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HAL Authorization

*Highlights (for review)

- Biochemical and functional characterisation of effluents from cooked ham plant
- 4 to 15 % weight of proteins including collagen can be recovered from effluents
- Even after cooking, proteins from ham broth still have functional properties
- Surface properties of proteins from effluent are comparable with milk proteins
- Effluent proteins used as functional ingredient lowers the cost of waste treatment

1 **Valorisation of cooked ham industrial by-products into functional ingredients**

2

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12

13 **Abstract**

14

15 The production of cooked ham at industrial scale induces the generation of two liquid by-products: pork
16 exudate (PE), collected before the salting, has 14% weight dry matter mainly rich proteins (90%); ham
17 broth (HB), released after cooking, has 8% dry matter including 50 % proteins. In this paper, biochemical,
18 rheological and functional properties of the proteins from these by-products are studied and compared to
19 sodium caseinate and whey protein isolate. PE can form gel by coagulation at 50°C and has good
20 emulsifying capacity (324 ± 8 mL oil per gram of protein) with high stability. Even after cooking that
21 alters proteins, HB has the ability to form gel at low temperature (10-18°C) and has good emulsifying
22 capacity (252 ± 7 mL oil per gram of protein) and foaming ability (ratio foam to initial volume: 177 ± 14).
23 Both by-products could then be valorised as functional ingredient for delicatessen products.

24

25 **Keywords:** cooked ham, by-products, proteins, gel, emulsion, foam

26

27 **1. Introduction**

28

29 Dealing with by-products is a major concern in the food processing industries (Mirabella et al. 2014). It is
30 especially the case in the meat-processing industry as they can be a source of protein for food or feed
31 purpose. These by-products can be found into solid form (carcass, skin) or in liquid form recovered in
32 wastewaters that need to be treated because of their high chemical or biological organic demand (Bull et
33 al. 1982). Solid waste could find a valorisation as energy vector after digestion (Hejnfelt and Angelidaki,
34 2009) or can be hydrolyzed by chemicals (Selmane et al. 2008) or enzymes (Rafieian et al. 2015) to
35 valuable functional proteins. Concerning the liquid waste, proteins from red blood cells (Gomez-Juarez et
36 al, 1999) or plasma (Penteado et al. 1979) were also found to have useful functional properties. As
37 slaughtering represents the main source of waste in meat industry, much interest was raised to deal with
38 these by-products (Arvanitoyannis and Ladas, 2008). There is actually less research on the potential uses
39 of by-products generated after another processing step of meat such as the cooking of hams in the
40 delicatessen trade.

41 In Europe, France is the leader in the production of cooked ham with particular stress on high quality
42 products such as Paris or York ham (Spencer, 2003). Each year, around 190000 tons of cooked ham are
43 produced but during the processing more than 13000 m³ of liquid by-products are generated. These by-
44 products, containing proteins salts and water, are poorly valorised and mainly rejected as effluents into
45 wastewaters inducing a non-negligible cost for treatment resulting from legal restrictions. In this paper,
46 two different effluents from cooked ham processing plants are investigated: pork exudate (PE) is minor
47 by-product recovered from the collecting tanks of pork chunks before salting and tumbling; ham broth
48 (HB) is collected at the end of the cooking and maturing of hams and represents 90% of the effluents of
49 the plant. The aim of this article is to characterize these effluents determine if they can easily processed
50 and if their proteins can be valorised as functional ingredients.

51

52 **2. Material and Methods**

53

54 2.1 Raw material characterisation

55 Effluents were collected on the ham production site and stored at 4°C or -16°C for respectively short or
56 mid-term conservation. For long-term conservation, effluents were transformed into powder by freeze-
57 drying (PL6000, Thermo Electron Corp.) or spray-drying (B290, Büchi). Water activity a_w was measured
58 post factum using an a_w -meter (Labmaster, Novasina Ag)

59 Dry matter of each effluent was first quantified then different assays were applied to determine their
60 biochemical composition. Protein content was obtained using a total nitrogen analyzer (TNM-1,
61 Shimadzu); collagen was specifically quantified by Lollar method (Lollar, 1958). The amount of fats and
62 soluble sugars were respectively measured via Bligh and Dyer (Bligh and Dyer, 1959) and Dubois
63 (Dubois et al. 1956) methods. Sodium chloride was determined by Charpentier-Volhard titration; other
64 salts such as phosphates or nitrates were not quantified specifically but are included in the ash
65 determination.

66

67 2.2 Protein qualitative analysis

68 Protein fraction of the effluents was also qualitatively analysed in order to determine the type, molecular
69 weight and composition in amino acids of the solubilised proteins.

70 Size-exclusion chromatography (SEC) was implemented with an Akta FPLC (Amersham Biosciences,
71 UK) device equipped with an UV/VIS detector. Samples of 0.1 mL filtered at 0.2 µm were loaded at 0.5
72 mL/min on a Superdex TM 200 column (GE Healthcare, Life Sciences) eluted with a 50 mM phosphate
73 buffer (pH 7) supplemented with 150 mM NaCl. Standard protein mixture (Bio-Rad Laboratories)
74 including thyroglobulin (670 kDa), γ -globulin (158 kDa), ovalbumin (44 kDa), myoglobin (17 kDa) and
75 B12 vitamin (1.35 kDa) was used for calibration. Eluted proteins were detected at 280 nm; quantitative
76 titration was also applied on each elution peak.

77 One-dimensional electrophoresis was also applied in order to determine molecular weight in denaturing
78 conditions. Precision Plus Protein™ (Bio-Rad Laboratories) was used as standard. The proteins fractions
79 were loaded at constant volume (20 µL) and proteins concentration (5mg.mL⁻¹) and separated on the 12%
80 SDS-PAGE. Gels were stained using Coomassie brilliant blue R-250.

81 For amino acids analysis, samples were submitted to basic (using NaOH 6 M) or acid (using HCl 5.5 M)
82 hydrolysis. Amino acids were then quantified by ion-exchange chromatography with post-column
83 ninhydrin detection (Hitachi L 8900).

84

85 2.3 Functional properties

86 As these by-products are derived from meat, investigations on the functional properties related to proteins
87 have been conducted. In particular, experiments on the capacity of gelling, emulsifying and foaming of the
88 proteins from the by-products were tested. The functional properties of PE and HB proteins were
89 compared with commercial ingredients used in food formulations such as sodium caseinate Na-cas and
90 whey proteins isolate WPI (Armor Protéines, France).

