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1 **Monitoring of acid-induced coagulation of dromedary and cow's milk using**
2 **different complementary analytical techniques**

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20

21 **Abstract**

22 This study investigates the acid-induced coagulation of dromedary and cow's milk using
23 rheological, light backscattering, and FT-MIR spectroscopy techniques. Throughout the acid-
24 induced coagulation process, dromedary milk exhibited higher values of pH than cow's milk.
25 Indeed, pH values of milk attained values of 3.94 and 3.84 at the end of the coagulation for
26 dromedary and cow's milks, respectively. Rheology measurements showed that, throughout
27 the acid-induced coagulation, dromedary milk significantly presented lower values of storage
28 modulus (G') and loss modulus (G'') than cow's milk. The type of milk significantly affects
29 the curd firmness of acid milk gels. Indeed, the lowest curd firmness of dromedary (8.71 Pa)
30 and cow's milk (11.37 Pa) was observed at $\text{pH} = 3.8$, and 3.94, respectively. The delta
31 backscattering (ΔBS) showed a sigmoidal increase at the initial steps of acid-induced coagula-
32 tion of dromedary milk.

33 **Keywords:** acid-induced coagulation; dromedary milk; rheology; light backscattering; FT-
34 MIR spectroscopy.

35 1. Introduction

36 More than 95% of dromedary camels (*Camelus dromedarius*) graze the arid and desert
37 lands of Tunisia (Zidi et al., 2018). According to recent FAO statistics (2019), the world pro-
38 duction of dromedary milk was around 2,700 3,111 thousand tonnes in 2016 2019, of which
39 only 1092 1095 tonnes were produced in Tunisia (FAOSTAT, 2019). The dromedary camel is
40 an exceptional animal that is capable to survive in hostile climatic conditions. Dromedary
41 milk has been known as a balanced food for human consumption especially for the population
42 residing in arid and semi-arid regions where the climatic conditions make it difficult to pro-
43 duce cow's milk. Dromedary milk is an important source of nutrients for the inhabitants of
44 arid and semi-arid areas where it is difficult to produce cow's milk due to climatic conditions.
45 In comparison with cows' milk, dromedary milk contains a higher content of β -casein and less
46 amount of κ -casein. Dromedary milk contains a higher amount of β -casein and a lesser con-
47 tent of κ -casein compared to cow's milk (Pathak, 2010). Additionally, β -lactoglobulin repre-
48 sents 50% of whey proteins for cows' milk but it is absent in dromedary milk (Felfoul, Jardin,
49 Gaucheron, Attia, & Ayadi, 2017). Moreover, dromedary milk contains a higher number of
50 protective proteins such as lactoferrin, lactoperoxidase, immunoglobulins, and lysozyme
51 (Pathak, 2010).

52 The preservation of dromedary milk products has aroused considerable interest in the last
53 few decades. This preservation can be realized by pasteurization and sterilization (Omar, Har-
54 bourne, & Oruna-Concha, 2018), Glucono-delta-lactone (GDL)-induced coagulation (Kamal,
55 Foukani, & Karoui, 2017; Zouari et al., 2018), enzymatic coagulation (Bouazizi et al., 2021)
56 and spray drying (Felfoul et al., 2020; 2021a). Dromedary milk cheese is preferred by con-
57 sumers and thus can compete with those made from cows' milk in the market. For instance,
58 only home production of cheeses made by dromedary milk is available in Tunisia and the

59 consumption is limited to people living in the South regions (Felfoul et al., 2021b; Bouazizi et
60 al., 2021).

61 Lactic acid fermentation is the most used acidification process to coagulate milk during
62 the manufacturing of dairy products. Fermented dromedary milk has a weak structure with a
63 watery consistency (Felfoul et al., 2021b). This trend was ascribed to the presence of
64 antibacterial agents (such as immunoglobulins, lactoferrin and lysozyme), the larger size of
65 casein micelles for dromedary milk (200–500 nm) in comparison with cow's milk (220–300
66 nm) (Kamal, Foukani, & Karoui, 2017), the absence of β -lactoglobulin in dromedary milk
67 (Felfoul et al., 2017), and the relative distribution of protein fractions into casein micelles (Al
68 haj & Al Kanhal, 2010). These factors contributed to a poor gelling behavior of dromedary
69 milk. The above-mentioned research studies depicted the difficulty in producing fermented
70 dromedary milk with an acceptable consistency. Therefore, the use of acidifiers is substantial
71 to improve the consistency of fermented dromedary milk.

72 To the best of our knowledge, very limited scientific research studies on the acid-induced
73 coagulation process of dromedary milk were found in the literature. Few studies comparing
74 the effect of ingredients, including polysaccharides on gel properties were reported (El-
75 Agamy et al., 2009). Most of the research studies have focused only on the texture and
76 sensory properties of the final product. Recent research studies on the GDL-induced
77 coagulation of dromedary and cow milk gels were focused on the rheological behavior
78 (Zouari et al., 2018). Kamal, Foukani and Karoui (2017) investigated the rheological and
79 structural properties of dromedary and cow's milk gels enriched with phosphate and calcium
80 during acid-induced gelation. In this context, this is the first study that investigates the acid-
81 induced coagulation of dromedary milk directly using HCl. Therefore, the objective of this
82 work was to better understand the behavior of dromedary milk during acid-induced
83 coagulation and to monitor dromedary and cow's milk gels formation during acidification by

84 HCl. using rheological, light backscattering, and mid-infrared spectroscopy. To extract
85 information from the data sets, principal component analysis (PCA) was applied.

86 **2. Materials and methods**

87 **2.1.Dromedary and cow's milk sourcing**

88 Dromedary milk (*Camelus dromedarius*) was collected from reared dromedaries of an
89 experimental station located in the south of Tunisia (Gabès governorate). Dromedary milk
90 used consisted of a pooled milk samples assembled from 24 different healthy dromedaries.
91 Dromedary milk was collected in sterile milking cans. Cow's milk was collected from 20 dif-
92 ferent Holstein cows. Cow's milk was derived from a local breeding located in Sfax region
93 (Sfax governorate, Tunisia). For the two milk species, sodium azide was added to the milk
94 (0.02 % w/v) to prevent the multiplication of bacteria. Milk samples were transported imme-
95 diately to the laboratory in Tunisia within 2 h and in France within 24 h using an icebox. Milk
96 samples were stored at -20 °C for further analyses. For acid-induced coagulation experiments,
97 milk samples were thawed at 4°C overnight. Milk freezing was necessary to ensure its preser-
98 vation for 2 weeks. No particle or aggregate was observed in milk samples after thawing. All
99 the analyses were carried out within 2 weeks.

100 Cow's milk was purchased from local breeding located in Sfax region (Sfax
101 governorate).

102 For the two milk species, sodium azide was added to the milk (0.02 % w/v) to prevent the
103 multiplication of bacteria.

104 **2.2.Acid coagulation monitoring**

105 The acidification of milk samples was carried out by adding defined amounts of HCl
106 (1 M) at the initial pH of both milk (pH = 6.5 and 6.68 for dromedary and cow's milk,
107 respectively) to reach pH = 4. Milk samples were then stirred at 200 rpm for 1 min for the
108 acidification monitoring. For each experiment, at least three replicates were performed. The
109 same milk sample was used for pH and acid-gel characterization.

110

111 **2.3. pH measurement**

112 The pH was determined, each 30 s, for all the milk samples during 3 h of the acid-
113 induced coagulation process held at 20 °C using a pH-meter (Metrohm pH meter, Apeldoorn,
114 The Netherlands) (Zouari et al., 2018). **The same milk sample was used for pH and acid-gel**
115 **characterization.** For each experiment, at least three replicates were performed

116 **2.4. Acid gel characterization by non-destructive methods**

117 **2.4.1. Light backscattering**

118 Acid-induced coagulation was monitored rapidly using a Turbiscan^{LAB} (Formulaction,
119 France) according to Felfoul et al. (2021). The apparatus is comprised of a detection head,
120 equipped with a near-infrared light source (880 nm), that moves up and down along a flat-
121 bottomed cylindrical cell. The light source scans the sample (around 20 mL) placed in a cell
122 with checking the quality of the meniscus (without air bubbles) from top to bottom and
123 **measures the percentage of backscattered or transmitted light** during 3 h at 20 °C incubation.
124 **For each experiment, three replicates were performed**

125 **2.4.2. Rheology measurement**

126 Rheology measurements of raw dromedary and cow's milk acid gel samples were
127 carried out according to Zouari et al. (2018) using a rotational rheometer (HAAKE® MARS
128 III, Thermo Fisher Scientific, Karlsruhe, Germany) equipped with a coaxial cylinder
129 measuring system (CC26/CCB26/SS) and a Peltier system, which maintains a constant
130 temperature at 20 °C throughout the acid-induced coagulation process. *In-situ* coagulation of
131 dromedary and cow's milk samples with HCl (1M), according to the procedure described in
132 section 2.2, was carried out in the cup of the rheometer and dynamic measurements (**storage**
133 **modulus, G'** and **loss modulus, G''**) in the linear viscoelastic region were **acquired recorded**
134 during 3 h with the strain and oscillation frequency values set at 1 % and 1 Hz, respectively.
135 **For each experiment, three replicates were performed (Zouari et al., 2018). The gelation time**

136 was experimentally determined as the time where the value of the storage modulus (G') was
137 equal to the value of the loss modulus (G''), as reported previously by Zouari et al. (2018).
138 Curd firmness was determined as the storage modulus (G') value after 3 h of the test
139 according to Kamal et al. (2017).

