 peptides/dextran (w/w) and stirred. This proportion was based on bibliography (Jung, Choi,
100 Kim, & Moon, 2006), where 10 kDa dextran was employed. The peptides/dextran mixture

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101 was then freeze dried to obtain a homogeneous dry powder; then the samples were placed
102 into hermetic vessels and dry-heated at 75 ºC for 3 hours in an oven (Gallenkamp, Germnay)
103 (Álvarez et al., 2012). After reaction, samples were removed and kept sealed under
104 refrigeration (4 ºC) until used for analysis. This process was carried out in duplicate.

105

### 106 2.3 Hydrolysates and glyco-peptides characterisation

#### 107 2.3.1 Protein content determination

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

111

#### 112 2.3.2 Ultra-filtration fractionation of hydrolysates (UF)

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

119

#### 120 2.3.3 Amino acid profile

121 Amino acid profile was analysed following the method reported by Hill (1965). Hide's
122 hydrolysates were hydrolysed in 6 mol/L HCl at 110 °C for 23 hours and the resulting
123 hydrolysates were quantified using a Jeol JLC-500/V amino acid analyser (Jeol (UK) Ltd.,
124 Garden city, Herts, UK) fitted with a Jeol Na+ high performance cation exchange column.
125 Norleucine was employed as internal standard.

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126 **2.3.4 Determination of free amino groups (FAG)**

127 To calculate the FAG as meq of leucine/g of sample (meq/g) a method with slight
128 modifications was followed (Anzani, Prandi, Tedeschi, et al., 2017). The OPA/NAC (o-
129 Phtalaldehyde/N-acetyl-cysteine) reagent (100 mL) was prepared by combining 10 mL of 50
130 mM OPA (in methanol) and 10 mL of NAC 50 mM (in methanol), 5 mL of 200 g/L SDS, and
131 75 mL of borate buffer (0.1 mol/L, pH 9.5). The OPA assay was carried out by the addition
132 of 20 µL of sample (diluted in water) to 2.4 mL of OPA/NAC reagent. The absorbance of this
133 solution was measured at 340 nm with JASCO B-530 UV–Vis-spectrophotometer (JASCO,
134 Oklahoma City, OK, U.S.A.). A standard curve was prepared using l-isoleucine (0–2
135 mg/mL).

136

137 **2.3.5 Size exclusion chromatography (SEC)**

138 Phosphate buffer (pH 7.0, 0.15 mol/L) was used as mobile phase with a flow of 0.35 mL/min
139 in a Waters HPLC (2795 Separation Module) system coupled to an AdvanceBio SEC 130A
140 2.7 µm 4.6x300 mm column attached to an AdvancedBio SEC130A 2.7 µm 4.6x50 mm
141 Guard. The result was monitored at 214 nm in a Photodiode Array Detector (Waters 2996)
142 and the retention time of each peak was evaluated using the Empower Pro 2 software (Waters
143 Corporation). A calibration curve was made using blue dextran (2000 kDa), albumin (66
144 kDa), carbonic anhydrase (29 kDa), lysozyme (14.3 kDa) and Vitamine B12 (1.3 kDa).

145

146 **2.4 Analysis of techno-functional properties**

147 **2.4.1 Solubility**

148 Exactly 0.5 g of the sample was dissolved in 10 mL of distilled water and pH adjusted
149 between 3 and 8 by addition of 1 mol/L NaOH or HCl. The solution was centrifuged at 2400g
150 for 30 min (Lynx6000, Thermo Fisher Scientific, Hempstead, UK) (Penteado, Lajolo, &

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151 Santos, 1979). The amount of soluble peptides before and after centrifugation was determined
152 by the Nanodrop ND1000 system (Thermo Fisher Scientific, Wilmington, DE, U.S.A.).
153 Solubility was calculated as follows:
154
155 $$\%S = (Pd/Pt) \times 100 \qquad \text{Equation 1}$$
156
157 Where Pd is the amount of soluble protein (g) and Pt the amount (g) the total protein used in
158 the assay.
159