91

92 2.3.1 Rheological behavior and gelling properties

93 A stress-controlled rheometer (AR-G2, TA Instruments, USA) equipped with a double gap Couette or
94 plate-plate system and a Peltier circulator for temperature control was used to determine two types of
95 properties.

96 Measurements in flow conditions were applied with shear rate in the range of 1 to 1000 s⁻¹ in order to
97 determine PE and HB viscosities in native solutions. Experiments were conducted at 20°C.

98 Dynamic oscillatory shear tests were carried out to obtain the gelling properties of the native solutions at
99 low and high temperature. Small amplitude deformation (1% strain) was applied at an oscillation
100 frequency of 1 Hz. For low temperature gelation, temperature was decreased from 20 to 1°C with a ramp
101 of -0.1°C.min⁻¹. Gelation temperature was detected at the crossing over on the curves of storage modulus
102 G' and loss modulus G'' versus temperature. For high temperature gelation, temperature was increased

103 from 20 to 90°C with a ramp of 0.5°C.min⁻¹. Gelation temperature was deduced from a rapid increase in
104 the storage modulus G' versus temperature curve.

105

106 *2.3.2 Solubility of dried powders*

107 After drying of the effluents, the solubility of the two protein powders was checked function of the pH.
108 The pH was adjusted in the range 3-11 using 1M solution of sodium hydroxide or hydrochloric acid and
109 measured with a Seven Easy pHmeter (Mettler Toledo).

110

111 *2.3.3 Surface properties*

112 Interfacial and surface tension were measured thanks to K12 tensiometer (Krüss GmbH, Germany)
113 equipped with a Wilhelmy plate. Experiments were conducted at 20°C with solutions that contain 2% of
114 proteins. Rapeseed oil was used as organic phase for interfacial tension measurements.

115 Protein surface hydrophobicity was evaluated according to a protocol derived from Kato and Nakai (1980)
116 work and using magnesium salt of 1-anilinonaphtalene-8-sulfonic acid (ANS) as extrinsic fluorescence
117 probe. Relative fluorescence intensity (RFI) was measured with a Flx spectrofluorimeter (Safas, Monaco).
118 Excitation and emission wavelengths of 390 and 470 nm and slit-widths of 10 nm were used. The
119 instrument standardization was carried out by measuring the RFI values of a solution of ANS in methanol
120 as described by Haskard and Li-Chan (1998).

121 5 mL protein solutions in 20 mM phosphate buffer (pH 7) were mixed with 25 µL ANS and incubated at
122 20°C and in the dark for 10 minutes before measuring the RFI. Surface hydrophobicity corresponded to
123 the initial slop estimated by linear regression analysis of the curve relative fluorescence intensity vs.
124 protein concentration. All measurements were done in triplicate for statistical purpose.

125

126 *2.3.4 Emulsifying and foaming properties*

127 Two different emulsifying properties were investigated. Emulsion capacity EC (expressed in gram of oil
128 per gram of protein) represents the maximum amount of oil that can be emulsified by a definite quantity of

129 proteins. Solutions of 1% wt. of proteins were prepared for this measurement. Emulsion stability ES
130 (expressed in percentage) refers to the level of emulsion remaining stable after 24 hours storage at room
131 temperature. Solutions of 2% wt. of proteins and a fraction of 50 % wt. of oil were used. Rapeseed oil was
132 again used as organic phase. Details on the applied protocols are available in Ursu et al. (2014).
133 Following the protocols described by Selmane et al. (2008), two foaming properties were studied.
134 Foaming ability FA (expressed in percentage) characterises the capacity of a solution of proteins to
135 produce foams and compares the volume of the foam to the initial volume of the solution after agitation
136 Ultra-Turrax T25 device (Ika-Werke GmbH, Germany). Foaming stability FS (expressed in minutes) is
137 the time necessary to reduce the volume of foam by half. All foaming experiments were made with
138 solution of proteins at 2 % wt.

139

140 **3. Results and discussion**

141

142 3.1 Biochemical characterisation of the effluents

143 The average chemical compositions of the two effluents were determined on different batches; results are
144 presented in Table 1.

145

146 PE has the higher content in dry matter (14%) mainly consisting of proteins (90%) and soluble minerals.

147 Moisture is more present in HB and protein percentage is lower (50%). Among the proteins, collagen

148 residues were not detected in the PE, as it is not soluble into water in its native form in the muscle. After

149 salting and cooking, collagen could be solubilised and represents around 12 % wt. of the proteins in HB.

150 Due to the meat salting before tumbling and cooking sodium chloride percentage is quite high in HB

151 (around 20 % wt. of the dry matter). Soluble sugars and a higher quantity of minerals are logically found

152 in HB in respect to PE as they are current additives during the salting in the production of standard quality

153 hams.

154

155 There is a large variety of proteins and their molecular weights profile is very different between the two
156 effluents (Figure 1). PE shows a majority, around 60% wt., of large proteins between 10 and 210 kDa and
157 the remaining 40% are low molecular weight (< 3 kDa) proteins. In pork muscle, soluble proteins in the
158 absence of salt are sarcoplasmic proteins and proteins from plasma. Thereby, the SEC profile of the PE is
159 representative of these proteins with for example myoglobin around 15 kDa that gives the pink color to the
160 PE solution and albumin at 66 kDa. On the other hand, HB has more low molecular proteins (< 3 kDa)
161 representing 70% wt. of the detected proteins, the other proteins (30%wt.) have molecular weight between
162 10 and 65 kDa. Proteins recovered in HB have undergone heat treatment between 70°C and 80°C for
163 many hours; it is not surprising then to find small proteins or subunits of myofibrillar proteins that have
164 not coagulated to form ham. Collagen does not appear on the chromatogram because it is retained by the
165 0.22 µm filter before injection into the SEC column. However, it is the case on the electrophoresis as light
166 band close to 150 and higher than 250 kDa can be seen (Figure 1b). The electrophoresis gels denote the
167 presence of some proteins fractions with molecular weight around 60, 35 and below 37 kDa: the same
168 signature that was found in the SEC profile. The electrophoresis profile for PE displays a large variety of
169 molecular weights between 20 and 100 kDa, PE is then composed of complex combination of different
170 protein units.

