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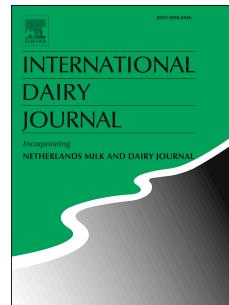
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# Accepted Manuscript

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1   **Rheological and sensory properties and aroma compounds formed during ripening of soft  
2   brined cheese made from camel milk**

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2526 ABSTRACT

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27

28 Protein degradation, rheological properties, sensory properties and the aroma profile of soft brined  
29 cheese made from camel milk using two levels of coagulant (camel chymosin) [55 and 85  
30 International Milk Clotting Units (IMCU) L<sup>-1</sup>] and two levels of brine (2% or 5% NaCl, w/w) were  
31 investigated over a ripening period of 60 d. Casein degradation in soft brined camel milk cheese  
32 significantly ( $p < 0.05$ ) increased during ripening and with increase of coagulant level. Young's  
33 modulus and stress at fracture significantly ( $p < 0.05$ ) increased with increasing level of salt in  
34 moisture in the cheese during ripening. However, cheese made with 85 IMCU L<sup>-1</sup> coagulant  
35 resulted in softening of cheese texture and higher salt uptake. Using descriptive sensory analysis,  
36 the experimental cheeses were described as salty, sour and firm. The volatile aroma compounds  
37 formed in soft ripened camel milk cheese are affected by ripening time, and coagulant and NaCl  
38 levels.

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41    **1. Introduction**

42

43           Production of cheese from camel milk has been difficult due to the lack of available  
44           coagulants able to specifically cleave camel κ-casein. With the availability of camel chymosin as  
45           coagulant (Kappeler et al., 2006) this is now changing and a few reports have been published  
46           recently on soft cheeses from camel milk (Benkerroum, Dehhaoui, El Fayq, & Tlaiha, 2011; Hailu,  
47           Seifu, & Yilma, 2014; Konuspayeva et al., 2016). The texture and appearance of cheese is as  
48           important as the flavour and it is one of the first properties that consumers use to identify and judge  
49           specific cheese varieties (Lawrence, Creamer, & Gilles, 1987). Cheese texture measurement can  
50           provide important information for new product development, product quality monitoring and about  
51           the structure of the product itself (i.e., elastic deformation, plastic deformation and hardness)  
52           (Essex, 1969). The quality of brined cheeses is affected by the duration of ripening due to  
53           proteolysis and lipolysis as well as changes in cheese appearance (Pappas, Kondyli, Voutsinas, &  
54           Mallatou, 1996).

55           Consumer studies (sensory evaluation) for food products are one of the central points for  
56           success in new food product development (Drake, 2007). Addition of NaCl to the cheese is  
57           necessary for the flavour and texture development of cheeses and it is also one of the sensory  
58           attributes perceived as a basic flavour (Guinee, 2004; Guinee & Fox, 2004). Firmness of brined  
59           cheese made from bovine milk increased with increasing the concentration of NaCl in the brine,  
60           salting time, and salt in moisture content of the cheese (Guinee & Fox, 2004; Prasad & Alvarez,  
61           1999). Salt affects the rheological properties of cheese by enhancing casein aggregation or  
62           hydration, causing cheese hardness and brittleness to increase with increase in NaCl concentration  
63           (Guinee & Fox, 2004; Pastorino, Hansen, & McMahon, 2003). The maturation of cheese involves  
64           complex biochemical changes due to microbiological and enzymatic processes that provide

65 different flavour characteristics (McSweeney, 2004; McSweeney & Sousa, 2000) and variations in  
66 texture (McSweeney, 2004). Biochemical changes such as lipolysis, proteolysis, metabolism of  
67 residual lactose, lactate, citrate, fatty acids and amino acids (McSweeney, 2004) result in  
68 development of different flavour compounds in cheese during ripening (McSweeney & Sousa,  
69 2000).

70 To date, there are no reports on the effect of NaCl and coagulant (camel chymosin)  
71 concentration on the casein degradation, rheology, sensory quality and aroma profile of soft brined  
72 cheese made from camel milk. Therefore, the aim of this study was to investigate these properties at  
73 two different levels of NaCl (2% and 5%, w/w) and coagulant (55 and 85 IMCU L<sup>-1</sup>) to better  
74 understand the structural changes and characterise the product over 60 d of ripening.

75

## 76 2. Materials and methods

77

### 78 2.1. Materials

79

80 Pooled camel milk samples were collected from 15 to 20 camels owned by pastoralists in the  
81 Erer Valley, Eastern Ethiopia, and transported in an icebox (4–5 °C) to Haramaya University for  
82 cheese making. Camel chymosin (Chy-Max® M; EC 3.4.23.4) with a strength of 1000 International  
83 Milk Clotting Units (IMCU) mL<sup>-1</sup> and freeze-dried starter culture (i.e., *Streptococcus thermophilus*  
84 STI-12) were obtained from Chr. Hansen, Hørsholm, Denmark. All chemicals used were analytical  
85 grade from Sigma Aldrich, Mannheim, Germany.

86

### 87 2.2. Experimental design

88

89       The rheological, sensory properties and aroma profiles of the cheese samples were evaluated  
90      over a ripening period of 60 d using a 2×2×3 factorial arrangement with completely randomised  
91      design. Four soft cheeses were made using coagulant concentrations of 55 or 85 IMCU L<sup>-1</sup> and  
92      NaCl levels of 2% or 5% (w/w) in solutions and sampled on 0, 30 and 60 d of ripening. Proteolysis  
93      was studied on 0, 20, 40 and 60 d of ripening (where 0 d is 24 h after manufacturing and  
94      immediately before the drained curd is transferred into the brine). The cheeses were made at  
95      Haramaya University, Ethiopia, and portions of each cheese samples were vacuum sealed in food  
96      grade plastic and frozen at -20 °C for volatiles analysis. The experiment was performed in  
97      duplicate.

