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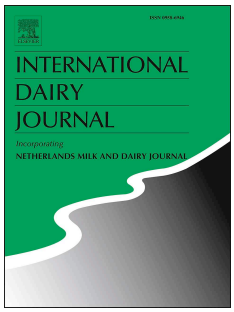

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Interaction between sodium chloride and texture in semi-hard Danish cheese as affected by brining time, DL-starter culture, chymosin type and cheese ripening

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ABSTRACT

Reduced NaCl in semi-hard cheeses greatly affects textural and sensory properties. The interaction between cheese NaCl concentration and texture was affected by brining time (0–28 h), DL-starter cultures (C1, C2, and C3), chymosin type (bovine or camel), and ripening time (1–12 weeks). Cheese NaCl levels ranged from <0.15 to 1.90% (w/w). NaCl distribution changed during ripening; migration from cheese edge to core led to a more homogeneous NaCl distribution after 12 weeks. As ripening time increased, cheese firmness decreased. Cheeses with reduced NaCl were less firm and more compressible. Cheeses produced with C2 were significantly firmer than those produced with C1; cheeses produced with C3 had higher firmness and compressibility. In NaCl reduced cheese, use of camel chymosin as coagulant resulted in significantly higher firmness than that given using bovine chymosin. Overall, cheese NaCl content is reducible without significant textural impact using well-defined starter cultures and camel chymosin.

1. Introduction

Dietary sodium, which is typically consumed as sodium chloride (NaCl), is an important ingredient, especially in pre-processed food, contributing to flavour and acting as a preserving agent (Mattes & Donnelly, 1991). The daily recommendation of NaCl intake for an adult is around 6 g per day (WHO, 2012), but the average daily intake in many European countries varies from 9 to 13 g NaCl per day (European Union, 2012). This elevated intake of NaCl can promote negative health consequences, such as hypertension, cardiovascular diseases and kidney failure (Appel et al., 2012; Frisoli, Schmieder, Grodzicki, & Messerli, 2012). Hence, there is a growing pressure for reducing the sodium content in processed foods. Within the dairy industry, cheese is an evident dairy product with potential for reduction in its sodium content. The salt content in cheese differs markedly with variety, from 0.5% (w/w) in cottage cheese to 4–6% (w/w) in feta cheese (Fox, Guinee, Cogan, & McSweeney, 2000).

Salt is a key ingredient in cheese. It is the major preservative, as it controls the water activity and thereby the microbial growth, protein hydration, enzymatic activity, but it also contributes to flavour formation and the textural properties of the cheese (Fox et al., 2000; Guinee, 2004; Pastorino, Hansen, & McMahon, 2003). Reducing the NaCl content in semi-hard cheeses, like Cheddar cheese, results in increased bitterness and unpleasant aftertaste together with decrease in salty taste and firmness (Johnson, Kapoor, McMahon, McCoy, & Narasimmon, 2009; Rulikowska et al., 2013; Schroeder, Bodyfelt, Wyatt, & McDaniel, 1988).

The majority of the cheeses produced in Denmark belong to the Danish semi-hard cheese types, e.g., Danbo and Samsoe, which unlike, for example, Cheddar cheese, are brined cheeses. These semi-hard cheeses contain, on average, 1.7–1.8% (w/w) NaCl, have a few round holes and are smear-ripened (Madsen & Ardö, 2001; Sørensen & Benfeldt, 2001). The cheeses are traditionally produced using bovine chymosin to coagulate the cheese milk along with mesophilic DL-starter cultures, i.e., containing *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris*, citrate-positive strains of *Lactococcus lactis*, and *Leuconostoc* ssp. (Daly, 1983; Jacob, Jaros, & Rohm, 2011). The salting of these Danish semi-

hard cheese types is done after pressing by immersion of the cheese into a saturated NaCl-solution for up to 28 h, i.e., brining. This differs from the salting process of Cheddar cheese, where the NaCl is added to the milled curd, mixed and then pressed (Grummer, Karalus, Zhang, Vickers, & Schoenfuss, 2012). The majority of studies on salt-reduced semi-hard cheese are on Cheddar and Gouda cheese. Research on textural properties of salt reduced brined semi-hard cheeses of Danbo type is lacking, and the literature on effect of brining mainly deals with mozzarella, halloumi, feta and soft cheese types (Ayyash & Shah, 2011; Hynes, Delacroix, Meinardi, & Zalazar, 1999; Katsiari, Voutsinas, Alichanidis, & Roussis, 1997; Thibaudeau, Roy, & St-Gelais, 2015). Hence, we need new knowledge on the effect of brining for salt uptake and salt distribution to be able to directly correlate salt-reduction to the cheese texture of such brined cheeses.

Both the starter cultures and coagulation enzyme affect texture and flavour formation in the cheese. The composition of the starter culture used in cheese production varies according to cheese type and is often a mixture of various strains to achieve the desired properties of the cheese (Beresford, Fitzsimons, Brennan, & Cogan, 2001). In Cheddar cheese, NaCl can influence the viability of the starter culture and the enzymatic activities, and the proteolysis of the caseins has been found to increase as NaCl content decreases (Mistry & Kasperson, 1998; Møller, Rattray, Høier, & Ardö, 2012). Autolysis of *Lc. lactis* is favoured by low NaCl content (0.17 M) and acidic pH (pH 5.4) (Ramírez-Nuñez, Romero-Medrano, Nevárez-Moorillón, & Gutiérrez-Méndez, 2011). We have previously shown that the NaCl content in Danish semi-hard cheese affects both the viability and autolysis of lactic acid bacteria, which depends to a high degree on the specific DL-starter culture (Søndergaard et al., 2015). Hence, to compensate for the environmental changes in the cheese caused by reducing NaCl, a DL-starter culture combining more specific bacteria strains could be thought to retain some of the traditional semi-hard cheese properties, e.g., texture and flavour.

The presence and the amount of chymosin in the cheese is reported to increase the proteolysis during ripening (Hynes et al., 2001), and this proteolysis is affected by NaCl in different ways when α_{S1}-casein and β-casein are considered (Noomen, 1978), which may impact the texture of the mature cheese.

Furthermore, camel chymosin has been shown to be an alternative to the traditional bovine chymosin. Camel chymosin has a 70% higher clotting activity towards bovine milk compared with bovine chymosin (Bansal et al., 2009; Jensen et al., 2015; Kappeler et al., 2006).

Previous studies comparing camel and bovine chymosin in Cheddar cheese showed firmer and less bitter cheeses when using camel chymosin (Bansal et al., 2009). Moynihan et al. (2014) also found less proteolysis occurring in mozzarella made using camel chymosin. The use of camel chymosin is a new approach to the aim of providing texture in reduced-salt cheese. It is hypothesised to result in cheeses with similar textural properties to those of cheeses made with bovine chymosin and normal NaCl level.

Many of the studies in this area vary according to cheese type, production methods, starter culture and chymosin type, which makes comparisons complicated. To our knowledge, no previous studies have evaluated the effect of NaCl content in brined Danish semi-hard cheeses in relation to cheese texture, and the present study brings novelty into the understanding this relationship.

The aim of this study was therefore to study the effect of NaCl reduction in Danish semi-hard cheese in relation to chemical composition and textural properties during cheese ripening. Additionally, three different DL-starter cultures and two different types of chymosin were used to investigate whether the DL-starter culture and/or the rennet type could counteract the consequences of reducing the NaCl content during processing of brined semi-hard Danish cheeses.

2. Materials and methods

2.1. Starter cultures and chymosin

Three different commercially available DL-starter cultures (C1, C2 and C3) were used (Chr. Hansen, Hørsholm, Denmark). All three starter cultures comprised of strains of *Lc. lactis* subsp. *lactis*, *Lc. lactis* subsp. *cremoris*, citrate-positive strains of *Lc. lactis* and *Leuconostoc* spp. The DL-starter culture C1 was a traditional DL-starter culture, propagated and produced as mixed-strain containing all these

organisms. The DL-starter culture C2 was composed of defined strains of the above-mentioned organisms, where strains have been isolated from a traditional DL-starter culture, grouped and grown separately, before combined into the final starter culture. Detailed description of these two DL-starter cultures can be found in Søndergaard et al. (2015). The DL-starter culture C3 was produced by isolating selected strains from the DL-starter culture C2. These selected strains had been grown separately before combined into the final DL-starter culture C3. The main difference between the DL-starter C2 and C3 was an increased level of *Lc. lactis* subsp. *lactis* in the DL-starter C3 (Chr. Hansen). Two commercially available chymosins (CHY-MAX M® and CHY-MAX plus®) were used (Chr. Hansen). The chymosins differ from each other according to their origin; CHY-MAX plus® contains bovine chymosin (BC), while CHY-MAX M® contains camel chymosin (CC).