171

172 The amino acid profiles (Table 2) of the two effluents are rather different: while the repartition is quite
173 homogeneous for PE (only glutamic acid is superior to 10%); three amino acids, glutamic acid, glycine
174 and histidine, represent 50% in HB. After basic hydrolysis of proteins, only traces of tryptophan were
175 detected, values are consequently not given in table 2. Concerning the other essential amino acids, their
176 sum reaches 50% of the content for PE which is in accordance with the value given for meat. In HB, the
177 total is a bit lower (45%) but it is mainly due to the high percentage of histidine (23.2%), value of
178 methionine is also superior to PE.

179

180 3.2 Functional properties

181 The two effluents are liquid in their native form at room temperature. Rheological measurements in flow
182 conditions at 20°C show that both exhibit a Newtonian behavior with low viscosity: PE is in the range of
183 3-10 cP according to the dry matter content and HB is in the range of 1.3-6.0 cP depending on the dry
184 matter. The effluents are nevertheless not always in a liquid state. After short-term storage at 4°C, HB
185 switches to a gel state. Typical oscillatory rheological measurements for a temperature range measurement
186 between 20 and 4°C presented on figure 2a show the cross-over point of storage modulus and loss
187 modulus. In the case of figure 2a, gelling point was measured just below 18°C. This value is sensitive to
188 the amount of collagen and can drop to 10°C with solutions containing less collagen. Gel strength is rather
189 low as storage modulus value at 4°C is of a few tens. Gel is reversible as HB becomes liquid when
190 temperature rises beyond gel point. PE does not follow the same tendency remaining liquid at 4°C (storage
191 temperature) but is much more sensitive to higher temperatures when its proteins can be subject to
192 degradation (figure 2b).

193

194 Proteins from PE have indeed the capacity to coagulate when temperature is raised beyond 45°C: there is a
195 sharp increase in the storage modulus between 45°C and 75°C before reaching a plateau with G' between
196 500 and 2000 Pa. This behaviour is consistent with the coagulation of sarcoplasmic proteins that starts to
197 coagulate between 40 and 60°C. After temperature ramp, PE forms a solid and elastic paste; state
198 transformation is irreversible as when temperature decreased to 20°C PE is still solid. Coagulation with
199 increase of temperature is not observed for HB: only a small increase of storage modulus is detected
200 beyond 70°C. Heat treatment (up to 90°C) has also a negative impact on HB properties as it could no more
201 form a gel at storage temperature (data not shown). In order to preserve the effluents from bacteria
202 development such as *Listeria*, drying must be applied if they are not used quickly after collection.
203 Considering the above rheological results, long high temperature treatment must be avoided. In this case,
204 freeze and spray-drying were used. With both methods, a_w was measured in the range of 0.15-0.25 which
205 is enough for long-term preservation. Whatever the method, the properties of proteins described below
206 were not affected by the drying process.

207

208 In order to test the functional properties of proteins from effluents, high solubility, expressed in percentage
209 of soluble proteins versus total proteins, must be achieved (Figure 3).

210 In native conditions, pH was measured close to 6.0 for HB and 5.0 for PE. The solubility of proteins from
211 HB is constant between 85 and 90% whatever the pH. This can be explained by the presence of salts that
212 induce a high ionic force; as a consequence acid or base cannot interact with charged groups of proteins.
213 PE exhibits a different behaviour: nearly a half of proteins are not soluble between pH equal to 6.0 and
214 9.0. In this range of pH, the colour of the solution changes from dark reddish to white. This is due to the
215 precipitation of myoglobin which is known to be insoluble close to pH equal to 7.0. Other sarcoplasmic
216 proteins also precipitate at neutral pH, this leads to a 30% reduction of solubility of proteins. Proteins of
217 both effluents were then used at native pH.

218

219 Solutions containing 2% wt. of proteins from HB and PE were tested for surface and interfacial tension
220 and compared with Na-Cas and WPI. Concerning surface tension (Figure 4a), equilibrium values are close
221 for HB and PE (between 49 and 50 mN/m). Proteins from both effluents have less ability to lower surface
222 tension than Na-Cas and WPI which have equilibrium values respectively at 45 and 42 mN/m. The
223 presence of other compounds (salts, sugars) in the solution containing effluent proteins may affect
224 negatively the behaviour of these proteins at the water-air interface. Equilibrium value is reached in a
225 short time for PE whereas the surface tension decreased more progressively for HB. Interfacial
226 measurements (Figure 4b) reveal similar behaviour for all the proteins: each kinetic has the same tendency
227 and equilibrium values are in the same range between 13 and 15 mN/m.

228

229 Results of other surface properties are presented in Table 3. Surface hydrophobicity is in the same range
230 for PE, Na-Cas and WPI. Typical values of surface hydrophobicity are of several hundreds for isolated
231 proteins. In this case, values are rather low as the solutions are a mixture of proteins that may interact with
232 each other and solubility is not complete (figure 3): this contributes to the decrease of fluorescence. It is

233 even lower for HB; this is mainly due to the presence of salts that also attenuates the fluorescence in the
234 solution. Proteins of PE and HB have good emulsifying properties: even if values of emulsion capacity are
235 lower than those of Na-Cas and WPI, they are in the same order of magnitude and are sufficient for future
236 application as oil represents nearly 80% of the volume of the emulsion when it is destabilised. Stability of
237 the emulsions with HB and especially PE are even higher than the commercial ingredients. About foaming
238 properties, HB has good foaming ability as it is just below those of Na-Cas and WPI but if level of
239 produced foam is important, the stability is poor as only 4 minutes are necessary to reduce the foam by
240 half. In this case, the presence of salt in the solution explains this value as salt is known to reduce the
241 stability of foams. On the other hand, PE has a rather low foaming ability, less than the half of Na-Cas and
242 WPI, but the stability of the generated foam is quite competitive with the commercial proteins.

243

244 **4. Conclusion**

245

246 In this work, two effluents of ham production at industrial scale were collected and characterised: ham
247 broth and pork exudate. Both by-products are a source of proteins with good nutritional properties.
248 Furthermore, their proteins have intrinsic functional properties such as emulsifying, foaming or gelling.
249 For pork exudate, containing native proteins, the presence of functionality could be expected and was
250 effectively demonstrated; it is more surprising for ham broth which is mainly composed of proteins
251 supposed to be degraded because of long heat treatment. Properties of proteins from both by-products are
252 indeed comparable to those of commercial dairy proteins that makes them good candidate in order to use
253 them as functional ingredient. In order to prevent bacteria development and contamination, ham broth and
254 pork exudate, having low viscosity can easily be processed into powders by spray-drying without losing
255 their functional properties. These proteins could not be used as widely as dairy proteins but could find
256 valorisation as functional ingredient for delicatessen products such as mousse or sausage or for domestic
257 animal preparations with a smell and taste of ham.

