



HAL
open science

Interfacial and foaming properties of two types of total proteose-peptone fractions

Gaoussou Karamoko, Sabine Danthine, Gilles Olive, Christophe Blecker

► **To cite this version:**

Gaoussou Karamoko, Sabine Danthine, Gilles Olive, Christophe Blecker. Interfacial and foaming properties of two types of total proteose-peptone fractions. *Food and Bioprocess Technology*, 2013, 6 (8), pp.1944-1952. 10.1007/s11947-012-0916-4 . hal-00724542

HAL Id: hal-00724542

<https://hal.science/hal-00724542>

Submitted on 21 Aug 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Food and Bioprocess Technology: An International Journal
INTERFACIAL AND FOAMING PROPERTIES OF TWO TYPES OF TOTAL
PROTEOSE-PEPTONE FRACTIONS
 --Manuscript Draft--

Manuscript Number:	FABT-2789R3
Full Title:	INTERFACIAL AND FOAMING PROPERTIES OF TWO TYPES OF TOTAL PROTEOSE-PEPTONE FRACTIONS
Article Type:	Original Research
Keywords:	Proteose-peptone, whey protein concentrate, kinetic adsorption, monolayer film, foaming properties, foamsan
Corresponding Author:	Gaoussou Karamoko, Ph. D. University of Liège Gembloux, BELGIUM
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of Liège
Corresponding Author's Secondary Institution:	
First Author:	Gaoussou Karamoko, Ph. D.
First Author Secondary Information:	
Order of Authors:	Gaoussou Karamoko, Ph. D. Sabine Danthine Giles Olive Christophe Blecker
Order of Authors Secondary Information:	
Abstract:	Total proteose-peptone (TPP) fractions were extracted from skimmed milk (UHT) and whey protein concentrate (WPC) on a laboratory scale. Protein solutions (0.1, 0.5 and 1 % w/w) were characterized as function of pH: 4.0, 4.6-4.7 (native pH) and 7.0. Their foaming capacities and stabilities were studied. Beforehand, the surface properties that govern them were investigated, notably the kinetics of adsorption and mechanical properties of monolayer films at the air-water interface involved in the formation and the stability of foams respectively. The TPP extracted from skimmed milk showed the lowest values as well as a significant reduction in surface tension and presented a good mechanically resistant film. The TPP extracted from WPC presented a better foaming capacity and stability which was unexpected. However, foaming properties and surface properties of TPP fractions depended on the pH. The considerable influence of extraction source and method on proteose-peptone's properties were highlighted.

1 **INTERFACIAL AND FOAMING PROPERTIES OF TWO TYPES OF TOTAL**
2 **PROTEOSE-PEPTONE FRACTIONS**

3
4
5 3
6
7
8 4 **Gaoussou Karamoko* , Sabine Danthine, Gilles Olive and Christophe Blecker**

9
10
11 5 University of Liege, Gembloux Agro-Bio Tech, Department of Food Sciences and
12
13 6 Formulation, Passage des Déportés 2, B-5030 Gembloux, Belgium.

14
15
16
17 7 *Corresponding author. Tel: +3281622564; fax: +3281601767.

18
19
20 8 E-mail adresse: karamgao@yahoo.fr

10 **Abstract**

1
2
3 11 Total proteose-peptone (TPP) fractions were extracted from skimmed milk (UHT) and
4
5 12 whey protein concentrate (WPC) on a laboratory scale. Protein solutions (0.1, 0.5 and 1 %
6
7 13 w/w) were characterized as function of pH: 4.0, 4.6-4.7 (native pH) and 7.0. Their foaming
8
9 14 capacities and stabilities were studied. Beforehand, the surface properties that govern them
10
11 15 were investigated, notably the kinetics of adsorption and mechanical properties of monolayer
12
13 16 films at the air-water interface involved in the formation and the stability of foams
14
15 17 respectively. The TPP extracted from skimmed milk showed the lowest values as well as a
16
17 18 significant reduction in surface tension and presented a good mechanically resistant film. The
18
19 19 TPP extracted from WPC presented a better foaming capacity and stability which was
20
21 20 unexpected. However, foaming properties and surface properties of TPP fractions depended
22
23 21 on the pH. The considerable influence of extraction source and method on proteose-peptone's
24
25 22 properties were highlighted.

26
27
28
29
30
31
32
33 23 **Keywords:** Proteose-peptone, whey protein concentrate, kinetic adsorption, monolayer film,
34
35 24 foaming properties, foamscan

36
37
38
39 25

26 **Introduction**

27 Foams are a subject of strong interest in a large number of industries where they play a
28 crucial role. In the food industry, a foaming formulation involves the use of surface-active
29 agents. During the foaming processes, these agents adsorb rapidly and form a film at the
30 surface of gas bubbles. Surface-active agents must simultaneously give abundant and stable
31 foams. Many researches are focused on the development of novel ingredients, including
32 proteins which can be used as foaming agents. Food processing industries use these protein
33 ingredients to improve the functional properties of a wide range of formulated food (Cassiani
34 et al., 2011). Among food proteins, whey proteins are considered as important and valuable
35 ingredients due to their high nutritional and functional properties (Blecker et al., 1997;
36 Mladen et al., 2009). These features confer to whey proteins ingredients high foaming and
37 stabilizing properties.

38 Among the whey components the total proteose-peptone fraction (TPP) could be of
39 interest. Indeed the TPP is a minor fraction of the heat-stable and acid-soluble protein which
40 is found in whey (Blecker et al., 2005). According to the literature, this fraction has
41 interesting functionalities such as surface properties, emulsifying properties and foaming
42 properties (Shimizu et al., 1989; Innocente et al., 1998a, 1998b).

43 However, previous works on the foaming property of the milk's TPP fraction are
44 controversial. Some authors such as Zhu and Damodaran (1994) have shown a negative effect
45 of the TPP on the foam property (foam stability) produced by whey due to certain competitive
46 actions of other proteins. On the other hand, the TPP fraction is considered responsible of
47 milk foaming capacity due to its component PP3 (Girardet and Linden, 1995). Moreover,
48 most of studies reported in the literature concerns the TPP extracted from milk.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49 In the light of these considerations, this study attempted to provide new information
50 about the foaming properties of TPP, especially the TPP extracted from whey. To reach this
51 goal, two types of TPP fractions were extracted in this work. The first one was extracted from
52 skimmed milk using a classic method according to Pâquet et al (1988) and the second was
53 extracted from whey protein concentrate according to a procedure which is feasible on an
54 industrial scale.

55 The interest of this work is to study the TPP extracted from whey protein concentrate
56 (78 % of protein), a commercially available by-product of cheese processing. The use of this
57 alternative source could allow an increased TPP production for industrial applications. Foam
58 properties were characterized for possible use in food applications requiring foam formation
59 such as whipped cream, ice cream, cakes and desserts.

60 In the literature, SDS-PAGE electrophoresis was used to characterize the TPP fraction's
61 components according to Laemmli's method (Pâquet et al., 1988). However several others
62 separation techniques were also used including liquid chromatography which allow a good
63 separation of the TPP's components. The hydrophobic interaction was most often used to
64 separate the TPP due to the hydrophobicity of some major components. In our case, Tricine
65 SDS-PAGE electrophoresis according to schägger (2006) was used to characterize the TPP,
66 due to it's high resolution, especially for the small proteins.

67 The aim of this study was to determine the ability of TPP fractions to form and stabilize
68 foams at several protein concentrations, at various pH, and to highlight the influence of the
69 extraction method on these properties.