2.2. Cheese manufacture and sampling

In total, four cheese experiments were performed to produce the described semi-hard Danish cheese types. Experiment 1 and 2 were performed to test the effect of starter culture, and experiments 3 and 4 to test the effect of the chymosin type. An overview of the experiments is shown in Table 1.

Milk for all cheese productions was fresh, pasteurised under high-temperature-short-time conditions (72 °C, 15 s) bovine milk standardised to a fat to protein ratio of 0.5 (1.88% fat and 3.75% protein).

In experiment 1, semi-hard Danish cheeses (type Samsoe, 30+) were manufactured at the Arla Foods R&D (Brabrand, Denmark), with brining times of 0, 6, 12, and 24 h in saturated NaCl solution. The manufacture of these cheeses is described in detail in Søndergaard et al. (2015). Sampling for all brining times was performed at 1, 2, 7 and 12 weeks of ripening with 3 cheese replicates of each treatment.

In experiment 2**,** semi-hard Danish cheeses (type Samsoe, 30+) were produced at Thise Dairy plant (Thise Dairy, Roslev, Denmark), with brining times of 0, 12, and 24 h, respectively. DL-starter

147 cultures C1 and C2 were used in both experiment 1 and 2. The manufacture of these cheeses is described
148 in detail in Søndergaard et al. (2015). Sampling for all brining times was performed at 1, 2, 7 and 12
149 weeks of ripening with 2 cheese replicates of each treatment.

150 In experiment 3, semi-hard Danish cheeses (type Danbo, 30+) were manufactured at Arla Foods
151 R&D (Brabrand, Denmark), with brining times of 6, 12, and 24 h. The procedure was similar to
152 Søndergaard et al. (2015), with some modifications. Two batches, each of 1000 L milk per day for two
153 days, were used for cheese production. Each day, the milk for one batch was coagulated by use of CHY-
154 MAX Plus® at a rate of 0.03% (w/w) (Chr. Hansen, Hørsholm, Denmark), and one batch was coagulated
155 using CHYMAX-M® at a rate of 0.01% (w/w) (Chr. Hansen, Hørsholm, Denmark). These levels of
156 chymosin correspond to equal levels of international milk clotting unit (IMCU) per L milk. The DL-starter
157 culture C3 used in this experiment was added at a rate of 0.008 % (w/w) together with 0.005% (w/w)
158 $CaCl_2$. Cheeses were placed in a saturated NaCl solution (23.3%, w/v) with 0.25% (w/v) $CaCl_2$ at 11.5 °C
159 for 6 h, 12 h and 24 h, respectively. The cheeses were smeared at the surface, cut in half (approximately
160 15 × 30 × 15 cm), vacuum packed and ripened at 13 °C. Sampling for all brining times was performed
161 after 1 and 12 weeks of ripening with 2 cheese replicates of each treatment.

162 In experiment 4, semi-hard Danish cheeses (type Danbo, 30+) were manufactured at Arla Foods
163 Dairy plant (Taulov, Denmark). The procedure was similar to Søndergaard et al. (2015), with some
164 modifications. Three batches each of 21,350 kg milk were used for production. The milk for one batch
165 was coagulated using CHY-MAX Plus® at a rate of 0.03% (w/w) (Chr. Hansen). Cheeses from this batch
166 were placed in a saturated NaCl solution (23.3%, w/v) with 0.25% (w/v) $CaCl_2$ at 11.5 °C for 28 h except
167 for the 0 h brining treatment, where this step was omitted. Two batches obtained coagulation using
168 CHYMAX-M® at a rate of 0.02% (w/w) (Chr. Hansen). The levels of chymosin correspond to equal
169 levels of IMCU. Cheeses from these batches were split in two parts which were either placed in a
170 saturated NaCl solution (23.3%, w/v) with 0.25% (w/v) $CaCl_2$ at 11.5 °C for 28 h or in the saturated NaCl
171 solution for either 10 h or 15 h (see Table 1 for overview). Due to practical issues, another commercial
172 DL-starter culture, the standard at Taulov Dairy, was used in the final trials, proven to give the same rate

of acidification, flavour development, and eye formation. The cheeses were produced in dimensions of 38 × 76 × 8.5 cm. The longitudes of the cheeses were smeared, and stored for 4 weeks at ~15 °C and relative humidity of 92–97%, then washed, coated with paraffin and further stored 3 weeks at ~8–9 °C. The cheeses were then cut into pieces of 15 × 9 × 4.25 cm, vacuum packed separately and stored at 3.5 °C until analysis after 12 weeks of ripening with 3 cheese replicates of each treatment.

2.3. Texture analysis by uniaxial compression

Textural properties of the cheeses were analysed by uniaxial compression analysis. Cheeses at the desired ripening time were stored for 24 h at 4°C before analysis. A lubricated cork borer, dipped in oil to minimize friction between cork borer and cheese, was slowly pressed through the cheese vertically to create cylindrical cheese pieces. Care was taken not to disrupt the cheese structure when using the cork borer. Cylindrical cheese samples with height (h) = 15 mm and diameter (d) = 15 mm from various locations (in experiments 1, 2, and 3, a total of 12 locations) in the cheese were used for textural analysis by uniaxial compression, and in experiment 4, as a result of the previous experiments only 2 locations were used for sampling (edge and core). The edge samples were taken at a position 1 cm from the surface of the cheese, while the core samples were taken in the centre of the cheese. The cheese cylinders were analysed immediately after cutting. Compression was performed until fracture of the cheese, or to a maximum distance of 12.5 mm, using a TA HDi Texture Analyzer (Stable Micro Systems, Godalming, UK) with a 100 kg load cell, 1 mN detection range, 75 mm diameter flat stainless steel plate and compression speed of 0.8 mm s^{-1}. Data were organised and initial data analysis was made in Texture Expert Exceed Version 2.63 (Stable Micro Systems, Godalming, UK). Recordings of force (N) and displacement (m) were converted into true axial stress σ (Equation 1) representing firmness of the cheese and Hencky strain ε (Equation 2) representing compressibility of the cheese according to the following:

$$\sigma = \frac{F}{A}\frac{H}{H_i}\,[\mathrm{Pa}] \quad (1)$$

198 $$\varepsilon = -ln\frac{H}{H_i}[-] \quad (2)$$

199 where F = force (N), A = initial end area of sample (m^2), H_i = initial sample height (m) and H = height
200 (m) (Hammershoj, Larsen, Ipsen, & Qvist, 2001). The σ_f and ε_f were obtained as sample stress and sample
201 strain, respectively, at the fracture point and used for further statistical analysis. For cheese samples,
202 which did not obtain a breakage point within the distance of analysis, the force at maximum distance was
203 used for further calculations.
204
205 *2.4. Cheese composition*
206
207 At all sampling times, composition of analysis (%) was performed according to International
208 Organization for Standardisation (ISO) methods for dry matter (ISO5534 and IDF004; ISO, 2004b), fat
209 (ISO1735 and IDF005; ISO, 2004a), protein (6.38×N; ISO8968-1 and IDF020-1; ISO, 2014), and NaCl
210 content (ISO5943 and IDF088; ISO, 2006). The pH of the grated cheese was measured by potentiometry.
211 All analyses were conducted at Eurofins Steins Laboratory (Holstebro, Denmark) on a mixture of grated
212 cheese from one whole cheese of each cheese replicate for all sampling times.
213
214 *2.5. Determination of NaCl content*
215
216 The compressed cylindrical cheese samples from texture analysis were collected afterwards and
217 used for analysis of the NaCl content. This approach was chosen to get a data set from the same position
218 in the cheese to correlate textural properties to NaCl concentration. This was determined using a
219 modification of the ISO method (ISO5943 & IDF088; ISO, 2006) based on potentiometric $AgNO_3$
220 titration of Cl^- ions. A mass of 1–1.5 g cheese from the compressed cylindrical cheese samples was
221 collected in a 100 mL tube and added 30 mL 55 °C milliQ water along with 20 mL 1 M sodium citrate.
222 The mixture was blended for 45 s with an Ultra Turrax homogeniser (IKA-Labortechnik, Janke & Kunkel

GmbH & Co., Staufen, Germany) at a speed of 10,000 rpm. To ensure that no sample mixture remained onto the homogenizer, 20 mL 55 °C milliQ water was used to wash off sample from the Ultra Turrax. The samples were then incubated at room temperature for 1 h after which time 10 mL 4 M HNO_3 was added. A Metrohm 862 compact titrosampler (Metrohm AG, Herisau, Switzerland) was used to determine the NaCl content potentiometrically using 0.1 M $AgNO_3$ solution as titrant. At the equivalence point of the titration, the amount of added silver nitrate was noted and used to calculate the concentration of NaCl in the sample solution (Equation 3):

$$NaCl(\%) = \frac{v(AgNO_3)\cdot c(AgNO_3)\cdot M(NaCl)\cdot 0.1}{m(sample)} \quad (3)$$

Two cheese cylinders from the textural analysis, one from the core and one from the edge, were analysed for salt content for all cheeses.