258

259 **Acknowledgement**

260

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263

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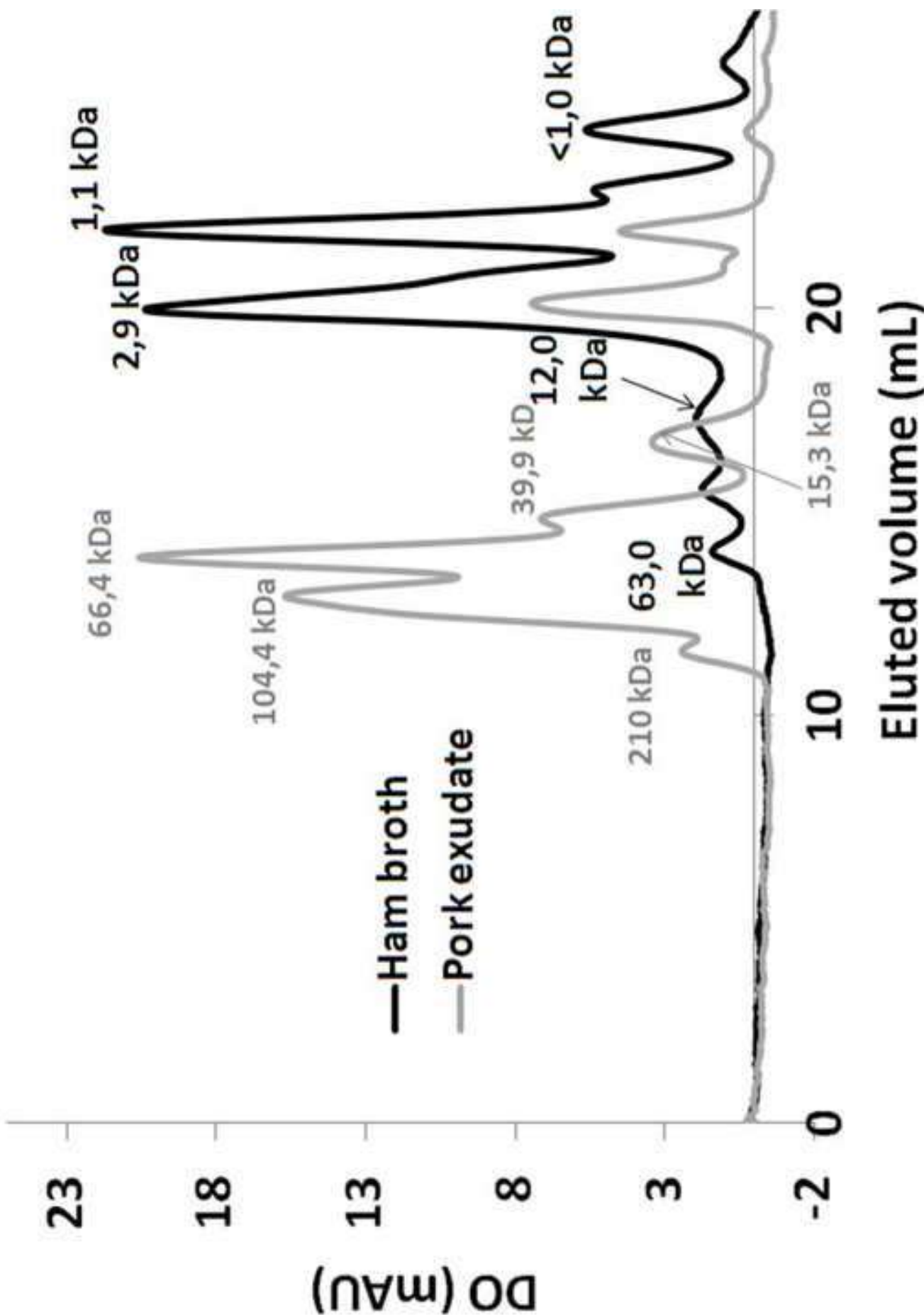
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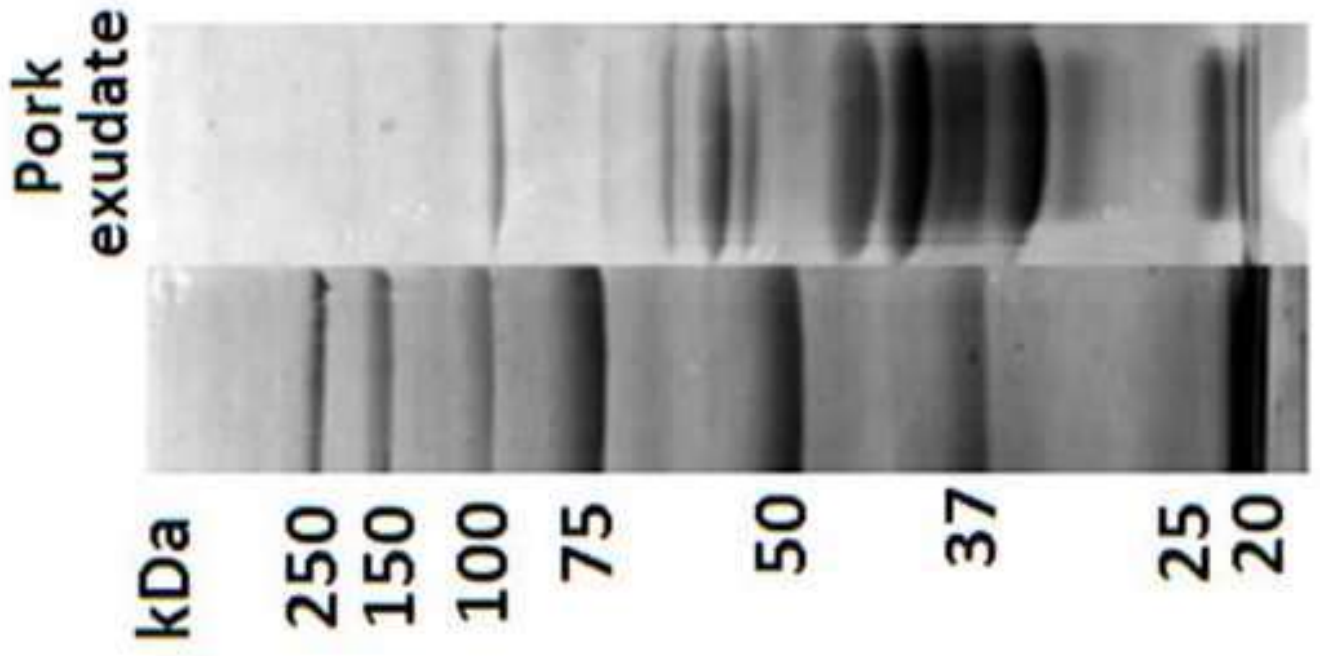
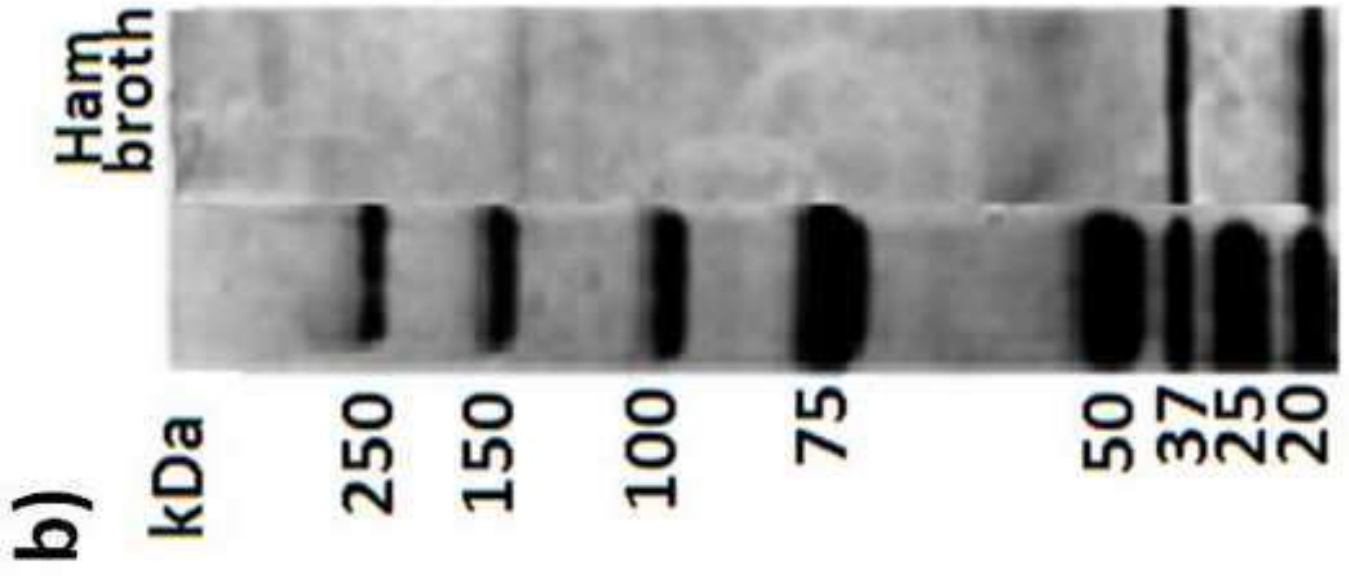
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Figure 1a

