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

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ABSTRACT

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

1. Introduction

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

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

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

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

2. Materials and methods

2.1. Materials

Pooled camel milk samples were collected from 15 to 20 camels owned by pastoralists in the Erer Valley, Eastern Ethiopia, and transported in an icebox (4–5 °C) to Haramaya University for cheese making. Camel chymosin (Chy-Max$^{@}$ M; EC 3.4 23.4) with a strength of 1000 International Milk Clotting Units (IMCU) mL^{-1} and freeze-dried starter culture (i.e., *Streptococcus thermophilus* STI-12) were obtained from Chr. Hansen, Hørsholm, Denmark. All chemicals used were analytical grade from Sigma Aldrich, Mannheim, Germany.

2.2. Experimental design

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

2.3. Cheese manufacturing

An Armfield FT20 (Armfield Ltd., Ringwood, Hampshire, UK) cheese vat was used for cheese making. Ten litres of pooled camel milk was pasteurised at 63 °C for 30 min and 0.02% $CaCl_2$ was added. After 20 min, the starter culture (*Str. thermophilus* STI-12) at a level of 75 U 1000 L^{-1} was added to the milk and acidified for 15–20 min at 36–38 °C. The coagulant Chy-Max®M was then added at a level of 55 IMCU L^{-1} or 85 IMCU L^{-1} and the milk was kept for 2 h at 36–38 °C. The cheese curd was sliced with a knife into 2 cm cubes and held for 10 min to facilitate whey drainage. After that the cheese curd was transferred to the mould and inverted twice every 2 h. The whey drainage in the mould was performed at 18–20 °C overnight and the curd was placed into a respective brine concentration of 2% or 5% NaCl (w/w). The mean pH of cheese curd during curd cutting and drainage steps of cheese manufacturing was 5.90 ± 0.10 and 5.79 ± 0.10, respectively. Similar volumes of cheese were brined in uniformly sized air tight transparent plastic

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

2.4. Physicochemical properties

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

2.5. Proteolysis study

2.5.1. Soluble nitrogen frication

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

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

2.5.2. Sodium dodecylsulphate polyacrylamide gel electrophoresis

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

The SDS-PAGE discontinuous buffer system of Schagger and Von Jagow (1987) was used and sample buffer was prepared (Grabski & Burgess, 2001) from 10× Tris/glycine/SDS electrophoresis buffer. Any kD™ Mini-Protean® TGX Stain-Free™ protein gels (Bio-Rad Laboratories Ltd.) were used for separating the caseins in the electrophoresis. Precision Plus Protein™ Unstained Protein Standards, Strep-tagged recombinant proteins (10–250 kDa) (Cat # 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^{-1} 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 (σ_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_2 flow of 100 mL

183 min^{-1} 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^{-1} (H_2 as carrier gas). The column temperature was programmed 10 min at 30 °C, from 30 °C to
191 240 °C at 8 °C min^{-1}, 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

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

2.9. Statistical analysis

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

3. Results and discussion

3.1. Physicochemical properties

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

3.2. Proteolysis

3.2.1. Soluble nitrogen fraction

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

255 with the lower coagulant level (55 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 α_{S1}-casein and β-casein degradation in soft brined cheese made from camel
261 milk is indicated in Fig. 1a, b. The degradation rate of α_{S1}-casein and β-casein was different and this
262 became more apparent after 20 d of ripening (Fig. 1). The effect of salt on α_{S1}-casein can be seen
263 from Fig. 1a, 20 d, lanes 2 and 4: cheeses made with 55 $IMCUL^{-1}$ and 85 $IMCUL^{-1}$ and both brined
264 in 5% salt have relatively higher intact α_{S1}-casein than those cheeses on the same ripening day
265 brined in 2% salt (i.e., lanes 1 and 3). The degradation of α_{S1}-casein and β-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 β-casein in
268 some lanes is believed to be as a result of some α_{S1}-casein fragments having the same
269 electrophoretic mobility as intact β-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 α_{S1}-casein is the preferred
272 substrate for chymosin hydrolysis during cheese maturation compared with β-casein and that
273 degradation of β-casein from bovine milk cheese by chymosin was reduced at ≥ 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 (σ_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^{-1} 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^{-1}, 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^{-1} (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 (σ_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^{-1} (Table 5).

316 By comparing the two cheeses made with different coagulant levels it could be concluded
317 that σ_f of cheese made with 85 IMCU L^{-1} 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 σ_f value at 85 IMCU L^{-1} 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).

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

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

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

Acetic acid is the volatile acid identified in the experimental cheese. It can be produced from 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^{-1} coagulant resulted in a higher degradation of caseins than
413 was obtained with 55 IMCU L^{-1} 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_{S1}-
415 casein remained more intact. The rheological properties (Y_m and σ_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 concertation and ripening time. Even though the Y_m and σ_f increased with increasing salt level, a
419 softer brined cheese texture was noted for cheese made with 85 IMCU L^{-1}. Furthermore, the σ_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^{-1}, 2% (w/w) NaCl; lane 2: 55 IMCU L^{-1}, 5% (w/w) NaCl; lane: 3, 85 IMCU L^{-1}, 2% (w/w) NaCl; lane 4: 85 IMCU L^{-1}, 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^{-1} coagulant; 2%, w/w, NaCl); LCHS (55 IMCU L^{-1} coagulant; 5%, w/w, NaCl); HCLS (85 IMCU L^{-1} coagulant; 2%, w/w, NaCl); HCHS (85 IMCU L^{-1} 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^{-1} brined in 2% (w/w) NaCl, (▲) 55 IMCU L^{-1} brined in 5% (w/w) NaCl, (■) 85 IMCU L^{-1} brined in 2% (w/w) NaCl and (◆) 85 IMCU L^{-1} brined in 5% (w/w) NaCl. Average values of 10 panellists' evaluations in triplicate.

Table 1

Composition of soft brined cheese made from camel milk over 60 d of ripening.[a]

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

[a] Values are least square means ± standard error (n = 8) of the four cheeses; means with the same superscript letter within a column are not significantly different ($p > 0.05$).

Table 2

Total nitrogen and soluble nitrogen fractions during ripening of 60 d of soft brined cheese made from camel milk. [a]

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

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

Table 3

Soluble nitrogen fractions of cheese made with different coagulant levels. [a]

Coagulant level	pH 4.4 SN	TCA-SN	PTA-SN
55 IMCU L^{-1}	11.64±1.66[b]	7.31±0.68[b]	6.42±0.58[b]
85 IMCU L^{-1}	19.39±1.66[a]	10.59 ±0.69[a]	8.68±0.56[a]

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

Table 4

Effect of ripening day on Young's modulus (Y_m), stress at fracture (σ_f) and salt in moisture (S/M) of cheeses. [a]

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

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

Table 5

Effect of coagulant and brine concertation on Young's modulus (Y_m), stress at fracture (σ_f) and salt in moisture (S/M) of cheese. [a]

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

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

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)				

Volatile aroma compounds identified from soft brined camel cheese ripened for 60 d. [a]

[a] Values in parenthesis are the numbers used to indicate the location of individual volatile compounds on the PCA plot of Fig. 3.