2.6. Microstructure by scanning electron microscopy

The network structure of cheeses after 12 weeks of ripening was studied by scanning electron microscopy (SEM). The procedure is described in detail in Søndergaard et al. (2015). A 1 mm^3 cube of cheese was fixed with 2.5% glutaraldehyde in 0.1 M piperazine-N,N'-bis(2-ethanesulfonic acid). The cheese sample was dehydrated by washing in ethanol in a series of stepwise increasing 10% concentrations from 10–100% ethanol each for 15 min. For the 20% ethanol step and onwards, the cheese sample was transferred to a critical point drying (CPD) capsule. The sample was washed in 100% dry ethanol for 2 × 15 min and stored at T = 4 °C for at least 1 h before CPD. The CPD procedure was performed with liquid CO_2 using a Leica CPD300 (Leica Microsystems, Heidelberg, Germany), and the dried samples were stored in a sealed container at room temperature until analysis. Before analysis, the sample was secured at an aluminium SEM stub with Ag paint and fractured in the horizontal plane. The free-break surface was thereby facing upwards, and the surface was covered with a thin layer of Au using an agar high resolution sputter-coater (Agar Scientific, Stansted, UK). The prepared sample was observed

at 3 kV with a Zeiss Supra 55VP FEG Scanning Electron Microscope (Carl Zeiss, Oberkochen, Germany), at a working distance of ~5 mm at magnifications ranging from 1000 to 95,000 ×. Several pictures were captured for each cheese sample. Each sample was analysed in duplicates and samples were taken from the core and edge of the cheeses.

2.7. Statistical analysis

Two-way ANOVA and three-way ANOVA were performed to determine significant differences ($P < 0.05$) among cheeses at different brining times, ripening time, depending on DL-starter culture and chymosin type. Differences were classified by the Ryan-Einot-Gabriel-Welsch multiple range test (SAS, version 9.3, SAS Institute Inc., Cary, NC). See Table 1 for the different variables and replicates.

3. Results and discussion

3.1. Chemical composition during ripening

Samples of all cheeses were collected during ripening for chemical compositional profiling. Fig. 1 shows the chemical composition for the semi-hard Danish cheeses produced in experiment 1, with DL-starter culture C1, during ripening. Similar trends were observed for the production of the cheeses in experiment 2, 3 and 4 and are therefore not shown. As expected, a significant increase ($P < 0.001$) in the total NaCl content was found with increased brining time (Fig. 1A). During the ripening period, the total NaCl content did not change significantly, which was also as expected. A small amount of Cl^- was detected in the non-brined cheeses. This is due to naturally occurring Na^+ and Cl^- ions present in the milk before cheese making (Belitz, Grosch, & Schieberle, 2004). Results shown in Fig. 1 are derived from cheeses produced from the same batch of milk, and the only difference among the cheeses was the brining time. Changes in the chemical composition are therefore caused by differences in the NaCl content. The

protein content as a function of brining time and ripening time is shown in Fig.1B. No significant difference in the protein content was observed between brining times after 1, 2, and 7 weeks of ripening.

As ripening time increased to 12 weeks, a significant decrease in the protein content for the non-brined cheeses was observed ($P < 0.05$). It is known that the water activity is higher and bacterial activity is increased, when the NaCl content is lowered, which might increase proteolysis (Guinee, 2004). It correlates with our previous results (Søndergaard et al., 2015), where the microbial activity in cheeses from experiments 1 and 2 were analysed. The cheese made using DL-starter culture C1 was found to have significantly higher number of colony forming units (cfu) per gram in the non-brined cheeses after 1 and 2 weeks of ripening (58 and 71%, respectively) compared with the cheeses treated 24 h in brine (21 and 22%, respectively), hence the proteolytic activity could likely be higher as the NaCl content decreased.

Proteolysis in non-brined cheeses is primarily due to primary proteolysis as NaCl-reduction accelerates the degradation of casein due to higher chymosin activity (Møller et al., 2012). With respect to non-starter lactic acid bacteria (NS-LAB), these were investigated and described in detail for cheeses produced in experiment 1 (Søndergaard et al., 2015). Here, the results for cheeses produced with C1 and C2 showed no significant influence of the NaCl concentration on the NS-LAB counts during the ripening period. However, the normal-salted cheeses had slightly lower NS-LAB counts after 2 weeks of ripening in cheeses produced with C1. Based on this we do not expect that the NS-LAB population will have a major impact on secondary proteolysis in this study.

The soluble peptides and free amino acids can to some extent be released from the protein matrix in the cheese and diffuse into the soluble fraction of the cheese, which may reduce the protein content in the cheese (Fox et al., 2000); however, as the protein content here was based on nitrogen analysis the peptides and amino acids still counted in the protein content (ISO8968-1 & IDF020-1, 2014). A more likely explanation could be the higher moisture content of non-brined cheeses (Fig. 1C), where the decrease in dry matter content with ripening time was more apparent for the non-brined cheeses ($P < 0.05$).

The content of dry matter, Fig. 1C, was found to increase as the brining time increased, which was expected ($P < 0.001$). This is caused by NaCl migrating from the rind into the cheeses during ripening and water was expelled from the cheese (Guinee, 2004).

Fig. 1D shows the development in pH during ripening. The pH after 1 week was lower than pH of the cheese ripened for 2–12 weeks. This is related to the degradation of lactose by the lactic acid bacteria of the DL-starter culture (McSweeney & Fox, 2004). Ripening times above 1 week resulted in significantly increase in pH with only small changes in pH from ripening weeks 2–12. This has been shown to be caused by rebalancing of calcium phosphate equilibrium in the cheeses, proteolysis of proteins and degradation of lactic acid (Hassan, Johnson, & Lucey, 2004; McMahon et al., 2014). Cheeses with 24 h of brining time had generally lower pH during ripening (Fig. 1D), which seems to be related to increased syneresis (Nielsen, 2006). Furthermore, a difference between starter cultures is reported, as for C1 the pH was lower with increased salt content, whereas for C2 the pH did not vary significantly as function of salt content (Søndergaard et al., 2015). This also contributes to the explanation of the interlinked effect between the specific DL-starter culture and its activity, the protein content and the resulting pH, as the accumulation of organic acids inhibits the growth of microorganisms, i.e., inevitably also the starter culture (Beresford et al., 2001).

Overall, these findings in brined semi-hard Danish cheese are in agreement with previous findings in dry-salted Cheddar cheese by Schroeder et al. (1988) and Rulikowska et al. (2013), who analysed the chemical changes in Cheddar cheese with reduced NaCl content during ripening.

3.2. NaCl distribution in the cheese

The Cl^- content in the cheeses was measured at various positions from the edge to the core of the cheeses as representative of the NaCl distribution in the cheeses during ripening. Fig. 2 shows the NaCl content in the cheese samples from the edge and core as a function of brining time for ripening times of 2, 7 and 12 weeks in experiment 2. Similar observations were found in experiments 1 and 3 (data not

shown). At 2 weeks of ripening, (Fig. 2A) a significant ($P < 0.01$) difference in the NaCl content between edge and core of brine treated cheese samples was observed, with samples from the edge having the highest content of up to 3% (w/w) NaCl with a gradient to the core of ~1.5% (w/w) for the 24 h brined cheese . At 7 weeks of ripening (Fig. 2B) a significant ($P < 0.05$) increase in NaCl concentration was found in the core, while the NaCl content in the edge decreased (NS), compared with 2 weeks of ripening.

For brining at both 12 h and 24 h, the difference in NaCl content between edge and core was still significant ($P < 0.05$) after 7 weeks of ripening. After 12 weeks (Fig. 2C), NaCl was equally distributed between edge and core of the cheeses with 12 h brining, but not for the 24 h brined cheeses. This diffusion of NaCl from edge to core of the cheese is driven by the concentration gradient (Geurts, Walstra, & Mulder, 1980). The time to reach NaCl equilibrium depends on cheese type, size and shape of the cheeses and ripening temperature. Sutherland (2002) observed similar results for 10 kg Gouda cheeses.