98

99      2.3. *Cheese manufacturing*

100

101       An Armfield FT20 (Armfield Ltd., Ringwood, Hampshire, UK) cheese vat was used for  
102      cheese making. Ten litres of pooled camel milk was pasteurised at 63 °C for 30 min and 0.02%  
103      CaCl<sub>2</sub> was added. After 20 min, the starter culture (*Str. thermophilus* STI-12) at a level of 75 U  
104      1000 L<sup>-1</sup> was added to the milk and acidified for 15–20 min at 36–38 °C. The coagulant Chy-  
105      Max®M was then added at a level of 55 IMCU L<sup>-1</sup> or 85 IMCU L<sup>-1</sup> and the milk was kept for 2 h at  
106      36–38 °C. The cheese curd was sliced with a knife into 2 cm cubes and held for 10 min to facilitate  
107      whey drainage. After that the cheese curd was transferred to the mould and inverted twice every 2  
108      h. The whey drainage in the mould was performed at 18–20 °C overnight and the curd was placed  
109      into a respective brine concentration of 2% or 5% NaCl (w/w). The mean pH of cheese curd during  
110      curd cutting and drainage steps of cheese manufacturing was 5.90 ± 0.10 and 5.79 ± 0.10,  
111      respectively. Similar volumes of cheese were brined in uniformly sized air tight transparent plastic

112 cans at 4–5 °C. The pH of the brine was adjusted using glucono- $\delta$ -lactone (Sigma-Aldrich) to the  
113 pH value of each cheese (i.e., 4.7–4.9).

114

115 *2.4. Physicochemical properties*

116

117 The composition of raw camel milk was analysed using a MilkoScan™FT1 (Foss, Hillerød,  
118 Denmark). The pH of cheese was measured by puncturing grated cheese with a calibrated pH meter  
119 electrode according to Ardö and Polychroniadou (1999). Total solids and ash content of cheese  
120 samples were measured using gravimetric methods. Fat content of the cheese samples was  
121 measured using the Gerber method (AOAC, 1995). NaCl content was determined using an  
122 automated potentiometric end point titrator (DL50, Mettler-Toledo A7S, Glostrup, Denmark)  
123 (ISO5943:IDF88, 2006). The protein content of the cheese was determined using Kjeldahl method  
124 (AOAC, 1995). Proximate analysis of the cheese samples was performed in duplicate.

125

126 *2.5. Proteolysis study*

127

128 *2.5.1. Soluble nitrogen fractionation*

129 Citrate dispersion and soluble nitrogen fractions, i.e., pH 4.4 soluble nitrogen (pH 4.4 SN),  
130 12% trichloroacetic acid soluble nitrogen (12% TCA-SN) and 5% phosphotungstic acid soluble  
131 nitrogen (5% PTA-SN), were prepared according to Ardö and Polychroniadou (1999). Grated  
132 cheese (12.5 g) was mixed with 50 mL of warm (40–50 °C) 0.5 M trisodium citrate solution in a 100  
133 mL beaker and stirred for 60 min until the cheese was completely dispersed. The warm cheese  
134 citrate dispersion was then cooled to room temperature and adjusted to 250 mL with distilled water  
135 and the soluble nitrogen fractions (i.e., pH 4.4 SN, 12% TCA-SN and 5% PTA-SN) were prepared

136 and analysed to evaluate proteolysis in the cheese accordingly. The protein content of the cheeses  
137 were determined using the Kjeldahl method (AOAC, 1995).

138

139 *2.5.2. Sodium dodecylsulphate polyacrylamide gel electrophoresis*

140

141 Protein degradation analysis was performed using sodium dodecylsulphate polyacrylamide  
142 gel electrophoresis (SDS-PAGE). The cheese sample for analysis was prepared by dissolving 1.2 g  
143 of cheese in 25 mL of 8 M urea at pH of 8.5 and homogenising for 2 min using a T25 digital Ultra-  
144 Turrax® (IKA® - Werke GmbH and Co.KG, Staufen im Breisgau, Germany) and the cheese extract  
145 was incubated for 2 h at 37 °C to complete solubilisation of casein. The completely solubilised  
146 casein in urea was centrifuged at 10,000 × g for 30 min at 4 °C and filtered using 11 µm pore size  
147 filter paper to remove fat and other insoluble matter. The filtrate solutions were dialysed overnight  
148 using dialysis membranes of 6–8 kDa cutoff (Spectra/Por® Membrane Dialysis Products, Spectrum  
149 Labs, Torrence, CA, USA) and the dialysed sample was lyophilised (CHRIST Beta 1 - 8 LD plus,  
150 Göttingen, Germany). The lyophilised protein sample was diluted 1:1 with 2× Laemmli sample  
151 buffer (Bio-Rad Laboratories Ltd., Hercules, CA, USA) and heated for 5 min at 95 °C to denature  
152 the protein (Park & Jin, 1998). The electrophoresis was operated with 10 µL of the sample.

153 The SDS-PAGE discontinuous buffer system of Schagger and Von Jagow (1987) was used  
154 and sample buffer was prepared (Grabski & Burgess, 2001) from 10× Tris/glycine/SDS  
155 electrophoresis buffer. Any kD™ Mini-Protean® TGX Stain-Free™ protein gels (Bio-Rad  
156 Laboratories Ltd.) were used for separating the caseins in the electrophoresis. Precision Plus  
157 Protein™ Unstained Protein Standards, Strep-tagged recombinant proteins (10–250 kDa) (Cat #  
158 1610363; Bio-Rad Laboratories Ltd.) was used for identification of respective caseins. Gel image

159 acquisition was performed using stain free, Gel Doc™ EZ System (Bio-Rad Laboratories Ltd.) and  
160 the relative intensity of each band was used to determine casein degradation.