140 **2.4.3. Mid-infrared measurements**

141 Mid-Infrared (MIR) measurements used in this study were performed according to
142 Karoui et al. (2006a). The MIR spectra were recorded at 20 °C between 4000 and 700 cm^{-1} at
143 a resolution of 4 cm^{-1} on a Fourier transform spectrometer IRTracer-100 (Shimadzu,
144 Duisburg, Germany). The attenuated total reflectance (ATR) cell used as a sampling
145 accessory (HATR, Pike technologies, Madison, USA) is a crystal made of zinc selenide (ZnSe)
146 with an incidence angle of 45° and a number of reflections of 10 and several reflections of 10.
147 Acidified milk was set on the crystal and spectra were recorded. The marked peaks represent
148 the absorption bands of bending and stretching vibrations for specific functional groups. To
149 improve signal-to-noise ratio, 32 scans were accumulated for each spectrum. The background
150 spectrum, which contains the absorptions of molecules present in the air, was scanned at the
151 beginning of measurement by pouring the ATR cell with distilled water. The same procedure
152 was used for scanning blank spectra, which contains the absorption of distilled water. A 2 ml
153 of sample was introduced into the ATR cell and after each measurement, the ATR crystal was
154 thoroughly washed with ethanol and distilled water and then dried (Karoui et al., 2006a). For
155 each experiment, three replicates were performed.

156 **2.5. Statistical analysis**

157 All experimental analyses and measurements of acid-induced coagulation were
158 conducted in triplicate on independently isolated preparations and were mentioned as mean
159 values \pm standard deviation of these three independent trials. Analysis of variance (ANOVA)

160 was carried out using the software XLSTAT 2016. Significant differences ($p < 0.05$) among
161 treatments were detected using Tukey's multiple range tests.

162 In order to compare the samples, MIR spectra were normalized by reducing the area
163 under each spectrum to a value of 1 according to Karoui et al. (2006b). Mainly the shift of the
164 peak maximum and the peak width changes in the spectra were considered following this
165 normalization. The PCA transforms the original variables into new axes called principal com-
166 ponents (PCs), which are orthogonal, so that the data sets presented on these axes are uncorre-
167 lated with each other (Blecker et al., 2012; Karoui et al., 2006a; Karoui et al., 2007a; Karoui
168 et al., 2007b). Therefore, PCA expresses as much as possible the total variation in the data set
169 in only a few PCs and each successively derived PC expresses decreasing amounts of the var-
170 iance. The multivariate statistical method was previously used to detect similarities among
171 different milk samples (Hammami et al., 2010; Karoui et al., 2008) reducing the dimension to
172 two or three PCs, while keeping most of the original information found in the data.

173 In a first step, principal component analysis (PCA) was separately applied to MIR
174 spectra of each acidified milk (dromedary and cow's milks) in order to monitor changes that
175 occurred in the network during acid-induced coagulation. Then, all the spectral collection
176 recorded on the studied acidified milk samples were pooled into one matrix, which was ana-
177 lyzed again by PCA. The objective of this new analysis was to determine the trajectory of the
178 investigated acidified milks together throughout acid-induced coagulation according to the
179 type of milk. The PCA was performed using MATLAB (Version 6.5, Release 12, The Math-
180 Works).

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186 **3. Results and discussion**

187 **3.1. Evolution of pH throughout acid-induced coagulation of dromedary and cow** 188 **milk**

189 Throughout the acid-induced coagulation process, acidified dromedary milk samples
190 exhibited higher pH values than acidified cow's milk (**Fig. 1**). **Table 1** summarized the pH
191 values of dromedary and cow's milk recorded each 15 min throughout acid-induced coagula-
192 tion. Indeed, the pH values of acidified milk attained values of 3.94 ± 0.01 and 3.84 ± 0.05
193 after 3h of acid-induced coagulation for dromedary and cow's milk, respectively (**Fig. 1**), in
194 agreement with previous investigation (Zouari et al., 2018). Differences in the pH values be-
195 tween dromedary and cow's milk could be explained by : i) their physicochemical disparities,
196 mainly in terms of micelle casein and whey protein composition (Al-haj & Al Kanhal, 2010),
197 ii) dissimilarities in the isoelectric point, which is close to 4.3 and 4.6 for dromedary and
198 cow's milk, respectively (Wangoh, Farah, and Puhan, 1998), iii) the higher β -casein level
199 (47%), and the lower κ -casein content (3.5%) for dromedary milk compared to cow's milk
200 (13%) (Omar, Harbourne, and Oruna-Concha, 2016) as well as the lack of β -lactoglobulin for
201 dromedary milk while it is the major whey protein for cow milk (Felfoul et al., 2017).

202 **3.2. Monitoring of acid-induced coagulation of dromedary and cow milk by rheologi-** 203 **cal measurements**

204 The time-course of rheological parameters (storage modulus, G' and loss modulus,
205 G'') during acid-induced coagulation of dromedary and cow's milk samples are shown in
206 **Figs. 2a and 2b**, respectively, and summarized in **Table 21**. In addition, **Fig. 2c** exhibited the
207 changes in the storage modulus (G') of both milk samples as a function of pH.

208 To the authors' knowledge, only a few studies have been reported on the evolution of
209 storage modulus (G') values during acid-induced coagulation of dromedary milk. Attia et al.

210 (2000) reported a final storage modulus (G') value of 16 Pa after 24 h of acidification of
211 dromedary skim milk at 20 °C with a GDL concentration of 1.05% (w/v). Recently, Zouari et
212 al. (2018) depicted that after 3h of coagulation at 45 °C with GDL concentration of 2.25 %
213 (w/v), storage modulus (G') value of dromedary gel milk was 35 times lower than the one
214 obtained for reconstituted cow's milk gel. Kamal, Foukani, & Karoui (2017) reported that the
215 final values of storage modulus (G') and loss modulus (G'') of dromedary milk gels were
216 significantly lower than those of cow milk gels during acid-induced coagulation following the
217 addition of calcium chloride (CaCl_2) and hydrogen phosphate dehydrate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$).

218 In the current study, an increase in the values of both moduli was observed for both
219 milk samples. In addition, similar trends were observed for the two milk samples during acid-
220 induced coagulation. However, for cow's milk, the storage modulus (G') was seen to decline
221 after ~10000 s, which could be due to the gel contraction (**Fig. 2b**). Acidified cow's milk sig-
222 nificantly presented the highest values of storage modulus (G') and loss modulus (G'') ($p <$
223 0.05) throughout the acid-induced coagulation. In addition, the curd firmness of acidified
224 cow's milk showed a higher value (i.e., 11.37 Pa) at pH = 3.94, while that of dromedary milk
225 gave a lower value of 8.71 ± 0.11 Pa at pH = 3.84 (**Table 21**). The results obtained from this
226 study showed that the type of milk significantly affects the curd firmness of acid-induced milk
227 gel samples. The low values of storage modulus (G'), loss modulus (G'') and curd firmness
228 for dromedary milk samples during acid-induced coagulation could be ascribed to the lack of
229 network structure through hydrophobic interactions that involved low energy and less re-
230 sistance to mechanical processing (Mahaut et al., 2000). In addition, the difference in the stor-
231 age modulus (G') values between dromedary and cow's milk gels could be attributed to the
232 difference in the micellar calcium phosphate distribution leading to higher establishment of
233 calcium bridges between milk proteins and the formation of more elastic cow milk gel (Guil-
234 laume et al., 2002). Indeed, the content of colloidal calcium bound to the micelles in drome-

235 dary milk was lower (35 % of the total calcium) than in cow's milk (65 %) (Yagil and Etzion,
236 1980; Yagil, 1994). Zidi et al. (2018) reported that dromedary milk gel showed a non-
237 viscoelastic behavior. As the pH is reduced, an increasing amount of calcium ions will be
238 available to bind with proteins, thereby promoting their aggregation.