3.3. NaCl and DL-starter culture

The cheeses in experiments 1, 2 and 3 with three different commercial DL-starter cultures (C1, C2 and C3) were analysed to evaluate the effect of the DL-starter culture on the chemical composition and textural properties of semi-hard cheeses. The mean NaCl contents of the cheeses with the three different DL-starter cultures are shown in Table 2 as a function of brining time after 12 weeks of ripening. The NaCl contents in the cheeses were analysed as an average of the entire cheese in contrast to the positional analysis, shown in Fig. 2. The DL-starter cultures did not affect the NaCl content of the cheeses significantly. Other factors may contribute to the final NaCl contents such as dairy factory, milk batch, pressing of the cheese, pore size and structure, brine saturation, etc. Furthermore, the cheeses produced with C3 had a tendency towards higher dry matter content, while no other differences in the chemical composition were observed (data not shown).

350 The most efficient brining was achieved during the first 6 h (experiments conducted with DL-
351 starter cultures C1 and C2) with a rate of 0.138±0.006% NaCl h^{-1}, while thereafter it decreased to
352 0.053±0.003% NaCl h^{-1} from 6–12 h and finally during the last 12 h of brining the rate was lowered to
353 0.036±0.002% NaCl h^{-1} for all starter cultures. This suggests that the brining process and NaCl uptake for
354 these semi-hard Danish cheeses occurred in a very consistent way, regardless of the above-mentioned
355 differences between the dairies, milk batches and DL-starter cultures.
356
357 *3.4. Cheese textural change during ripening*
358
359 The cheese firmness, illustrated as axial stress, and compressibility, illustrated as Hencky strain,
360 for experiment 2 with DL-starter culture C1 and C2 according to ripening time are shown in Fig. 3. The
361 firmest cheeses were found with ripening of 1 week for all brining times and starter cultures, Fig.3A. As
362 the ripening time increased, the firmness decreased significantly ($P < 0.01$) for all cheeses. These results
363 are in agreement with Murtaza et al. (2014), who followed the texture profile in Cheddar cheeses with
364 various NaCl content during ripening. The firmness is correlated to the proteolysis, i.e., increased
365 proteolysis during ripening results in decreased firmness of the cheeses (Fox, 1989; McSweeney, 2004).
366 The cheese network of caseins is weakened by the proteolytic degradation into peptides, and as a result,
367 the texture becomes softer over time.
368 The decrease in firmness was most pronounced for C2 with a brining time of 24 h while cheeses
369 subjected to 0 h and 12 h of brining showed similar decreases in firmness. For both DL-starter cultures,
370 the relative loss in cheese firmness during 12 weeks of ripening was highest for the non-brined cheeses
371 with 68–72% loss relative to week 1. During the same period, the 24 h brined cheeses had a textural loss
372 of 36–49%. It is noteworthy, that this was mainly caused by differences in the initial cheese firmness, as
373 the actual decrease in stress was 19.4 ± 0.5 kPa (all cheeses produced with C1) and 27.0 ± 3.3 kPa (all
374 cheeses produced with C2) during ripening regardless of brining time. This suggests a very similar
375 development in cheese structure and therefore firmness during ripening.

376 Overall, the use of DL-starter culture C2 generally resulted in significantly ($P < 0.05$) firmer
377 cheeses compared with C1, regardless of brining time. From the standard deviation bars of Fig. 3, it is
378 clear that large variations between samples were observed, especially in week 1 of the ripening period,
379 while the variation between samples decreased as ripening time increased. This is due to that the mean
380 value was generated from samples from both edge and core. As shown in Fig. 2 there were large
381 variations in NaCl content among samples from edge and core of the cheese, until final ripening stage was
382 reached, which resulted in variations in firmness.

383 The compressibility is given as Hencky strain as function of brining time, ripening and starter
384 culture (Fig. 3B). For the present semi-hard Danish cheeses, a high Hencky strain value indicated a highly
385 compressible or elastic cheese, while a low Hencky strain correlated with a cheese that fractured at low
386 compression distance and was observed as more brittle. This is consistent with previous findings for
387 Gouda cheese (Luyten, 1988). Throughout the ripening time, small variations for all salted cheeses
388 occurred, but these were not significant. The non-brined cheeses increased in compressibility for ripening
389 times of 2–7 weeks ($P < 0.01$). For these cheeses, there was often not detected a fracture point of the
390 cheese cylinder during the textural compression analysis. The samples were very elastic and could be
391 compressed >83% without breaking during the analysis. These non-brined cheeses were also more prone
392 to temperature, which made them lose their cylindrical structure very quickly, while all salted cheeses
393 retained their shape at room temperature.

394 Generally, it is found that the compressibility decreases during ripening for cheeses like Cheddar
395 and Gouda (Luyten, 1988; Zoon, 1993). Furthermore, Watkinson et al. (2001) observed an increase in
396 Hencky strain during ripening of Gouda cheeses. In this study, the compressibility appeared unaffected by
397 the changes occurring in the cheese during ripening of salted cheeses.

398 In comparison, the stress at fracture and Hencky strain values of 7 week ripened Danbo (30+)
399 cheeses is reported to be 92 kPa and 1.10 (-), respectively, by Madsen and Ardö (2001), which is
400 somewhat higher in stress at fracture than observed in the present study, where 7 week ripened cheeses
401 had values of ~45 kPa (Fig. 3). Their compressibility levels are, however, comparable with levels

presented in Fig. 3B. The cheese firmness may be affected by a range of processing parameters, although the dry matter content was ~47% in both studies. This is illustrated for the textural analysis in experiment 3, which resulted in much firmer reference cheeses (24 h brining, culture C3, bovine chymosin) after 12 weeks ripening with fracture stress values of 100 kPa and Hencky strain of 1.09 (-) (data not shown).

Søndergaard et al. (2015) analysed the number of viable lactic acid bacteria (LAB), the extent of autolysis and also determined free amino acids of the cheeses as used in experiment 1 and 2. For the DL-starter culture C1, growth was found to be more affected by the NaCl concentration as compared with the DL-starter culture C2. Elevated levels of free amino acids have previously been found to increase stress and decrease strain due to binding of water to peptide bonds in the cheese matrix (Børsting et al., 2012; McSweeney, 2004)**,** which can relate to the observed variation in texture between C1 and C2.

SEM micrographs of cheeses from experiment 2 (DL-starter cultures C1 and C2) brined for either 0 h or 24 h after 12 weeks of ripening are shown in Fig. 4. The holes in the protein matrix originate from fat and water, which were removed during sample preparation. Variations in the number and size of voids in the cheese matrix can be observed. Fig. 4B and Fig. 4D show cheeses with 24h brining time. These had a more clearly structured protein matrix with many and smaller voids than in Fig. 4A and Fig. 4C, which are micrographs of non-brined cheeses. The protein matrix of the non-brined cheeses appeared less defined, which was seen by fewer and slightly larger voids. Comparing DL-starter culture C1 and C2, there was a tendency towards a more defined protein matrix when using C1. However, this was not confirmed with certainty by the SEM analysis. These microstructural observations support the chemical and textural results as the less defined protein matrix structure visualized by the SEM would be expected to result in softer and more compressible cheese texture as observed.

3.5. Textural change as an effect of NaCl

Cheese samples used for textural analysis were also analysed for NaCl content to explore the correlation between NaCl content and textural properties. Fig. 5 shows the correlation between textural

properties and NaCl content for cheeses from experiment 2, for both DL-starter cultures C1 and C2, during ripening of cheese samples from both edge and core. For the non-brined cheeses, there was not always a detectable fracture point of the cheese cylinders, when performing the texture analysis. These samples were so elastic that they could be compressed without breaking, and they were therefore not included in Fig. 5.

The firmness, given as axial stress, as a function of NaCl content is shown in Fig. 5A–C. An increase in firmness was observed with increasing NaCl content. This was expected, as NaCl is a major contributor to the formation of a strong gel network (Guinee, 2004; Mistry & Kasperson, 1998; Schroeder et al., 1988). After 2 weeks of ripening (Fig. 5A), large variations in texture were found between samples. During further ripening, (Fig. 5B,C), these variations became less pronounced and after 12 weeks of ripening there was a linear correlation with a regression coefficient of $R^2 = 0.75$. These observations could be related to the results shown in Fig. 2, which showed large differences in NaCl between edge and core in early ripening, while this became less pronounced during ripening.

The compressibility of the cheese, given as Hencky strain, (Fig. 5D–F) decreased linearly as the NaCl content increased. The fracture point of the cheese sample thereby occurred at a shorter distance in the textural compression analysis, which means that the samples became less elastic and more brittle. As for the firmness, the compressibility showed large variations among samples after 2 weeks of ripening and this became less during ripening. The correlation coefficients were generally low with R^2-values between 0.12-0.47, and the fit was poorest for the 2 weeks ripened cheeses. Especially for low salt content cheeses, the variations in Hencky strain at 7 and 12 weeks of ripening were very high. In perspective, a cheese with 0.5-1% (w/w) NaCl could have been useful to include to complete the picture.