161

162 *2.6. Texture analysis*

163

164 Uniaxial compression was made using a TA.XT plus Texture Analyzer (Stable Micro  
165 Systems Ltd., Godalming, UK). Cylindrical cheese samples 20 mm high and 13 mm diameter were  
166 prepared by vertically punching the cubic cheese samples at 5 °C gently using a lubricated cork  
167 borer, and analysed immediately. The compression test was performed with 25 mm diameter  
168 cylindrical probe, at 0.83 mm sec<sup>-1</sup> speed to 70% of the initial height of the sample (Wium, Gross,  
169 & Qvist, 1997). The compression probe was lubricated with oil between each analysis. Stress at  
170 fracture ( $\sigma_f$ ) (i.e., stress corrected for the change in sample area) and Young's modulus ( $Y_m$ ) (i.e.,  
171 the slope at the initial 5% of compression) were determined to characterise the firmness and the  
172 initial structure of the cheese, respectively (O'Callaghan & Guinee, 2004). Rheological analysis  
173 was performed in triplicate.

174

175 *2.7. Analysis of volatile compounds*

176

177 Volatile compounds were analysed by dynamic headspace sampling/gas chromatography-  
178 mass spectrometry (DHS/GC-MS). The frozen cheese samples were grated and 20 g of the sample  
179 was transferred into a glass flask equipped with purge head and 60 mL of cold tap water was added  
180 to the flask and mixed gently. The samples were equilibrated for 10 min at 37 °C in a water bath  
181 (Julabo, Buch and Holm, Copenhagen, Denmark) and the volatile compounds were collected in  
182 Tenax-TA traps (Buchem bv, Apendoorn, The Netherlands) by purging with a N<sub>2</sub> flow of 100 mL

183 min<sup>-1</sup> for 30 min at 37 °C. The trapped volatile compounds were thermally desorbed (Turbo Matrix  
184 350, Perkin Elmer, Shelton, WA, USA) and then cryo-focused in a Tenax TA cold trap (30 mg held  
185 at 5 °C), which was subsequently heated at 300 °C for 4 min (secondary desorption, outlet split  
186 1:10). The desorbed compounds were analysed using a GC-MS (7890A GC-system interfaced with  
187 a 5975C VL MSD with Triple-Axis detector; Agilent Technologies, Palo Alto, CA, USA).  
  
188 Compounds were separated on a DB-Wax capillary column 30 m long × 0.25 mm internal diameter,  
189 0.50 µm film thicknesses. The pressure of the column was 2.4 psi with an initial flow rate of 1.2 mL  
190 min<sup>-1</sup> (H<sub>2</sub> as carrier gas). The column temperature was programmed 10 min at 30 °C, from 30 °C to  
191 240 °C at 8 °C min<sup>-1</sup>, and finally 5 min at 240 °C. The mass spectrometer was operated in electron  
192 ionisation mode at 70 eV and the mass to charge values between 15 and 300 were scanned. MSD  
193 Chemstation (Version E.02.00; Agilent Technologies) was used for data analysis. The commercial  
194 data base (Wiley 275.L, HP product no. G1035A) was used to match the spectra with the respective  
195 compounds. The peak areas were used to determine the relative abundance of each volatile in the  
196 experimental cheese samples. The GC-MS analysis was performed in duplicate.  
  
197

198 2.8. *Descriptive sensory analysis*

199  
200 The descriptive sensory analysis was performed using 10 trained panellists (Delahunty &  
201 Drake, 2004). Panel recruitment and training in identifying the sensory descriptors for the cheese  
202 was according to the procedure described by Hootman (1992). Panellists were trained for each  
203 descriptive vocabulary of the four basic sensory modalities, i.e., aroma [buttery, silage, smoky,  
204 mouldy (musty) and pungent], appearance (watery, shiny, mouldy, openness and mottling), texture  
205 (crumbly, firmness, grainy, rubbery and smoothness), taste (sour, sweet, salty and bitter) (Lawlor &  
206 Delahunty, 2000). Cheese samples were provided to the panellists and they were asked to scale the

207 intensity of each descriptor on a 15 cm non-structured line scale. A feedback on the consensus and  
208 repeatability of their assessment was given to panels by analysing the data with PanelCheck  
209 software (V1.4.0; Copenhagen, Denmark) during the training and between each assessment to  
210 improve their rating capacity. The cheese samples were coded with three digit codes and provided  
211 to the panel randomly (Ritvanen et al., 2005). A two way analysis of variance (ANOVA) was  
212 performed to identify significant ( $p < 0.05$ ) product effects (Losó, Gere, Györey, Kókai, & Sipos,  
213 2012). The evaluation of the final product was performed independently at 20–22 °C in triplicate.  
214 Warm spring water was served for rinsing the mouth between each sample.

215

216 2.9. *Statistical analysis*

217

218 Physicochemical properties, soluble nitrogen fractions and texture data were analysed using  
219 SAS version 9.4 (SAS Institute, Cary, NC, USA). The general linear model (PROC GLM)  
220 procedure of SAS was used to determine significant ( $p < 0.05$ ) differences. Least square means with  
221 significant ( $p < 0.05$ ) differences were computed with multiple comparison tests (SHEFFE option in  
222 PROC GLM). The descriptive sensory profile data were analysed using PanelCheck software  
223 (V1.4.0) according to the work flow of Losó et al. (2012). Attributes with significance level  
224 difference above 5% ( $p < 0.05$ ) of product effect were considered in the analysis of the difference  
225 between each cheese samples. A spider web plot was used to characterise sensory attributes of the  
226 individual cheese samples and to indicate the score provided for significant attributes of each  
227 samples (Losó et al., 2012). Volatile aroma compounds data were auto-scaled and principal  
228 component analysis (PCA) was made using LatentiX 2.12 (Latentix<sup>TM</sup> 2016, Copenhagen,  
229 Denmark).