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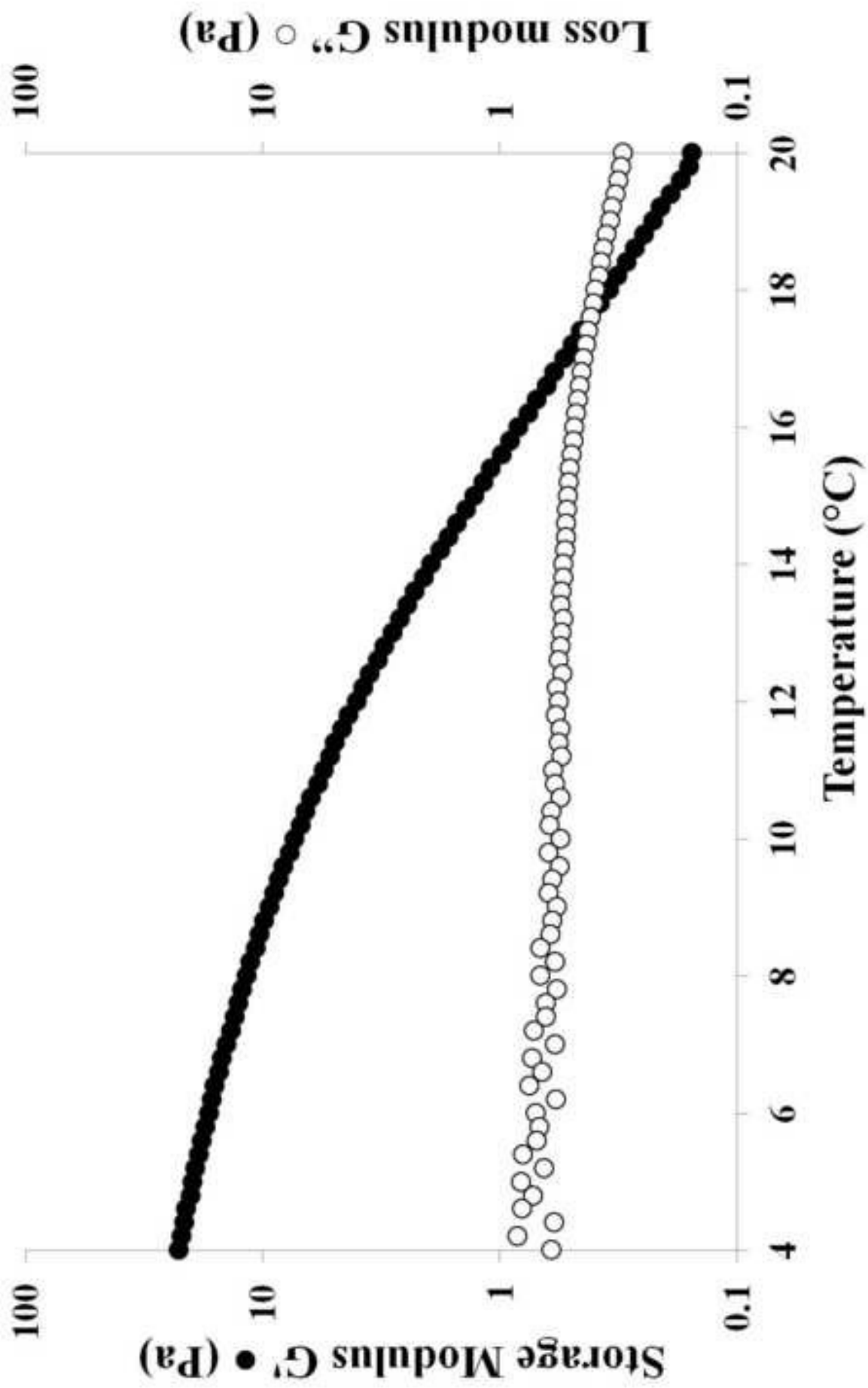