70

71 **1. Materials and methods**

72 **1.1. Extraction of total proteose-peptone fractions**

73 **1.1.1 Extraction from milk**

74 Milk total proteose-peptone fraction (milk TPP) was extracted from UHT skimmed milk
75 according to the procedure described by Pâquet et al. (1988) with some modifications. Three
76 batches of ten litres of commercial skimmed milk (Everyday, Colruyt, Belgium) were used to
77 extract the TPP. The process consisted in heating one litre of skimmed milk at 90 - 95 °C
78 during 30 min and then cooling it down in an ice bath to 20 °C during 40 min. pH was then
79 adjusted at 4.6 with 1M HCl to induce isoelectric precipitation. Samples were centrifuged at
80 5000 g for 30 min at 20 °C (Centrifuge Beckman Model J2-21, Indianapolis, USA).
81 Ammonium sulfate (304 g) was added to the supernatant (800 mL) to obtain a half-saturated
82 solution that was then shaken and centrifuged (5000 g for 10 min at 20 °C). The pellet was
83 dispersed, exhaustively dialyzed and finally lyophilized. The TPP yield was stored at 4 °C
84 until analysis.

85 **1.1.2 Extraction from whey protein concentrate (WPC)**

86 Whey protein concentrate (WPC) (Lacprodan 80) was obtained from Arla Foods
87 Ingredients (Viby J, Denmark). 2.85 Kg of commercial WPC were used. WPC was dispersed
88 into distilled water (95 g.L⁻¹) by stirring at room temperature for 2 hours to ensure good
89 dispersion. Whey total proteose-peptone (WPC TPP) was then extracted from the dispersion
90 using the same procedure described for extraction from milk without the ammonium sulfate
91 addition. The TPP yield was stored at 4 °C until analysis.

92 **1.2. Chemical composition of TPP**

93 The TPP fractions obtained were analyzed for ash (AOACI, 2000), lipid by modified
94 mojonnier method (AOACI, 2000) and lactose (Miller, 1959). The determination of protein
95 content was performed by the Lowry method as described by Lowry et al. (1951).

96 **1.3. Electrophoretic Analysis using Tricine-SDS-PAGE**

97 TPP molecular weight distribution was evaluated by Tricine-SDS-PAGE
98 electrophoresis following the method of Schägger (2006). The Tricine-SDS-PAGE was
99 performed with a vertical electrophoresis apparatus model 2001 (Amersham-Pharmacia,
100 Uppsala, Sweden) with a 16 % polyacrylamide separating gel (16 % T, 3 % C where T is the
101 total concentration of acrylamide/bis-acrylamide monomers and C is the amount of
102 crosslinker relative to the total concentration) and thickness of 1.5 mm. Tricine-SDS-Page
103 uses two buffers and offers a high resolution, especially in the small protein and peptide
104 range. The electrophoresis buffers were tris-HCl buffer (anode buffer) and Tricine-tris buffer
105 (cathode buffer). 500 µl of sample (1-3 % w/v of proteins) were treated with a equivalent
106 volume of sample buffer (150 mM Tris-HCl, pH 7.0, 12 % SDS; 6 % mercaptoethanol, 30 %
107 glycerol, 0.05 % Coomassie blue), and then heated at 37 °C for 15 min before electrophoresis.
108 Electrophoresis was performed at an initial voltage of 30 V for stacking and at a constant
109 voltage of 90 V for separation. The protein bands were fixed in a solution consisting of 50 %
110 methanol, 10 % acetic acid, and 100 mM ammonium acetate for 60 min, before staining by
111 0.025 % coomassie dye in 10 % acetic acid for 24 h. The gel destaining was performed in 10
112 % acetic acid.

113 **1.4. Dynamic Drop Volume Method**

114 TPP samples were prepared in ultra-pure water (ultra-pure water system Milli.Q50,
115 Millipore). TPP solutions of 0.1, 0.5 and 1% (w/w) were prepared at various pH values: 4.0,
116 4.6-4.7 (native pH) and 7.0. Automated drop volume tensiometer TVT1 (LAUDA,
117 Königshöfen, Germany) was used to perform dynamic measurements for surface tension

118 measurements at air-water interface. The experiments resulted in the measurement of surface
119 tension as a function of the drop formation time (Innocente et al. 1998). All experiments were
120 performed at $25 \pm 0.5^\circ\text{C}$. Each measurement was repeated three times. Equilibrium interfacial
121 tension (γ_e) and the initial adsorption rate (v) were determined by extrapolated values of γ vs.
122 $t^{-1/2}$ at $t \rightarrow \infty$ and the tension (γ) versus time (t) curve slope at initial time (t_0), respectively
(Blecker et al., 2002; Innocente et al., 2011).

1.5. Adsorption Kinetics Recording

125 The surface tension-time dependence of TPP solutions was measured with the
126 automatic drop tensiometer TRACKER (IT Concept, Longessaignes, France) forming a rising
127 protein solution bubble of at the tip of 5 μL a curved capillary in an empty cell (containing
128 air). The surface tension was measured and recorded every second. All measurements were
129 performed at $25 \pm 0.5^\circ\text{C}$.

1.6. Mechanical properties of monolayer film

131 Protein samples were prepared by dissolving the lyophilized material in ultra-pure
132 water (ultra-pure water system Milli.Q50, Millipore). Protein solution (1 mg.mL^{-1}) were
133 characterized at various pH values: 4.0, 4.6-4.7 (native pH) and 7.0. Measurements of the
134 surface pressure (π) versus surface area were performed on Langmuir-type film balance
135 LFW2 (LAUDA GmbH, Königshöfen, Germany) of which the principle and the experimental
136 condition were described by Innocente et al. (1998). Schematically this technique involved
137 the measurement of the surface tension of an aqueous surface under different compression
138 conditions in the presence and absence of a surface monolayer, and may be described by the
139 following equation:

$$140 \quad \pi = \gamma_0 - \gamma, \quad (1)$$

141 where π is the surface pressure, γ_0 and γ are the surface tension of the clean surface and the
142 surface containing the monolayer, respectively. The elasticity (ϵ) of the protein film at the air-

143 water interface, defined as the variation in surface pressure for a variation in the surface unit
144 area, was then calculated for every point of the isotherm π/A by means of the following
145 equation:

$$\varepsilon = - A (d\pi/dA), \quad (2)$$

147 where $d\pi/dA$ is the surface pressure derivative in surface area (Damodaran, 1994). All
148 experiments were performed at $25 \pm 0.5^\circ\text{C}$.

149 **1.7. Foaming properties**

150 Foaming capacity and foam stability of total proteose-peptone were determined with a
151 Foamscan (IT Concept Instrument, Longessaignes, France). The system includes a transparent
152 column of glass, with at its base a porous frit through which the gas is injected. The volume of
153 liquid and foam are measured with a conductivity meter, composed of a series of two
154 electrodes located at the base of the column and four electrodes placed at the top of the
155 column. 25 ml of protein solution was aerated at a flow of $100 \text{ mL}\cdot\text{min}^{-1}$. The evolution of the
156 foam was monitored to assess foam stability and its height was continuously measured by
157 image analysis. A fritted glass with a porosity of 4 (P4) was used and the aeration time was 60
158 seconds. Measurements were performed at 25°C . The foam capacity (FC) was determined
159 by:

$$160 \quad \text{FC} = V_f/V_g, \quad (3)$$

161 Where V_f is the final foam volume and is V_g the final gas volume injected. Foam stability
162 (FS) was determined as the time needed to drop to half the final foam volume ($T_{1/2}$).