230

## 231    3.     Results and discussion

232

233    3.1.    *Physicochemical properties*

234

235              The mean values of the composition of the camel milk used for cheese making were: pH  
236         $6.45 \pm 0.07$ ;  $3.49 \pm 0.09$  g fat  $100 \text{ g}^{-1}$  cheese;  $2.93 \pm 0.34$  g protein  $100 \text{ g}^{-1}$  cheese;  $11.65 \pm 0.28$  g  
237        total solids  $100 \text{ g}^{-1}$  cheese;  $4.08 \pm 0.44$  g lactose  $100 \text{ g}^{-1}$  cheese. These values were in the range for  
238        camel milk composition reported previously (Konuspayeva, Faye, & Loiseau, 2009). The  
239        composition of the experimental cheeses is indicated in Table 1 and were within the range of  
240        compositional properties reported for soft brined cheeses (Anifantakis, 1996; Prasad & Alvarez,  
241        1999).

242

243    3.2.    *Proteolysis*

244

245    3.2.1.    *Soluble nitrogen fraction*

246              The soluble nitrogen fractions of cheese were used as an index of proteolysis. Total nitrogen  
247        (TN) and all the three types of soluble nitrogen fractions generated from the cheese were all  
248        significantly ( $p < 0.05$ ) affected by ripening time (Table 2). A similar rate in development of  
249        soluble nitrogen fractions has been reported for brined cheese varieties from milk of other species  
250        (Anifantakis, 1996). Nitrogen fractions of Feta cheese made from ewes' milk increased throughout  
251        the ripening period (Katsiari, Alichanidis, Voutsinas, & Roussis, 2000). Increasing the coagulant  
252        level resulted in a significantly ( $p < 0.05$ ) higher value for soluble nitrogen fractions (i.e., pH 4.4,  
253        TCA and PTA) (Table 3). A significantly ( $p < 0.001$ ) higher formation of soluble nitrogen fractions  
254        was recorded for cheese made with a higher coagulant level ( $85 \text{ IMCU L}^{-1}$ ) than for cheese made

255 with the lower coagulant level ( $55 \text{ IMCU L}^{-1}$ ) (Table 3). The formation rate of TCA soluble  
 256 nitrogen fractions in our study is in general agreement with findings for soft brined cheese made  
 257 from other milk types (Anifantakis, 1996; Mallatou, Pappa, & Boumba, 2004).

258

259 *3.2.2. Casein proteolysis*

260 The extent of  $\alpha_{S1}$ -casein and  $\beta$ -casein degradation in soft brined cheese made from camel  
 261 milk is indicated in Fig. 1a, b. The degradation rate of  $\alpha_{S1}$ -casein and  $\beta$ -casein was different and this  
 262 became more apparent after 20 d of ripening (Fig. 1). The effect of salt on  $\alpha_{S1}$ -casein can be seen  
 263 from Fig. 1a, 20 d, lanes 2 and 4: cheeses made with  $55 \text{ IMCUL}^{-1}$  and  $85 \text{ IMCUL}^{-1}$  and both brined  
 264 in 5% salt have relatively higher intact  $\alpha_{S1}$ -casein than those cheeses on the same ripening day  
 265 brined in 2% salt (i.e., lanes 1 and 3). The degradation of  $\alpha_{S1}$ -casein and  $\beta$ -casein was highest  
 266 during the later stage of ripening and a number of degradation fragments with relative high mobility  
 267 appeared during later period of ripening (Fig. 1). The increase of the band intensity of  $\beta$ -casein in  
 268 some lanes is believed to be as a result of some  $\alpha_{S1}$ -casein fragments having the same  
 269 electrophoretic mobility as intact  $\beta$ -casein lanes (Fig. 1). Similar effects of ripening day for soft  
 270 brined (Teleme cheese) cheese made from ewes' milk have been reported by Mallatou et al. (2004).  
 271 For bovine caseins, Fox (1989) and Guinee and Fox (2004) indicated that  $\alpha_{S1}$ -casein is the preferred  
 272 substrate for chymosin hydrolysis during cheese maturation compared with  $\beta$ -casein and that  
 273 degradation of  $\beta$ -casein from bovine milk cheese by chymosin was reduced at  $\geq 5\%$  NaCl. The level  
 274 of NaCl affected individual caseins by hydrating the casein (Guinee, 2004), which determined the  
 275 accessibility of caseins to proteinases.