Figure 2a
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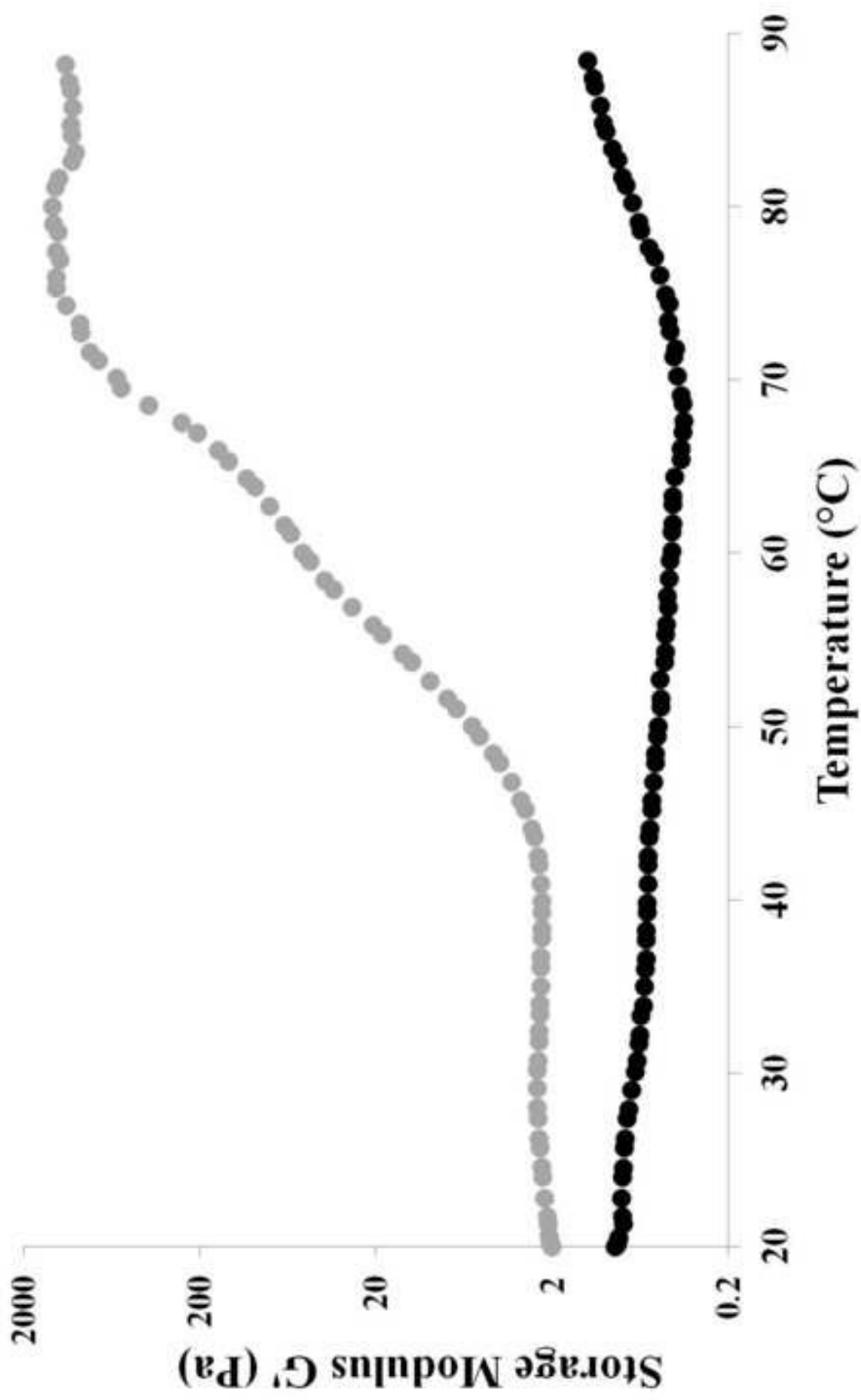