163 Measurements were performed in triplicate with fresh solution. Protein solutions were
164 prepared according to the sample preparation of the surface tension study. The speed of
165 decreasing (Sd) after switching off the bubbling was calculated using equation 4.

$$(FV_{450s} - FV_{60s}) / (450 - 60)$$

166 Sd = $\frac{\quad}{60}$ (4)
1 167
2
3 168

4 169 **1.8. Statistical analysis**

5

6 170 The data shown are the average of triplicate values. Statistical analyses were carried out
7
8
9 171 using a SPSS for windows, version 11.0 (SPSS Inc., 1999). The data were subjected to
10
11 172 analysis of variance (ANOVA) using the general linear model option (DUNCAN test) to
12
13
14 173 determine significant differences between samples ($P < 0.05$).
15

16 174 **2. Resultats and Discussion**

17
18

19 175 Total proteose-peptone (TPP) fraction is usually extracted from milk, with a yield
20
21
22 176 around 1 g.L⁻¹ (Mati et al., 1991). In this study, the modification of Pâquet's procedure
23
24 177 allowed to increase the quantity of TPP extracted from milk to 1.56 g.L⁻¹ of milk (i.e. 1.56 g /
25
26
27 178 130 g dry matter). The extraction of TPP from the alternative source WPC (78 % of protein)
28
29 179 reached 19.13 g / 95 g of WPC. The use of WPC allowed to increase the TPP production by a
30
31
32 180 process potentially transposable on an industrial scale. However, the composition of TPP
33
34 181 fractions (Table 1) showed a fundamental difference between the two types of TPP in terms of
35
36 182 protein content. Milk TPP contains more protein than WPC TPP (79.15 % versus 65.20 % of
37
38
39 183 protein). The lipid content of milk TPP was relatively greater than in WPC TPP, while the
40
41 184 lactose content was more important in WPC TPP than milk TPP. It should be noted that
42
43
44 185 lactose represents a big part of nonprotein component that does not affect the interfacial
45
46 186 properties of the extract but could instead affect the foam stability (Zhu and Damodaron,
47
48
49 187 1994).
50

51
52 188 The protein analysis on milk TPP by Tricine SDS-PAGE (Figure 1), presented the same
53
54 189 bands as described in the literature, notably component PP3 also named LP28 (28 kDa)
55
56
57 190 according to (Kanno, 1989), LP18 (high hydrophobic component of 18 kDa) (Sorensen and
58
59
60
61
62
63
64
65

191 Petersen, 1993), component PP5 (14.3 kDa) (Kollar and Brunner., 1970) and component
192 PP8S (9.9 kDa) (Kollar and Brunner., 1970). The band corresponding to component PP8 F as
193 referred to by Kollar and Brunner (1970) was not observed on the gel due to its very low
194 molecular weight (4 kDa). The LP18 (18kDa) was the high hydrophobic component of the
195 FHPP of TPP. LP18 hydrolyzed by plasmin from LP28 in milk was absent in the extract of
196 WPC (WPC TPP). However, a difference in thickness and intensity was observed with regard
197 to the bands PP5 and PP3. Thus, the PP3 band in milk TPP appears stronger and more intense
198 than the PP3 band in WPC TPP. The PP5 band was thinner in milk TPP than WPC TPP. In
199 view of these observations, it could be said that milk contained more PP3 and less PP5
200 compared to WPC TPP, this depends on milk's evolution before undergoing heat treatments
201 such as UHT or whey processing.

2.1. Dynamic surface tension

203 Adsorption kinetics at the air-water interface of TPP fractions extracted from skimmed
204 milk and WPC were compared by using dynamic surface tension technique. Many factors
205 may affect this property at the interface such as concentration, pH, and temperature. Among
206 these, the concentration and pH were specifically considered. Therefore, Figure 2 represents
207 the surface tension as a function of drop formation time for both different fractions at 1 %
208 (w/w), and at various pH. Surface tension value decrease with the increase in drop formation
209 time, because molecules have more time to position themselves uniformly at the interface
210 where they can develop their surfactant activity (Innocente et al., 2011). At all tested
211 concentrations and pH, the surface tension reduction was more pronounced for milk TPP in
212 comparison to WPC TPP. This suggested that milk TPP had a higher surface activity than
213 those extracted from WPC. This difference in behavior could be related to the hydrophobic
214 fraction commonly called HFPP which contains LP18 and LP28 in milk TPP according to the

215 electrophoresis. Moreover, it was demonstrated that the surface-active properties of the TPP
216 fractions are in part governed by the physicochemical properties of PP3 such as aggregation
217 properties and surface hydrophobicity (Innocente et al., 1998a; Piquet et al., 1988). Therefore,
218 the relative percentage of PP3 in each fraction might certainly explain the lower interfacial
219 activity of WPC TPP compared to milk TPP.

220 The discussions hereafter are focused on the 1 % (w/w) solutions because the
221 difference between the samples was not significant at 0.1 % and 0.5 % ($P \geq 0.05$). It appeared
222 that the milk TPP lowered the surface tension when the pH increased. Conversely, for WPC
223 TPP the lowest value of surface tension was observed at pH 4.0 or native pH (4.7). These
224 differences are due to the composition of each fraction. The LP18 observed in milk TPP
225 composition could be at the origin of these behaviors with regards to pH. Indeed, LP18
226 comprises four isoelectric isoforms (pI 5.0 - 8.0) (Inagaki et al., 2010). At pH 7.0 milk TPP
227 presented a net charge because this pH is in this range, which explained the lowest surface
228 tension value at pH 7.0. Whereas, the pI of TPP is 4.0 to 5.0 according to Pâquet et al. (1988).
229 So, WPC TPP presented a net charge at pH 4.0 and native pH (4.70), which explained the
230 lowest surface tension values obtained at these pH compared to pH 7.0.

231 The analysis of adsorption kinetic curves was quantified by determining the initial
232 adsorption rate and equilibrium surface pressure (Table 2) to explain the reduction of surface
233 tension. Thus, the initial adsorption rate is equivalent to the slope of the tangent to the curve at
234 the start of the kinetics; it characterizes the velocity at which molecules lower the surface
235 tension at the beginning adsorption. This initial adsorption rate has been determined using the
236 automatic drop tensiometer tracker (Table 2) which was used as a complement to TVT. The
237 initial surface tension value at $t = 0$ corresponding to the water surface tension, was equal to
238 72.00 mN.m^{-1} . Equilibrium surface pressure value ($\pi_e = \gamma_0 - \gamma_e$, where γ_0 is the initial surface
239 tension and γ_e is the equilibrium surface tension) characterizes the maximum reduction of

240 surface tension induced by the surfactant molecules, in our case the dispersion of the
241 proteose-peptone. The highest values of initial adsorption rate and equilibrium surface
242 pressure were observed for milk TPP ($P < 0.05$). Equilibrium pressure values obtained
243 correspond to a reduction of the initial surface tension value of 32 to 35 % for milk TPP and
244 29 to 30 % for WPC TPP. This indicated that milk TPP caused a rapid reduction of interfacial
245 tension that reached the equilibrium values faster compared to WPC TPP. Many authors have
246 shown that the adsorption rate is correlated with the foaming capacity of proteins (German et
247 al., 1985; Dagorn-Scaviner, 1986) and the development of an important surface pressure is
248 required to obtain good stability of dispersed systems (Nakai et al., 1980). Overall, these
249 findings suggested a better foaming capacity for milk TPP compared to WPC TPP.

250 **2.2. Study of monolayer films**

251 Mechanical properties of the monolayer films formed by TPP fractions were
252 investigated in addition to the adsorption kinetics study. This was done using a Langmuir film
253 balance to determine the compression isotherms in order to understand the foaming
254 properties. The compression isotherms obtained at the air-water interface represent the surface
255 pressure against surface area (square meter per milligram of proteins). During the
256 compression of film, the surface pressure increases.