The higher number of samples depicted in Fig. 5 revealed novel information on the texture in cheese core and cheese edge when salt migrated during ripening. At the very beginning of ripening (Fig. 5A,D), the core and edge of brined cheese were clustered based on the textural properties, while increased ripening time resulted in more textural uniformity between the edge and core samples (Fig. 5C,F).

453 The relationship between firmness and compressibility and NaCl content for cheeses produced
454 with DL-starter cultures C1, C2 and C3 and ripened for 12 weeks are shown in Table 2. Experiment 3
455 cheeses with DL-starter culture C3 were produced at same dairy plant but at a different time, compared
456 with cheeses produced with the C1 and C2 DL-starter cultures from experiment 1.

457 Comparing the DL-starter cultures, all cultures had similar tendencies to increase firmness with
458 increased NaCl content (Table 2). However, the DL-starter culture C3 in experiment 3 produced much
459 firmer cheeses at comparable brining hours than C1 and C2 in experiment 1, which resulted in 2-fold
460 higher axial stress values for C3 compared with C1, regardless of NaCl content. The usage of starter
461 culture C3 showed higher variations in firmness, which was found relating to variations between
462 replicates. The variations were relatively lower for the DL-starter cultures C1 and C2. However, as
463 explained earlier it is noted that the experimental set-up did vary for the cheeses produced with C1 and C2
464 as compared with the cheeses produced with C3.

465 The compressibility, given as Hencky strain, for cheeses produced with C2 tended to be lower
466 than cheeses produced with C3, while cheeses produced with C1 had the highest compressibility, i.e., they
467 were more elastic.

468 C1 resulted in cheeses with lower firmness and higher compressibility compared with C2 and C3
469 at similar brining times resulting in NaCl concentrations within a range of 0.11% at 6 h, 0.13% at 12 h,
470 and 0.20% at 24 h brining time (Table 2). This indicates that the more defined DL-starter cultures,
471 represented by C2 and especially C3, might result in firmer and more brittle cheeses. C3 resulted in the
472 most firm cheeses, but these cheeses had also larger compressibility compared with the cheeses produced
473 with C2. This indicates that the casein network of cheeses produced with C2 was more compact and
474 therefore broke more easily.

475 Scientific studies on the relationship between NaCl content and cheese texture for brined semi-
476 hard Danish cheeses are not available. However, for Cheddar cheese made from buffalo milk a reduction
477 of NaCl content from 2.5% to 0.5% (w/w) resulted in lower hardness and crumbliness of the cheese
478 textual properties (Murtaza et al., 2014). In another study, NaCl in Cheddar cheese was reduced from

2.3% to 0.9% (w/w); however, by maintaining an equal moisture content of 37.6 ± 0.1%, the textural properties of the cheeses in the range from 0.9–1.7% (w/w) NaCl were kept similar (Møller, Rattray, Bredie, Høier, & Ardö, 2013). Also, replacing NaCl partly by other salts like KCl, $MgCl_2$ and $CaCl_2$ is reported to alter the hardness of Cheddar cheese in ways of both increased hardness and decreased hardness, even though the salt-to-moisture relationship and water activity was maintained at the same level (Grummer et al., 2012). The general trend of reducing NaCl in Cheddar cheese is a parallel change in textural properties (Floury et al., 2009; Rulikowska et al., 2013; Saint-Eve, Lauverjat, Magnan, Déléris, & Souchon, 2009), unless the NaCl reduction is substituted with other salts and/or moisture management is addressed.

3.6. Cheese textural effects of chymosin type

Cheeses of experiment 3 and 4 were analysed with regard to the effect of the origin of chymosin, camel (CC) or bovine (BC) on the chemical composition and textural properties of the cheese. However, as the choice of DL-starter culture varied between the cheese productions, the experiments cannot be compared directly. Table 3 shows the chemical composition and textural properties of the cheeses made with either chymosin type CC or BC for both experiments 3 and 4 after 12 weeks of ripening. Again, a significant increase in NaCl content was observed as the brining time increased ($P < 0.05$), but no differences were found when comparing the chymosin types at equal brining times. The NaCl uptake in the cheeses was thus apparently not affected by the chymosin type. The dry matter content increased as brining time increased, but no significant differences between BC and CC cheeses were observed. The firmness of the cheeses with 6 h of brining for CC cheeses produced significantly ($P < 0.05$) firmer cheeses compared with BC cheeses at equal brining times (Table 3). This is in agreement with Elagamy (2000), who observed that CC activity was less affected by low NaCl concentrations, while at high NaCl concentration both chymosin types were more equally affected. At brining times of < 12 h , there was a significant textural effect of CC resulting in firmer cheeses than BC (experiment 3, Table 3), whereas at

505 brining times longer than 10 h, a tendency towards firmer cheeses with CC compared with BC was
506 observed; however, this effect was not significant. In experiment 4, the CC renneted cheese brined for 15
507 h had an axial stress level comparable with the BC renneted cheese brined for 28 h (Table 3) even at a
508 NaCl content that was reduced by 18%. Firmer cheeses are generally found when using CC compared
509 with BC (Bansal et al., 2009; Børsting et al., 2012; Govindasamy-Lucey, Lu, Jaeggi, Johnson, & Lucey,
510 2010; Moynihan et al., 2014). It was therefore expected that the CC would result in firmer cheeses, as the
511 amount of enzymes added corresponded to equal enzymatic activities (IMCU per mL milk). Different
512 results among studies are most likely caused by variations in cheese type, DL-starter culture and amount
513 of chymosin added.

514 The compressibility decreased as the NaCl content increased. For experiment 3, no significant
515 differences in compressibility were found between chymosin types. In experiment 4, a significantly lower
516 ($P < 0.001$) compressibility was observed for CC compared with BC at comparable brining times of 28 h.
517 Furthermore, for practical reasons, it was decided to not include a control treatment (0 h brining) in
518 experiment 3, and only for the BC treatment in experiment 4. Basic knowledge on non-brined cheeses
519 textural properties was obtained in experiments 1 and 2, and since the perspective for the Danish dairies is
520 to reduce salt in cheese rather than avoiding salt in cheese, it was prioritised to include more treatments
521 with reduced salt rather than with no salt in experiments 3 and 4.

522 SEM micrographs of cheeses from experiment 4 with 28 h of brining and ripened for 12 weeks
523 are shown in Fig. 6. The structure of CC cheese (Fig. 6B) appears finer stranded and more compact than
524 the BC cheese (Fig. 6A) and contains many small pores, while the BC cheese appears to contain more
525 open network of relatively larger pores. Since this is the first time SEM images of salt reduced semi-hard
526 brined cheeses are reported, we cannot compare to other studies. The structure show some agreement with
527 Weijers, van de Velde, Stijnman, van de Pijpekamp, and Visschers (2006), who observed that gels
528 composed of relatively thin network strands and small homogeneous pores are more brittle and would
529 fracture at low strain values, while gels that fracture at high strain values are composed of thicker strands
530 and relatively larger homogeneous pores.

4. Conclusions

Overall, this study has provided new knowledge on the effect of NaCl, DL-starter culture and chymosin type on the textural properties and chemical composition of Danish semi-hard cheeses. Shorter brining time reduced the NaCl content with a significant influence on firmness, compressibility and chemical composition of the cheeses. Cheese firmness increased and compressibility decreased linearly as the NaCl content increased. The three different DL-starter cultures influenced the textural properties of the cheeses. The most defined DL-starter culture, i.e., C3, produced significantly firmer cheeses while retaining a relative compressible cheese structure. The firmness was higher for cheeses made using camel chymosin at low NaCl content than for cheeses renneted with bovine chymosin. The compressibility of the cheeses was not significantly affected by chymosin type. However, the DL-starter culture may interact with the chymosin type in relation to cheese textural compressibility.

It therefore seems possible to reduce the NaCl content in semi-hard cheeses without compromising the textural properties by use of well-defined DL-starter cultures and camel chymosin. The cheese experiments performed at industrial scale provided novel insight into controlling cheese texture by brining under conditions that are readily applicable by the dairy industry. As the NaCl content also has an effect on the activity of the DL-starter cultures and the flavour formation, it is of importance to obtain knowledge on these parameters.

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Figure legends

Fig. 1. Composition of semi-hard Danish cheeses from experiment 1 with DL-starter culture C1. A: NaCl content, B: protein content, C: dry matter and D: pH are shown as a function of ripening times (week) for brining times of 0 h (black), 6 h (dark grey), 12 h (grey) and 24 h (white). The results are shown as the mean ± SD, n = 3. Bars with different letters above differ significantly ($P < 0.05$).