276

277 *3.3. Rheological properties*

278

279        Young's modulus ( $Y_m$ ) and stress at fracture ( $\sigma_f$ ) of soft brined cheeses made from camel  
280    milk increased significantly ( $p < 0.05$ ) with increasing salt in moisture (S/M) content and ripening  
281    time which were the highest on 60 d of ripening (Table 4).  $Y_m$  of the cheeses was not significantly  
282    affected ( $p > 0.05$ ) by coagulant level at 2% NaCl in the brine (Table 5). These results are in  
283    agreement with those of Kaya (2002) and Prasad and Alvarez (1999) who reported that cheese  
284    hardness increases at higher salt (NaCl) levels. The observed increase in rheological properties  
285    during ripening might be as a result of the increase in S/M of the cheese. The initial structure of a  
286    rennet coagulated cheese during deformation is mainly due to the para-casein strand matrix  
287    (O'Callaghan & Guinee, 2004) and addition of NaCl will affect the rheological properties of the  
288    cheese by hydration effects on the para-casein (Guinee & Fox, 2004; O'Callaghan & Guinee, 2004).  
289    Similarly, the proteolysis study showed that the level of salt has variable effect on the degradation  
290    of the individual caseins from soft brined camel milk cheese (Fig 1). The ratio of viscous to elastic  
291    character of a cheese can be influenced by degree of changes in casein hydration and aggregation,  
292    which could be due to the salt added to the cheese (Guinee & Fox, 2004). Increasing the salt  
293    concentration promotes protein hydration and expansion (Pastorino et al., 2003) and also increases  
294    the integrity and formation of continuous junctions of the para-casein matrix which is maintained by  
295    hydrophobic and electrostatic attraction between aggregates in cheese matrix (O'Callaghan &  
296    Guinee, 2004). The salt uptake of the cheese is determined by cheese shape, size, distance between  
297    cheese cubes, salting temperature, length of salting time and brine concentration (Guinee & Fox,  
298    2004).

299        Significantly ( $p < 0.05$ ) lower  $Y_m$  was observed for cheese made with 85 IMCU L<sup>-1</sup> and 2%  
300    of NaCl % (w/w) in brine but  $Y_m$  increased with increasing level of NaCl concentration in the brine  
301    for cheeses made at both coagulant levels (Table 5). O'Callaghan and Guinee (2004) and Lucey,  
302    Johnson, and Horne (2003) indicated that extent of hydrolysis of the para-casein matrix determines

303 the rheological properties of cheese. The accessibility of these caseins for protease activity could be  
304 higher at the lower salt level (Guinee, 2004). Prasad and Alvarez (1999) also reported that  
305 increasing rennet concentration resulted in a weak texture for Feta type cheese. Therefore, at a  
306 coagulant concentration of 85 IMCU L<sup>-1</sup>, the para-casein strands in brined camel milk cheese might  
307 be hydrolysed and result in a softening of the cheese texture.

308 The S/M content of the cheese was also significantly ( $p < 0.05$ ) higher for cheese made with  
309 85 IMCU L<sup>-1</sup> (Table 5) and this is in agreement with Prasad and Alvarez (1999) who also found that  
310 salt uptake of cheese was influenced by the concentration of rennet. Guinee (2004) also reported  
311 that the migration of NaCl to the cheese would be affected by structure of the cheese matrix, again  
312 the hydrolysis of para-casein at a higher coagulant level could have facilitated the salt uptake of  
313 brined camel milk cheese. The firmness ( $\sigma_f$ ) of cheeses was significantly ( $p < 0.05$ ) affected by  
314 coagulant level and NaCl concentration in the brine and this effect was more pronounced for cheese  
315 made using 55 IMCUL<sup>-1</sup> (Table 5).

316 By comparing the two cheeses made with different coagulant levels it could be concluded  
317 that  $\sigma_f$  of cheese made with 85 IMCU L<sup>-1</sup> was not dependent on NaCl concentration in the brine and  
318 S/M content of the cheeses (Table 5). S/M content of cheese is significantly ( $p < 0.05$ ) higher on 60  
319 d for both cheeses (Table 4). As indicated by O'Callaghan and Guinee (2004) at large deformations,  
320 the interior structure, i.e., the moisture and fat globules in the matrix, is responsible for the  
321 rheological properties of the cheese. Therefore, the variations in NaCl concentration which would  
322 lead to variation in level of moisture and other components between the interior and exterior part of  
323 brined cheeses in different brine might affected the rheology of the cheeses. Guinee (2004)  
324 indicated exchange of components between the interior and exterior part of the cheese results in  
325 moisture and other component variation during ripening. These migration of salt is also assisted by  
326 possible degradation of casein strands matrixes by the coagulant (Anifantakis, 1996), which could

327 explain the observed non-significant ( $p > 0.05$ ) difference in  $\sigma_f$  value at 85 IMCU L<sup>-1</sup> at both NaCl  
328 concentrations (Table 5).

329

330 *3.4. Volatile compounds*

331

332 A total of 40 volatile aroma compounds were identified in the experimental cheese samples  
333 (Table 6). The groups of these compounds were aldehydes (9), alcohols (14), ketones (10), esters  
334 (4), sulphur compounds (2), and volatile acids (1). These groups of volatile compounds were also  
335 reported for cheese made from bovine milk by Curioni and Bosset (2002) and McSweeney and  
336 Sousa (2000). To gain an overview of the data, a PCA was carried out (Fig. 2) and most of the  
337 variation was explained by PC1 that can be interpreted as variation due to ripening time. All 0 d  
338 samples are placed to the left in the plot (negative scores in PC1), 30 d samples have slightly  
339 positive values, and most of the 60 d samples have high positive scores in PC1. This is because  
340 aroma compounds generated from 0 d samples are due to starter culture degradation of lactose;  
341 while degradation of protein and fat are the main contributors for aroma compounds in later stage of  
342 ripening which result in the development of ester and sulphur compounds.

343 Nine aldehydes were identified in the experimental cheeses and the degradation of amino  
344 acids can lead to formation of aldehydes via transamination or by Strecker degradation (Ardö,  
345 2006). The straight chain aldehydes can provide green and herbaceous aroma notes to cheese  
346 (Curioni & Bosset, 2002). Alcohols identified in the experimental cheeses can be biosynthesised via  
347 many metabolic pathways in general and they are reported as main aroma compounds of soft cheese  
348 (Ardö, 2006; Curioni & Bosset, 2002). Phenylethanol and 2-propanol were seen to appear at the end  
349 of positive axis of PC1 in the current study (Fig. 2). Phenylethanol, 3-octanol and 2-propanol may  
350 provide a pleasant aroma of rose flower and fruity notes to the cheese (Curioni & Bosset, 2002).