257 In generally, when the film molecules undergo a compression, the surface area occupied
258 per mg of protein decreases. The surface pressure increased from a value A_0 (initial area),
259 linearly up to a transition zone which was observed around an inflection point. After this, the
260 surface pressure increased to a maximum value at the end of compression. Milk TPP formed
261 films with a higher mechanical resistance with regard to surface pressure, compared to WPC
262 TPP. The compression isotherms were recorded at different pH, it is clear from Figure 3 that
263 they present the same profiles at the considered pH. The surface pressure changed with the

264 pH. Monolayer films of milk TPP presented a better resistance at pH 7.0 than at pH 4.0, and
265 inversely for the WPC TPP ($P < 0.05$). These results are in agreement with those of the
266 kinetics of adsorption at air-water interface.

267 The compression isotherms reflect the mechanical properties of the monolayer whereas
268 the stability of the film is a phenomenon linked to the forces of cohesion in the monolayer as
269 well as the intermolecular interactions within the film, and between the molecules of the film
270 and the aqueous phase. These interactions influence the mechanisms governing the film
271 instability leading to a break (Baglioni et al., 1980). Hence, the film elasticity which measures
272 the film resistance towards a change in surface can be considered as a parameter quantifying
273 the intermolecular interaction and the film-subphase interaction (Rodriguez-Patino et De la
274 Fuente Feria, 1995) allowing to explain the stability mechanism of film. The elasticity was
275 determined in the area above the transition zone because it corresponds to the location of the
276 condensed film during the film stability (Gaines, 1966). Maximum elasticity values obtained
277 at the different pH are shown in Table 3. The highest values were observed for the TPP WPC
278 spread at pH 4 ($13.15 \text{ mN}\cdot\text{m}^{-1}$), whereas milk TPP obtained its highest value at pH 7.0 (11.62
279 $\text{mN}\cdot\text{m}^{-1}$). This trend inversement has already been mentioned for adsorption kinetics and
280 compression isotherms. In view of the results presented in Table 3, WPC TPP films were
281 more elastic than milk TPP film except at pH 7.0 ($P < 0.05$). The molecules of WPC TPP film
282 were more resistant to conformational change compared to those of milk TPP film except at
283 pH 7.0. Such a property is required for good foam stability (Poré, 1976). The differences
284 between milk TPP and WPC TPP films could again be explained by considering differences
285 in their compositions.

286 2.3. Foaming properties

287 Foam capacities (FC) of proteose-peptone fractions at 1 % (w/w) were compared at
288 various pH as depicted in Figure 4. Generally, the foaming capacity increased with increasing
289 concentration of protein. The foaming capacity of milk TPP was lower than that of WPC TPP
290 at all pH studied. The milk TPP foaming power increased with the increasing of pH, whereas
291 the WPC TPP foaming power decreased with increasing pH. This effect of pH on foaming
292 capacity of each fraction was correlated with the adsorption rate obtained for each fraction
293 (see paragraph 2.1). This is in accordance with German et al (1985) and Dagorn-Scaviner
294 (1986) which showed a correlation between the adsorption rate and the foaming capacity.

295 The foaming inability of milk TPP can be explained by the quantity of fat in its
296 composition. The quantity of lipid is greater in milk TPP than in WPC TPP (Table 1). The
297 presence of small lipid quantities can have a strong destabilizing effect on foaming (Huppertz,
298 2010). A lower FC of milk TPP induced a collapse of the air bubble during foam formation.
299 This is probably due to the weak mechanical strength of the lipid-protein interaction.

300 Foam stability (FS) was characterized by the half-life of foam volume. The effect of pH
301 on this parameter is represented in Figure 5. As in the case of foaming capacity, the effect of
302 pH on foam stability was correlated with the surface pressure obtained in Adsorption kinetics
303 study. This was mentioned by Nakai et al. (1980) which say that the development of an
304 important surface pressure lead to a good stability of dispersed systems.

305 In general, the increasing concentration of TPP improved foam stability (data not
306 shown). WPC TPP capacity to stabilize foam was greater compared to milk TPP (at all pH),
307 as the time needed to reduce the foam volume to half of the maximum volume ($T_{1/2}$) was
308 higher. This indicates the higher resistance of WPC TPP film to the coalescence of bubbles.
309 The evolution of foam volume of TPP fraction at 1 % during the first 450 seconds as function
310 of pH is represented in Figure 6. The difference in foam behavior was demonstrated by the
311 speed of decrease after switching off the bubbling (Table 4). The highest value of decrease

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

312 speed was observed for milk TPP foam; this indicated a greater ability to stabilize for WPC
313 TPP. The better foaming properties of WPC TPP were essentially due to the lower quantities
314 of lipids in WPC TPP composition, as it is well known that lipids destabilize foam (Huppertz,
315 2010).

316 **3. Conclusion**

317 Variations in functional and surface properties of the two TPP fractions are due to
318 several factors (heat treatments, UHT or whey processing), extraction source, extraction
319 method and the composition of each fraction (lipids and proteins). Milk TPP exhibited a
320 higher surface activity and a better monolayer film resistance to mechanical disturbances.
321 Whereas the best foaming properties were exhibited by WPC TPP. This best performance of
322 WPC TPP compared to milk TPP is due to the lipid fraction of milk TPP which prevents them
323 to foam. It is conceivable they could both be used as surface-active agents in food
324 applications requiring foam formation. On the other hand, the extraction from WPC would be
325 interesting because it is advantageous in terms of high TPP production yield and economical
326 compared to TPP extracted from milk.

328 **References**

1
2 329 Albrecht, O., and Sackmann, E. (1980). A precision Langmuir film balance measuring
3
4
5 330 system. *Journal of Physics E: Scientific Instruments*, 13(5), 512-515.

6
7 331 Andrews, A. T. (1983). Proteinases in normal bovine milk and their action on caseins.
8
9 332 *Journal of Dairy Research*, 50(1), 45-55.

10
11 333 Association of Official Analytical Chemists International. 2000. *Official Methods for*
12
13
14 334 *Analysis. Fat in milk (modified mojonier method)-method 989.05; 33.2.26, ash of dried milk*
15
16 335 *method 930.30; 33.5.05. AOACI 17th edn, Gaithersburg, MD.*

17
18
19 336 Baglioni, P., Gabrielli, G., Guarini, G. T. (1980). On the mechanism of collapse of
20
21 337 monolayers of macromolecular substances II. Cellulose acetate. *Journal of Colloid and*
22
23 338 *Interface Science*, 78(2), 347-355.

24
25
26 339 Bastiaens, A., Paquot, M., Marbaise, M., Deroanne, C. (1990). Foaming capacity of
27
28 340 protein isolates: effect of the electro-kinetic potential of protein dispersions. *Sciences des*
29
30 341 *aliments*, 10(4), 773-783.

31
32
33 342 Blecker, C., Anceau, C., Danthine, S., Deroanne, C., Laloux, J., Sindic, M., Van Reusel,
34
35
36 343 A. (2005). S'adapter aux exigences du marché, innover dans les productions animales et
37
38 344 maîtriser la qualité. Cas du lait et de la viande. Communication orale « Dixième Carrefour des
39
40 345 *Productions Animales* », Gembloux, Belgique, 26 janvier 2005.

41
42
43 346 Boyd, J. V., Mitchell, J. R., Irons, L., Musselwhite, P. R., Sherman, P. (1973). The
44
45 347 mechanical properties of milk protein films spread at the air-water interface. *Journal of*
46
47 348 *Colloid and interface Science*, 45(3), 478-486.

48
49
50 349 Blecker, C., Paquot, M., Lamberti, I., Sensidoni, A., Lognay, G., and Deroanne, C.
51
52 350 (1997). Improved emulsifying and foaming of whey proteins after enzymic fat hydrolysis.
53
54 351 *Journal of Food Science*, 62(1), 48-52/74.

