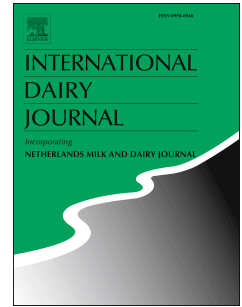


# Journal Pre-proof

Evaluation of production of Cheddar cheese from micellar casein concentrate

Bozhao Li, David S. Waldron, John T. Tobin, Surabhi Subhir, Alan L. Kelly, Paul L.H. McSweeney



PII: S0958-6946(20)30081-9

DOI: <https://doi.org/10.1016/j.idairyj.2020.104711>

Reference: INDA 104711

To appear in: *International Dairy Journal*

Received Date: 5 December 2019

Revised Date: 6 March 2020

Accepted Date: 7 March 2020

Please cite this article as: Li, B., Waldron, D.S., Tobin, J.T., Subhir, S., Kelly, A.L., McSweeney, P.L.H., Evaluation of production of Cheddar cheese from micellar casein concentrate, *International Dairy Journal*, <https://doi.org/10.1016/j.idairyj.2020.104711>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Published by Elsevier Ltd.

**Evaluation of production of Cheddar cheese from micellar casein concentrate**

Bozhao Li<sup>a</sup>, David S. Waldron<sup>a</sup>, John T. Tobin<sup>b</sup>, Surabhi Subhir<sup>b</sup>, Alan L. Kelly<sup>a\*</sup>, Paul L. H.

McSweeney<sup>a</sup>

<sup>a</sup> *School of Food and Nutritional Sciences, University College Cork, College Road, Cork, T12 K8AF, Ireland.*

<sup>b</sup> *Department of Food Chemistry and Technology, Teagasc Food Research Centre, Fermoy, Co. Cork, P61 C996, Ireland.*

\*Corresponding author: Tel: +353-21-4903405

Email address: [a.kelly@ucc.ie](mailto:a.kelly@ucc.ie) (A. L. Kelly)

---

**ABSTRACT**

The production of Cheddar cheese using micellar casein concentrate (MCC), a novel milk ingredient powder with a high casein content (~92%), was evaluated. Four types of Cheddar cheese were manufactured and ripened for 180 days from the following starting materials: standardised control milk (control), skim milk with cream (SC), reconstituted MCC with cream (MC) and reconstituted low-heat skim milk powder with cream (PC). Only minor differences were found in composition between treatments, but MC cheese showed higher levels of proteolysis compared with other treatments, linked to significantly higher plasmin and chymosin activities. No differences were observed in hardness between treatments (60, 120 and 180 days), but the springiness and cohesiveness of MC and PC cheeses were significantly higher than that of the control and SC cheeses at 60, 120 and 180 days. The use of casein-dominant dairy streams has the potential for production of Cheddar cheese with tailored functionality.

---

## 1. Introduction

Global milk production was approximately 843 million tonnes in 2018 (FAO, 2019); among dairy products (i.e., cheese, casein and butter), cheese production used the highest proportion of milk. Whey protein powder, production of which is traditionally associated with the manufacture of cheese, represents 57% of the market for global protein supplement for exercising and nutrition, while there is a huge market for whey protein powders in infant formula. Cheese output is increasing at a rate of 2% yearly while the demand for whey protein has been growing at 6–7% yearly (Hoogwegt Group, 2019). Nowadays, whey protein with high quality is required as it can be used to manufacture a range of food ingredients or products with nutrition and functional properties (Boland, 2011). Kelly (2019) reported that the ultrafiltration (UF) properties of sweet and acid whey are influenced by their high or low pH, respectively. However, microfiltration (MF) permeate made directly from skim milk is considered to be an ideal whey source for whey protein ingredient production. Therefore, recovering whey protein from milk rather than cheese whey could improve the whey quality and is an option for whey protein ingredients manufacture.

During membrane filtration of skim milk, native micellar casein is concentrated in the retentate, which could be recovered and concentrated to produce micellar casein concentrate (MCC). This is a novel dairy ingredient powder with a high casein fraction of 85–95% of total protein which may be used in functional and nutritional applications (Crowley et al., 2018). As the traditional way to manufacture Cheddar cheese is followed by

whey protein manufacture, the concept of recovering whey protein before Cheddar cheese manufacture is of interest.

In terms of the protein ingredients being used for Cheddar cheese manufacture, low heat skim milk powder (LHSMP) can be used to enhance cheese yield giving a constant cheese production throughout the year (Freeman, Bucy, & Hassan, 1970). LHSMP is produced from skim milk using low temperatures during manufacture and is mostly used for condensed milk, UHT-treated fluid milk and ice-cream (Augustin & Margetts, 2003). Unlike reconstituted medium- and high-heat skim milk powder that have impaired rennet coagulation ability, the rennet coagulation properties of LHSMP are good as the whey protein is not highly denatured (Ménard, Camier, & Guyomarc'h, 2005). With only physical separation processing, the micellar casein in MCC may have better rennet coagulation properties compared to LHSMP. The rennet coagulation properties and cheese-making potential of MCC may be better than those of LHSMP.

Two main factors that influence cheese yield are lactation and seasonality. The gross composition of bovine milk varies with the stage of lactation. Kuchtík, Šustová, Urban, and Zapletal (2008) reported that protein and casein content increase and lactose content decreases through lactation. After 200 days lactation, cows are in the late lactation stage, which requires the udder tissue to repair and recover for the next lactation. During late lactation, milk yield decreases dramatically, which may influence cheese yield, unless milk is standardised. Also, at specific times of the year, the milk volume decreases, and the composition and rennet coagulation properties of milk are significantly influenced (Freeman et al., 1970; O'Brien, Mehra, Connolly, & Harrington, 1999). Both cheese yield and

manufacturing efficiency are influenced by seasonal variation of milk protein and fat composition (Barbano & Sherbon, 1984).

To solve the problem of low cheese yield, one solution is adding LHSMP to low-protein milk to increase the protein composition (Freeman et al., 1970). Another possibility may be making Cheddar cheese with MCC that is manufactured as a co-product of high-quality whey protein.

This study evaluated the production of Cheddar cheese from micellar casein concentrate, with LHSMP used for comparison. The consequence of this manufacture was evaluated concerning composition, proteolysis, texture and functionality.

## **2. Materials and methods**

### *2.1. Preparation of cheese milk*

Micellar casein concentrate (MCC) powder was obtained from Teagasc (Moorepark, Fermoy Co. Cork, Ireland); whereby pasteurised bovine skim milk was microfiltered at 50 °C using 0.14 µm TAMI Isoflux ceramic membranes (TAMI, Lyons, France). MF was performed in a batch mode involving two diafiltration steps to produce a final 3× MF retentate (liquid MCC) which was evaporated at 65 °C using a pilot plant single-effect falling-film evaporator (Anhydro F1 Laboratory; Copenhagen, Denmark). This was followed by spray-drying of the evaporated liquid MCC in a single-stage spray dryer (Anhydro Laboratory Spray Dryer, SPX Flow Technology, Denmark) equipped with nozzle

atomisation at inlet and outlet temperatures of 185 and 85 °C, respectively. The protein content (91.6% casein) of MCC was determined by the Kjeldahl method (IDF, 1986). MCC powder and low heat skim milk powder (LHSMP; WPNI=6.0) (Uelzena, Uelzen, Germany) were rehydrated using a Silverson mixer at 55 °C for 2 h.

Raw milk was obtained from a local farm in Cork, Ireland. Skim milk and cream were separated from raw milk using a separator. Composition of milk samples was measured by MilkoScan™ Mars (Foss, Hillerød, Denmark) and casein content was calculated by multiplying the casein percentage of protein of the milk sample (an estimated percentage of 78% for milk and LHSMP milk and 91.6% for MCC). Control milk was prepared by combining raw milk and skim milk to a casein: fat ratio of 0.7:1. Both reconstituted MCC and reconstituted LHSMP were made up to the same casein level as skim milk. Reconstituted MCC and LHSMP and skim milk were blended with cream and lactose according to the same standardization ratio (casein: fat of 0.70:1.00) and lactose content (~5%) as control milk to obtain three more vats: skim milk with cream (SC), reconstituted MCC milk with cream (MC) and reconstituted LHSMP milk with cream (PC). All milk samples were pasteurised at 72 °C for 15 s (Microthermics Inc., Raleigh, NC, USA) and stored at 4 °C until analysis and cheese-making.

## 2.2. *Rennet coagulation properties*

Dynamic oscillatory analysis (small amplitude oscillatory measurement) of renneted control milk, SC milk, MC milk and PC milk was performed using a Peltier concentric

cylinder geometry, which comprised of an aluminium conical rotor [42.01 mm (h) by 28.02 mm (d)], on an AR-G2 controlled stress rheometer (Waters TA Instruments, Leatherhead, Surrey, UK). Aliquots (25 mL) of each sample were pre-warmed in a water bath at 32 °C for 15 min, and 80 µL of a 1:10 (v/v) dilution of Maxiren™ (Chr Hansen A/S, Hørsholm, Denmark) was added. The sample was placed immediately in the preheated cup (32 °C), the frequency of oscillation was set at 0.6283 rad s<sup>-1</sup>, and the storage modulus, G' of the sample was recorded continuously as a function of time at a low-amplitude shear strain (0.01) over 90 min. Each sample was analysed in triplicate (Ibáñez, Waldron, & McSweeney, 2015).