160 **2.4.2 Water- and oil-holding capacities**

161 The methods of Vioque, Sánchez-Vioque, Clemente, Pedroche, and Millán (2000) were used
162 with modifications. Ten g of distilled water or rapeseed oil were mixed with 0.5 g of sample,
163 held for 30 min, stirred twice gently, and centrifuged at 2,700g for 30 min (Lynx6000). The
164 volume of the supernatant was weighed again. The water- (WHC) or oil-holding capacities
165 (OHC) were expressed as following:
166
167 $$\text{WHC/OHC}= ((initial\ weight - weight\ supernatant))/(grams\ protein\ employed)$$
168 Equation 2
169

170 **2.4.3 Lowest gelation concentration value (LGC)**

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

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176

177 **2.4.4 Foam capacity and stability**

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

183

184 $Foam\ capoacity\ (\%) = (foam\ volume)/(liquid + foam\ volume)$ Equation 3

185

186 **2.4.5 Emulsifying capacity**

187 Sample solutions ranging between 2 and 10 g/L where added with 13 mL of rapeseed oil.
188 After stirring the samples for 2 min at 15000 rpm with a homogenizer (omni-prep
189 Homogenizer, OMNI Inc, Kennesaw, Georgia, U.S.A) the samples were centrifuged at
190 1200 × g for 10 min (Lynx6000) (Inklaar & Fortuin, 1969). The percentage of emulsification
191 was calculated as:

192

193 $\%E = (Ve/Va\ ) \times 100$ Equation 4

194

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

196

197 **2.5 Statistical analysis**

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

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202

203 ## 3 Results and discussion

204 ### 3.1 Molecular weight and amino acid profile of hydrolysates

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

212

213 ### 3.2 Hydrolysates' functional properties

214 The protein content of the freeze dried hydrolysates was of 79.8±0.3 and 85.1±0.2 g/100g, for
215 1:3 and 1:2 ratios respectively. Such powders were employed to analyse the functional
216 properties, as summarized in Table 1 and Figure 2. No significant differences ($p>0.05$),
217 regarding the functional properties, were found between both samples, with the exception of
218 foam capacity which was higher for hydrolysate 1:3 ($p<0.05$); as shown in Figure 3. The
219 reduced efficiency of small peptides as emulsifiers was related to the charge repulsion which
220 prevented the peptides agglomerating to produce a layer around fat globules. Foam stability is
221 also linked to the molecular properties, as small peptides do not have the strength needed to
222 maintain stable foams (Karami & Akbari-adergani, 2019). Solubility was found to be 100%
223 in the range of pH analysed; which is a positive characteristic that may play an important role
224 in using hide hydrolysates as food ingredients in liquid formats or for easy incorporation into
225 food matrices. Precisely, due to its high solubility, these peptides have no WHC since, under
226 experimental conditions; a solution is formed rather than gel. Short peptides are unable to

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227 create a hydrophilic matrix that can cause water retention. OHC of hydrolysates (Table 1)
228 when compared to other proteins extracted from meat co-products performed poorly
229 (Alvarez, Drummond, & Mullen, 2018). Finally, the most relevant functionality for collagen
230 derived products, gel formation, was completely lost due to the hydrolysis. Short peptide
231 chains are not able to generate enough intermolecular bonds to establish a 3-dimensional
232 protein structure; therefore, water cannot be entrapped and retained to form a gel.
233 Since no main differences were found in the functional properties, size distribution or amino
234 acid profile between both hydrolysates, the one generated at 1:2 ratio was selected for further
235 research, given the fact that water consumption is minimised.
236

237 **3.3 Changes in free amino groups and molecular size**

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

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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

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276 O'Mahony (2016), on whey protein isolate treated with maltodextrin. After hydrolysis and the
277 following glycation of the commercial gelatin powders, the solubility between pH 3 and 8
278 was maximum for all the samples assayed. This increase in protein solubility after
279 conjugation might be attributed to an increase in the hydration of the collagen proteins due to
280 the covalent bonging of hydrophilic dextran, and modification of the net charge of the
281 protein, contributing to greater repulsion between the protein molecules (O'Mahony, Drapala,
282 Mulcahy, & Mulvihill, 2017).