351 All ketones except 3-octanone have negative PC1 loadings, indicating that they have the  
352 highest concentration in the early stages of ripening (Fig. 2). McSweeney and Sousa (2000) and  
353 Urbach (1997) reported that ketones are mostly produced by adjunct cultures, mainly from moulds,  
354 and are highly abundant in later stage of mould ripened cheeses. 3-Octanone is known for its green  
355 plant/musty/mouldy aroma (Urbach, 1997); 2,3-butanedione (diacetyl) and acetoin (3-hydroxy-2-  
356 butanone) provide buttery flavour to the cheese (Curioni & Bosset, 2002).

357 The two ester compounds (i.e., ethyl 3-methylbutanoate and ethyl hexanoate) were shown to  
358 be most abundant in later stage of the ripening (Fig. 2) as they had positive loadings in PC1. Esters  
359 can be formed either from purely chemical interaction between alcohol and free fatty acids  
360 (McSweeney & Sousa, 2000) that could result in either ethyl hexanoate or ethyl acetate (Marilley &  
361 Casey, 2004). Esters can also be formed from the catabolism of amino acids and lactose  
362 fermentation (Curioni & Bosset, 2002). Ethyl hexanoate or esters provide a fruity/pineapple flavour  
363 to cheese (Curioni & Bosset, 2002; Yuceer et al., 2009). The observed variance in ester compounds  
364 explained by PC2 (Fig. 2) could be due to the fact that the level of the coagulant added resulted in  
365 higher amounts of substrate (amino acids) (Smit, Smit, & Engels, 2005) and salt added to cheeses  
366 could also affect the coagulant proteolysis activity (Guinee & Fox, 2004).

367 Two sulphur compounds (i.e., dimethylsulphide and dimethyldisulphide) identified in  
368 cheese could originate from sulphur containing amino acids such as methionine and cysteine  
369 (McSweeney & Sousa, 2000). Dimethyldisulphide had positive loading value (PC1 and PC2),  
370 which shows that the production of this compound is higher in later stage of ripening and for higher  
371 coagulant level (85 IMCU L<sup>-1</sup>). These sulphur compounds can bestow garlic or onion flavour to  
372 cheese (McSweeney & Sousa, 2000).

373 Acetic acid is the volatile acid identified in the experimental cheese. It can be produced from  
374 fatty acids (C4–C12) (Curioni & Bosset, 2002), from citrate metabolism (Kondyli, Massouras,

375 Katsiari, & Voutsinas, 2003) and also from amino acid, i.e., threonine, catabolism (Ardö, 2006).  
376 The increase of acetic acid during the ripening in this study is in agreement with the reports of  
377 Curioni and Bosset (2002) and Kondyli et al. (2003). Acetic acid provides a flavour note of sour or  
378 vinegar (Molimard & Spinnler, 1996; Yuceer et al., 2009).

379 The overall different patterns observed in development of aroma compounds identified in  
380 the current experimental cheeses could be explained by the regulatory effect of salt on proteolytic  
381 enzymes (Guinee & Fox, 2004) and the proteolysis effect of coagulant that can determine the  
382 availability of amino acids for further degradations. Such variation in flavour compounds might be  
383 due to the variation in environmental condition such as pH, temperature, water activity and  
384 precursor availability that affect the rate of flavour compounds formation from amino acid (Ardö,  
385 2006).

386

387 *3.5. Descriptive sensory evaluation*

388

389 Descriptive sensory profiles of cheese samples are shown in Fig. 3 and only the attributes  
390 for which the panellists could significantly ( $p < 0.05$ ) differentiate between samples along the  
391 ripening period are shown as spider web plots. Sensory attributes [i.e., aroma (silage,  
392 mouldy/musty), taste (saltiness, sweet, sour), appearance (watery) and texture (firmness)] were  
393 significantly ( $p < 0.05$ ) differentiated by the panellists and varied between cheeses (Fig. 3). During  
394 the ripening period, the intensity of silage and mouldy (musty) aroma showed a slight increase (Fig.  
395 3a, c). The response from the panellists generally agrees with the aroma compounds identified and  
396 their patterns from the experimental cheeses, as the overall concentration of aroma compounds  
397 generated from the cheeses increased during ripening as indicated by the first principal component  
398 (PC1) (Fig. 3). This is due to the fact that different flavour compounds were generated during the

399 ripening of the cheese as a result of complex biochemical reactions (McSweeney & Sousa, 2000;  
400 Urbach, 1993; 1997). The panellists provide a higher score for saltiness, sourness, silage, mouldy  
401 and firmness for cheeses made with the higher salt (5% NaCl) level on 30 d and 60 d (Fig. 3b,c),  
402 hence these attributes were dependent on salt concentration. The sensory attributes of the  
403 experimental cheeses resembled most brined cheese made from milk of other species which can be  
404 characterised as salty, sour and firm (Anifantakis, 1996). Closely looking into the sensory  
405 descriptors provided by the panellists and the specific volatiles generated from the cheeses, it can be  
406 concluded that the sensory descriptors provided by the panellists would precisely describe the  
407 cheeses even though cheese flavour is a balance between each volatile and the threshold of  
408 detection level.