### 2.3. *Cheese manufacture*

Cheddar cheeses were made from 20 L control, SC, MC and PC milks which were prepared, pasteurised and stored at 4 °C overnight before cheese-making. Cheddar-type cheeses were manufactured according to a standard Cheddar cheese manufacture protocol (Fox, Guinee, Cogan, & McSweeney, 2000). An aliquot of 6 g (0.03%, w/w) of Cheddar cheese starter culture (R604Y Chr. Hansen Ltd., Little Island, Co. Cork, Ireland) was added into each sample and allowed ripen for 30 min, followed by 0.09% (v/w) of 1 mol L<sup>-1</sup> CaCl<sub>2</sub> and 60 IMCU L<sup>-1</sup> rennet being added to all samples. Whey was drained when the pH dropped to 6.2 and curd was cheddared. When the pH decreased to 5.4, the curd was milled into small pieces and salted at a level of 2.5% (w/w) NaCl. The curds were then wrapped in cheesecloth and moved to 2-kg circular moulds, which were pressed at a pressure of 2.5 kg



cm<sup>-2</sup> for 14 h. The cheeses were then removed, vacuum-packed and ripened at 8 °C for 180 days.

#### 2.4. *Compositional analysis*

Gross composition of cheeses was analysed at 14 days old. Moisture was determined by oven-drying method (IDF 1982), protein (%N×6.38) by the Kjeldahl method (IDF 1986) and salt was analysed by titration with AgNO<sub>3</sub> (Fox, 1963). The pH was measured at 14, 30, 60, 120 and 180 days of ripening by using a calibrated pH probe placed in contact with dry grated cheeses. All results were determined in triplicate.

#### 2.5. *Microbiological analysis*

Enumeration of starter lactic acid bacteria (LAB) was performed using LM 17 agar plates (Terzaghi & Sandine 1975), incubated for 3 days at 30 °C. Enumeration of non-starter lactic acid bacteria (NSLAB) was performed on Rogosa agar plates (Rogosa, Mitchell, & Wiseman, 1951), incubated anaerobically for 5 days at 30 °C. Enumeration of LAB and NSLAB were performed in duplicate after 60 and 90 days of ripening.

#### 2.6. *Analysis of proteolysis*

Urea-polyacrylamide gel electrophoresis (urea-PAGE) of cheese samples was used to study the proteolysis of  $\alpha_{S1}$ - and  $\beta$ -casein (CN) during ripening using the method of Andrews (1983) with modifications of Shalabi and Fox (1987). The pH 4.6-soluble and insoluble fraction were prepared (Kuchroo & Fox, 1982), and peptide profiles of pH 4.6-soluble fractions filtered through 0.22  $\mu\text{m}$  cellulose acetate filters (Sartorius GmbH, Gottingen, Germany) were analysed by reverse-phase high-performance liquid chromatography (HPLC) using an ultra-performance liquid chromatography (UPLC) Waters Acquity UPLC H-Class Core System (Waters Corp, Milford, MA, USA), with a Waters Acquity UPLC TUV Detector (dual-wavelength; Waters Corp) operated by Empower 3 software (Waters Corp), following the method of Mane, Ciocia, Beck, Lillevang, and McSweeney (2019).

For determination of free amino acids (FAA), frozen pH 4.6-soluble fractions were de-proteinised by mixing equal volumes of 24% ( $\text{w v}^{-1}$ ) trichloroacetic acid (TCA) and sample and following the method of Fenelon and Guinee (2000).

## 2.7. *Plasmin activity*

The plasmin activity of cheese samples at 180 days of ripening was measured using the coumarin peptide method (Richardson & Pearce, 1981). A standard curve of the emission intensity at 460 nm was constructed using 7-amido-4-methyl coumarin (AMC), and results expressed in  $\text{nmol AMC min}^{-1} \text{mL}^{-1}$ , which was defined as one unit of plasmin activity.

## 2.8. *Residual coagulant assay*

Grated cheese samples (50 mg) were extracted by dissolving cheese in 1 mL of 0.1 M trisodium citrate, followed by 30 min incubation at 37 °C. Fat was separated by centrifugation at  $1000 \times g$  (Sigma 1-16K, Harz, Germany) for 1 min, and the aqueous layer was used for analysis. An aliquot of 70  $\mu\text{L}$  citrate dispersion of cheese was incubated with 30  $\mu\text{L}$  of  $1 \text{ mg mL}^{-1}$  aqueous solution of a synthetic heptapeptide substrate (Pro-Thr-Glu-Phe-[NO<sub>2</sub>-Phe]-Arg-Leu) in 400  $\mu\text{L}$  0.1 M sodium formate buffer, at 37 °C, pH 3.2, for 24 h. The mixture was heated at 70 °C for 10 min to stop the reaction, followed by centrifugation at  $16,000 \times g$  for 10 min, and the supernatant was used for UPLC analysis. Substrate and product levels were determined using the reversed-phase HPLC system described above, following the method of Hurley, O'Driscoll, Kelly, and McSweeney (1999).

## 2.9. *Texture profile analysis*

Texture profile analysis was performed using a Texture Analyzer TA-XT2i (Stable Micro Systems, Godalming, Surrey, UK) at 60, 120, and 180 days of ripening. Cheese samples were cut into 20 mm height, 20 mm diameter cylinders and kept at 4 °C overnight. Cheese cylinders were compressed to 25% of the initial height in two continuous compressions with a speed of  $1 \text{ mm s}^{-1}$ . Hardness, cohesiveness and springiness were

measured (Truong, Daubert, Drake, & Baxter, 2002), and five cheese samples were measured for each treatment.

### 2.10. *Meltability*

Meltability was analysed using the Schreiber meltability test as described by Altan, Turhan, and Gunasekaran (2005). Cheese samples were heated at 232 °C for 5 min, and meltability was measured as the percentage increase in diameter of the original samples. Analyses were performed in triplicate at 60, 120, 180 days of ripening.

### 2.11. *Dynamic small amplitude oscillatory rheology*

The rheological properties of meltability of cheese after 180 days ripening were analysed with a controlled stress AR-G2 rheometer (TA Instruments, Waters LLC, Leatherhead, Surrey, UK) according to the method of Ibáñez et al. (2015). Storage modulus ( $G'$ ), loss modulus ( $G''$ ), and loss tangent (LT) were measured during heating. The maximum LT ( $LT_{\max}$ ) and the temperature where  $LT = 1$ , which are indicators of melting, were also recorded. Each sample was analysed in triplicate.

### 2.12. *Colour measurements*

Colour values were measured using a Konika-Minolta colourimeter CR400 (Konika-Minolta Optics Inc., Osaka, Japan) at 14, 30, 60, 120, 180 days of ripening. The measurement used the CIELAB system based on illuminant D65 and a visual angle of 2°. Five random readings were taken on fresh-cut cheese at 20 °C. The Euclidean distance between the colour of control cheese and that for other treatments was calculated by:

$$\Delta E_{ab}^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where  $\Delta L^* = L^*_{\text{sample}} - L^*_{\text{control}}$ ,  $\Delta a^* = a^*_{\text{sample}} - a^*_{\text{control}}$  and  $\Delta b^* = b^*_{\text{sample}} - b^*_{\text{control}}$  (Mahy, Van Eyckden, & Oosterlinck, 1994).

### 2.13. Statistical analysis

Control, SC, MC and PC cheeses were made in three independent trials, 4 × 3 blocks. Statistical analysis was carried out using one-way analysis of variance (ANOVA) with R project for Windows version i386 3.4.0 to establish significant differences between samples. The probability level used for statistical significance was  $P < 0.05$ .

## 3. Results and discussion

### 3.1. Rennet coagulation properties

The rennet coagulation properties of milk samples are shown in Table 1. During the first 10 min, G' value of all the treatments was low and constant, after which the G' value of

all samples increased, with the MC and PC samples exhibiting a slower rate of increase compared to other samples (Fig. 1). The gel strength ( $G'$  value) at 90 min of MC and PC samples was significantly ( $P < 0.05$ ) lower than that of other samples. The gel strength of MC and PC kept increasing and did not reach a constant level during the analysis, while control and SC reached stable values at 45 min. The loss tangent at 90 min of MC sample was significantly ( $P < 0.05$ ) higher than that of the other treatments.

Martin, Williams, and Dunstan (2010) reported that, during the manufacture of milk protein concentrate (MPC), casein micelles were not damaged; however, the minerals removed through diafiltration and decreased calcium ionic strength could depress rennet coagulation. The rennet coagulation ability of reconstituted MPC could be restored by adding extra calcium ions. The production of MCC involves two diafiltration steps, which may reduce soluble calcium levels; thus, 0.09% (v/w) of  $1 \text{ mol L}^{-1} \text{ CaCl}_2$  was added to all of the batches before cheese manufacture.

### 3.2. *Cheese composition and pH*

The composition of cheese at 14 days of ripening and pH of cheese samples at 14, 30, 60, 120 and 180 days of ripening are shown in Table 2; the results presented are means of data from the three trials. Guinee, Mulholland, Kelly, and O'Callaghan (2007) stated that different casein to fat ratio can influence the protein and fat content of cheese. As the casein: fat ratio of milk was adjusted to 0.7 for all samples, no statistically significant differences ( $P >$

0.05) were found in cheese protein and fat contents between all treatments, and the use of MCC or LHSMP for Cheddar cheese manufacture did not affect these parameters.

The moisture content of PC cheese was significantly ( $P < 0.05$ ) higher than that of SC and MC cheese; no significant differences ( $P > 0.05$ ) were found for moisture content between control, SC and MC cheese. The salt content of MC cheese was significantly ( $P < 0.05$ ) higher than that of control and SC cheese. The pH of MC cheese was significantly ( $P < 0.05$ ) higher than that of other treatments, the reason for which is not clear. No significant differences ( $P > 0.05$ ) were found for moisture in non-fat substances (MNFS) and fat in dry matter (FDM) for all treatments; MNFS is an important parameter for Cheddar cheese quality, so it is important that all batches of cheese sample had similar levels of MNFS (Bogenrief & Olson, 1995; Fox et al., 2000).