352 Brnčić , M., Bosiljkov, T., Ukrainczyk, M., Tripalo, B., Rimac Brnčić, S., Karlović, S.,
1
2 353 Karlović, D., Ježek, D., Vikić Topić, D. (2011). Influence of whey protein addition and feed
3
4 354 moisture content on chosen physicochemical properties of directly expanded corn extrudates.
5
6 355 Food and Bioprocess Technology, 4(7), 1296-1306. DOI 10.1007/s11947-009-0273-0.
7
8
9 356 Cassiani, D. M., Yamul, D. K., Conforti, P. A., Pérez, V. A., Lupano, C. E. (2011).
10
11 357 Structure and functionality of whey protein concentrate-based products with different water
12
13 358 contents. Food and Bioprocess Technology, DOI 10.1007/s11947-011-0680-x.
14
15
16 359 Dagorn-Scaviner, C. (1986). Etudes des proprietes tensioactives des gliadines du pois.
17
18 360 Application aux isolats protéiques utilisés comme agents d'émulsification ou de moussage.
19
20 361 Thèse de Doctorat, Université de Nantes.
21
22
23 362 Gaines, G. L. (1966). Insoluble Monolayers at Liquid-gaz Interface. Prigogine,
24
25 363 Interscience, New York, p 1-386.
26
27
28 364 German, J. B., O'Neil, T. E., Kinsella, J. E. (1985). Film forming and foaming behavior
29
30 365 of food proteins. *Journal of the American Oil Chemists' Society*, 62(9), 1358-1366.
31
32
33 366 Girardet, J. M., and Linden, G. (1995). PP3 component of bovine milk: a
34
35 367 phosphorylated whey glycoprotein. *Journal of Dairy Research*, 63(2), 333-350.
36
37
38 368 Huppertz Thom. (2010). Foaming properties of milk: A review of the influence of
39
40 369 composition and processing. *International Journal of Dairy Technology*, 64(4), 477-488.
41
42
43 370 Inagaki, M., Nakaya, S., Nohara, D., Yabe, T., Kanamaru, Y., Suzuki, T. (2010). The
44
45 371 multiplicity of n-glycan structures of bovine milk 18 kDa lactophorin (milk glyCAM-1).
46
47 372 *Bioscience: Biotechnology and Biochemistry*, 74(2), 447-450.
48
49
50 373 Innocente, N., Corradini, C., Blecker, C., and Paquot, M. (1998a). Dynamic surface
51
52 374 properties of the proteose-peptone fraction of bovine milk. *Journal of Dairy Science*. 81(7),
53
54 375 1833-1839.
55
56
57
58
59
60
61
62
63
64
65

376 Innocente N., Corradini C., Blecker C., and Paquot M. (1998b). Emulsifying properties
1 of the total fraction and hydrophobic fraction of bovine milk proteose-peptone. International
2 377 Dairy Journal. 8(12), 981-985.
3
4 378

5
6
7 379 Innocente, N., Biasutti, M., and Blecker, C. (2011). HPLC profile and dynamic
8
9 380 properties of the proteose-peptone fraction from bovine milk and whey protein concentrate.
10
11 381 International Dairy Journal, 21(4), 222-228.
12

13
14 382 Kamikaze, N., K., K., Gonçales, M., M., Zaia, C., T., B., V., Zaia, D., A., M. (2003).
15
16 383 Determination of total protein in cow milk powder sample s: a comparative study between the
17
18 384 Kjeldahl method and spectrophotometric methods. Journal of Food Composition and
19
20 385 Analysis, 16(4), 507-516.
21

22
23
24 386 Kanno, C., 1989. Purification and separation of multiple forms of lactophorin from
25
26 387 bovine milk whey and their immunological and electrophoresis properties. Journal of Dairy
27
28 388 Science., 72(4), 883-891.
29

30
31 389 Kollar, C. K., and Brunner, J. R. (1970). Proteose-peptone fraction of bovine milk:
32
33 390 lacteal serum component 5 and 8 casein associated glycoprotein. Journal of Dairy Science.
34
35 391 53(8), 997-1008.
36

37
38
39 392 Lowry O. H., Rosebrough N. J., Farr A. L., and Randall R. J. (1951). Protein
40
41 393 measurement with the folin phenol reagent. Journal of Biological Chemistry. 193, 265-275.
42

43 394 Mati, A., Girardet, J. M., Xenakis, D., Linden, G. (1991). Isolement et Caractérisation
44
45 395 de la fraction hydrophobe des Protéose-peptones des laits Bovin, Ovin et Caprin. Lait, 71(3),
46
47 396 259-273.
48

49
50
51 397 Pâquet, D., Nejjar, Y., & Linden, G. (1988). Study of hydrophobic protein fraction of
52
53 398 isolated from milk proteose-peptone. Journal of Dairy Science, 71(6), 1464-1471.
54

55
56 399 Pâquet, D. (1989). Review: The proteose-peptone fraction of milk. Milk, 69(1), 1-21.
57
58
59
60
61
62
63
64
65

400 Poré J. (1976). In: Dispersion Aqueuses, Suspension, Emulsions, Mousses. Le cuir,
1
2 401 Paris, p 184.
3

4 402 Rodriguez-Patino and De la Fuente Feria. (1995). Destabilization of monoglyceride
5
6
7 403 monolayers at the air-water interface: structure and stability relationships. In: Food
8
9
10 404 Macromolecules and Colloids, The Royal Society of Chemistry, Cambridge, eds. E. Dickson,
11
12 405 and D. Lorient, p 109-113.
13

14 406 Nakai, S., Ho, L., Kato, A., Tung, M. A. (1980). Relationships between hydrophobicity
15
16
17 407 and emulsifying properties of some plant proteins. Canadian Institute of Food Science
18
19 408 Technology Journal, 13, 23-27.
20

21 409 Schägger H. (2006). Tricine-SDS-PAGE. Nature. Publishing Group. 1(1). 16-22.
22
23

24 410 Sorensen, E. S., & Petersen, T. E., 1993. Purification and characterization of three
25
26
27 411 proteins isolated from the proteose-peptone fraction of bovine milk. Journal of Dairy
28
29 412 Research., 60(2), 189-197.
30

31 413 SPSS Inc. (1999). SPSS. Chicago:SPSS.
32
33

34 414 Shimizu, M., Yamauchi, K., Saito, M. (1989). Emulsifying properties of the proteose-
35
36 415 peptone fraction obtained from bovine milk. Milchwissenschaft, 44(8), 497-500.
37

38 416 Zhu, H., and Damodaran, S. (1994). Proteose Peptones and Physical Factors Affect
39
40
41 417 Foaming Properties of Whey Protein Isolate. Journal of Food Science, 59(3), 554-560.
42
43

44 418
45
46

47 419
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

420 **List of Tables**

421 **Table 1:** Composition, expressed in percentage of dry material, of powders of total proteose-
422 peptone fractions extracted from WPC and milk.

423 **Table 2:** Equilibrium surface pressure (π_e) and Initial adsorption rate (V) at air-water interface
424 for total proteose-peptone fractions at 1 % (w/w) of protein at various pH.

425
426 **Table 3:** Maximum elasticity value of total proteose-peptone fractions films at various pH.

427
428 **Table 4:** Speed of decreasing ($\text{cm}^3 \cdot \text{min}^{-1}$) after switching off the bubbling of total proteose-
429 peptone fractions foams at 1 % (w/w) at various pH.

430
431 **List of Figures**

432 **Figure 1:** Tricine-SDS-PAGE electrophoresis of proteose-peptones fractions, WPC and milk.
433 M: Marker, I: WPC, II: Residual WPC after TPP extraction, III: WPC TPP, IV: Milk TPP and
434 V: Milk protein.

435 **Figure 2:** Surface tensions as a function of time for 1 % of total proteose-peptone fractions
436 (Milk TPP (♦) and WPC TPP (◊)) using the drop volume method. (A) pH 4.0, (B) native pH,
437 and (C) pH 7.0.