409

410 **4. Conclusion**

411

412 Cheese making with 85 IMCU L<sup>-1</sup> coagulant resulted in a higher degradation of caseins than  
413 was obtained with 55 IMCU L<sup>-1</sup> coagulant. Similar to the case for brined cheese from bovine milk,  
414 β-casein in brined camel cheese was clearly seen to be degraded in later stage of ripening while α<sub>S1</sub>-  
415 casein remained more intact. The rheological properties ( $Y_m$  and  $\sigma_f$ ) and the accumulation of  
416 individual volatile aroma compounds of brined camel cheeses were dependent on the time of  
417 ripening, salt and coagulant levels. S/M content of cheese increased with increase in brine  
418 concentration and ripening time. Even though the  $Y_m$  and  $\sigma_f$  increased with increasing salt level, a  
419 softer brined cheese texture was noted for cheese made with 85 IMCU L<sup>-1</sup>. Furthermore, the  $\sigma_f$  was  
420 not dependent on NaCl concentration at a higher coagulant concentration. The developments of  
421 volatile aroma compounds from the cheeses were mainly influenced by ripening time. However,  
422 both salt and coagulant level also exerted a significant effect on the development of different

423 volatile aroma compounds, which could be due to the proteolysis effect of coagulant and salt on  
424 proteolytic enzymes that could influence the production of free amino acids. The overall volatile  
425 aroma compounds generated in the experimental cheeses were similar to that of brined cheeses  
426 made from other milk types. The trend in the development of these volatiles also explains the  
427 variation in sensory descriptors provided by the panellists. Soft brined camel milk can be described  
428 with salty, sour and firm sensory descriptors.

429

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431

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438

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## Figure legends

**Fig. 1.** Electrophoresis images of casein from soft brined cheese made from camel milk.

Casein from cheese made with different level of coagulant and salt level and sampled at (a) 0 and 20 d and (b) 40 and 60 d. For each day data set, lane 1: 55 IMCU L<sup>-1</sup>, 2% (w/w) NaCl; lane 2: 55 IMCU L<sup>-1</sup>, 5% (w/w) NaCl; lane 3, 85 IMCU L<sup>-1</sup>, 2% (w/w) NaCl; lane 4: 85 IMCU L<sup>-1</sup>, 5% (w/w) NaCl.

**Fig. 2.** PCA plots (scores, top; loadings, bottom) for the volatile aroma compounds identified from soft brined camel milk cheese. The four cheese types are designated as LCLS (55 IMCU L<sup>-1</sup> coagulant; 2%, w/w, NaCl); LCHS (55 IMCU L<sup>-1</sup> coagulant; 5%, w/w, NaCl); HCLS (85 IMCU L<sup>-1</sup> coagulant; 2%, w/w, NaCl); HCHS (85 IMCU L<sup>-1</sup> coagulant; 5%, w/w, NaCl) and ripening day is indicated. Average peak areas of each compounds were used to compute principal component analysis. Numbers in the plot represents individual volatile compounds listed in Table 6.

**Fig. 3.** Sensory descriptors for soft brined cheese made from camel milk on 0 d (a), 30 d (b) and 60 d (c) of ripening. Soft cheese made with (●) 55 IMCU L<sup>-1</sup> brined in 2% (w/w) NaCl, (▲) 55 IMCU L<sup>-1</sup> brined in 5% (w/w) NaCl, (■) 85 IMCU L<sup>-1</sup> brined in 2% (w/w) NaCl and (◆) 85 IMCU L<sup>-1</sup> brined in 5% (w/w) NaCl. Average values of 10 panellists' evaluations in triplicate.

1 **Table 1**2 Composition of soft brined cheese made from camel milk over 60 d of ripening.<sup>a</sup>

3

4

Day	pH	Fat (g 100 g <sup>-1</sup> )	Total solids (g 100 g <sup>-1</sup> )	Protein (g 100 g <sup>-1</sup> )	Ash (g 100 g <sup>-1</sup> )
0	4.73±0.02 <sup>a</sup>	26.0±0.4 <sup>a</sup>	45.42±0.64 <sup>a</sup>	20.37±0.93 <sup>a</sup>	1.48±0.15 <sup>b</sup>
30	4.46±0.02 <sup>b</sup>	23.81±0.4 <sup>b</sup>	38.36±0.64 <sup>b</sup>	17.37±0.93 <sup>ab</sup>	3.07±0.15 <sup>a</sup>
60	4.53±0.02 <sup>c</sup>	24.0±0.4 <sup>b</sup>	36.46±0.64 <sup>b</sup>	14.39±0.93 <sup>b</sup>	2.94±0.15 <sup>a</sup>

5

6 <sup>a</sup> Values are least square means ± standard error (n = 8) of the four cheeses; means with the same  
7 superscript letter within a column are not significantly different (*p* > 0.05).

8

9

10 **Table 2**

11

12 Total nitrogen and soluble nitrogen fractions during ripening of 60 d of soft brined cheese made  
13 from camel milk.<sup>a</sup>

14

Day	TN	pH 4.4 SN	TCA-SN	PTA-SN
0	3.19±0.139 <sup>a</sup>	11.70±1.848 <sup>b</sup>	4.17±0.981 <sup>b</sup>	3.34±0.760 <sup>b</sup>
20	2.65±0.127 <sup>ab</sup>	12.87±1.653 <sup>b</sup>	5.64 ±0.885 <sup>b</sup>	5.16±0.724 <sup>b</sup>
40	2.43±0.127 <sup>bc</sup>	16.27±1.848 <sup>ab</sup>	11.73±0.981 <sup>a</sup>	9.919±0.842 <sup>a</sup>
60	2.07±0.142 <sup>c</sup>	23.98±1.653 <sup>a</sup>	14.27±1.034 <sup>a</sup>	11.79±0.887 <sup>a</sup>

15

16 <sup>a</sup> Abbreviations are: TN, total nitrogen; pH 4.4 SN, pH 4.4 soluble nitrogen; TCA-SN,  
17 trichloroacetic acid soluble nitrogen; PTA-SN, phosphotungstic acid soluble nitrogen. Values (%)  
18 are least square means ± standard error (n = 8) of the four cheeses; means with the same superscript  
19 letter within a column are not significantly different ( $p > 0.05$ ).