239 The storage modulus (G') value of dromedary milk gel showed initially an average
240 value of 2.50 ± 0.01 Pa and increased significantly ($p < 0.05$) during acid-induced coagulation
241 (**Fig. 2a**). This increase continued progressively until reaching a pH value of 4.26 ± 0.06 dur-
242 ing the first hour. In the 60 – 72 min range, the storage modulus (G') values remained con-
243 stant with a slight decrease of pH value from 4.26 ± 0.06 to 4.17 ± 0.03 . Beyond 72 min, a
244 rapid increase in the storage modulus (G') value of dromedary milk gel was observed till the
245 end of the acid-induced coagulation (180 min) reaching a final pH value of 3.84 ± 0.02 (**Fig.**
246 **2a**). From **Fig. 2b**, it was observed that the storage modulus (G') values of cow's milk gel
247 increased rapidly during the first 20 min, corresponding to a pH value of 4.90 ± 0.02 . This
248 trend could be related to the dissociation and rearrangement of casein that increased with the
249 solubilization of colloidal calcium phosphate during acidification (Gastaldi et al., 1996). After
250 the first 20 min, the storage modulus (G') values showed a small increase until 170 min with
251 the decrease of pH from 4.90 ± 0.02 to 3.86 ± 0.02 , which could be ascribed to the reincorpo-
252 ration of casein into the micelle structure (Gastaldi et al., 1996). Beyond 170 min, the storage
253 modulus (G') values showed a slight decrease until the end of gelation (180 min) reaching a
254 pH value of 3.94 ± 0.03 which was interpreted as gel contraction (Beltrán-Espinoza et al.,
255 2021) (**Fig. 2b**). The increase in storage modulus (G') values for both milk samples is due to
256 the fusion of casein micelles, as well as to the dissociation and rearrangement of caseins as
257 discussed by Pang et al. (2014). Our results agreed with the findings of Zouari et al. (2018)
258 who pointed out that the increase of storage modulus (G') values is related to the intensive
259 restructuring of the protein network that occurs before at pH higher than pH 4.4 for reconsti-

260 tuted cow's milk while reconstituted dromedary milk exhibited only one step for the evolution
261 of storage modulus (G') after reaching pH 4.7.

262 The storage and loss moduli (G' and G'') at the gel point as well as the gelation times
263 of the acidified dromedary and cow's milk samples were determined in **Table 21**. The ob-
264 tained results showed that : (i) the storage modulus (G') as well as the loss modulus (G'')
265 were significantly lower for dromedary milk than for cow's milk : 1.50 ± 0.58 and 1.06 ± 0.18
266 Pa vs 6.74 ± 0.35 and 2.19 ± 0.10 Pa, respectively; ii) the gelation time of dromedary milk
267 samples was longer than cow's milk (8.33 ± 0.15 min vs 5.20 ± 0.05 min, respectively); and
268 (iii) the gelation pH was higher for cow's milk ($\text{pH} = 5.51 \pm 0.02$) than for dromedary milk
269 ($\text{pH} = 5.12 \pm 0.06$), in agreement with the findings of Zouari et al. (2018). At the gelation
270 point, the gelation pH and curd firmness were significantly higher for cow's milk than for
271 those obtained with dromedary milk. During this time, the pH of each milk sample decreased
272 drastically (**Fig. 1**), and significant physicochemical changes occurred (**Fig. 2c**). The delay in
273 the onset of gelation for dromedary milk could be due to the time required by the acidified
274 dromedary milk to reach the gelation pH (i.e., $\text{pH} = 5.12 \pm 0.06$) in comparison to cow's milk.
275 The results obtained in the present study for cow's milk acid gels agreed with previous find-
276 ings (Lucey, Mishra, Hassan, & Johnson, 2005; McMahon, Du, McManus, & Larsen, 2009).
277 For instance, Zouari et al. (2018) reported that the gel point of reconstituted dromedary milk
278 occurred at lower pH (i.e., $\text{pH} = 4.46$) than that of reconstituted cow's milk (i.e., $\text{pH} = 5.06$),
279 in line with the findings of the current study.

280 The maximum curd firmness rate throughout the coagulation process is very im-
281 portant. The results illustrated in **Table 21** showed that the type of milk presented a signifi-
282 cant effect on the curd-firming rate. The maximum curd firmness was 0.0008 ± 0.00 and 0.01
283 ± 0.00 Pa s^{-1} for respectively dromedary and cow's milk during acid-induced coagulation.
284 There was a significant difference in the curd-firming rate between both studied milk samples.

285 The lower curd-firmness rate and storage modulus (G') values for dromedary milk compared
286 with those of cow's milk could be explained by the decrease in the colloidal calcium phos-
287 phate cross-linking leading to the dispersion of casein as described by Mizuno and Lucey
288 (2007).

289 The difference observed in the gelling behavior between dromedary and cow's milk
290 during acid-induced coagulation could be explained by many factors: i) the larger size of
291 dromedary casein micelles compared to cow's milk (200–500 nm vs 220–300 nm, respective-
292 ly), which could affect the rheological properties of milk gels (Glantz et al. 2010); ii) the low-
293 er content of κ -casein in dromedary milk as compared with cow's milk (3.5 % vs 13 %, re-
294 spectively), which could contribute to the poor gelling ability of dromedary milk (Zouari et
295 al., 2018); iii) the richness in β -casein in dromedary milk compared to cow's milk (47 % vs 39
296 %, respectively), which helps maintain the integrity of casein micelle during acid-induced
297 coagulation, *via* colloidal calcium phosphate bonds in the first step of acidification and/or
298 more hydrophobic interactions at $\text{pH} < 5.0$ (Kappeler et al., 1998) ; and iv) the absence of β -
299 lactoglobulin and a higher richness in α -lactalbumin in dromedary milk compared to cow's
300 milk : 50% vs 25% of the total whey protein, respectively (Atri et al., 2010), thus indicating
301 lower whey protein-calcium ions electrostatic interactions for dromedary milk under acidifi-
302 cation.

303 **3.3. Monitoring of acid-induced coagulation of dromedary and cow milk by turbiscan** 304 **light scattering**

305 **Figs. 3a and 3b** exhibited the evolution of the profiles acquired throughout the acid-
306 induced coagulation process of dromedary and cow's milk, respectively. The changes in delta
307 backscattering (ΔBS) values were attributed to the variation in the molecular weight, particle
308 size, number of colloidal casein aggregates, and protein crosslinking, which occur during milk
309 coagulation (McMahon, Brown, & Ernstrom, 1984).

310 As for the infrared light delta backscattering (Δ BS) signal, a sigmoidal increase was
311 observed at the initial steps of acid-induced coagulation of dromedary milk followed by a
312 stable response ratio during the gel formation phase, and finally, an increase of the signal was
313 again observed (**Figs. 3a and 3b**). In the current study, the Δ BS measured at 880 nm was
314 found to be most responsive during the initial stage of acid-induced coagulation of dromedary
315 milk. In the study of Mains et al. (2017), the infrared light backscatter profiles obtained for
316 yogurt fermentation showed an increase in the light backscatter as the fermentation pro-
317 gressed. As the pH drops from 6.0 to 5.0, the electrostatic attraction between the casein mi-
318 celles increased causing aggregation thus resulting in increasing the light backscattering
319 (Mains et al., 2017). These results confirmed the observation found in the current study
320 whereby an increase in the Δ BS response was recorded as the dromedary milk gel formation
321 progressed.

322 For dromedary milk, throughout the 3 h of acid-induced coagulation, Δ BS increase of
323 20 and 18 % were observed respectively at the bottom (height at 0 – 10 mm) and the top
324 (height at 30 – 42 mm) parts. This finding indicates that dromedary milk has slight
325 coagulation at the bottom part and creaming at the top part. Δ BS decrease of 9 % was
326 observed at the middle part (height at 10 – 30 mm) when scanned during acid-induced
327 coagulation of dromedary milk (**Fig. 2a 3a**). The Δ BS change was different in cow and
328 dromedary milk. Indeed, Δ BS decreased by 14% at the bottom part (height at 0 – 10 mm) for
329 cow's milk, indicating a major clarification when scanned for 3 h of acid-induced coagulation.
330 Δ BS increased by 29% at the top part (height at 30 – 42 mm) for cow's milk, indicating a
331 large amount of creaming (**Fig. 2b 3b**). Therefore, the results of the Δ BS profile indicated the
332 coagulation of dromedary milk and the sedimentation of cow's milk.