438 **Figure 3:** Isotherms of compression of total proteose-peptone fractions (— Milk TPP) and (—
439 WPC TPP) monolayers spread on aqueous solutions at (A) pH 4.0, (B) native pH and (C) pH
440 7.0. Registered at $25 \pm 0.5^\circ\text{C}$. Spread 50 μL of solution. Average of three repetitions.

441 **Figure 4:** Foaming capacity at various pH of total proteose-peptone fractions 1%. (♦) Milk
442 TPP and (◊) WPC TPP at various pH.

443 **Figure 5:** half-life of the foam related to various pH of total proteose-peptone fractions at 1%
444 (w/w): (♦) Milk TPP and (◊) WPC TPP.

445 **Figure 6:** Evolution of total proteose-peptone fractions foam (♦ Milk TPP) and (◊ WPC TPP)
446 at 1% (w/w) over time (450s) at (A) pH 4.0, (B) native pH and (C) pH 7.0.

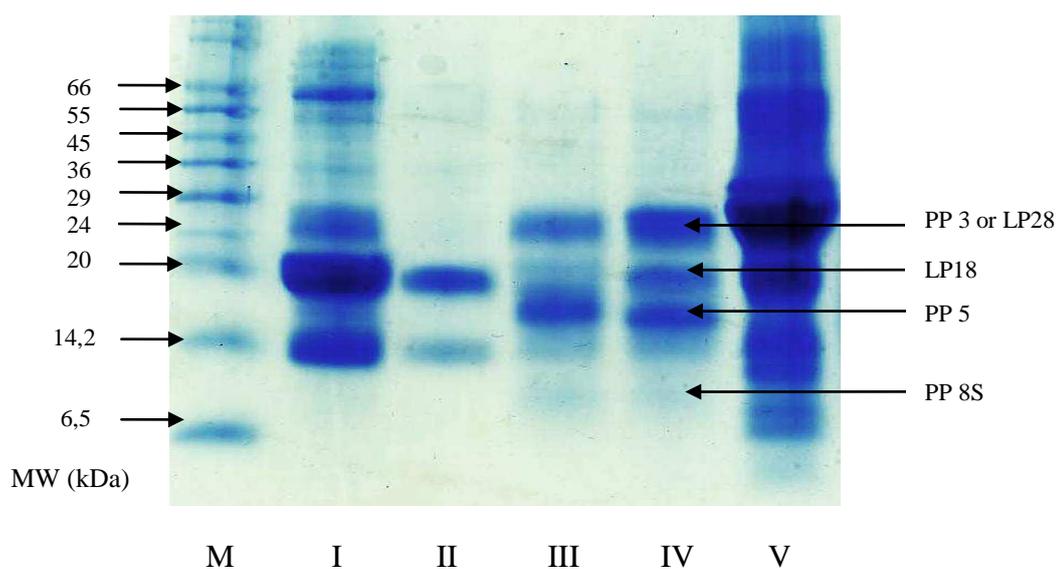


Figure 1: Tricine-SDS-PAGE electrophoresis of proteose-peptones fractions, WPC and milk. M: Marker, I: WPC, II: Residual WPC after TPP extraction, III: WPC TPP, IV: Milk TPP and V: Milk protein.

Figure 2

[Click here to download Figure: Figure 2.doc](#)

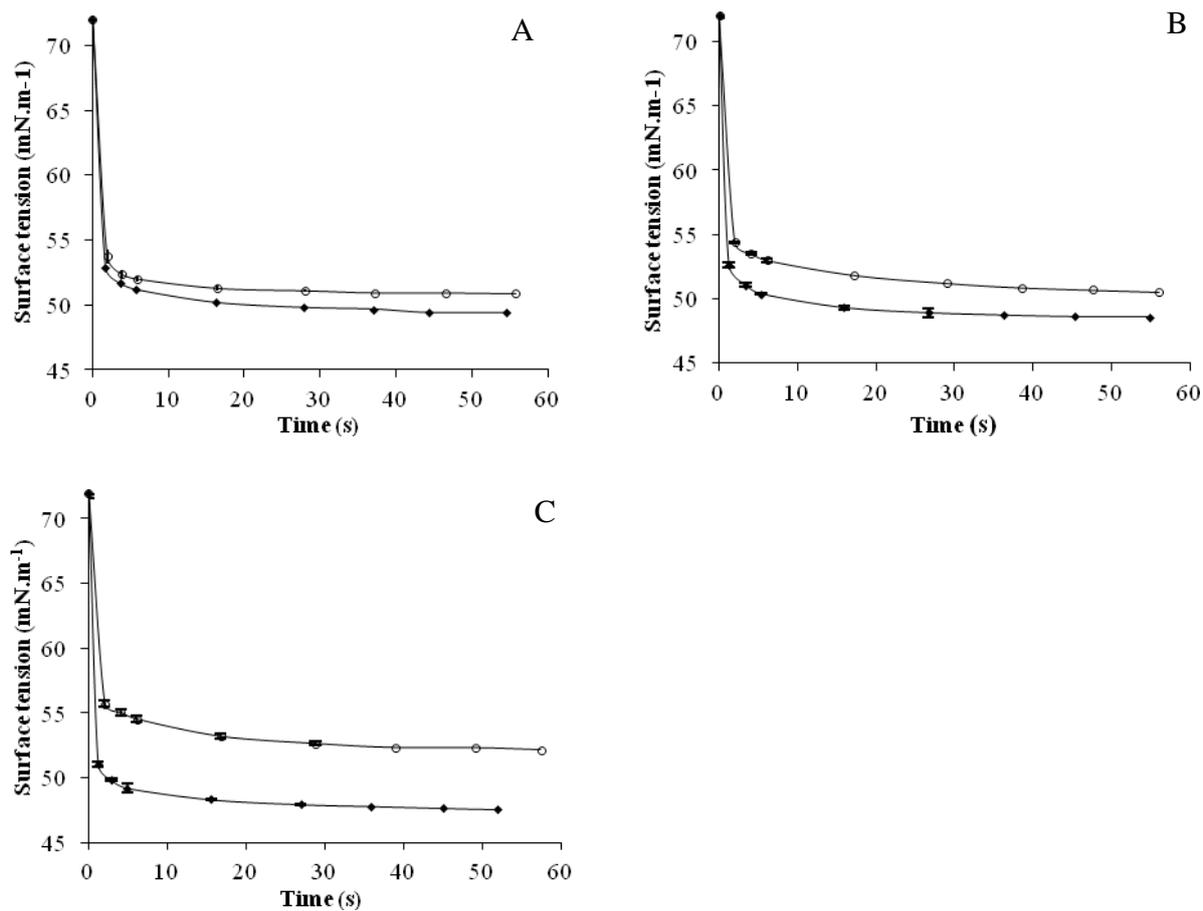


Figure 2: Surface tensions as a function of time for 1 % (w/w) of total proteose-peptone fractions (Milk TPP (◆) and WPC TPP (○)) using the drop volume method. (A) pH 4.0, (B) native pH, and (C) pH 7.0.