Figure 2b

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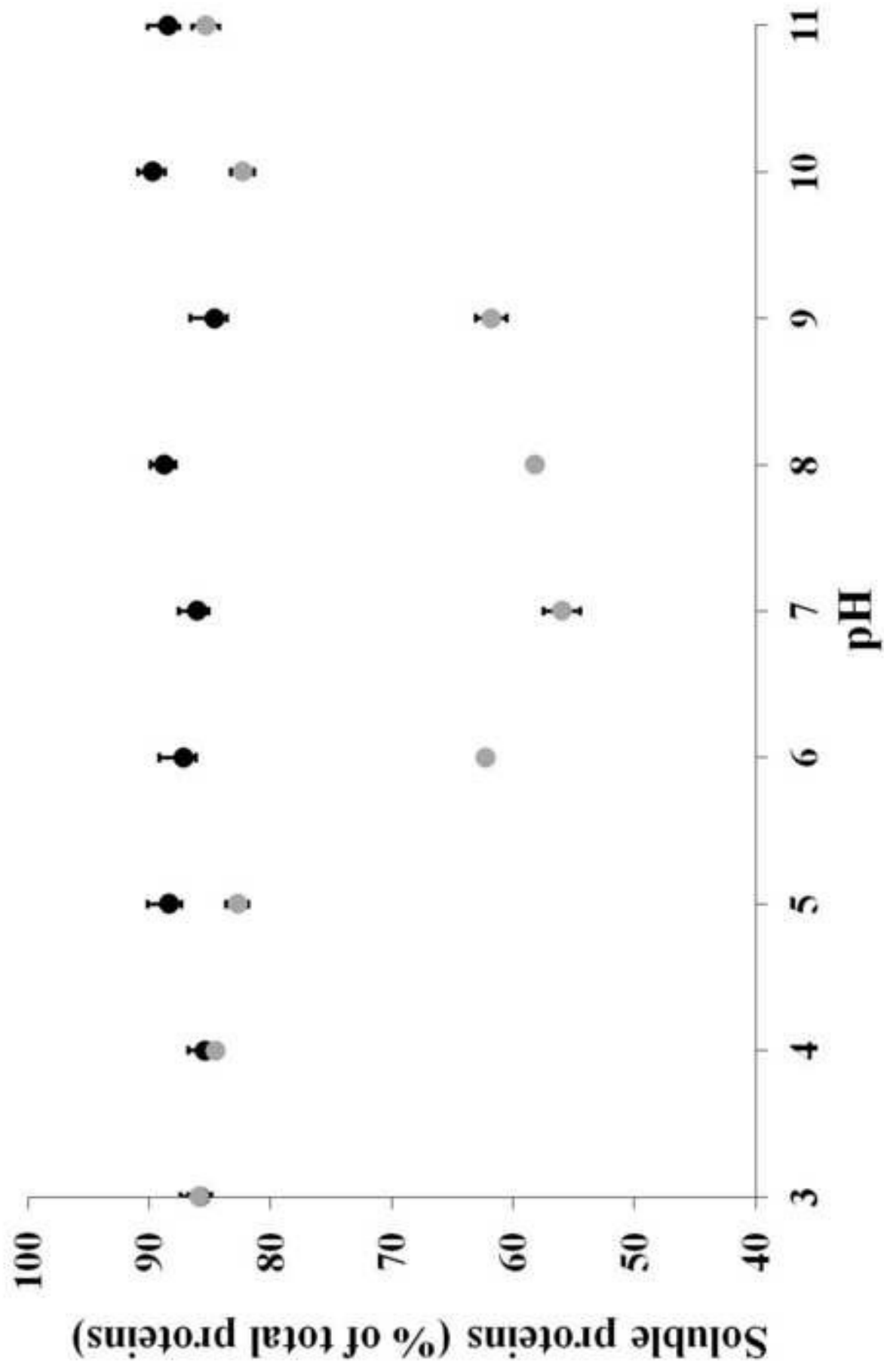


Figure 3
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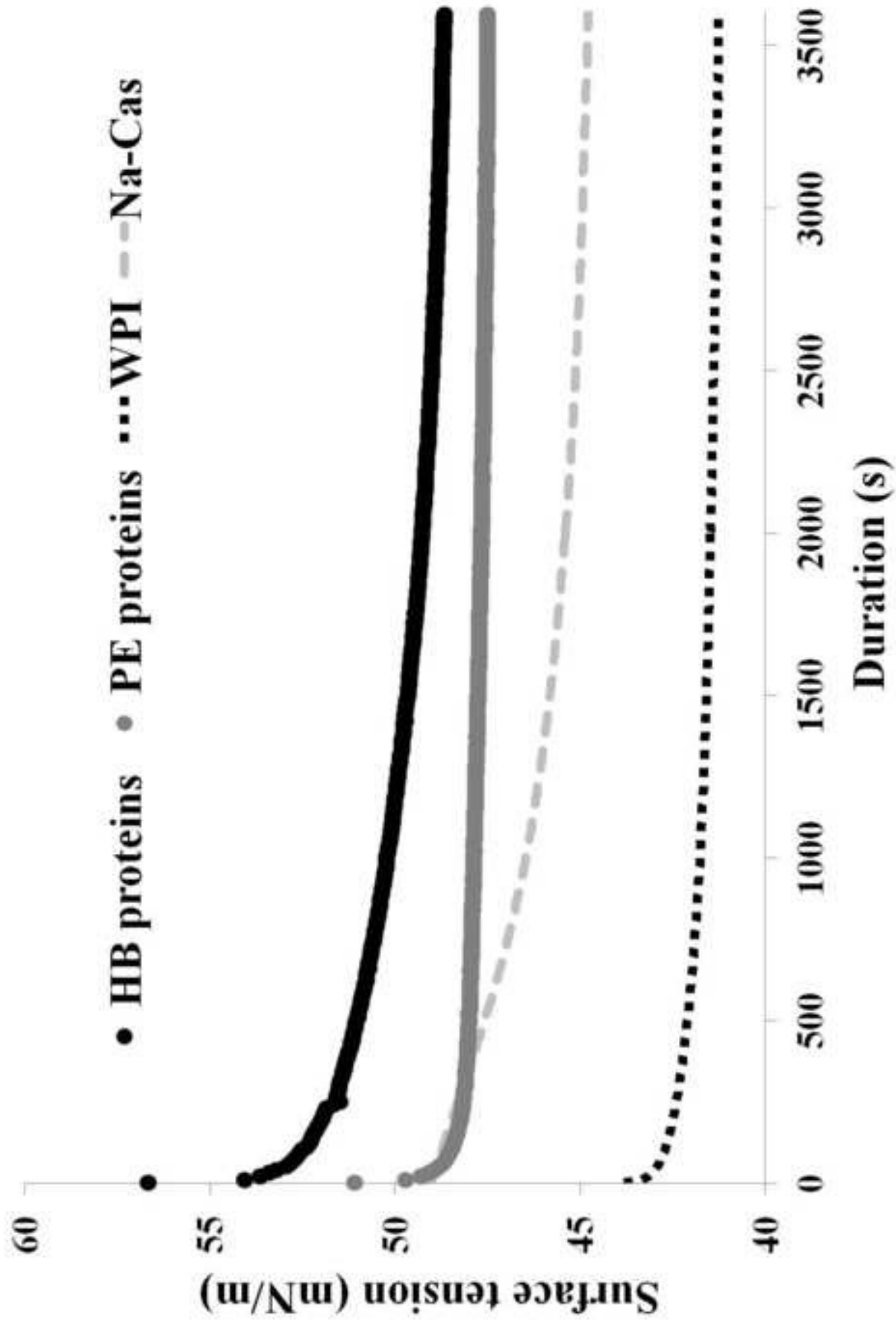