### 3.3. *Proteolysis*

Table 2 shows the level of pH 4.6-SN/TN (%) of control, SC, MC and PC cheeses at 180 days of ripening; pH 4.6-SN/TN is an index of proteolysis (Sousa, Ardo, & McSweeney, 2001). Significantly ( $P < 0.05$ ) higher pH 4.6-SN/TN levels were found in MC cheese than in SC and PC cheese. In addition, the levels of pH 4.6-SN/TN were significantly ( $P < 0.05$ ) lower in PC than control cheese. The value (23%) found at 180 days of ripening in this study was comparable with previous studies on Cheddar cheese (Lucey, Mishra, Hassan, & Johnson, 2005; O'Mahony, Lucey, & McSweeney, 2005). The main agent producing pH 4.6-soluble nitrogen is the coagulant (O'Keeffe, Fox, & Daly, 1978), while Farkye and Fox (1992)

reported that plasmin, the principal indigenous milk proteinase, is also important for primary proteolysis in Cheddar cheese during ripening.

Urea-PAGE electrophoretograms of cheese samples during ripening are shown in Fig. 2. All cheese samples showed break-down of  $\beta$ - and  $\alpha_{S1}$ -CN, but lower levels of intact  $\beta$ - and  $\alpha_{S1}$ -CN were apparent during ripening in MC cheese. Due to the action of plasmin,  $\beta$ -CN is hydrolysed to  $\beta$ -CN (f29–209),  $\beta$ -CN (f106–209) and  $\beta$ -CN (f108–209) (Eigel et al., 1984). The levels of  $\beta$ -CN (f106–209) and  $\beta$ -CN (f108–209) of MC cheese were higher than other treatments during ripening, which suggests higher plasmin activity. Also, in Fig. 2, the MC cheese showed the lowest level of intact  $\alpha_{S1}$ -CN level, followed by PC cheese, control and SC cheese from 60 to 180 days of ripening; at 180 days of ripening, no intact  $\alpha_{S1}$ -CN was found in MC and PC samples. Residual chymosin in cheese hydrolyses  $\alpha_{S1}$ -CN to  $\alpha_{S1}$ -CN (f24–199) and  $\alpha_{S1}$ -CN (f102–199) and, as the residual chymosin activity increases, the breakdown of  $\alpha_{S1}$ -CN increases (Sheehan, Wilkinson, & McSweeney, 2008). The faster breakdown of  $\alpha_{S1}$ -CN of MC and PC samples may indicate higher residual chymosin activity in these samples.

The peptide profiles of the pH 4.6-soluble extracts for control, SC, MC and PC cheeses at 180 days of ripening were generated by ultra-performance liquid chromatography (Fig. 3). The highest peak areas were observed at the retention time of 1 to 5 min for all treatments. The peak area of peptides that were eluted later (28–45 min retention time) was higher in MC sample compared to the other treatments. Peptides in the pH 4.6-soluble extracts reflect the effect of proteinases and peptidases of starter (Fox & McSweeney, 1997). The number of peptide peaks for all treatments was similar, which suggests that the proteolysis for all



treatments broadly followed similar pathways, but differences in peak area indicate that there was more extensive proteolysis in MC cheese compared to the other treatments.

The individual FAA levels of all treatments at 120 and 180 days of ripening are shown in Fig. 4 (A and B). The major FAA determined in cheese were glutamic acid, valine, leucine, phenylalanine, histidine and lysine; Bansal et al. (2009) also reported that glutamic acid, valine, leucine, phenylalanine histidine and lysine are the principal FAA in Cheddar cheese after 180 days of ripening. No significant differences ( $P > 0.05$ ) were found between treatments at 120 days of ripening. At 180 days of ripening, significantly ( $P < 0.05$ ) higher concentrations of threonine, serine, glycine, alanine, valine, methionine, isoleucine, phenylalanine, histidine and proline were found in control and SC cheese than that of MC and PC cheese. No significant differences ( $P > 0.05$ ) were found between treatments in the levels of aspartic acid, glutamic acid, leucine, tyrosine and arginine. From 120 to 180 days of ripening, the concentrations of glutamic acid, alanine, leucine, tyrosine and phenylalanine increased significantly ( $P < 0.05$ ) for all treatments, while only control and SC cheese had a significant increase in the concentration of threonine, glycine, valine and isoleucine. The total FAA levels of all treatments at 120 and 180 days of ripening are shown in Fig. 4 (C). No significant differences ( $P > 0.05$ ) were found between all treatments at 120 days of ripening, but significantly higher ( $P < 0.05$ ) levels of total FAA were found in control cheese than in MC and PC cheeses at 180 days of ripening. No significant differences ( $P > 0.05$ ) were found between control and SC cheese at 180 days of ripening.

Fox and McSweeney (1996) reported that peptidases of starter and non-starter lactic acid bacteria are the principal agents releasing FAA in Cheddar cheese during ripening. No

significant ( $P > 0.05$ ) differences were found for the numbers of starter bacteria and NSLAB between treatments in this study (result not shown). Therefore, the release of the FAA by starter and NSLAB should not have been an influence in this regard, and so the reasons for this difference is not clear.

#### 3.4. *Plasmin and residual chymosin activities in cheese*

The plasmin activity of MC cheese was significantly ( $P < 0.05$ ) higher than that of the other cheese batches (Table 3) which is consistent with the result of urea PAGE electrophoresis. The plasmin activity of PC cheese was significantly ( $P < 0.05$ ) lower than that of the other cheese samples. As the pH increases, the hydrolysis of  $\beta$ -CN increases since plasmin has an alkaline optimum pH (Watkinson et al., 2001).

Aaltonen and Ollikainen (2011) reported that in diafiltration, with the removal of whey protein, the concentration of inhibitors of both plasmin and plasminogen activators decreases, which promotes the conversion from plasminogen to plasmin and thus increases plasmin activity. In this study, MCC was made using microfiltration, which may have enhanced the plasmin activity of retentate by removing inhibitors in the permeate, resulting in the higher plasmin activity and more  $\beta$ -CN breakdown. MC cheese showed significantly ( $P < 0.05$ ) higher residual chymosin activity compared to the other treatments (Table 3), which is consistent with the faster breakdown of  $\alpha_{S1}$ -CN in that cheese. No significant differences ( $P > 0.05$ ) were found between the residual chymosin activities of control and SC cheese, so the recombination of fat and skim milk did not affect the retention of coagulant during cheese manufacture. The

residual chymosin activity of PC cheese was significantly ( $P < 0.05$ ) lower than that of control cheese. The MC cheese apparently retained more coagulant compared with control, SC and PC cheeses; the percentage of retained chymosin in cheese curd depends on the pH at the curd-cutting stage, pH at whey drainage, cooking temperature and method (Hurley et al., 1999).

### 3.5. *Texture profile analysis*

No significant changes ( $P > 0.05$ ) were found in the hardness of MC and PC cheeses from 60 to 180 days of ripening (Fig. 5). The hardness of control cheese significantly ( $P < 0.05$ ) decreased between 60 and 120 days of ripening, while that of SC cheese significantly ( $P < 0.05$ ) increased. At 60 days of ripening, the hardness of control cheese was significantly ( $P < 0.05$ ) higher than that of SC and PC cheeses. No significant differences ( $P > 0.05$ ) were observed in the hardness of the cheeses made by different treatments at 120 and 180 days of ripening. Chevanan and Muthukumarappan (2007) reported that the contents of calcium, phosphate, and residual lactose affect the texture profile of Cheddar cheese. The cheese-milk of SC, MC and PC samples was reconstituted according to the composition of control cheese-milk, which would not influence lactose content. In this study, extra calcium chloride was added to all treatments, but the addition of calcium chloride does not cause significant changes to the texture of ripened Cheddar cheese (Soodam, Ong, Powell, Kentish, & Gras, 2015).

From 60 days to 180 days of ripening, the springiness and cohesiveness of MC cheese decreased, and the springiness and cohesiveness of MC and PC cheeses were significantly ( $P < 0.05$ ) higher than that of control and SC cheese at 60 and 180 days of ripening. At 120 days of ripening, the springiness of MC cheese was significantly ( $P < 0.05$ ) higher than that of the other treatments and the cohesiveness of MC and PC cheeses was significantly ( $P < 0.05$ ) higher than that of control and SC cheese. Everard et al. (2006) found that higher pH of Cheddar cheese was associated with increased springiness and cohesiveness; the pH of MC cheese was significantly ( $P < 0.05$ ) higher than that for the other treatments (Table 2), which may explain the increases in springiness and cohesiveness. O'Mahony et al. (2005) reported that, as levels of secondary proteolysis increase, the charged groups released from peptides would associate with free water, which may lead to increased cohesiveness and springiness during ripening.

### 3.6. Meltability

Meltability (percentage increase in diameter) of Cheddar cheeses was determined by the Schreiber method; results are shown in Fig. 6. The four types of cheeses showed increases in meltability between 60 and 180 days. At 60 and 120 days of ripening, no significant differences ( $P > 0.05$ ) for meltability were found between control and SC cheese. Significantly ( $P < 0.05$ ) higher meltability was found in control cheese compared to SC cheese. At 60, 120 and 180 days of ripening, the meltability of MC cheese was significantly higher ( $P < 0.05$ ) than that of cheese from the other treatments. At 120 and 180 days of

ripening, the meltability of PC cheese was significantly lower ( $P < 0.05$ ) than that of cheese from the other treatments.