283 **3.4.2 WHC and OHC**

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

291

292 **3.4.3 Foam capacity and foam stability**

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

297 In the case of the bovine gelatine, the foam capacity is not modified after glycation ($p>0.05$);
298 however, the stability at 45 minutes of analysis was negatively affected ($p<0.05$). In the case
299 of the porcine gelatines, glycation and the glycation after the hydrolysis also negatively
300 affected the foam capacity and stability ($p<0.05$) when compared to the control; however,

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301 foam was more stable within each treatment. Similar negative results were obtained for the
302 hide hydrolysates, since no improvement was observed after Maillard reaction (Figure 3b).
303 The same outcome was found out or by Corzo-Martínez, Sánchez, Moreno, Patino, and
304 Villamiel (2012) when β-lactoglobulin was conjugated with galactose.
305

306 **3.4.4 Emulsifying capacity**

307 Emulsifying properties play an important role in food systems, as they contribute directly to
308 texture and sensory properties of food. There are several examples in literature showing an
309 increase of the emulsion activity and stability after conjugation with dextran (Cheng, Tang,
310 Xu, Wen, & Chen, 2018; Zhang, Yu, Wang, Wang, & Zhang, 2019). The increment depends
311 on the Hydrophilic-Lipophilic Balance (HLB) of the protein-polysaccharide conjugates. In
312 this sense, the saccharides attract water molecules around the oil droplet, while the
313 hydrophobic residues of the protein molecules are attached on the oil droplets inhibiting the
314 oil droplets coalescence (Doost et al., 2019). The commercial gelatines behaved in a different
315 way, as previously noticed in the foaming properties (Figure 6).
316 In the case of bovine gelatines, the glycation did not significantly affect the emulsifying
317 capacity and stability ($p>0.05$). By contrast, after the glycation of the porcine commercial
318 gelatines, a dramatic decrease in emulsifying properties was observed. Glycated hydrolysed
319 samples showed no emulsifying capacity. Hydrolysed hides after glycation performed equally
320 poor as emulsifier agents; as glycation had no positive effect. It is likely that the short
321 peptides forming part of the glycoprotein had not enough hydrophobic groups to stabilise the
322 emulsion.
323

324 **3.4.5 Gelling properties**

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

330

331 **4. Conclusions**

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

348 The application of peptides recovered from bovine hides conjugated with functional prebiotic
349 carbohydrates in liquid food matrix, will be a possible avenue to add value to this product;

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350 based on its high solubility and the low interaction with other ingredients (water, fat or large
351 proteins) present in the formulation.

352

353 **Acknowledgments**

354 "This work was supported by the Department of Agriculture, Food and the Marine (DAFM)
355 under the National Development Plan2007–2013 funded by the Irish Government."
356 This research has been carried out with the financial support by Cluster Agrifood Project 4
357 "Sustainability of the Italian food chain", (Project So.FI.A CTN01_00230_450760).

358

359 **Conflict of interest**

360 The authors declare that they have no conflict of interest.

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480 **Figure Captions**

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

485

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

492

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

499

500 Figure 4: Emulsifying capacity of bovine (A) and porcine (B) collagen. Circles represent
501 native collagen, squares collagen glycated; and triangles hydrolysed and glycated collagen.
502 Data within group sharing in common capital letter are not significantly different ($p<0.05$)
503 Data within same sampling time in common lower-case letter are not significantly different
504 ($p<0.05$)

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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)[a]<br>0.96 ±0.04 g oil/g protein (hydrolysates 1:3)[a] |
| Gelling properties<br>(20-120 g/L of protein) | No gel was obtained |
| Emulsifying capacity | Less than 10% of added oil |

Data within columns sharing superscript are not significantly different ($p<0.05$)

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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±0.24 $^{A}$ | 1.60±0.01 $^{B}$ | 0.12±0.01 $^{A}$ | 0.03±0.01 $^{B}$ | 74.31±0.83 $^{a}$ |
| **Hydrolysed bovine gelatin** | 7.85±0.82 $^{A}$ | 5.00±0.02 $^{B}$ | 1.20±0.12 $^{A}$ | 0.77±0.03 $^{B}$ | 36.30±2.64 $^{b}$ |
| **Porcine gelatine** | 4.37±0.70 $^{A}$ | 0.54±0.01 $^{B}$ | 0.09±0.01 $^{A}$ | 0.01±0.01 $^{B}$ | 87.58±2.87 $^{a}$ |
| **Hydrolysed porcine gelatine** | 6.05±0.72 $^{A}$ | 4.08±0.39 $^{B}$ | 0.93±0.11 $^{A}$ | 0.63±0.06 $^{B}$ | 32.51±1.36 $^{b}$ |
| **Hydrolysate 1:2** | 8.86±0.73 $^{A}$ | 7.49±0.56 $^{A}$ | 1.36±0.11 $^{A}$ | 1.15±0.08 $^{A}$ | 15.46±1.03 $^{d}$ |

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 ($p < 0.05$).