333 **Fig. 3c** showed the visual observation of gel formation during acid-induced
334 coagulation of dromedary and cow's milk. As observed, the formation of acid gels started

335 from the first hour of acidification and continued until the end of the coagulation process (3
336 h). Three phases were found to appear at the end of the acid-induced coagulation process for
337 dromedary milk: i) the protein aggregation that has occupied the bottom of the tube; ii) the
338 expelled whey obtained due to the syneresis phenomenon in the middle of the tube; and iii)
339 the large cream layer formation at the top of the tube. As for cow's milk, the acid-induced
340 coagulation process occurred throughout the whole tube induced only a particle size variation
341 leading to protein aggregation. Syneresis phenomenon was not very clear on the tube, and a
342 very thin cream layer was observed at the top of the tube at the end of the acid-induced
343 coagulation (180 min). From these observations, it can be concluded that the **dromedary acid**
344 **gel was more organized and structured than that of cow's milk gel.** **dromedary milk acid gel**
345 **and cow milk acid gel are differently organized.**

346 The drop of pH from pH = 6.7 to pH = 6.0 induced the dissolution of only a small
347 amount of colloidal calcium phosphate, thus a very little change in the structure of the casein
348 molecules is obtained. From pH = 6.0 to pH = 5.0, most of the structural changes of the casein
349 occurred. The drop in pH values during coagulation induced some changes in the environment
350 of the milk and promoted electrostatic interaction between casein molecules, causing them to
351 become highly attracted to each other and aggregate. When this phenomenon occurred, the
352 Δ BS profile increased. Below At pH values lower than pH = 5.0, a slight increase in the elec-
353 trostatic attraction between the casein molecules could occur as the pH approach the isoelec-
354 tric point of the casein (pH = 4.6 for cow's milk) and the endpoint of the acid gelation (pH =
355 3.94 ± 0.05). This confirms the significant sedimentation observed during the decrease of pH
356 of cow's milk. For dromedary milk, these mechanisms could not occur for many reasons: i)
357 the lower isoelectric point (pH = 4.3 vs pH = 4.6 for cow's milk) as well as the difference in
358 casein proportions compared to cow's milk; ii) few hydrophobic regions are exposed to the
359 surface of casein micelle, thus the mineral balance equilibrium could not be achieved, and iii)

360 the richness of dromedary milk in β -casein, which could contribute to maintaining the casein
361 micelles integrity. The drop of pH during acid coagulation of dromedary milk provokes the
362 aggregation and the fusion of only smaller caseins, confirming the obtained Δ BS results,
363 which indicates slight coagulation at the bottom part of dromedary milk.

364 The results of the present study focusing on the use of HCl to obtain acidified milk gel
365 from dromedary milk showed some differences in gelling properties with acid-induced drom-
366 edary milk gels produced using GDL, which was investigated previously in the literature
367 (Zouari et al., 2018; Kamal et al., 2017). The use of HCl as acidifier reduced the gelation time
368 and increased the gelation rate and curd firmness for dromedary milk gel compared with
369 GDL-acidified dromedary milk gels. Indeed, Zouari et al. (2018) reported higher gelation time
370 of around 37.08 ± 338 min and lower curd firmness of 1.74 ± 0.44 Pa for acid milk gel ob-
371 tained by using GDL as acidifier than those reported in the current study with HCl as acidifier
372 of dromedary milk, about 8.33 ± 0.15 min and 8.71 ± 0.11 Pa (Table 2), respectively. Moreo-
373 ver, Kamal et al. (2017) also investigated the rheological properties of dromedary milk using
374 GDL as acidifier. The authors showed higher gelation time (58.5 ± 2.125 min vs 8.33 ± 0.15),
375 lower gelation rate (0.009 ± 0.001 Pa min⁻¹ vs 0.048 ± 0.001) and lower curd firmness value
376 (1.610 ± 0.110 Pa vs 8.71 ± 0.11 Pa) than those obtained in the current study.

377 Therefore, the comparison of acid-induced coagulation of dromedary milk using HCl
378 and GDL indicated that from a technological point of view, the use of HCl as acidifier is a
379 better method than GDL. Indeed, HCl can produce better milk gels with better gelling behav-
380 iour, which presents a great interest in dairy industry for obtaining dairy ingredients such as
381 caseins or caseinates. Furthermore, both methods are not able to produce milk gels with simi-
382 lar gelling and quality properties as cow's milk. Indeed, a significant delay in the gelation
383 onset and decreases of the gel firmness were observed for dromedary milk compared to cow's

384 milk. Thus, a combination of both methods could be a better solution to obtain a final drome-
385 dary milk gel with similar gelling properties as cow's milk.

386

387

388 **3.4. Monitoring of acid-induced coagulation of dromedary and cow milk by Mid-** 389 **infrared spectroscopy**

390 **Figs. 4a and 4b** show the MIR spectra recorded on dromedary and cow's milk during
391 acid-induced coagulation. Considering the whole spectral range, a similar spectra shape was
392 observed for the investigated milk samples. The spectra were dominated by one strong band ~
393 1042 (dromedary milk) and 1038 cm^{-1} (cow's milk) corresponding to the absorbance of the
394 lactose due to the hydroxyl group (O – H) as depicted by Souhassou et al. (2018).

395 Other peaks were observed for the Amide I, Amide II, and Amide III at 1635, 1538, and
396 1235 cm^{-1} , respectively. The Amide I is characterized by C = O and C – N stretching
397 vibrations and N – H bending vibration detected at 1635 cm^{-1} . The Amide II is characterized
398 by the stretching vibrations of C = N, N – H and C – N functional groups and bending
399 vibration of N – H group that is observed at ~1538 cm^{-1} . The Amide III represented the
400 stretching vibrations of C – N, N – H and C – N functional groups and bending vibration of N
401 – H group observed at ~ 1235 (Moskal et al., 2018). The band at 1150 (dromedary cow's
402 milk) and 1155 cm^{-1} (cow's dromedary milk) is generally associated with the phosphate group
403 (O = P – O) of the casein proteins (Etzion et al., 2004).

404 The Amide I region was used to investigate the secondary structure of proteins during
405 acid-induced coagulation of dromedary and cow's milk. The secondary structure of milk
406 proteins was determined using the second derivative method. The percentage of β -sheet
407 ranged from 30.50 to 30.90 %, disorderly structure from 12.40 to 12.60 %, α -helix from 13.90
408 to 14.00 %, and β -turns from 43.10 to 42.40%, for cow and dromedary milk, respectively.

409 Lipids absorb at 1745 (fat A), 2849 (fat B), 1460 (fat C) and 1070 cm^{-1} , corresponding to
410 C = O (carbonyl) stretching, C – H (alkyl) stretching, C – H bending and C – O stretching
411 vibrations, respectively as depicted by Aernouts et al. (2011).

412 During acid-induced coagulation, the absorbance of all spectra bands increased for both
413 milk samples. However, the absorbance of bands for dromedary milk was higher than for
414 cow's milk throughout the acid-induced coagulation, except for the band assigned to lactose.
415 The increase in the intensity could be attributed to the acid-induced changes in milk
416 components.

417 In order to extract the information from the data set, the PCA was applied to the spectra
418 acquired on dromedary and cow's milk during acid-induced coagulation. The similarity map
419 (**Fig. 4c**) showed clear discrimination between dromedary and cow's milk samples that
420 enabled us to separate the milk samples into two groups: one for dromedary milk and another
421 group for cow's milk. Indeed, the PC2 accounting for 1.7% of the total variation separated the
422 samples into two groups: the first group is composed of dromedary milk having positive
423 scores; and the second group is formed by cow's milk samples exhibiting negative scores. The
424 PC1 accounting for 97.6% of total variance allowed a separation of milk samples during
425 coagulation. A clear separation was observed for dromedary milk and cow's milk. Indeed, for
426 dromedary milk, during the first 105 minutes of acid-induced coagulation, the samples
427 presented negative scores according to the PC1, while the others exhibited positive values.
428 For cow's milk, during 120 minutes of acid-induced coagulation, the samples exhibited
429 negative values according to the PC2, whereas the others presented positive scores.

430 **4. Conclusion**

431 This study evaluated the acid-induced coagulation of dromedary and cow's milks following
432 HCl addition. (1M). The pH values of acidified gels attained values of 3.94 and 3.84 at the
433 end of the 3h incubation duration for dromedary and cow's milks, respectively. The results

434 obtained from rheology measurements showed that the gelation time of dromedary milk
435 samples was longer than cow's milk, while the gelation pH was higher for cow's milk (pH =
436 5.51) than for dromedary milk. (pH = 5.12). Results of the delta backscattering (Δ BS) profile
437 indicated the slight coagulation of dromedary milk and the significant sedimentation of cow's
438 milk. Finally, the FT-MIR spectra showed clear discrimination between dromedary and cow's
439 milk during acid-induced coagulation.