The lower meltability of PC cheese may be caused by the changes of casein in protein during drying of milk powder. Moiseev, Suchkova, and Iakovchenko (2017) reported that mozzarella cheese made with reconstituted non-fat milk powder has lower meltability than the control, due to the drying process of non-fat milk powder decreasing the stability and dispersity of casein micelles and promoting demineralisation of calcium salts. Dave et al. (2003) found that the meltability of mozzarella cheese is related to the breakdown of ( $\alpha_{S1}$ -CN and  $\alpha_{S1}$ -CN (f24-199)) and breakdown of intact  $\beta$ -casein, while Bogenrief and Olson (1995) reported that hydrolysis of  $\beta$ -casein increases the meltability of Cheddar cheese. Hydrolysis of  $\beta$ -casein is primarily related to the action of plasmin (Eigel, McMahon, Oberg, & Broadbent 1984) and, although both MC and PC cheeses were made from powder, and showed a higher breakdown of  $\alpha_{S1}$ -casein and  $\beta$ -casein, the meltability of MC cheese was significantly higher than that of PC.

### 3.7. *Dynamic small amplitude oscillatory rheology*

The results of the rheology of meltability of all treatments at 180 days of ripening are shown in Table 4. Higher LT value indicates a higher extent of melting (Lucey, Johnson, & Horne, 2003). MC cheese showed significantly higher ( $P < 0.05$ )  $LT_{max}$  values than PC cheese, followed by control and SC cheese. The temperature at  $LT_{max}$  of MC and PC cheeses was significantly higher ( $P < 0.05$ ) than that of control and SC cheese. The  $LT_{max}$  is an

indicator of cheese meltability (Mounsey & O’Riordan, 1999); the more thermal energy needed to melt cheese, the higher the  $LT_{max}$  temperature of cheese will be.

In the Schreiber test for cheese meltability, MC cheese also exhibited the highest percentage increase in diameter (Fig. 4). There were differences between Schreiber melting test and dynamic small amplitude rheology for control, SC and PC cheese. Cooke, Khosrowshahi, and McSweeney (2013) stated that differences between results generated by those tests may be linked to the more complete fat melting during the higher temperature of the Schreiber melting test. MC cheese exhibited the lowest temperature when  $LT = 1$  compared with the rest of the treatments ( $P < 0.05$ ). At the point of  $LT = 1$ , cheese is considered to start transforming from a solid to a viscous form (Gunasekaran & Ak, 2002). MC cheese thus commenced melting at the lowest temperature and had the highest meltability compared to the other treatments, which may be linked to the more extensive proteolysis of casein.

### 3.8. Cheese colour

The colour values of samples at 180 days of ripening are shown in Table 5. Whiteness ( $L^*$  values) of control, SC cheese was significantly higher ( $P < 0.05$ ) than that of MC cheese at 180 days of ripening. The greenness of MC and PC cheeses was significantly lower (higher  $a^*$  values) ( $P < 0.05$ ) than that of control or SC cheese at 180 days of ripening. The yellowness ( $b^*$  values) of MC cheese was significantly lower ( $P < 0.05$ ) than that of SC cheese at 180 days of ripening. Ibáñez et al. (2015) reported that the whiteness of cheese may

be related to proteolysis; lower whiteness of MC cheese may thus be attributed to higher proteolysis. The  $\Delta E_{ab}^*$  values are also shown in Table 5. Sharma and Bala (2003) reported that  $\Delta E_{ab}^*$  of above 2.3 leads to a just noticeable difference (JND) and, on this basis, overall, no JND was found between control cheese and the other treatments. Overall, no visible difference was found between the cheeses made from protein ingredient and control and SC cheese.

#### 4. Conclusions

Cheddar cheeses were made from standardised cheese milk, skim milk with cream, reconstituted MCC with cream and reconstituted LHSMP with cream according to the same casein, fat and lactose content. Only minor differences in composition were found with cheese made from reconstituted MCC with added cream compared with control cheese. The cheese manufactured with MCC had significantly higher ( $P < 0.05$ ) levels of pH 4.6-SN/TN than cheese manufactured with skim milk or LHSMP with cream. The level of intact  $\beta$ - and  $\alpha_{S1}$ -CN of MC cheese was lower than rest treatments, consistent with significantly ( $P < 0.05$ ) higher plasmin and chymosin activity. No significant difference ( $P > 0.05$ ) were found in hardness between all treatments. Significantly higher ( $P < 0.05$ ) springiness and cohesiveness were found in cheese manufactured from MCC and LHSMP powder, meltability and maximum loss tangent in MC cheese were significantly ( $P < 0.05$ ) higher than that of the other treatments. No overall differences of colour were found between all treatments. Use of novel casein-dominant dairy streams such as MCC has potential for production of Cheddar cheese with tailored

functionality. The results of this study suggest that using reconstituted LHSMP with cream for the manufacture of Cheddar cheese may result in changes in functionality, while using reconstituted MCC with cream for the manufacture of Cheddar cheese may be more feasible.

### Acknowledgements

The authors gratefully acknowledge Department of Agriculture, Food and the Marine, Ireland and Teagasc, Ireland for providing the samples of MCC for Cheddar cheese manufacture.

### References

- Aaltonen, T., & Ollikainen, P. (2011). Effect of microfiltration of milk on plasmin activity. *International Dairy Journal*, 21, 193–197.
- Altan, A., Turhan, M., & Gunasekaran, S. (2005). Short communication: comparison of covered and uncovered Schreiber test for cheese meltability evaluation. *Journal of Dairy Science*, 88, 857–861.
- Andrews, A. T. (1983). Proteinases in normal bovine milk and their action on caseins. *Journal of Dairy Research*, 50, 45–55.
- Augustin, M. A., & Margetts, C. L. (2003). Powdered milk | Milk powders in the marketplace. In B. Caballero, P. Finglas, & F. Toldra (Eds.), *Encyclopedia of food sciences and nutrition* (2nd edn., pp. 4694–4702). London, UK: Academic.



- Bansal, N., Drake, M. A., Piraino, P., Broe, M. L., Harboe, M., Fox, P. F., et al. (2009). Suitability of recombinant camel (*Camelus dromedarius*) chymosin as a coagulant for Cheddar cheese. *International Dairy Journal*, *19*, 510–517.
- Barbano, D. M., & Sherbon, J. W. (1984). Cheddar cheese yields in New York. *Journal of Dairy Science*, *67*, 1873–1883.
- Bogenrief, D. D., & Olson, N. F. (1995). Hydrolysis of  $\beta$ -casein increases Cheddar cheese meltability. *Milchwissenschaft*, *50*, 678–682.
- Boland, M. (2011). Whey proteins. In G. O. Phillips & P. A. Williams (Eds.), *Handbook of food proteins* (pp. 30–55). Cambridge, UK: Woodhead Publishing.
- Chevanan, N., & Muthukumarappan, K. (2007). Effect of calcium and phosphorus, residual lactose, and salt-to-moisture ratio on the melting characteristics and hardness of Cheddar cheese during ripening. *Journal of Food Science*, *72*, 168–176.
- Cooke, D. R., Khosrowshahi, A., & McSweeney, P. L. H. (2013). Effect of gum tragacanth on the rheological and functional properties of full-fat and half-fat Cheddar cheese. *Dairy Science & Technology*, *93*, 45–62.
- Crowley, S. V., Burlot, E., Silva, J. V. C., McCarthy, N. A., Wijayanti, H. B., Fenelon, M. A., et al. (2018). Rehydration behaviour of spray-dried micellar casein concentrates produced using microfiltration of skim milk at cold or warm temperatures. *International Dairy Journal*, *81*, 72–79.
- Dave, R. I., McMahon, D. J., Oberg, C., & Broadbent, J. R. (2003). Influence of coagulant level on proteolysis and functionality of Mozzarella cheeses made using direct acidification. *Journal of Dairy Science*, *86*, 114–126.

- Eigel, W. N., Butler, J. E., Ernstrom, C. A., Farrell, H. M., Harwalkar, V. R., Jenness, R., et al. (1984). Nomenclature of proteins of cow's milk: Fifth revision. *Journal of Dairy Science*, 67, 1599–1631.
- Everard, C. D., O'Callaghan, D. J., Howard, T. V., O'Donnell, C.P., Sheehan, E. M., & Delahunty, C. M. (2006). Relationships between sensory and rheological measurements of texture in maturing commercial Cheddar cheese over a range of moisture and pH at the point of manufacture. *Journal Texture Studies*, 37, 361–382.
- FAO. (2019). Dairy market review, March 2019. Rome, Italy: Food and Agriculture Organisation.
- Farkye, N., & Fox, P. F. (1992). Contribution of plasmin to Cheddar cheese ripening: effect of added plasmin. *Journal of Dairy Research*, 59, 209.
- Fenelon, M. A., & Guinee, T. P. (2000). Primary proteolysis and textural changes during ripening in Cheddar cheeses manufactured to different fat contents. *International Dairy Journal*, 10, 151–158.
- Fox, P. F. (1963). Potentiometric determination of salt in cheese. *Journal of Dairy Science*, 46, 744–745.
- Fox, P. F., & McSweeney, P. L. H. (1996). Proteolysis in cheese during ripening. *Food Reviews International*, 12, 457–509.
- Fox, P. F., & McSweeney, P. L. H. (1997). Rennets: their role in milk coagulation and cheese ripening. In B. A. Law (Ed.), *Microbiology and biochemistry of cheese and fermented milk* (2<sup>nd</sup> edn., pp. 1–49). London, UK: Blackie Academic and Professional.