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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

| | WHC | OHC |
|---|---|---|
| Bovine gelatine | 5.54±0.33[a] | 1.20±0.08[b] |
| Bovine gelatine glycated | 1.40±0.03[b] | 0.77±0.03[c] |
| Hydrolysed bovine glycated | 1.22±0.01[b] | 0.86±0.04[c] |
| Porcine gelatine | 5.38±0.53[a] | 1.54±0.08[a] |
| Porcine gelatine glycated | 1.28±0.03[b] | 0.78±0.03[c] |
| Hydrolysed porcine glycated | 1.25±0.03[b] | 0.85±0.06[c] |
| Hydrolysate 1:2 | ND | 0.89±0.05[c] |
| Hydrolysate 1:2 glycated | 1.21±0.01[b] | 0.90±0.04[c] |
| Dextran | 1.56±0.03[b] | 0.53±0.01[d] |

Data within columns sharing superscript are not significantly different ($p<0.05$). ND: not detected as sample was too soluble

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Figure 1

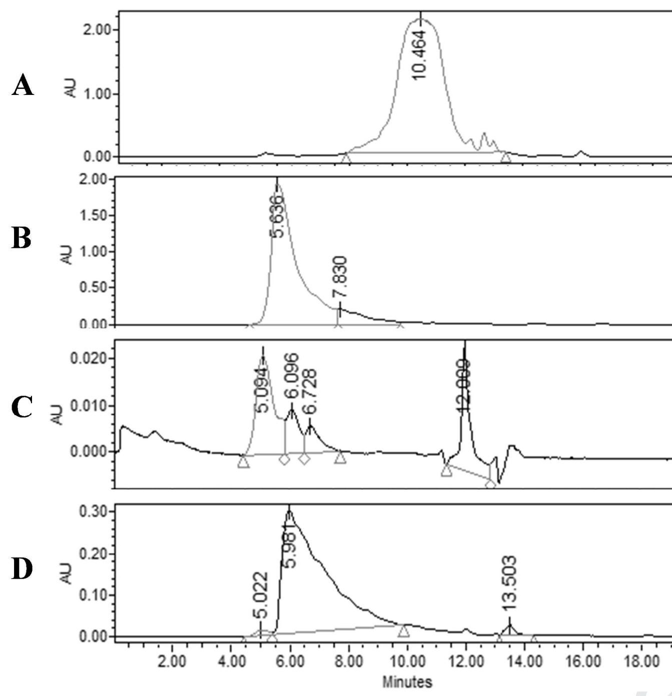


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Figure 2

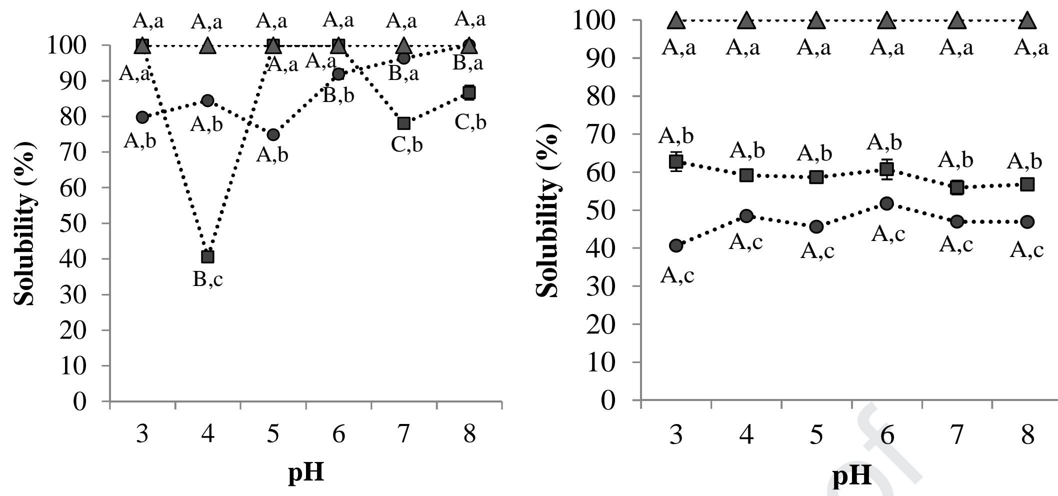


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Figure 3

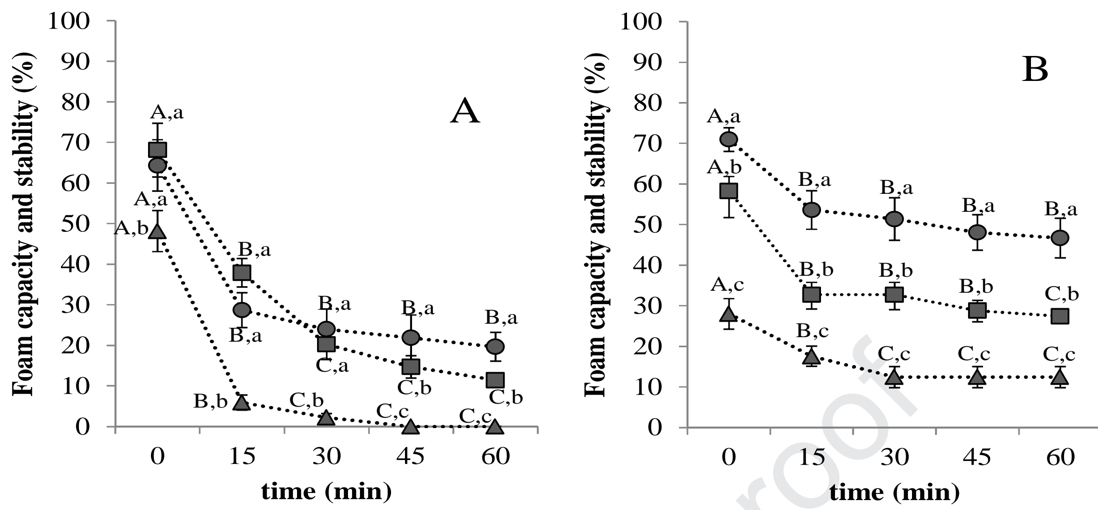


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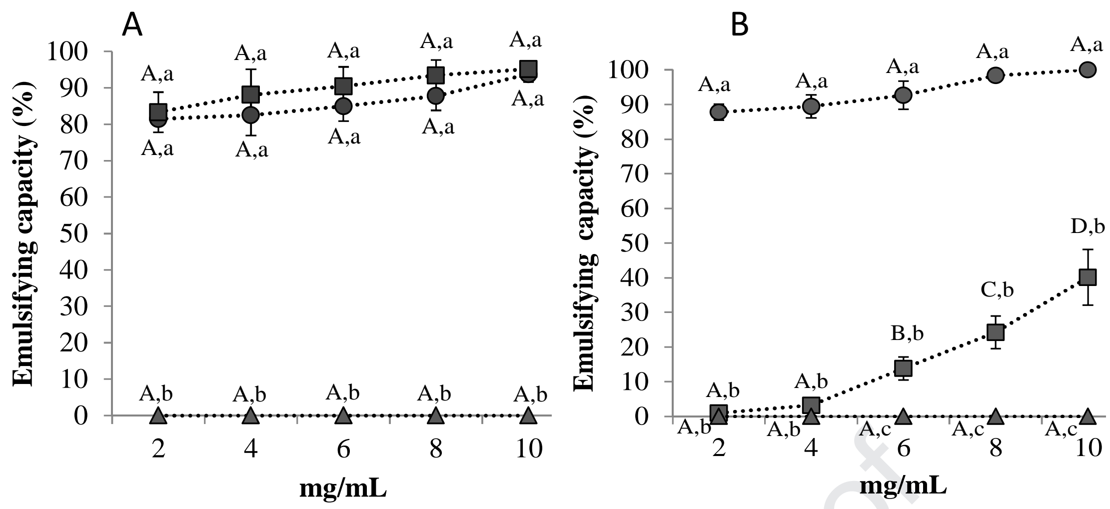
Figure 4

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- 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

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