Fig. 2. NaCl distribution in semi-hard Danish cheeses from experiment 2 during ripening as a function of brining time, for samples from core (open circles) and edge (filled circles), with ripening times of 2 weeks (A), 7 weeks (B) and 12 weeks (C). Values are shown as mean ± SD, n = 6; values with different letters above are significantly different by a 3-factor interaction ($P < 0.05$).

Fig. 3. Textural parameters Axial stress (A) and Hencky strain (B) for cheeses from experiment 2, produced with DL-starter culture C1 (■, ■, ■) and C2 (■,■,□) in combination with brining times of 0 h (■, ■), 12 h (■, ■), and 24 h (■, □) according to ripening times (week). Values are shown as mean ± SD, n = 24.

Fig. 4. Experiment 2. Scanning electron microscopy images (2000 × magnification) of semi-hard Danish cheeses after 11 weeks of ripening. A) DL-starter culture C1 with 0 h brining, B) DL-starter culture C1 with 24 h brining, C) DL-starter culture C2 with 0 h brining and D) DL-starter culture C2 with 24 h brining.

Fig. 5. Correlations from experiment 2 of semi-hard Danish cheese NaCl content with textural properties; Axial stress (A+B+C) and Hencky strain (D+E+F) at fracture for ripening periods of 2 weeks (A+D), 7 weeks (B+E), and 12 weeks (C+F) for samples from core (open circles) and edge (filled circles). Linear regressions and the corresponding regression coefficient are given.

Fig. 6. Scanning electron microscopy images (5000 × magnification) of semi-hard Danish cheeses from experiment 4 receiving 28 h of brining and 12 weeks of ripening, using bovine chymosin (A) or camel chymosin (B).

Table 1

Schematic overview of the four cheese experiments along with the main parameters studied, time of analysis during ripening and number of replicates. [a]

Experiment	Chymosin type	DL-starter culture	Brining time (h)	Ripening time (weeks)	Cheese replicates	Positions for texture sampling
1	BC	C1	0, 6, 12, 24	1, 2, 7, 12	3	12
		C2	0, 6, 12, 24	1, 2, 7, 12	3	12
2	BC	C1	0, 12, 24	1, 2, 7, 12	2	12
		C2	0, 12, 24	1, 2, 7, 12	2	12
3	BC	C3	6, 12, 24	1, 12	2	12
	CC	C3	6, 12, 24	1, 12	2	12
4	BC	Commercial used at Taulov dairy	0, 28	12	12	2
	CC		10, 15, 28	12	12	2

[a] Abbreviations are: BC, bovine chymosin; CC, camel chymosin; C1, C2 and C3, DL-starter cultures originating from Chr. Hansen A/S.

Table 2

Content of NaCl (%) and textural properties by axial stress (kPa) and Hencky strain (-) of semi-hard Danish cheeses after 12 weeks of ripening from experiment 1 and 3. [a]

Parameters	Brining time (h)				F-test
	0	6	12	24	
C1 - experiment 1					P-value
NaCl (%)	0.21±0.03^{d}	1.00±0.09^{c}	1.30±0.15^{b}	1.70±0.10^{a}	<0.001
Stress (kPa)	-*	25.9±9.1^{b}	33.3±1.8^{b}	48.6±18.3^{a}	<0.001
Strain (-)	-*	1.34±0.17^{a}	1.26±0.18^{a}	1.11±0.14^{b}	<0.05
C2 - experiment 1					
NaCl (%)	0.19±0.03^{d}	1.05±0.05^{c}	1.39±0.07^{b}	1.83±0.14^{a}	<0.001
Stress (kPa)	19.8±4.8^{c}	36.5±11.9^{b}	48.4±16.1^{ab}	53.6±18.1^{a}	<0.001
Strain (-)	1.37±0.34^{a}	1.24±0.13^{b}	1.19±0.06^{b}	1.06±0.10^{c}	<0.001
C3 - experiment 3					
NaCl (%)	-**	1.11±0.04^{c}	1.43±0.13^{b}	1.90±0.18^{a}	<0.05
Stress (kPa)	-**	54.5±18.4^{b}	82.5±31.8^{a}	95.9±23.9^{a}	<0.001
Strain (-)	-**	1.30±0.10^{a}	1.25±0.07^{a}	1.07±0.11^{b}	<0.001

[a] The cheeses were produced with bovine chymosin and DL-starter cultures C1, C2 and C3. Values are means ± standard deviation, n=6 (NaCl content), n=36 (textural analysis exp. 1), and n=24 (textural analysis exp. 3); values within a row with different superscript letters differ significantly at the level of given *P*-value. A single asterisk indicates no textural analysis was performed; a double asterisk indicates no non-brined cheeses were produced using DL-starter culture C3.

Table 3

Experiment 3 and 4, effect of chymosin type and brining time used for semi-hard Danish cheeses on NaCl content, dry matter, pH, and textural properties after 12 weeks of ripening. [a]

Chymosin type	Brining time (h)	NaCl (%, w/w)	Dry matter (%, w/w)	pH	Axial stress (kPa)	Hencky strain (-)
Experiment 3						
BC	6	1.11±0.03^{c}	45.9±0.5^{b}	5.51±0.04^{a}	54.5±18.4^{c}	1.31±0.10^{a}
BC	12	1.43±0.12^{ab}	48.5±1.1^{a}	5.51±0.05^{a}	82.5±31.8^{b}	1.25±0.07^{a}
BC	24	1.90±0.18^{a}	49.2±0.1^{a}	5.37±0.06^{a}	95.9±23.9^{a}	1.08±0.11^{b}
CC	6	1.17±0.04^{bc}	47.6±0.1^{ab}	5.53±0.02^{a}	77.7±25.9^{b}	1.33±0.07^{a}
CC	12	1.48±0.70^{ab}	48.7±0.1^{a}	5.48±0.02^{a}	88.0±35.5^{b}	1.25±0.10^{a}
CC	24	1.79±0.21^{a}	49.6±0.8^{a}	5.47±0.10^{a}	91.6±36.3^{ab}	1.05±0.12^{b}
Experiment 4						
BC	0	0.08±0.05^{c}	46.3±0.1^{a}	5.41±0.02^{b}	28.5±4.5^{c}	1.65±0.12^{a}
BC	28	1.51±0.11^{a}	47.8±0.1^{a}	5.52±0.02^{a}	66.0±16.3^{a}	1.23±0.05^{b}
CC	10	1.20±0.08^{b}	46.5±0.1^{a}	5.55±0.02^{a}	54.0±8.1^{b}	1.16±0.08^{c}
CC	15	1.23±0.11^{b}	47.7±0.1^{a}	5.58±0.02^{a}	62.8±7.6^{a}	1.11±0.08^{c}
CC	28	1.53±0.12^{a}	47.4±0.0^{a}	5.53±0.02^{a}	70.6±10.8^{a}	1.09±0.07^{c}

[a] Abbreviations are: BC, bovine chymosin; CC, camel chymosin. Values are least squares-means ± standard deviation (n = 4, chemical analysis; n = 24, textural analysis); values within a column with different superscript letters differ significantly ($P < 0.05$)