- Fox, P. F., Guinee, T. P., Cogan, T. M., & McSweeney, P. L. H. (2000). *Fundamentals of cheese science*. Gaithersburg, MD, USA: Aspen Publishers.
- Freeman, T. R., Bucy, J. L., & Hassan, Z. (1970). Use of nonfat dry milk in the production of Cheddar cheese. *Journal of Dairy Science*, *53*, 727–733.
- Guinee, T. P., Mulholland, E.O., Kelly, J., & O’Callaghan, D. J. (2007). Effect of protein–to–fat ratio of milk on the composition, manufacturing efficiency and yield of Cheddar cheese. *Journal of Dairy Science*, *90*, 110–123.
- Gunasekaran, S., & Ak., M. M. (2002). *Cheese rheology and texture*. Boca Raton, FL, USA: CRC Press LLC.
- Hoogwegt Group. (2019). Market matters demand for whey products looks bright. Available at: [https://hoogwegt.com/media/2612/horizons\\_june-2019.pdf](https://hoogwegt.com/media/2612/horizons_june-2019.pdf) [Accessed 2 Nov. 2019].
- Hurley, M. J., O’Driscoll, B. M., Kelly, A. L., & McSweeney, P. L. H. (1999). Novel assay for the determination of residual coagulant activity in cheese. *International Dairy Journal*, *9*, 553–558.
- Ibáñez, R. A., Waldron, D. S., & McSweeney, P. L. H. (2015). Effect of pectin on the composition, microbiology, texture, and functionality of reduced–fat Cheddar cheese. *Dairy Science & Technology*, *96*, 297–316.
- IDF. (1982). *Determination of the total solids content of cheese and processed cheese. IDF standard 4A*. Brussels, Belgium: International Dairy Federation.

- IDF. (1986). *Determination of nitrogen content (Kjeldahl method) and calculation of crude protein content. IDF standard 20A*. Brussels, Belgium: International Dairy Federation.
- Kelly, P. (2019). Manufacture of whey protein products: concentrates, isolate, whey protein fractions and microparticulated. In H. C. Deeth & N. Bansal (Eds.), *Whey proteins* (pp. 97–122). London, UK: Elsevier Applied Science.
- Kuchroo, C. N., & Fox, P. F., (1982). Soluble nitrogen in Cheddar cheese; comparison of extraction procedures. *Milchwissenschaft*, *37*, 331–335.
- Kuchtík, J., Šustová, K., Urban, T., & Zapletal, D. (2008). Effect of the stage of lactation on milk composition, its properties and the quality of rennet curdling in East Friesian ewes. *Czech Journal of Animal Science*, *53*, 55–63.
- Lucey, J. A., Johnson, M. E., & Horne, D. S. (2003). Invited review: perspectives on the basis of the rheology and texture properties of cheese. *Journal of Dairy Science*, *86*, 2725–2743
- Lucey, J. A., Mishra, R., Hassan, A., & Johnson, M. E. (2005). Rheological and calcium equilibrium changes during ripening of Cheddar cheese. *International Dairy Journal*, *15*, 645–653.
- Mahy, M., Van Eyckden, L., & Oosterlinck, A., (1994). Evaluation of uniform color spaces developed after the adoption of CIELAB and CIELUV. *Color Research and Application*, *19*, 105–121.

- Mane, A., Ciocia, F., Beck, T. K., Lillevang, S. K., & McSweeney, P. L. H. (2019). Proteolysis in Danish Blue cheese during ripening. *International Dairy Journal*, *97*, 191–200.
- Martin, G. J. O., Williams, R. P. W., & Dunstan, D. E. (2010). Effect of manufacture and reconstitution of milk protein concentrate powder on the size and rennet gelation behaviour of casein micelles. *International Dairy Journal*, *20*, 128–131.
- Ménard, O., Camier, B., & Guyomarc'h, F., (2005). Effect of heat treatment at alkaline pH on the rennet coagulation properties of skim milk. *Lait*, *85*, 515–526.
- Moiseev, N., Suchkova, E., & Iakovchenko, N. (2017). Possibility of using reconstituted milk in manufacture of cheese with cheddaring and cheese curd stretching. *Agronomy Research*, *15*, 1358–1368.
- Mounsey, J. S., & O’Riordan, E. D. (1999). Empirical and dynamic rheological data correlation to characterize melt characteristics of imitation cheese. *Journal of Food Science*. *64*, 701–703.
- O’Brien, B., Mehra, R., Connolly, J. F., & Harrington, D. (1999). Seasonal variation in the composition of Irish manufacturing and retail milks. *Irish Journal of Agricultural and Food Research*, *38*, 53–64.
- O’Keeffe, R. B., Fox, P. F., & Daly, C. (1978). Proteolysis in Cheddar cheese: role of coagulant and starter bacteria. *Journal of Dairy Research*, *45*, 465–477.
- O’Mahony, J. A., Lucey, J. A., & McSweeney, P. L. H. (2005). Chymosin mediated proteolysis, calcium solubilisation, and texture development during the ripening of Cheddar cheese. *Journal of Dairy Science*, *88*, 3101–3114.

Richardson, B. C., & Pearce, K. N. (1981). The determination of plasmin in dairy products.

*Journal of Dairy Science and Technology*, *16*, 209–220.

Rogosa, M., Mitchell, J. A., & Wiseman, R. F. (1951). A selective medium for the isolation and enumeration of oral and faecal lactobacilli. *Journal of Bacteriology*, *62*, 132–133.

Shalabi, S. I., & Fox, P. F. (1987). Electrophoretic analysis of cheese, comparison of methods. *Irish Journal of Food Science and Technology*, *11*, 135–151.

Sharma, G., & Bala, R. (2003). *Digital color imaging handbook* (1st edn.). Boca Raton, FL, USA: CRC Press LLC.

Sheehan, J. J., Wilkinson, M. G., & McSweeney, P. L. H. (2008). Influence of processing and ripening parameters on starter, non-starter and propionic acid bacteria and on the ripening characteristics of semi-hard cheese. *International Dairy Journal*, *18*, 905–917.

Soodam, K., Ong, L., Powell, I. B., Kentish, S. E., & Gras, S. L. (2015). Effect of calcium chloride addition and draining pH on the microstructure and texture of full fat Cheddar cheese during ripening. *Food Chemistry*, *181*, 111–118.

Sousa, M. J., Ardo, Y., & McSweeney, P. L. H. (2001). Advances in the study of proteolysis during cheese ripening. *International Dairy Journal*, *11*, 327–345.

Terzaghi, B. E., & Sandine, W. E. (1975). Improved medium for lactic streptococci and their bacteriophages. *Applied Microbiology*, *29*, 807–813.

Truong, V. D., Daubert, C. R., Drake, M. A., & Baxter, S. (2002). Vane rheometry for textural characterization of cheddar cheeses: correlation with other instrumental and sensory measurements. *LWT – Food Science and Technology*, 35, 305–314.

Watkinson, P., Coker, C., Crawford, R., Dodds, C., Johnston, K., McKenna, A., et al. (2001). Effect of cheese pH and ripening time on model cheese textural properties and proteolysis. *International Dairy Journal*, 11, 455–464.

Journal Pre-proof

**Figure legends**

**Fig. 1.** Storage modulus ( $G'$ ) of rennet gel of control (●), SC (■), MC (▼) and PC (▲) samples, under strain of 0.01 and frequency of 1 Hz.

**Fig. 2.** Urea-polyacrylamide gel electrophoretograms of control (C), skim milk with cream (SC), reconstituted micellar casein concentrate with cream (MC) and reconstituted low heat skim milk powder with cream (PC) cheeses at 60, 120 and 180 days of ripening. Sodium caseinate (S) was used as standard on both sides of the gel. The figure corresponds to data for one of the three cheese replicates; all replicates were similar.

**Fig. 3.** Ultra-performance liquid chromatograms (UPLC profiles,  $C_8$  column) of pH 4.6-soluble extracts from control (A), SC (B), MC (C) and PC (D) cheeses at 180 days of ripening, at wavelength of 214 nm. The figure corresponds to data for one of the three cheese replicates; all replicates were similar.

**Fig. 4.** Individual FAA results of cheese samples at 120 (A) and 180 (B) days of ripening and total FAA results of cheese samples at 120 and 180 days of ripening (C). Control (■), SC (■), MC (□) and PC (▨). Values are means of three independent trials; means not sharing a common letter differ ( $P < 0.05$ ). Error bars indicate standard deviation.

**Fig. 5.** Changes in (A) TPA hardness, (B) springiness and (C) cohesiveness of cheese samples from 60 to 180 days of ripening. Control (■), SC (■), MC (□) and PC (▨). Values are means of three independent trials; means at the same time point not sharing a common letter differ ( $P < 0.05$ ). Error bars indicate standard deviation.



**Fig. 6.** Meltability (diameter increase, %) of cheese samples from 60 to 180 days of ripening Control (■), SC (■), MC (□) and PC (▨). Values are means of three independent trials; means at the same time point not sharing a common letter differ ( $P < 0.05$ ). Error bars indicate standard deviation.