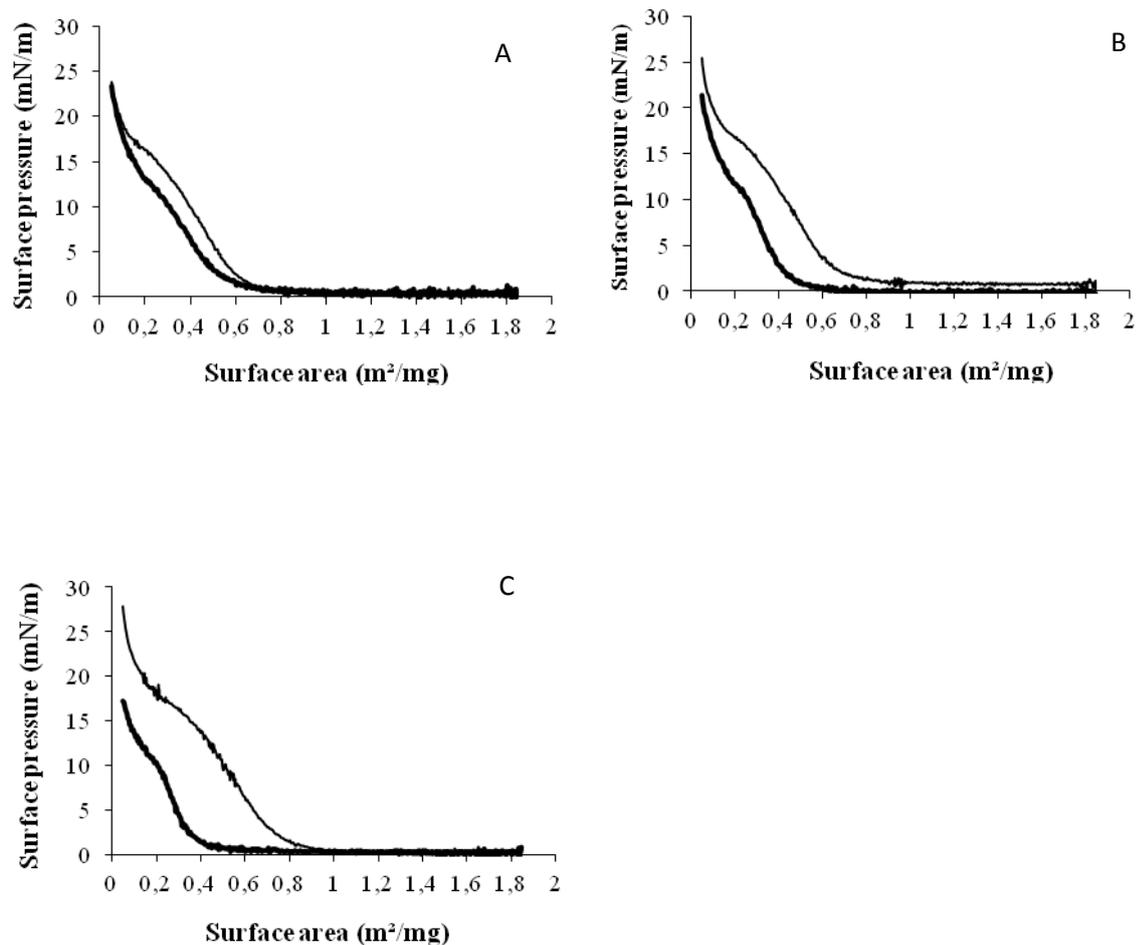


Figure 3: Isotherms of compression of total proteose-peptone fractions (Milk TPP) and (WPC TPP) monolayers spread on aqueous solutions at (A) pH 4.0, (B) native pH and (C) pH 7.0. Registered at $25 \pm 0.5^\circ\text{C}$. Spread $50 \mu\text{L}$ of solution. Average of three repetitions.

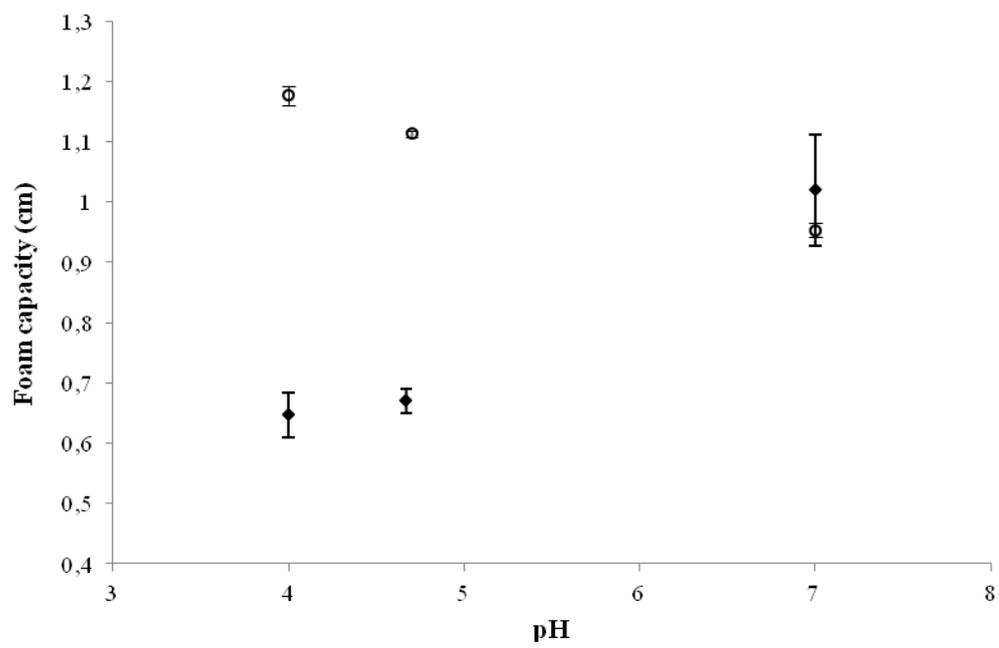


Figure 4: Foaming capacity at various pH of total proteose-peptone fractions 1%. (♦) Milk TPP and (○) WPC TPP at various pH.

Figure 5

[Click here to download Figure: Figure 5.doc](#)

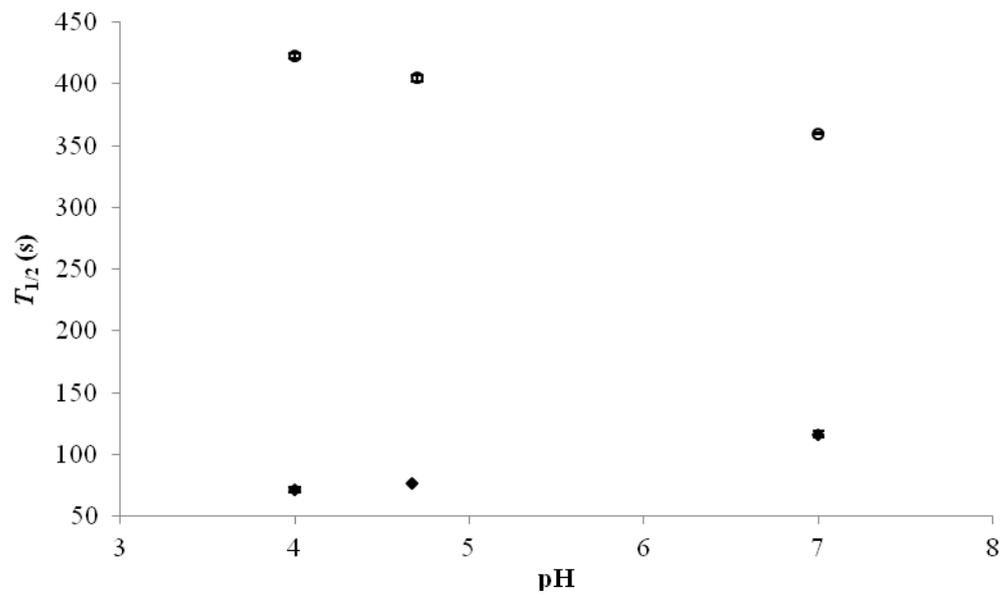


Figure 5: half-life of the foam related to various pH of total proteose-peptone fractions at 1%: (◆) Milk TPP and (○) WPC TPP.