Fig. 1

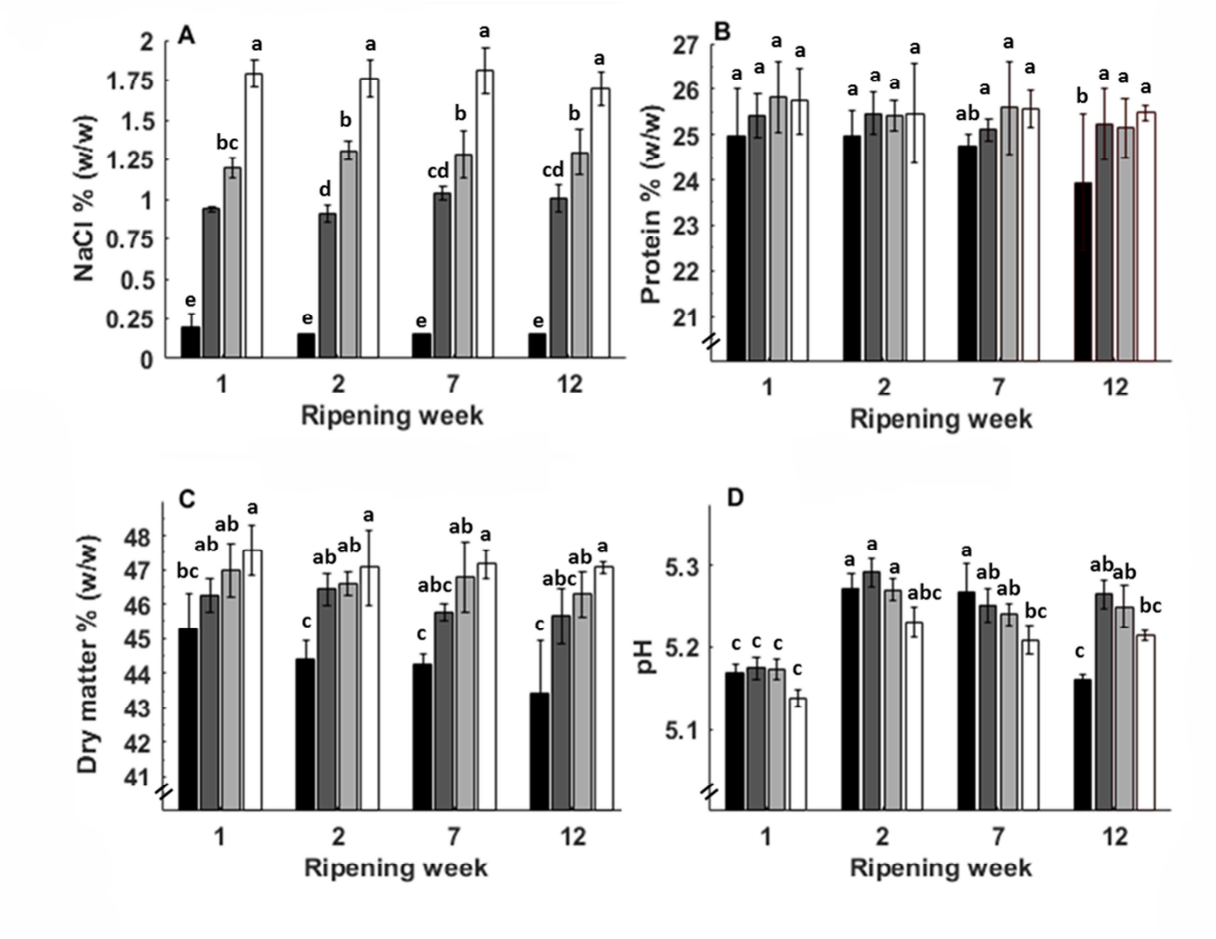

Fig. 2

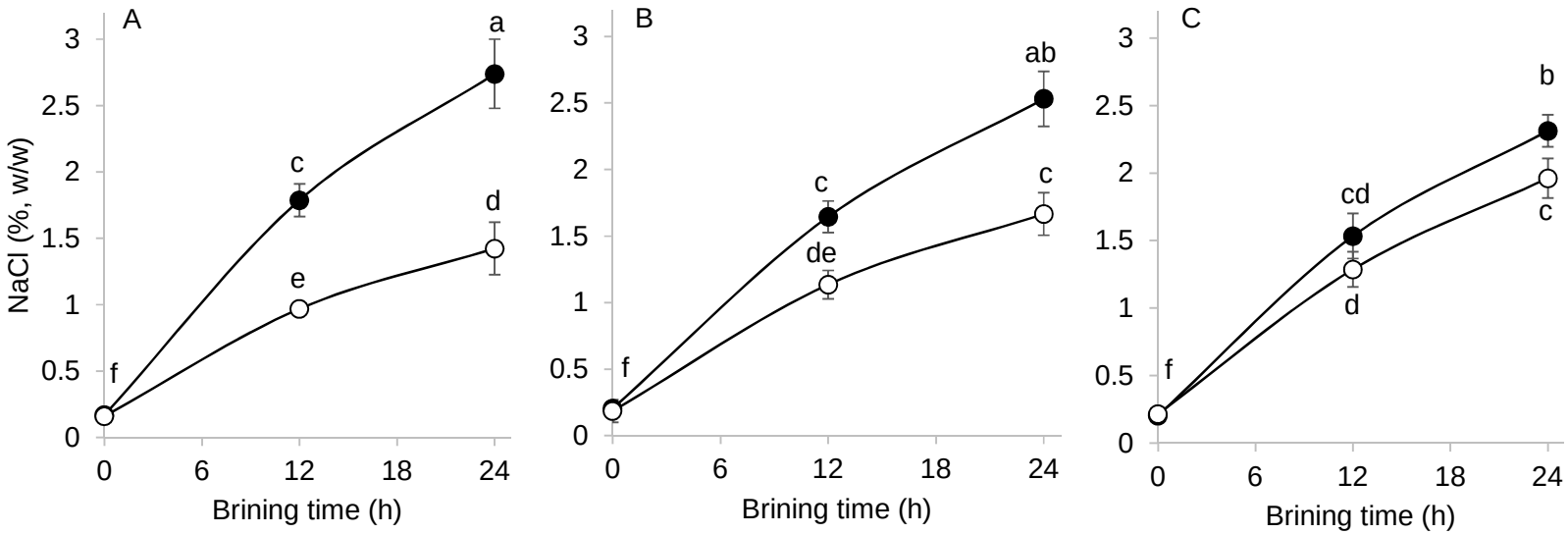
A
B
C
NaCl (%, w/w)
Brining time (h)
Brining time (h)
Brining time (h)
0
0.5
1
1.5
2
2.5
3
0
6
12
18
24
a
c
d
e
f
ab
c
de
f
b
cd
c
d
f

Fig. 3

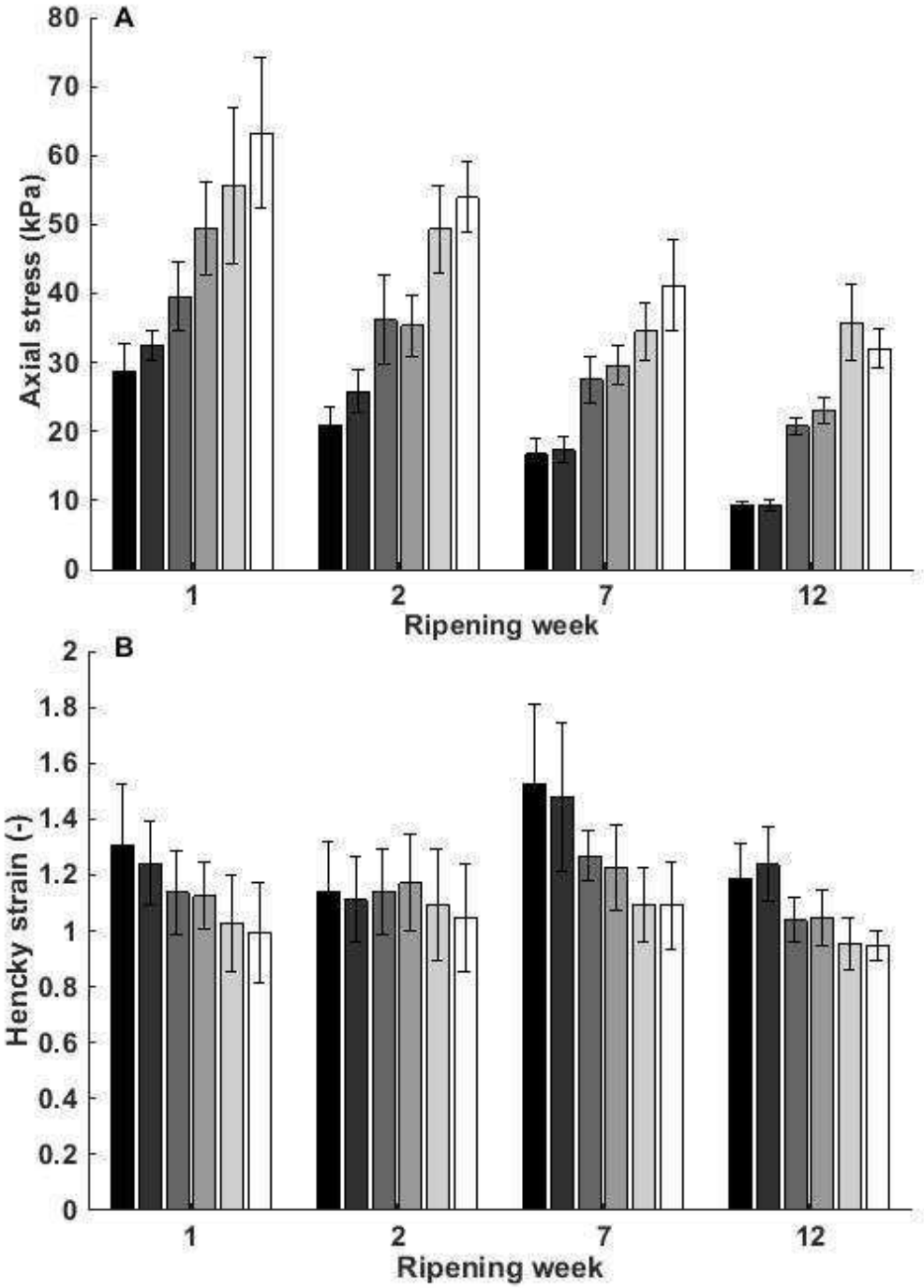
A
Axial stress (kPa)
80
70
60
50
40
30
20
10
0
1
2
7
12
Ripening week
B
Hencky strain (-)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1
2
7
12
Ripening week