Journal Pre-proof

**Table 1**

Rheological properties of rennet-induced coagulum made from control, skim milk with cream (SC), reconstituted micellar casein concentrate with cream (MC) and reconstituted low heat skim milk powder with cream (PC). <sup>a</sup>

Sample	Gelation time (min)	G' at 90 min (Pa)	Loss tangent at 90 min
Control	7.81 <sup>a</sup> (0.58)	143.3 <sup>bc</sup> (31.66)	0.26 <sup>a</sup> (0.003)
SC	7.78 <sup>a</sup> (0.71)	169.5 <sup>c</sup> (17.68)	0.27 <sup>b</sup> (0.028)
MC	9.29 <sup>a</sup> (0.73)	74.23 <sup>a</sup> (6.71)	0.29 <sup>c</sup> (0.002)
PC	8.79 <sup>a</sup> (1.15)	91.04 <sup>ab</sup> (10.26)	0.27 <sup>ab</sup> (0.001)

<sup>a</sup> Gelation time refers to the time when G' value was higher than 1. Values are means, with standard deviation in parenthesis, of three replicate trials; means within the same column not sharing a common superscript letter differ significantly ( $P < 0.05$ ).

**Table 2**

Composition of cheeses made from control, skim milk with cream (SC), reconstituted micellar casein concentrate with cream (MC) and reconstituted low heat skim milk powder with cream (PC).<sup>a</sup>

Parameter	Control	SC	MC	PC
Moisture (%)	38.41 <sup>ab</sup> (0.41)	37.41 <sup>a</sup> (0.41)	37.80 <sup>a</sup> (1.43)	39.31 <sup>b</sup> (1.14)
Fat (%)	35.25 <sup>a</sup> (2.47)	35.75 <sup>a</sup> (2.13)	35.50 <sup>a</sup> (2.75)	34.84 <sup>a</sup> (2.12)
Protein (%)	23.85 <sup>a</sup> (0.71)	24.12 <sup>a</sup> (0.95)	23.44 <sup>a</sup> (0.95)	23.73 <sup>a</sup> (1.47)
Salt (%)	1.57 <sup>ab</sup> (0.11)	1.48 <sup>a</sup> (0.10)	1.70 <sup>c</sup> (0.10)	1.66 <sup>bc</sup> (0.07)
MNFS (%)	59.33 (0.65)	58.22 (0.65)	58.61 (2.21)	60.33 (1.75)
FDM (%)	59.50 (3.00)	58.14 (1.87)	59.22 (2.94)	58.31 (2.59)
pH at 14 days	5.03 <sup>a</sup> (0.09)	5.05 <sup>a</sup> (0.07)	5.23 <sup>b</sup> (0.14)	5.06 <sup>a</sup> (0.11)
pH at 30 days	5.07 <sup>ab</sup> (0.04)	5.01 <sup>a</sup> (0.04)	5.26 <sup>c</sup> (0.04)	5.14 <sup>b</sup> (0.08)
pH at 60 days	5.07 <sup>a</sup> (0.07)	5.11 <sup>ab</sup> (0.07)	5.20 <sup>b</sup> (0.07)	5.12 <sup>ab</sup> (0.03)
pH at 120 days	5.13 <sup>a</sup> (0.09)	5.17 <sup>a</sup> (0.06)	5.40 <sup>b</sup> (0.04)	5.25 <sup>ab</sup> (0.03)
pH at 180 days	5.04 <sup>a</sup> (0.03)	5.07 <sup>a</sup> (0.04)	5.31 <sup>b</sup> (0.10)	5.17 <sup>ab</sup> (0.07)
pH 4.6-SN/TN	23.01 <sup>bc</sup> (1.22)	22.21 <sup>ab</sup> (1.23)	25.89 <sup>c</sup> (1.65)	19.71 <sup>a</sup> (2.03)

<sup>a</sup> Data taken for moisture, fat, protein, salt, moisture in non-fat substance (MNFS) and fat in dry matter (FDM) at 14 days of ripening, pH values at 14–180 days of ripening, and pH 4.6-SN/TN levels at 180 days of ripening. Values are means, with standard deviation in parenthesis, of three independent trials; means within the same row not sharing a common superscript letter differ significantly ( $P < 0.05$ ). There were no significant differences found for MNFS or FDM.

**Table 3**

Plasmin activity and residual chymosin activity of cheeses made from control, skim milk with cream (SC), reconstituted micellar casein concentrate with cream (MC) and reconstituted low heat skim milk powder with cream (PC) at 180 days of ripening. <sup>a</sup>

Treatment	Plasmin activity (nmol AMC mL <sup>-1</sup> min <sup>-1</sup> )	Residual chymosin activity (% of control)
Control	0.572 <sup>b</sup> (0.121)	100 <sup>b</sup>
SC	0.656 <sup>b</sup> (0.185)	106.04 <sup>b</sup> (4.89)
MC	1.116 <sup>c</sup> (0.024)	132.58 <sup>c</sup> (3.81)
PC	0.396 <sup>a</sup> (0.042)	80.74 <sup>a</sup> (6.61)

<sup>a</sup> Data are means, with standard deviation in parenthesis, from three independent trials; means within the same column not sharing a similar superscript letter differ significantly ( $P < 0.05$ ).

**Table 4**

Rheological properties of cheeses made from control, skim milk with cream (SC), reconstituted micellar casein concentrate with cream (MC) and reconstituted low heat skim milk powder with cream (PC) at 180 days of ripening. <sup>a</sup>

Sample	LT <sub>max</sub>	Temperature at LT <sub>max</sub> (°C)	Temperature at LT = 1 (°C)
Control	1.25 <sup>a</sup> (0.31)	70.55 <sup>a</sup> (0.70)	62.02 <sup>c</sup> (0.58)
SC	1.42 <sup>a</sup> (0.33)	71.36 <sup>a</sup> (2.49)	61.80 <sup>b</sup> (2.88)
MC	2.93 <sup>b</sup> (0.33)	77.76 <sup>b</sup> (1.91)	55.24 <sup>a</sup> (1.57)
PC	1.92 <sup>c</sup> (0.18)	75.64 <sup>b</sup> (4.75)	58.93 <sup>b</sup> (1.58)

<sup>a</sup> Rheological parameters assessed by dynamic small amplitude oscillatory rheology. Data are means, with standard deviation in parenthesis, from three independent trials; means within the same column not sharing a similar superscript letter differ significantly ( $P < 0.05$ ).

**Table 5**

CIE LAB colour values of cheeses made from control, skim milk with cream (SC), reconstituted micellar casein concentrate with cream (MC) and reconstituted low heat skim milk powder with cream (PC) after 180 days of ripening. <sup>a</sup>

Sample	L*	a*	b*	$\Delta E^*_{ab}$
Control	81.05 <sup>b</sup> (1.81)	-4.44 <sup>a</sup> (0.23)	36.72 <sup>ab</sup> (2.42)	–
SC	80.91 <sup>b</sup> (1.19)	-4.36 <sup>a</sup> (0.32)	37.43 <sup>b</sup> (1.53)	1.82
MC	79.34 <sup>a</sup> (1.56)	-3.88 <sup>b</sup> (0.29)	35.56 <sup>a</sup> (1.43)	0.15
PC	79.74 <sup>ab</sup> (0.85)	-3.40 <sup>c</sup> (0.54)	36.07 <sup>ab</sup> (0.89)	1.47

<sup>a</sup> CIELAB data are means, with standard deviation in parenthesis, of five replicate analyses from three independent trials with  $\Delta E^*_{ab}$ , calculated from CIELAB results, between control and each treatment after 180 days of ripening. Means within the same column not sharing a similar superscript letter differ significantly ( $P < 0.05$ ). The just noticeable differences of  $\Delta E^*_{ab}$  was approximately 2.3.