Figure 6

[Click here to download Figure: Figure 6.doc](#)

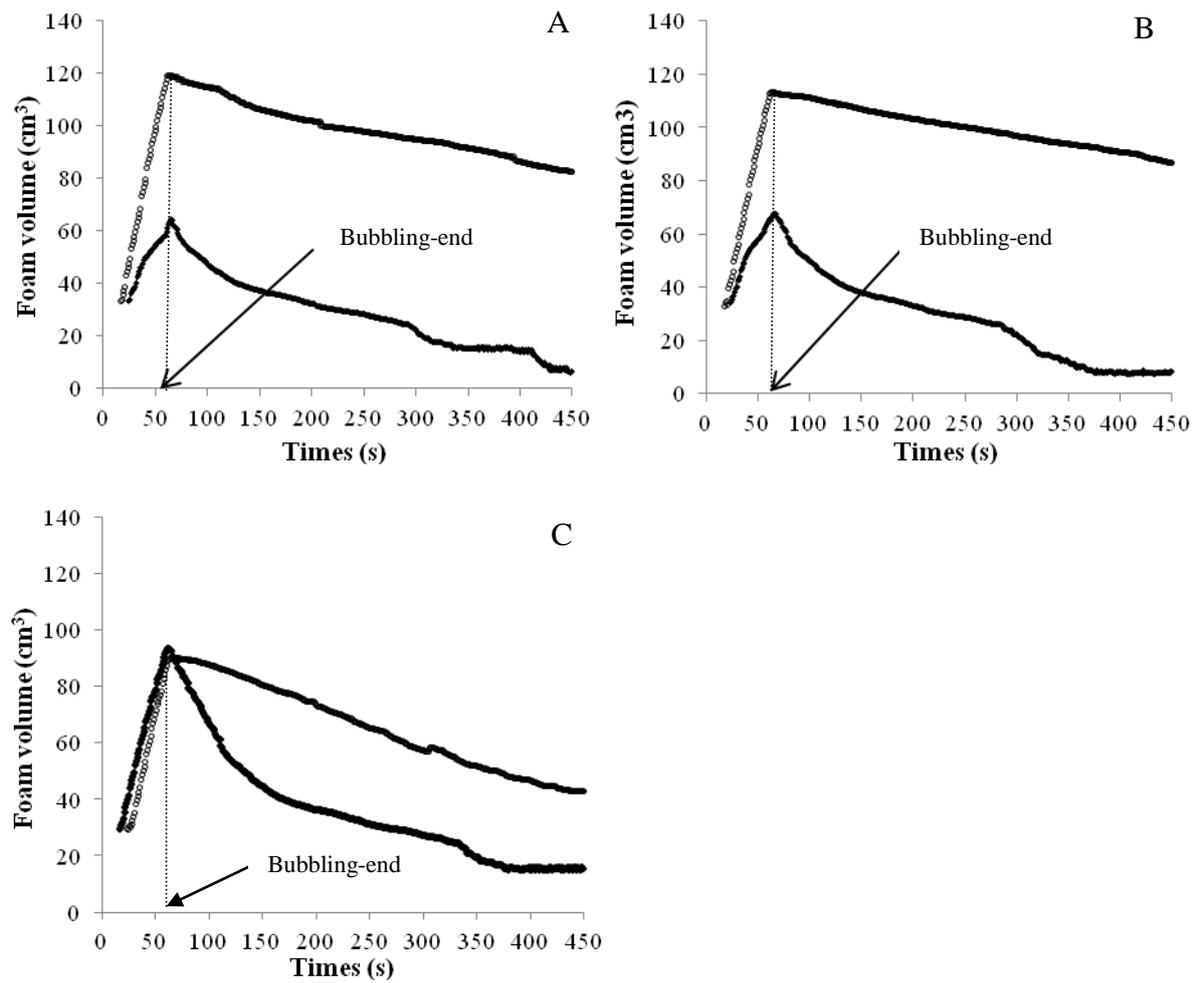


Figure 6: Evolution of total proteose-peptone fractions foam (\blacklozenge Milk TPP) and (\circ WPC TPP) at 1% (w/w) for the first 450s at (A) pH 4.0, (B) native pH and (C) pH 7.0.

Table 1: Composition (% of dry material) of freeze dried TPP fractions extracted from WPC and milk.

	WPC TPP	Milk TPP
	(Dry matter : 91.40 % \pm 0.04)	(Dry matter : 91.75 % \pm 0.10)
Protein	65.20 % \pm 0.03 ^a	79.15 % \pm 0.10 ^b
Ash	3.50 % \pm 0.15 ^a	2.85 % \pm 0.13 ^a
Lipid	4.60 % \pm 0.08 ^a	6.55 % \pm 0.05 ^b
Lactose	26.22 % \pm 0.09 ^b	10.52 % \pm 0.07 ^a

^a Values within the same line followed by the same superscript letters do not differ significantly at $P < 0.05$ (Duncan test). Mean of triplicates.

Table 2: Equilibrium surface pressure (π_e) and Initial adsorption rate (V) at air-water interface for total proteose-peptone fractions at 1 % (w/w) of protein at various pH.

	pH	V (mN.m ⁻¹ .s ⁻¹)	π_e (mN.m ⁻¹)
Milk TPP	4.00	11.39 ± 0.12 ^d	23.39 ± 0.24 ^d
	4.67	15.60 ± 0.15 ^e	24.11 ± 0.03 ^e
	7.00	17.37 ± 0.19 ^f	24.88 ± 0.12 ^f
WPC TPP	4.00	9.61 ± 0.18 ^c	21.66 ± 0.09 ^c
	4.70	8.80 ± 0.09 ^b	21.29 ± 0.13 ^b
	7.00	7.95 ± 0.26 ^a	20.94 ± 0.06 ^a

^a Values within the same column followed by the different superscript letters are differ significantly at P < 0.05 (Duncan test). Mean of triplicates.

Table 3: Maximum elasticity value (ϵ) of total proteose-peptone fractions films at various pH.

	pH	ϵ (mN.m ⁻¹ .s ⁻¹)
Milk TPP	4.00	10.19 ± 0.01 ^b
	4.67	10.22 ± 0.27 ^b
	7.00	11.62 ± 0.12 ^c
WPC TPP	4.00	13.15 ± 0.09 ^e
	4.70	12.32 ± 0.14 ^d
	7.00	9.05 ± 0.09 ^a

^a Values within the same column followed by the same superscript letters do not differ significantly at $P < 0.05$ (Duncan test). Mean of triplicates.

$\epsilon = -A (d\pi/dA)$.

Table 4: Speed of decreasing ($\text{cm}^3 \cdot \text{min}^{-1}$) after switching off the bubbling of total proteose-peptone fractions foams at 1 % (w/w) at various pH.

	pH	Speed of decreasing ($\text{cm}^3 \cdot \text{min}^{-1}$)
Milk TPP	4.00	8.84 ± 0.72
	4.67	9.16 ± 1.06
	7.00	12.06 ± 1.55
WPC TPP	4.00	5.75 ± 0.77
	4.70	4.15 ± 2.99
	7.00	7.40 ± 0.31

Means of triplicates.

ABSTRACT

Total proteose-peptone (TPP) fractions were extracted from skimmed milk (UHT) and whey protein concentrate (WPC) on a laboratory scale. Protein solutions (0.1, 0.5 and 1 % w/w) were characterized as function of pH: 4.0, 4.6-4.7 (native pH) and 7.0. Their foaming capacities and stabilities were studied. Beforehand, the surface properties that govern them were investigated, notably the kinetics of adsorption and mechanical properties of monolayer films at the air-water interface involved in the formation and the stability of foams respectively. The TPP extracted from skimmed milk showed the lowest values as well as a significant reduction in surface tension and presented a good mechanically resistant film. The TPP extracted from WPC presented a better foaming capacity and stability which was unexpected. However, foaming properties and surface properties of TPP fractions depended on the pH. The considerable influence of extraction source and method on proteose-peptone's properties were highlighted.