Fig. 4

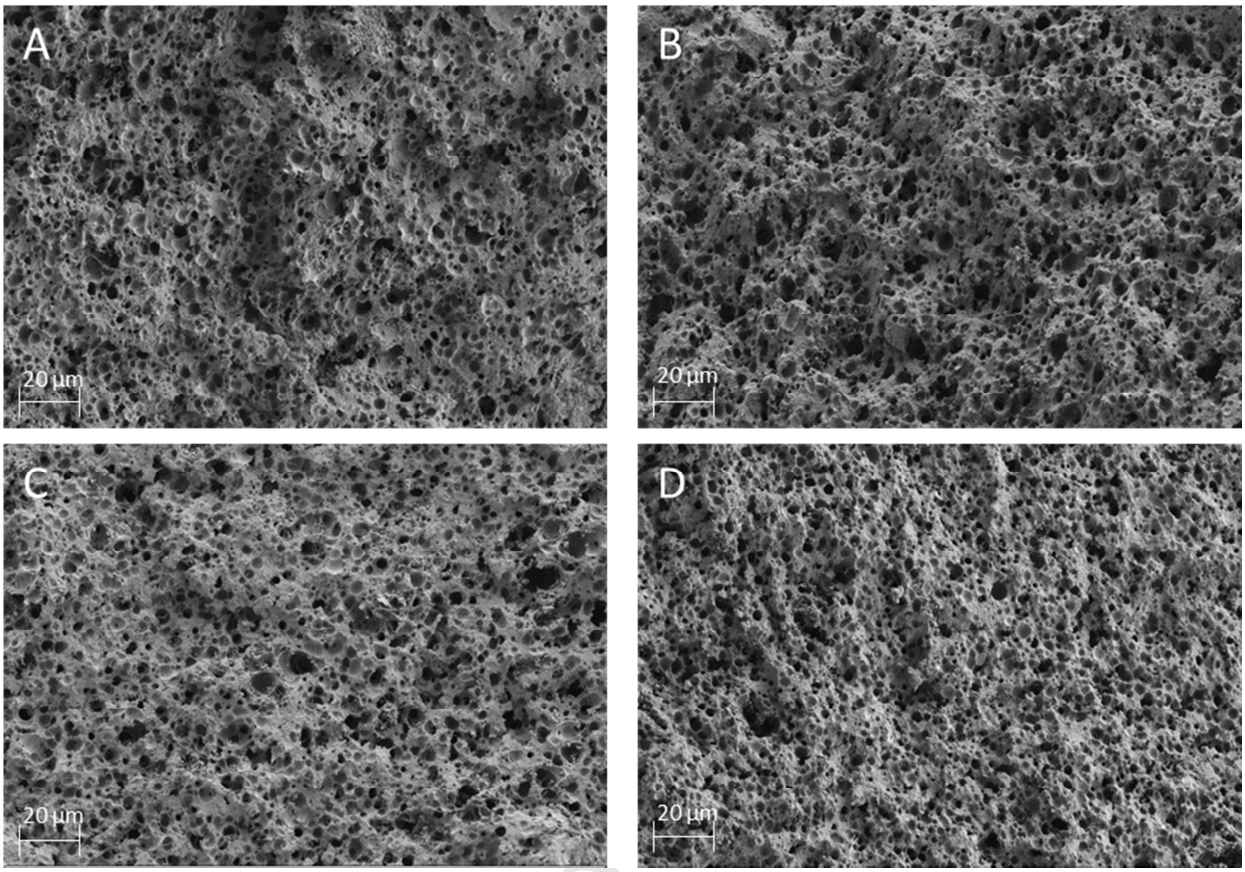

Fig. 5

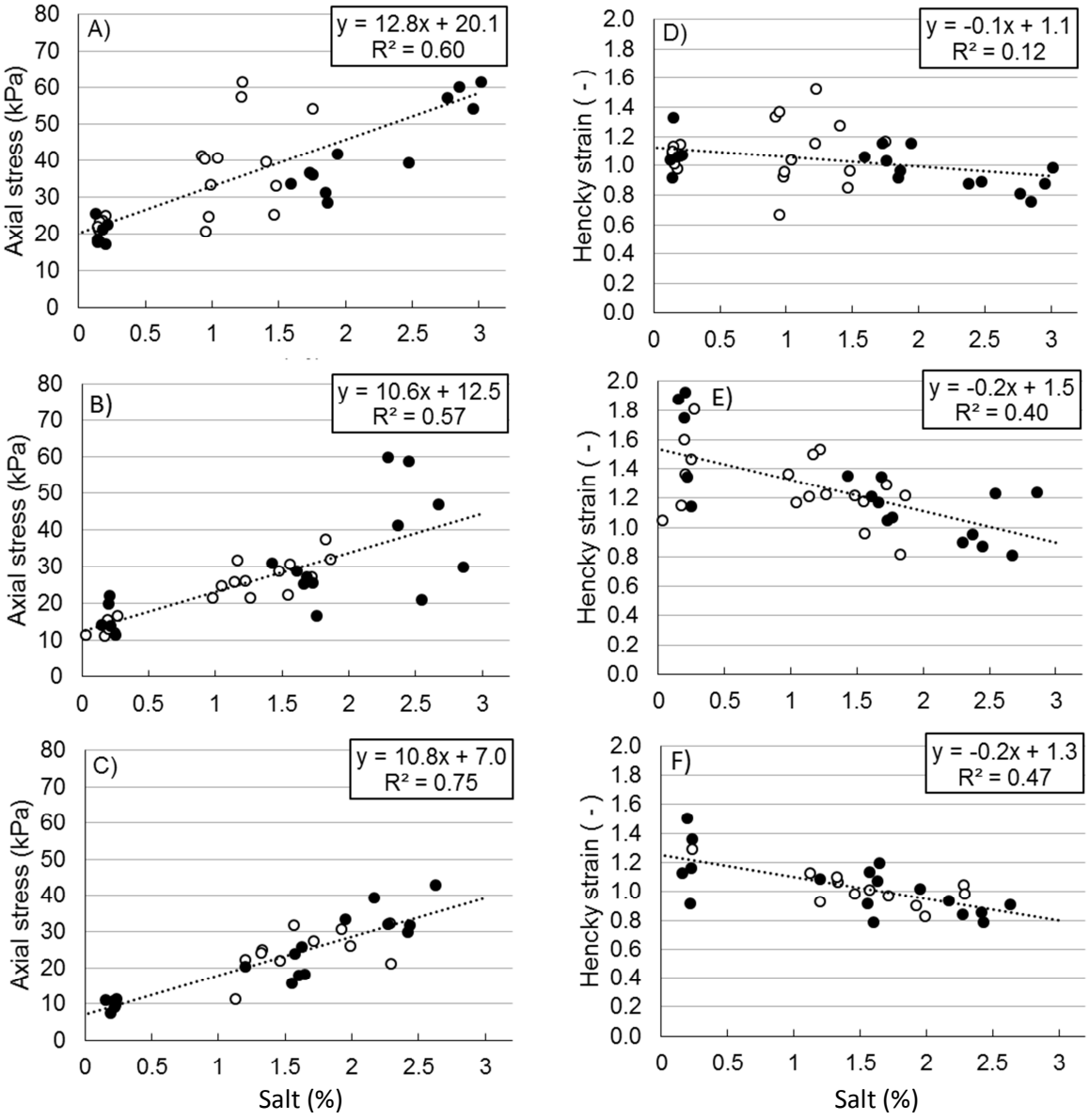
A)
y = 12.8x + 20.1
R² = 0.60
B)
y = 10.6x + 12.5
R² = 0.57
C)
y = 10.8x + 7.0
R² = 0.75
D)
y = -0.1x + 1.1
R² = 0.12
E)
y = -0.2x + 1.5
R² = 0.40
F)
y = -0.2x + 1.3
R² = 0.47
Axial stress (kPa)
Hencky strain (-)
Salt (%)

Fig. 6