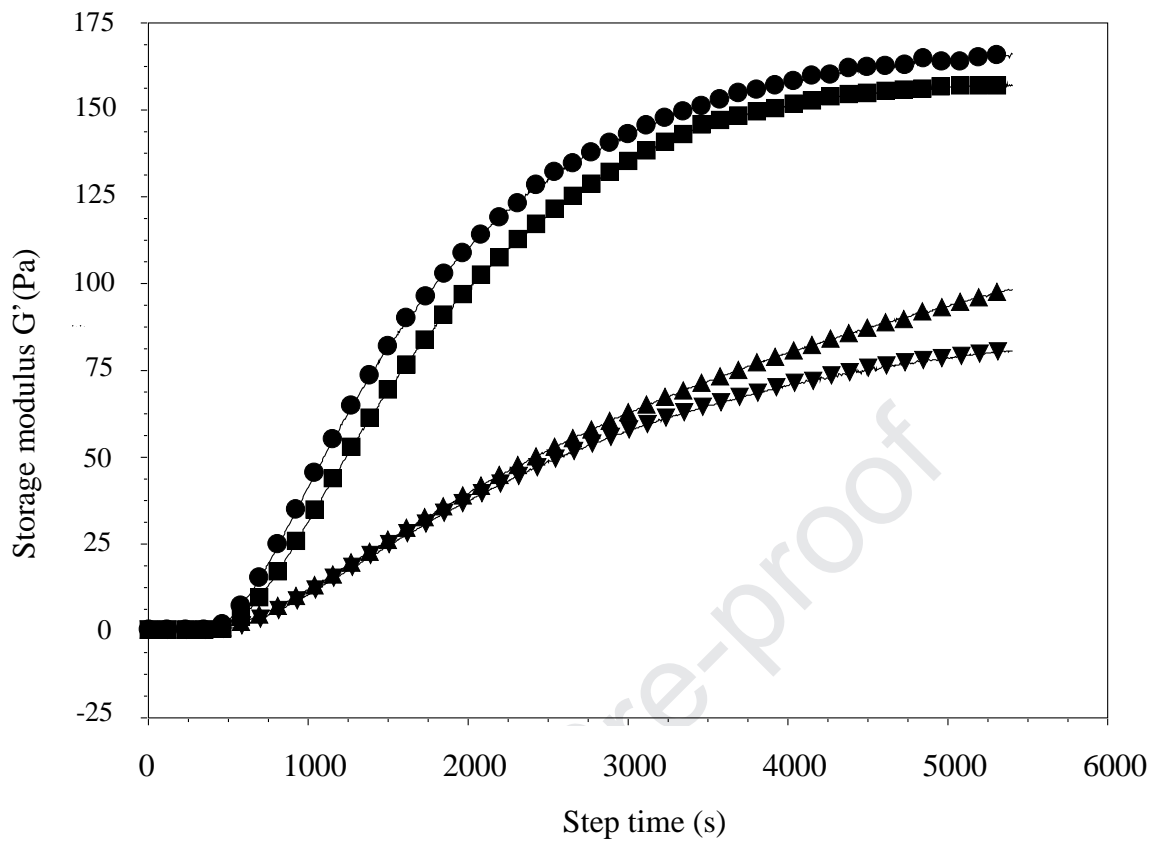
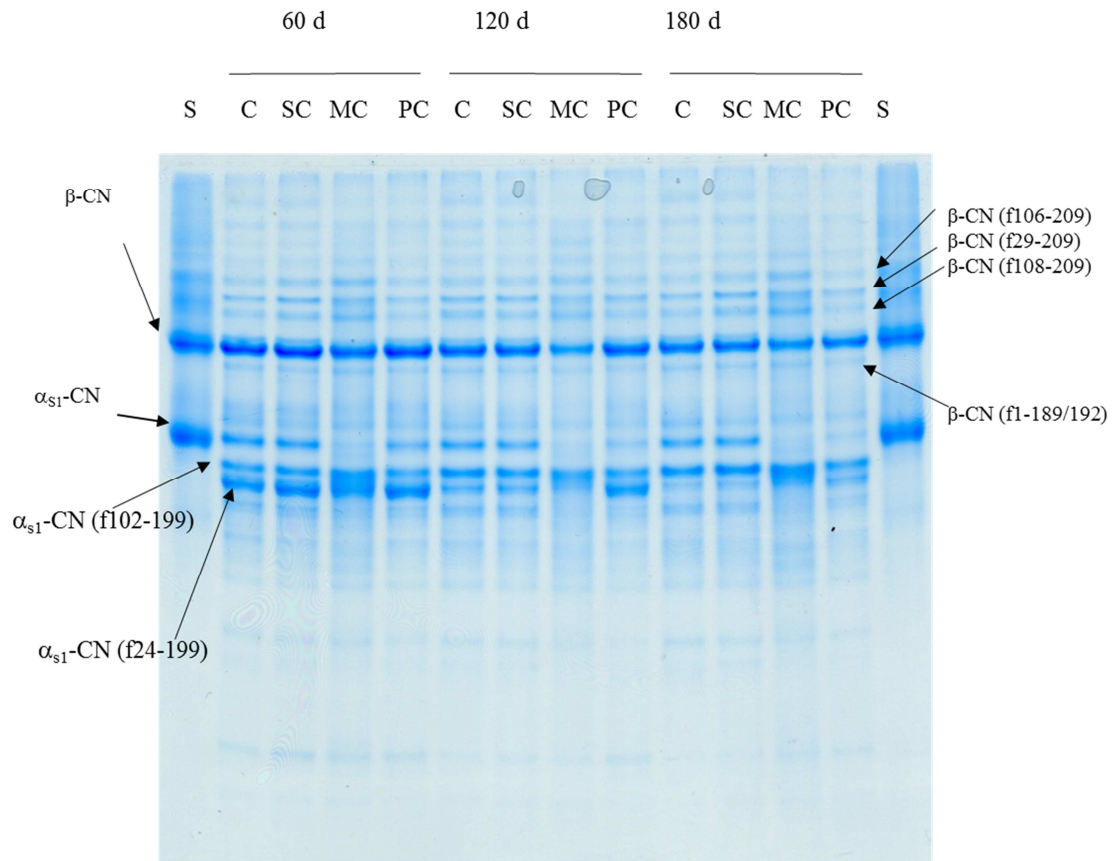