440 Dromedary milk can be a good alternative to cow's milk and its derivative products are of
441 great interest to dairy industry since cow's milk is associated to different health problems,
442 especially allergy. In this study, dromedary milk was used to produce acid milk gels for its
443 nutritional and therapeutic properties. The purpose of the acid-induced coagulation of
444 dromedary milk by using HCl was to expand the camel dairy industry with the production of
445 several dairy products such as cheese and some dairy ingredients such as caseins and
446 caseinates. The results obtained from the current study showed that rheology, light scanning,
447 and FT-MIR techniques present a potential to evaluate the structure at the macroscopic and
448 molecular levels and the interactions of the different components in milk. Results of the
449 rheology showed that the gelation time of dromedary milk samples was longer than cow's
450 milk, while the gelation pH was higher for cow's milk than for dromedary milk. Results of the
451 delta backscattering profile indicated the slight coagulation of dromedary milk and the
452 significant sedimentation of cow's milk. The obtained findings highlighted the importance to
453 produce dromedary milk gels, which can help its industrialization to overtake the problems
454 associated to the seasonality of dromedary milk production.

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463 **Conflicts of interest**

464 On behalf of the authors and as the corresponding author of this publication, I confirm that
465 there are no known conflicts of interest associated with this publication and that there has
466 been no financial support for this work that could have influenced its outcome.

467 **Author contributions**

468 **Imène Felfoul:** Conceptualization; Investigation; Formal analysis; Writing original draft.

469 **Alia Bouazizi:** Investigation; Formal analysis.

470 **Hamadi Attia:** Funding acquisition; Supervision.

471 **Romdhane Karoui:** Conceptualization; Investigation; Formal analysis; Supervision;
472 Validation; Writing-review & editing.

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617

618 **Figure captions**

619 **Fig. 1.** Evolution of pH values during acid-induced coagulation of dromedary and cow's milk.

620 **Fig. 2.** Evolution of storage modulus (G') and loss modulus (G'') during acid-induced
621 coagulation of dromedary (aA) and cow's (bB) milk. Evolution of storage modulus (G') of
622 dromedary and cow's milk samples during acidification as a function of pH 4 (cC). One
623 sample of each pooled milk types was analyzed in triplicate.

624 **Fig. 3.** Delta backscattering profiles of dromedary (aA) and cow's (bB) milk throughout acid-
625 induced coagulation and visual observation (cC) during acid-induced coagulation. The data
626 are reported as a function of time (0–180 min). The blue line was detected at 0 min and the
627 red line was detected at 3 h. The 0 – 42 mm height on the x-coordinates represented the
628 bottom to top of sample cells.

629 **Fig. 4.** (aA) Averaged absorbance of the FT-MIR spectroscopy spectra recorded on
630 dromedary milk during acid-induced coagulation. (bB) Averaged absorbance of the FT-MIR
631 spectroscopy spectra recorded on cow's milk during acid-induced coagulation. (cC) Principal
632 component analysis similarity map determined by PC1 and PC2 of FT-MIR spectra scanned
633 on dromedary and cow's milk during acid-induced coagulation (3h).

Figure 1:

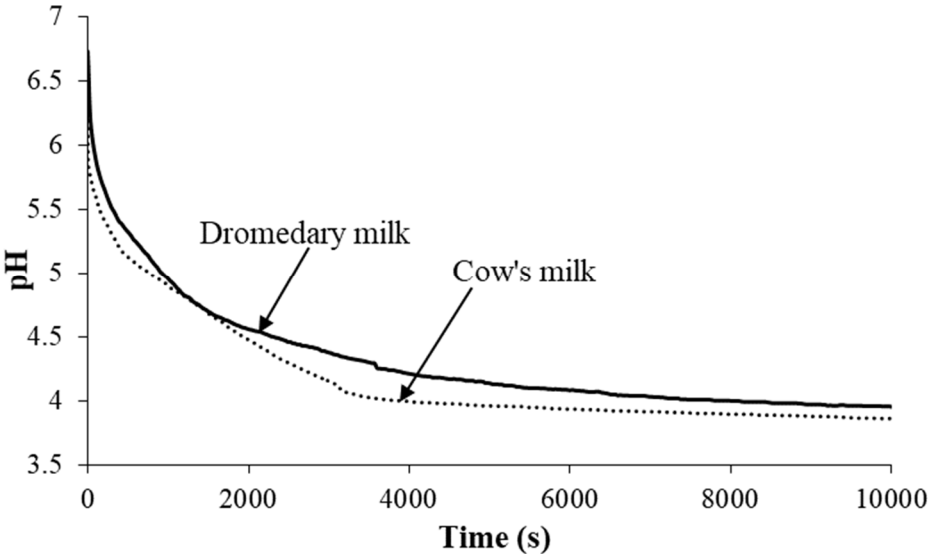


Figure 2a:

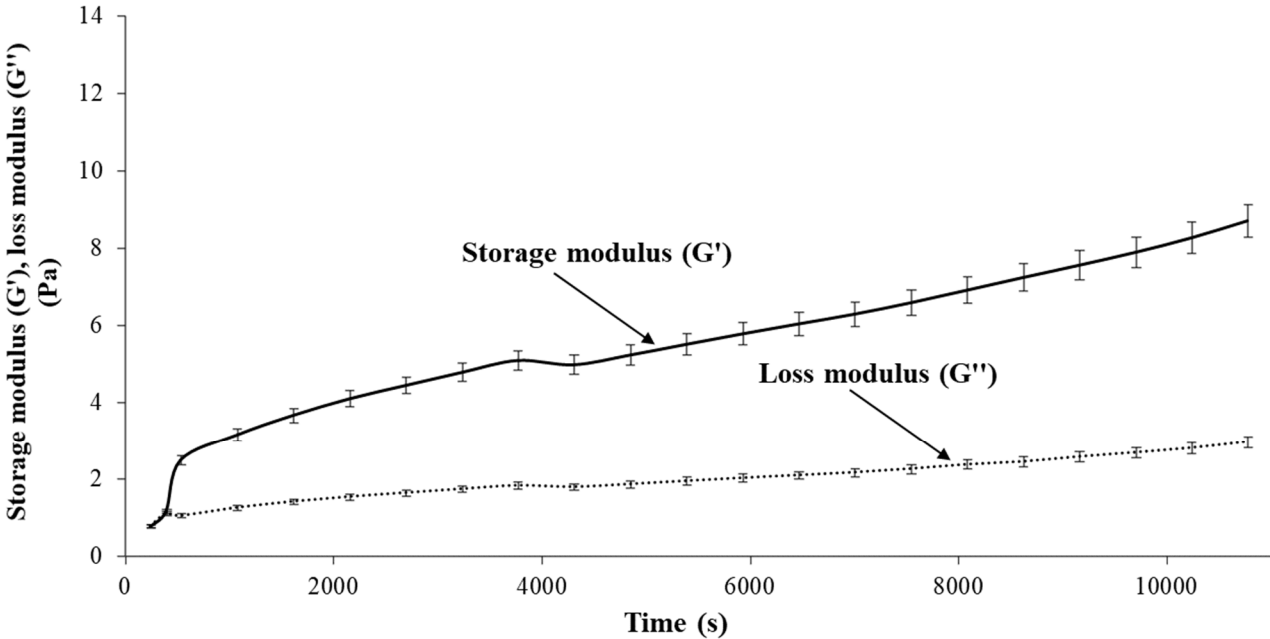


Figure 2b:

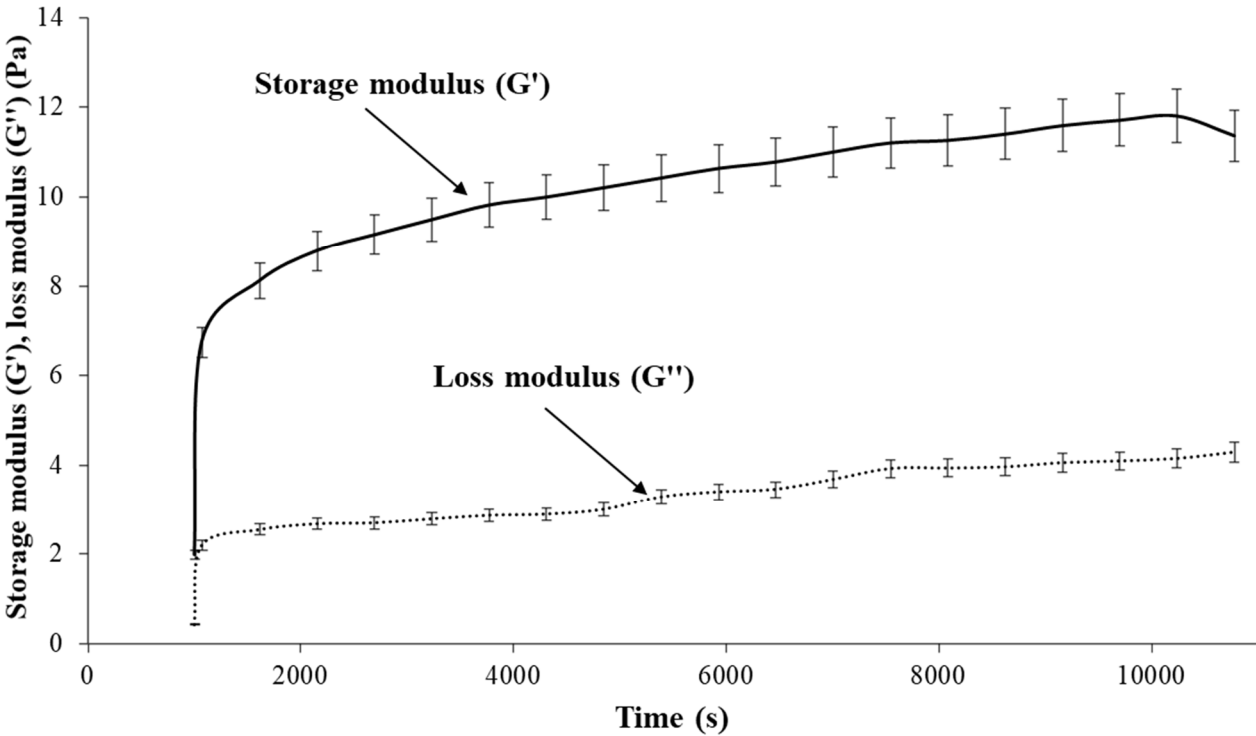


Figure 2c:

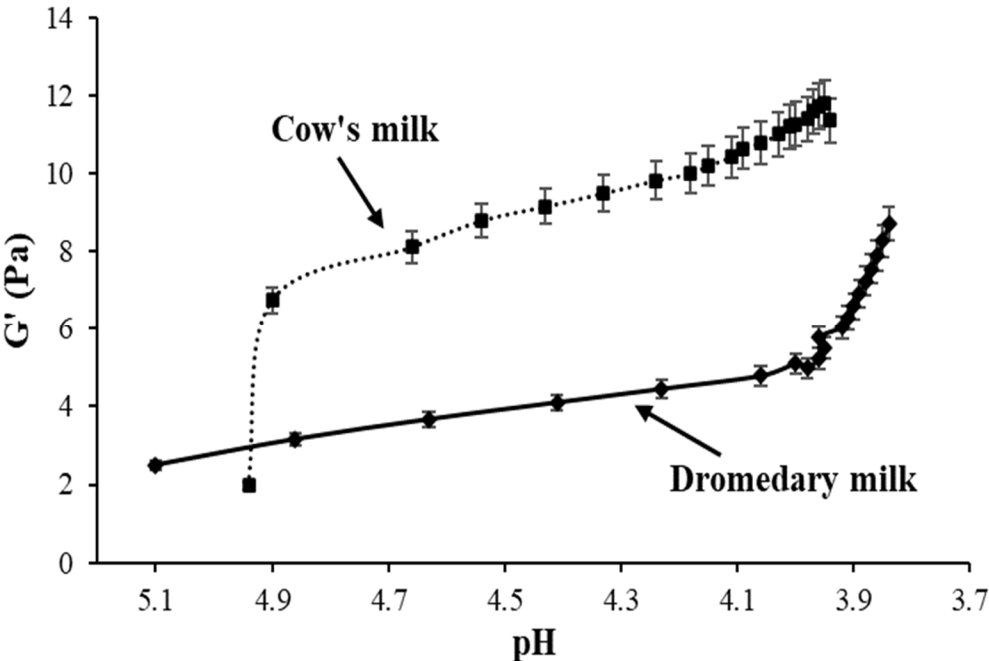


Figure 3a:

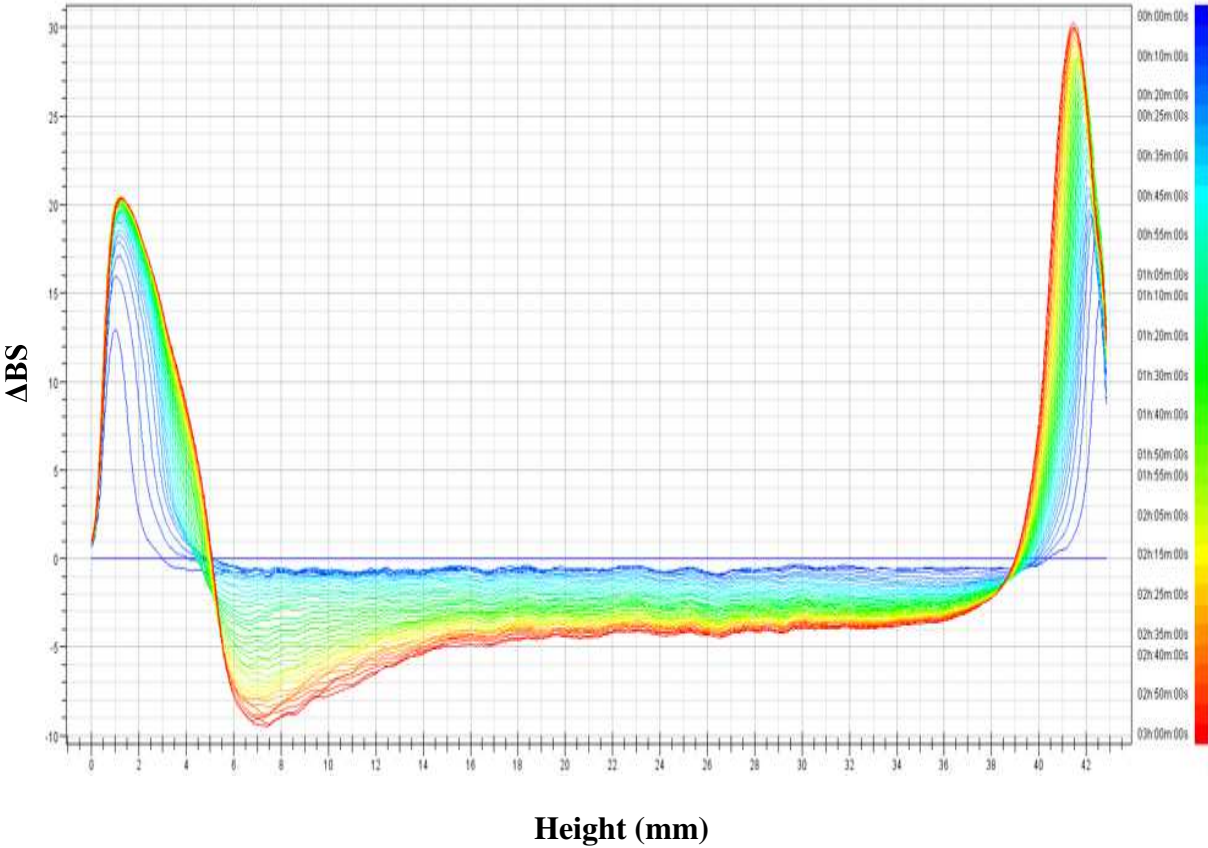


Figure 3b:

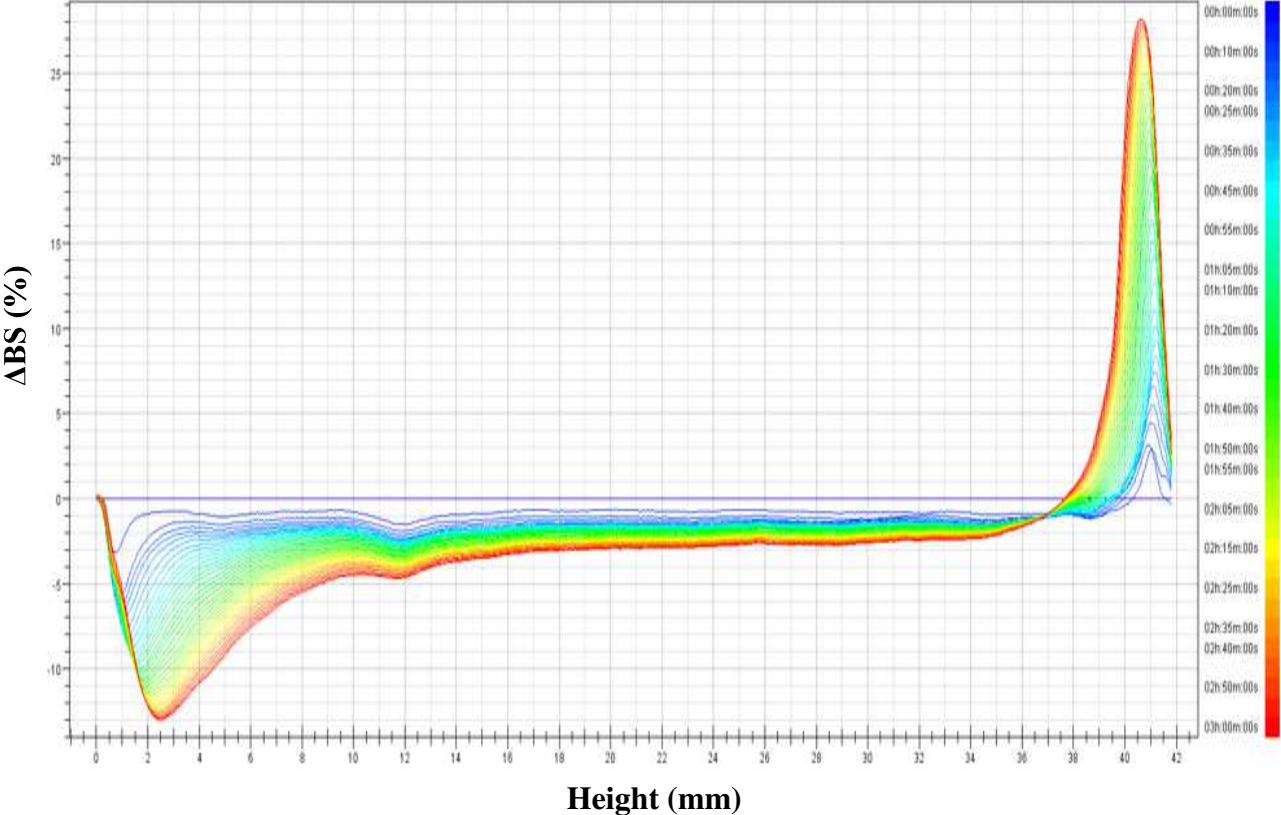


Figure 3c:

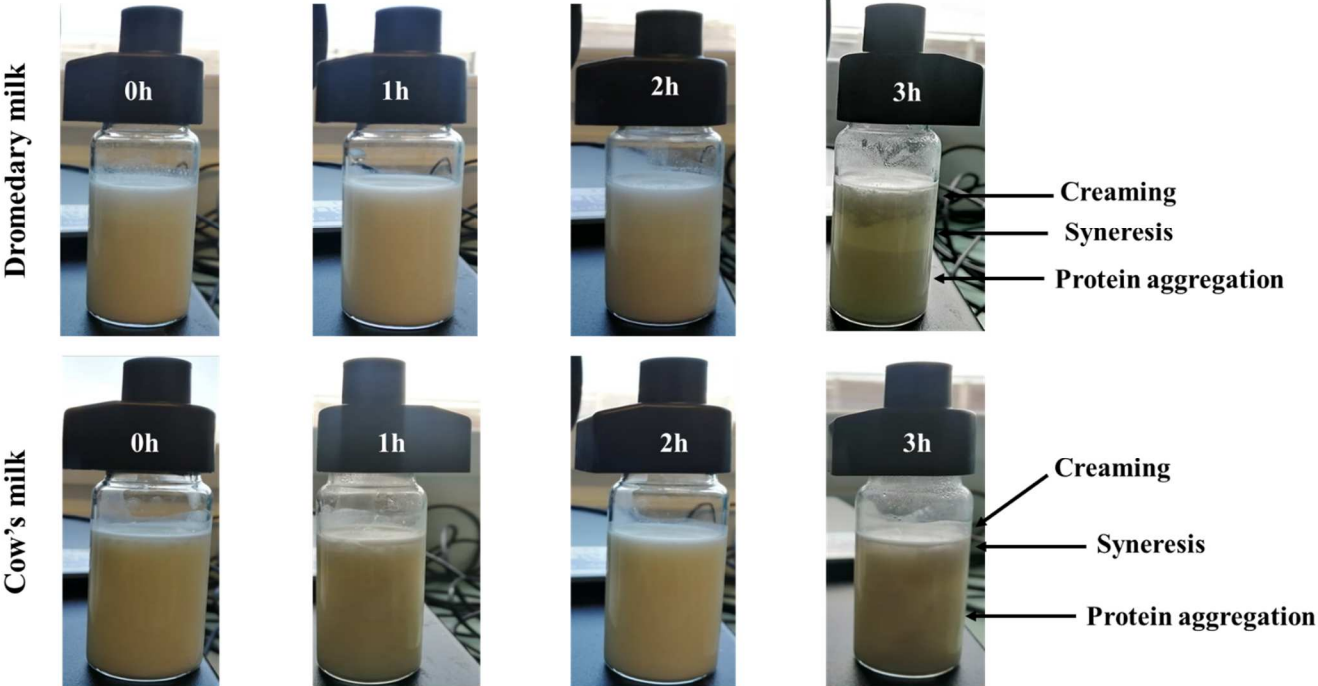


Figure 4a:

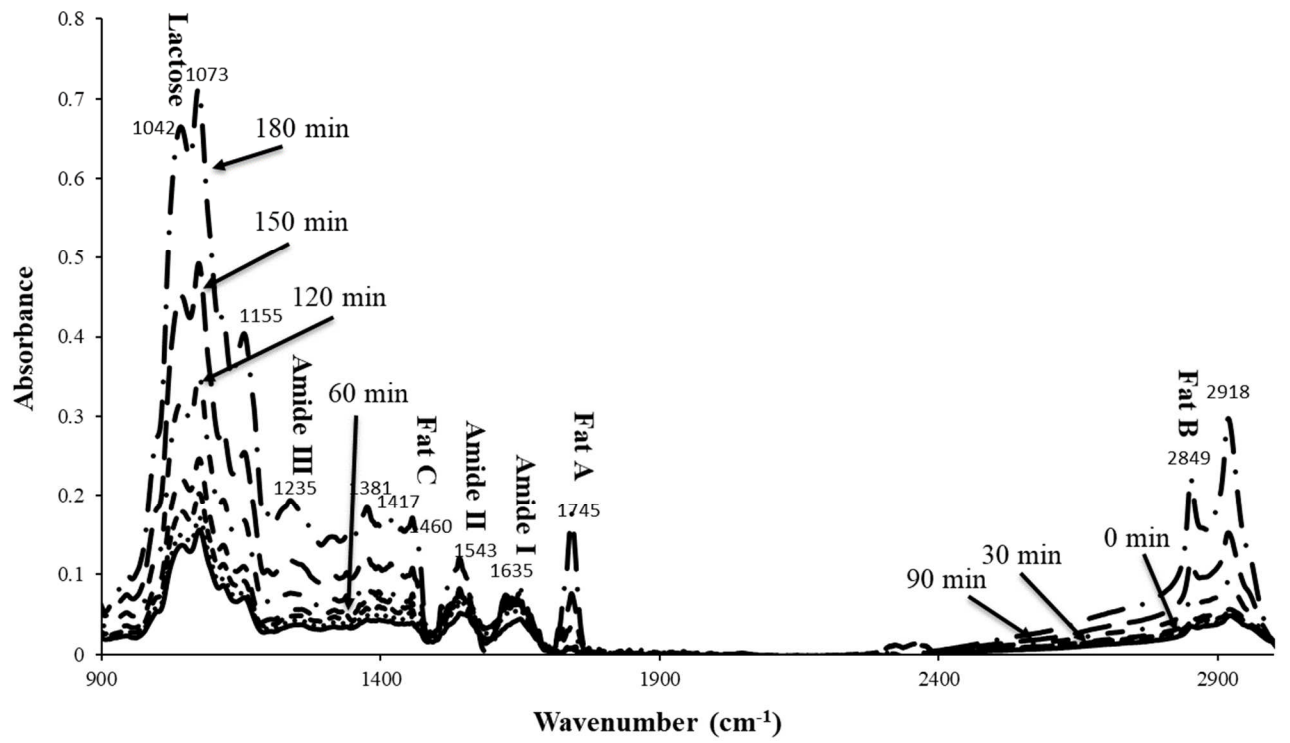


Figure 4b:

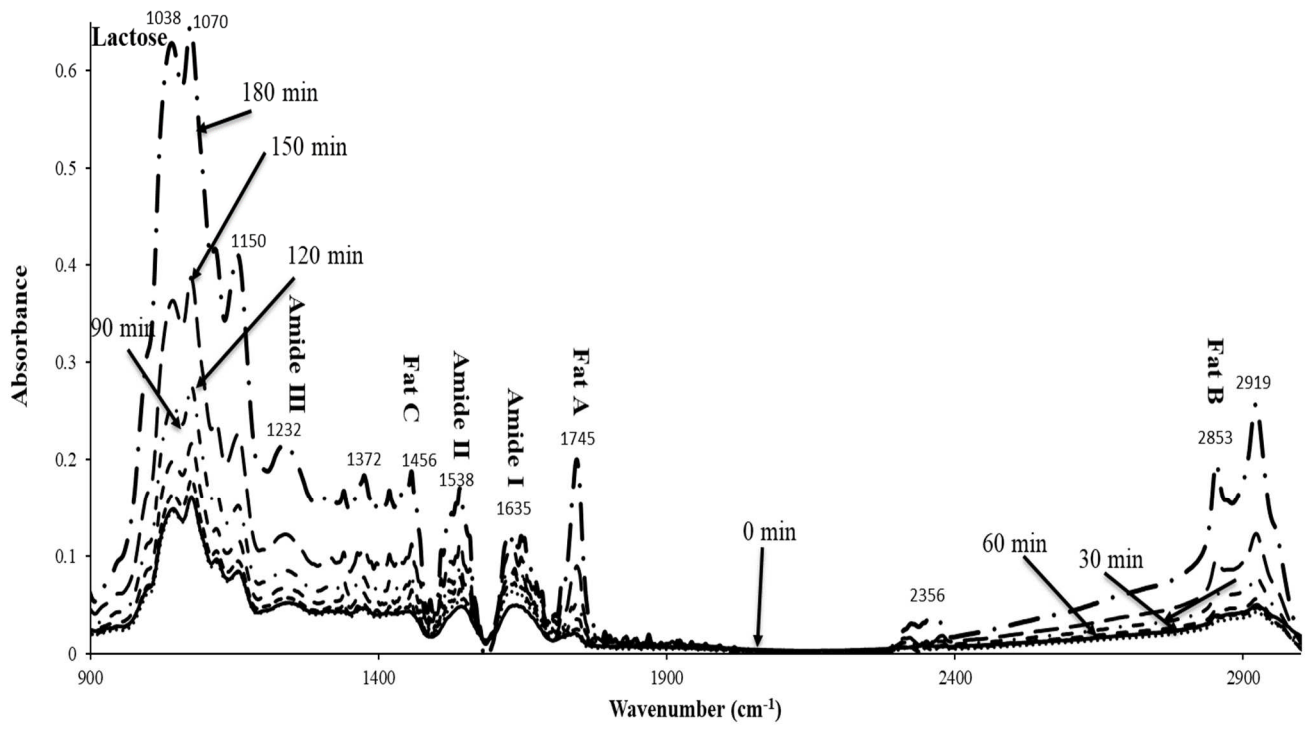


Figure 4c:

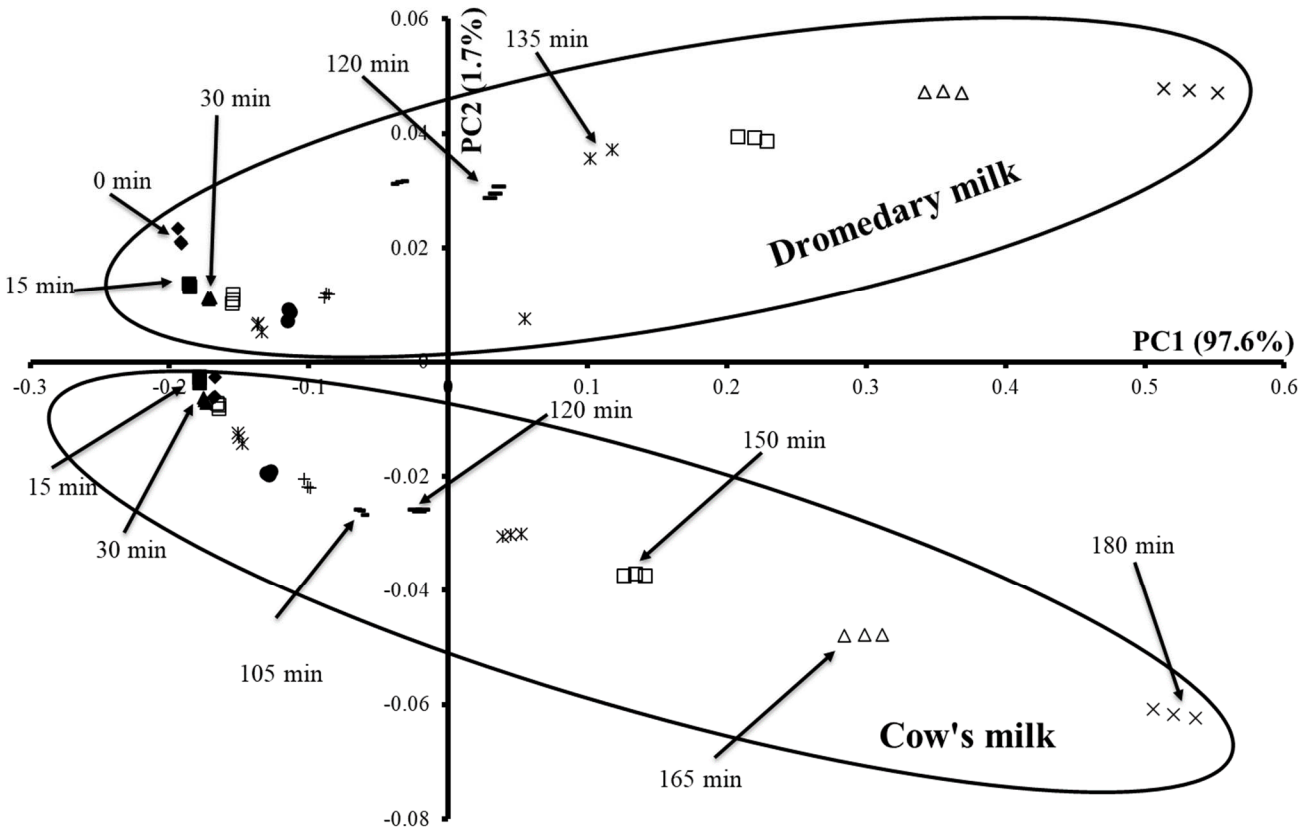


Table 1. Evolution of pH values during acid-induced coagulation of dromedary and cow's milk.

Time (min)	Dromedary milk	Cow's milk
0	6.72 ± 0.01 ^{a,A}	6.61 ± 0.04 ^{b,A}
15	5.01 ± 0.08 ^{a,B}	4.94 ± 0.07 ^{b,B}
30	4.61 ± 0.08 ^{a,C}	4.55 ± 0.04 ^{b,C}
45	4.43 ± 0.06 ^{a,D}	4.23 ± 0.01 ^{b,D}
60	4.26 ± 0.06 ^{a,E}	4.01 ± 0.08 ^{b,E}
75	4.17 ± 0.03 ^{a,F}	3.97 ± 0.04 ^{b,E}
90	4.11 ± 0.02 ^{a,G}	3.95 ± 0.02 ^{b,E}
105	4.07 ± 0.01 ^{a,H}	3.93 ± 0.02 ^{b,E}
120	4.02 ± 0.01 ^{a,H}	3.91 ± 0.01 ^{b,F}
135	4.00 ± 0.01 ^{a,H}	3.89 ± 0.02 ^{b,F}
150	3.97 ± 0.02 ^{a,H}	3.87 ± 0.02 ^{b,F}
165	3.96 ± 0.02 ^{a,H}	3.86 ± 0.02 ^{b,F}
180	3.94 ± 0.03 ^{a,H}	3.84 ± 0.02 ^{b,F}

Means with different superscripted lowercase letters in the same line were significantly different according to Tukey's HSD test ($p < 0.05$).

Means with different superscripted uppercase letters in the same column were significantly different according to Tukey's HSD test ($p < 0.05$).

Table 2. Rheological parameters (~~gelation time, maximum curd firming and curd firmness~~) of dromedary and cow's milk ~~throughout acid-induced coagulation.~~

	Parameters	Dromedary milk	Cow's milk
Gelation	Gelation G' (Pa)	1.50 ± 0.58 ^b	6.74 ± 0.35 ^a
	Gelation G'' (Pa)	1.06 ± 0.18 ^b	2.19 ± 0.10 ^a
	Gelation time (min)	8.33 ± 0.15 ^a	5.20 ± 0.05 ^b
	Gelation pH	5.12 ± 0.06 ^b	5.51 ± 0.02 ^a
Firmness	Maximum curd firming rate (Pa s ⁻¹)	8.08 10 ⁻⁴ ± 0.00 ^b	1.0 10 ⁻² ± 0.00 ^a
	Curd firmness (Pa)	8.71 ± 0.11 ^b	11.37 ± 1.44 ^a
	Firmness pH	3.84 ± 0.01 ^b	3.94 ± 0.05 ^a

Means with different superscripted letters in the same line were significantly different according to ~~Duncan's~~ Tukey's HSD test ($p < 0.05$).