20

21

22 **Table 3**23  
24 Soluble nitrogen fractions of cheese made with different coagulant levels.<sup>a</sup>  
25

Coagulant level	pH 4.4 SN	TCA-SN	PTA-SN
55 IMCU L <sup>-1</sup>	11.64±1.66 <sup>b</sup>	7.31±0.68 <sup>b</sup>	6.42±0.58 <sup>b</sup>
85 IMCU L <sup>-1</sup>	19.39±1.66 <sup>a</sup>	10.59 ±0.69 <sup>a</sup>	8.68±0.56 <sup>a</sup>

26  
27 <sup>a</sup> Abbreviations are: pH 4.4 SN, pH 4.4 soluble nitrogen; TCA-SN, trichloroacetic acid soluble  
28 nitrogen; PTA-SN, phosphotungstic acid soluble nitrogen; ICMU, international milk clotting units.  
29 Values (%) are least square means ± standard error (n = 12) of the cheese from both brine  
30 concentrations; means with the same superscript letter within a column are not significantly  
31 different ( $p > 0.05$ ).  
32  
33  
34

35 **Table 4**

36 Effect of ripening day on Young's modulus ( $Y_m$ ), stress at fracture ( $\sigma_f$ ) and salt in moisture (S/M)  
 37 of cheeses.<sup>a</sup>

Day	$Y_m$ (Kpa)		$\sigma_f$ (Kpa)		S/M (g 100g <sup>-1</sup> )	
	2% NaCl	5% NaCl	2% NaCl	5% NaCl	2% NaCl	5% NaCl
0	12.40±3.96 <sup>d</sup>	10.97±3.96 <sup>d</sup>	9.34±1.12 <sup>b</sup>	11.02±1.12 <sup>b</sup>	0.46±0.04 <sup>d</sup>	0.51±0.04 <sup>d</sup>
30	30.96±4.43 <sup>d</sup>	60.13±3.96 <sup>c</sup>	12.34±1.12 <sup>b</sup>	22.56±1.25 <sup>a</sup>	3.38±0.04 <sup>c</sup>	5.71±0.04 <sup>b</sup>
60	104.25±4.83 <sup>b</sup>	232.76±4.85 <sup>a</sup>	25.96±1.37 <sup>a</sup>	23.97±1.37 <sup>a</sup>	3.35±0.04 <sup>c</sup>	6.20±0.04 <sup>a</sup>

38

39 <sup>a</sup> Values are least square means ± standard error (n = 4) of the two cheeses; means with the same  
 40 superscript letters for each variable in column are not significantly different ( $p < 0.05$ ).

41

42

43 **Table 5**

44 Effect of coagulant and brine concentration on Young's modulus ( $Y_m$ ), stress at fracture ( $\sigma_f$ ) and salt  
 45 in moisture (S/M) of cheese.<sup>a</sup>

46

Coagulant (IMCU L <sup>-1</sup> )	$Y_m$ (Kpa)		$\sigma_f$ (Kpa)		S/M (g 100 g <sup>-1</sup> )	
	2% NaCl	5% NaCl	2% NaCl	5% NaCl	2% NaCl	5% NaCl
55	54.44±3.49 <sup>bc</sup>	134.54±3.49 <sup>a</sup>	14.89±1.0 <sup>b</sup>	22.62±1.05 <sup>a</sup>	2.39±0.03 <sup>c</sup>	3.84±0.03 <sup>b</sup>
85	43.97±3.49 <sup>c</sup>	68.03±3.49 <sup>b</sup>	16.88±1.0 <sup>b</sup>	15.75±1.05 <sup>b</sup>	2.40±0.03 <sup>c</sup>	4.40±0.03 <sup>a</sup>

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48 <sup>a</sup> Values in the table are least square means ± standard error (n=6) of the two cheeses during  
 49 ripening; means with the same superscript letters for each variable in column are not significantly  
 50 different ( $p > 0.05$ ).

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59 **Table 6**

Aldehydes	Alcohols	Ketone	Esters	Sulphur compounds	Volatile acid
Acetaldehyde (1)	2-Propanol (10)	Acetone (24)	Ethyl acetate (34)	Dimethylsulphide (38)	Acetic acid (40)
2-Methylpropanal (2)	Ethanol (11)	2-Butanone (25)	Ethyl hexanoate (35)	Dimethyldisulphide (39)	
2-Methylbutanal (3)	2-Butanol (12)	2,3-Butanedione (26)	Ethyl-4-ethoxybenzoate (36)		
3-Ethylbutanal (4)	Propanol (13)	2,3-Pentanedione (27)	Ethyl-3-methylbutanoate (37)		
Hexanal (5)	1-Pentanol (14)	2-Heptanone (28)			
Heptanal (6)	1-Hexanol (15)	3-Octanone (29)			
Nonanal (7)	3-Octanol (16)	Acetoin (30)			
Benzaldehyde (8)	1-Heptanol (17)	2-Nonanone (31)			
Benzeneacetaldehyde (9)	2-Octen-1-ol (18)	Acetophenone (32)			
	1-Nonanol (19)	2-Hydroxy-3-pentanone(33)			
	Phenol (20)				
	2-Methyl-1-propanol (21)				
	Phenylethanol (22)				
	3-Methyl-1-butanol (23)				

60 Volatile aroma compounds identified from soft brined camel cheese ripened for 60 d.<sup>a</sup>

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62 <sup>a</sup> Values in parenthesis are the numbers used to indicate the location of individual volatile compounds on the PCA plot of Fig. 3.