Figure 4a
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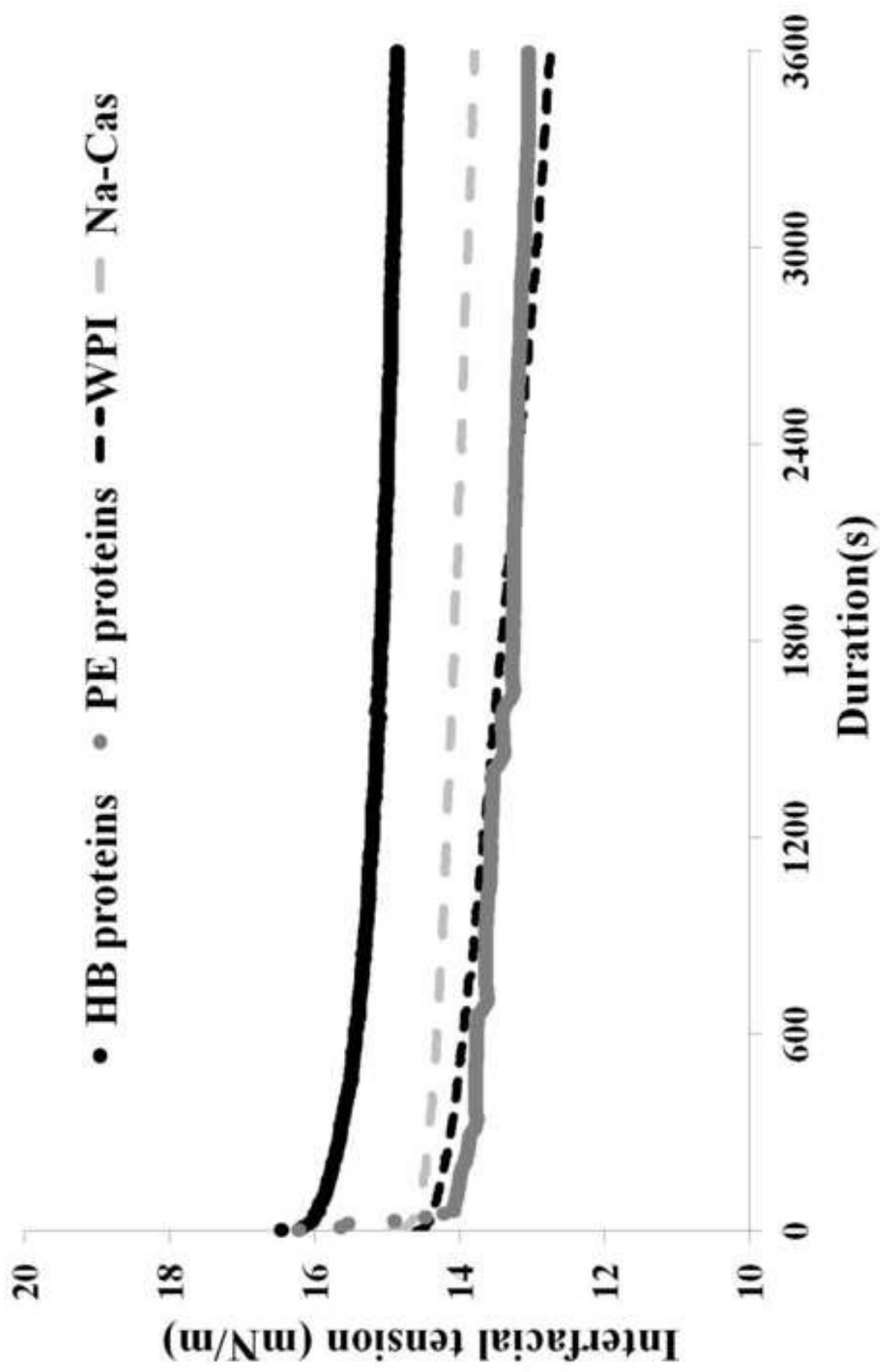


Figure 4b
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Figure Captions

Figure 1. a) Size-exclusion chromatographs of PE and HB; b) Electrophoresis of the two effluents

Figure 2. a) Evolution of visco-elastic properties of HB in the range 4-20°C; b) Evolution of storage modulus of HB (●) and PE (●) with temperature increase

Figure 3. Solubility of proteins from HB (●) and PE (●) versus pH

Figure 4. Evolution of surface tension (a) and interfacial tension (b) of solutions at 2% wt proteins from HB, PE, Na-cas and WPI versus time at 20°C

Table 1. Average composition in dry matter of the collected effluents

Ingredients (%)	PE	HB
Dry matter	14 ± 2	8.0 ± 0.5
Proteins <i>with collagen</i>	89 ± 3 <i>ND*</i>	48 ± 4 <i>7 ± 1</i>
Fat	0.9 ± 0.1	10 ± 2
Soluble Sugars	ND	4 ± 1
Sodium Chloride	0.7 ± 0.1	20 ± 5
Residual ash	8 ± 2	25 ± 2

*ND : non detected

Table 2. Average composition in amino acids (% of total amino acids) from proteins of the by-products

Amino Acids	PE	HB
Asp	9.5	4.4
Thr*	5.2	2.2
Ser	3.7	2.7
Glu	10.6	13.3
Gly	5.4	13.5
Ala	6.2	6.6
Val*	7.1	3.6
Ileu*	4.8	1.5
Leu*	7.4	3.0
Tyr	3.1	1.0
Phe*	7.8	1.8
His*	6.6	23.2
Lys*	8.6	4.3
Arg	5.0	4.3
Pro	4.7	6.8
Cys	1.8	2.4
Met*	2.3	5.5

* : essential amino acids

Table 3. Functional properties of proteins from PE and HB compared to commercial proteins

Functional properties	PE	HB	Na-Cas	WPI
Surface hydrophobicity	66 ± 3	6 ± 2	56 ± 3	44 ± 1
EC (mL oil/ g of protein)	324 ± 8	252 ± 7	408 ± 19	356 ± 4
ES (%)	94 ± 6	77 ± 3	63 ± 1	61 ± 1
FA (%)	98 ± 3	177 ± 14	238 ± 3	217 ± 7
FS (min)	16 ± 1	4 ± 1	10 ± 1	29 ± 3