Figure 1



21

22

23

24

25

26

27

28

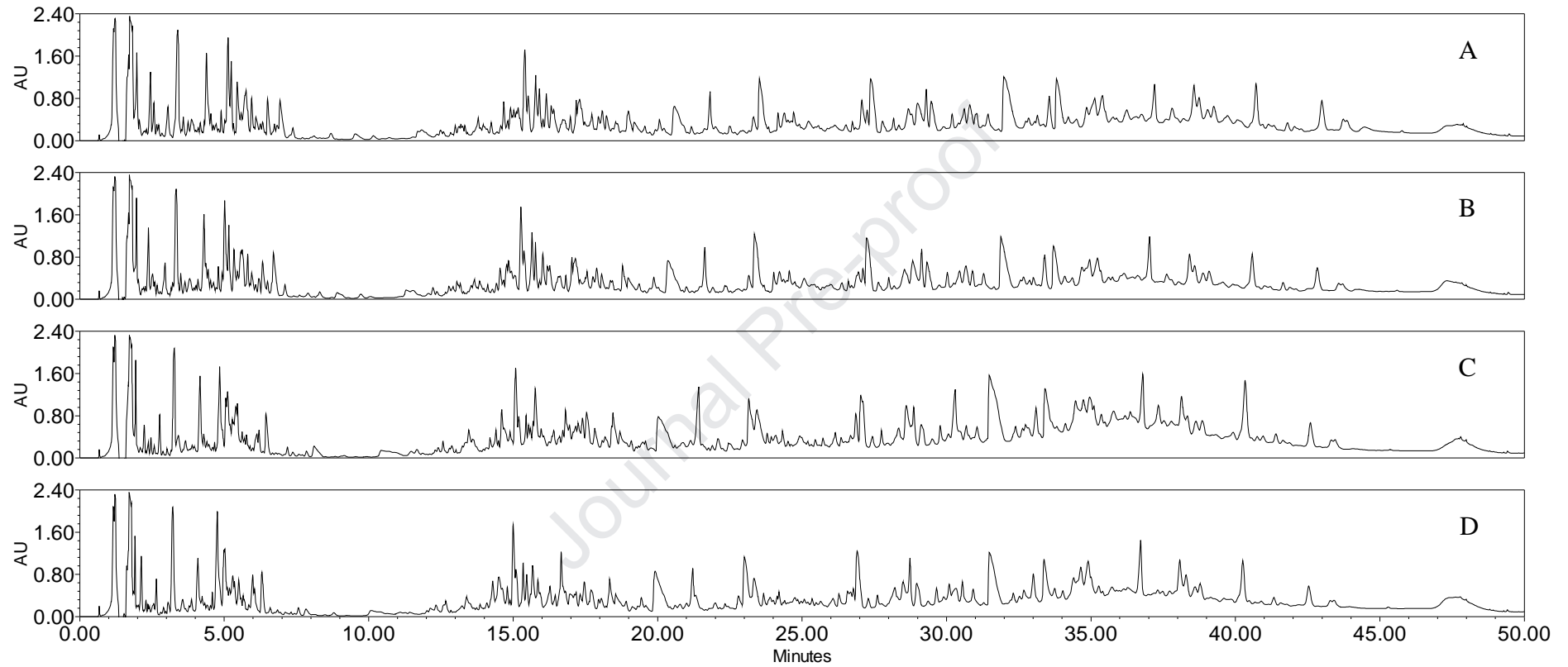
29 **Figure 2.**

30

31

32





33

34

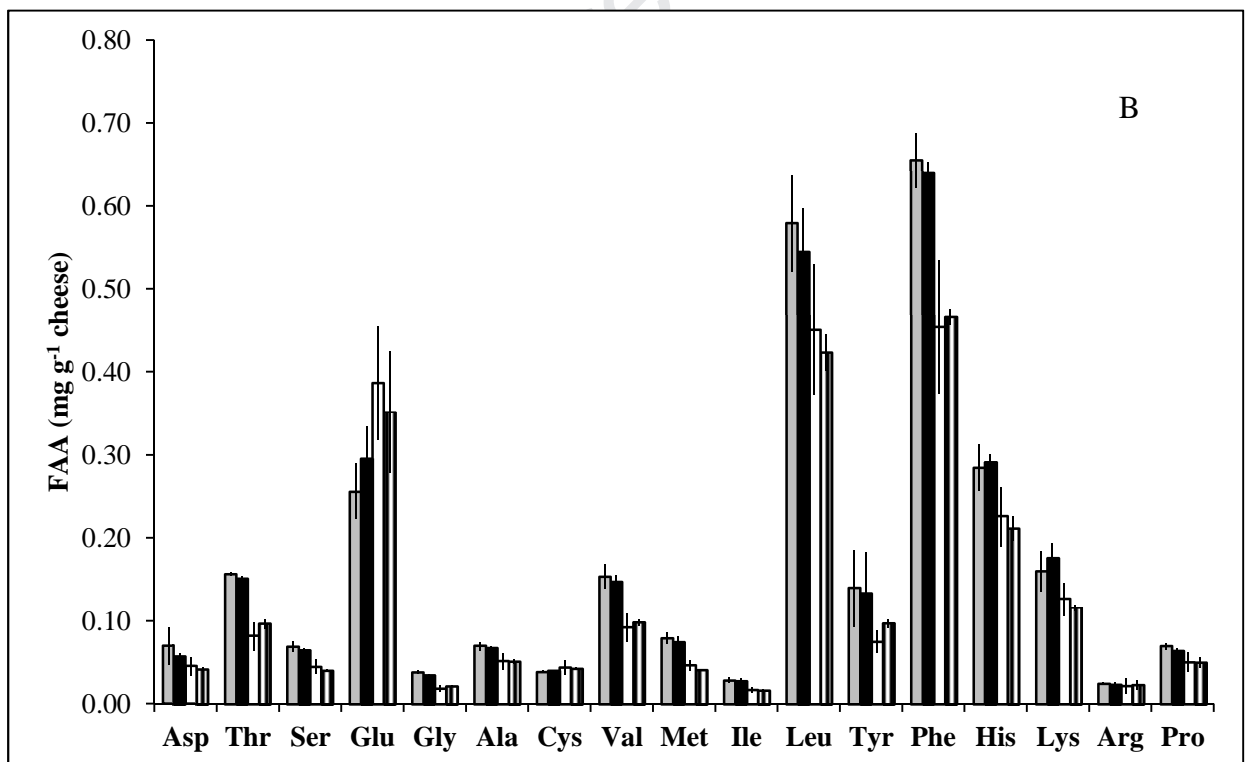
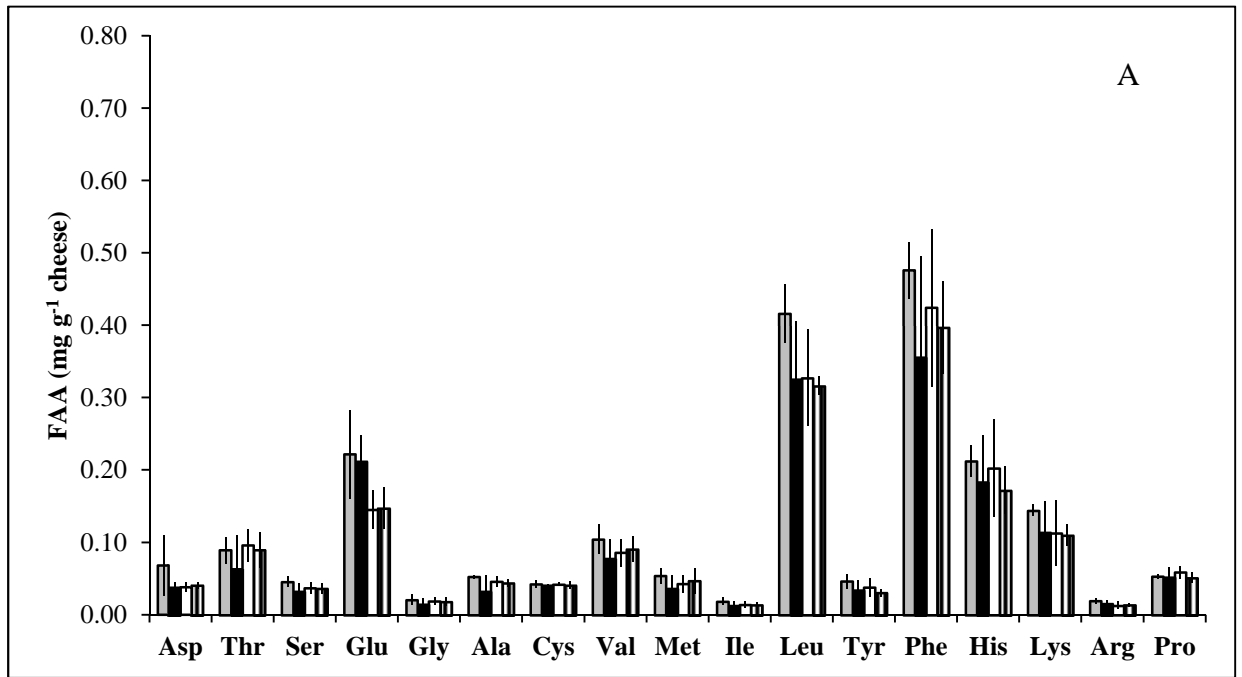
35 **Figure 3**

36

Journal Pre-proof

A

B



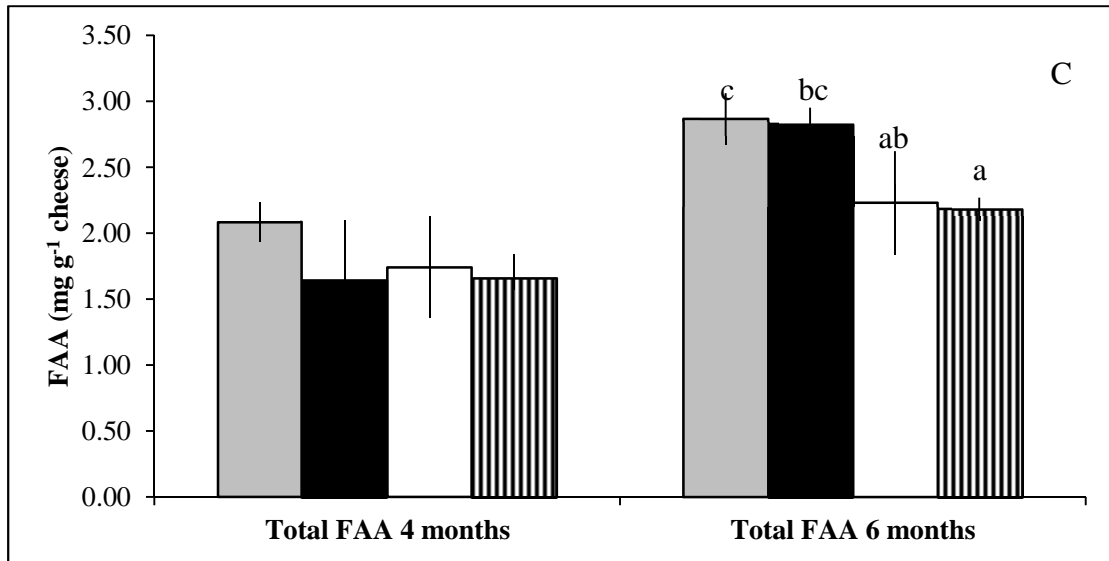


Figure 4

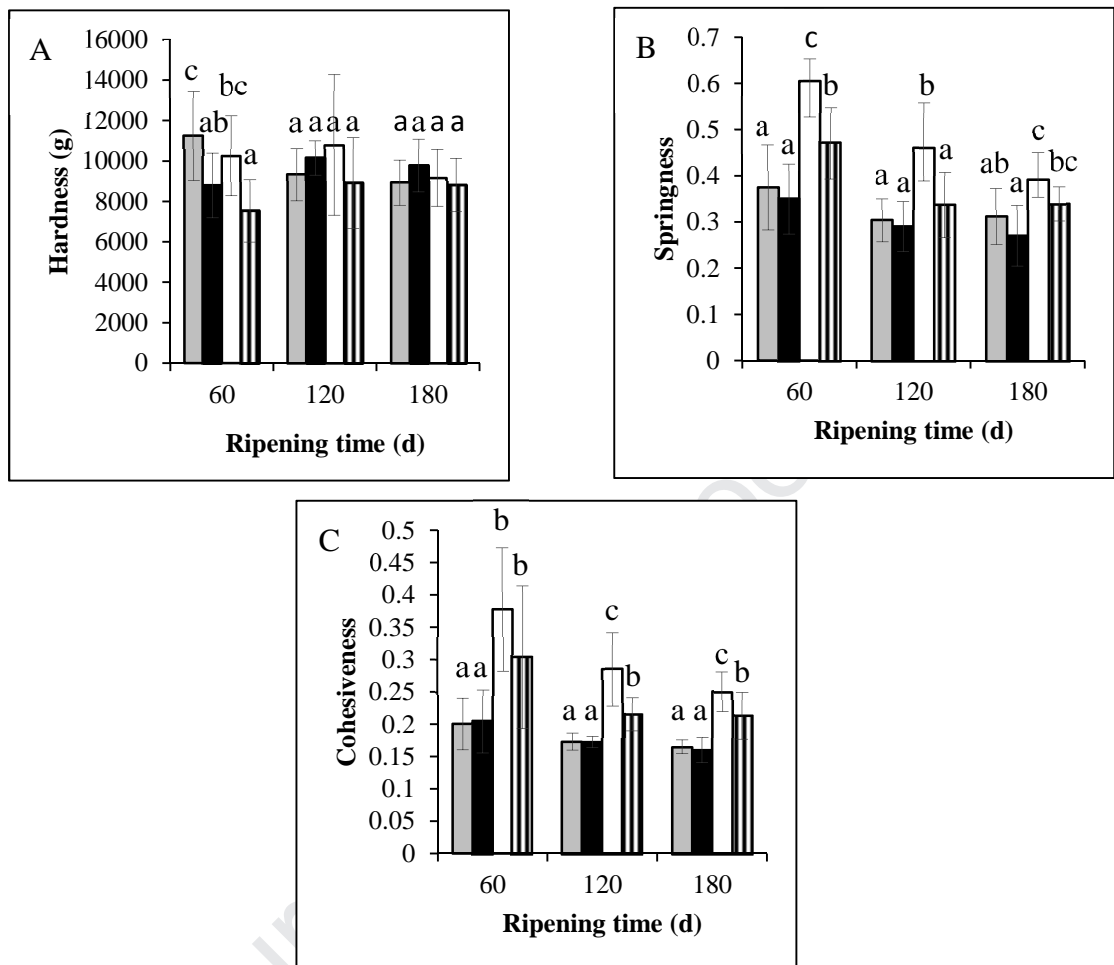


Figure 5

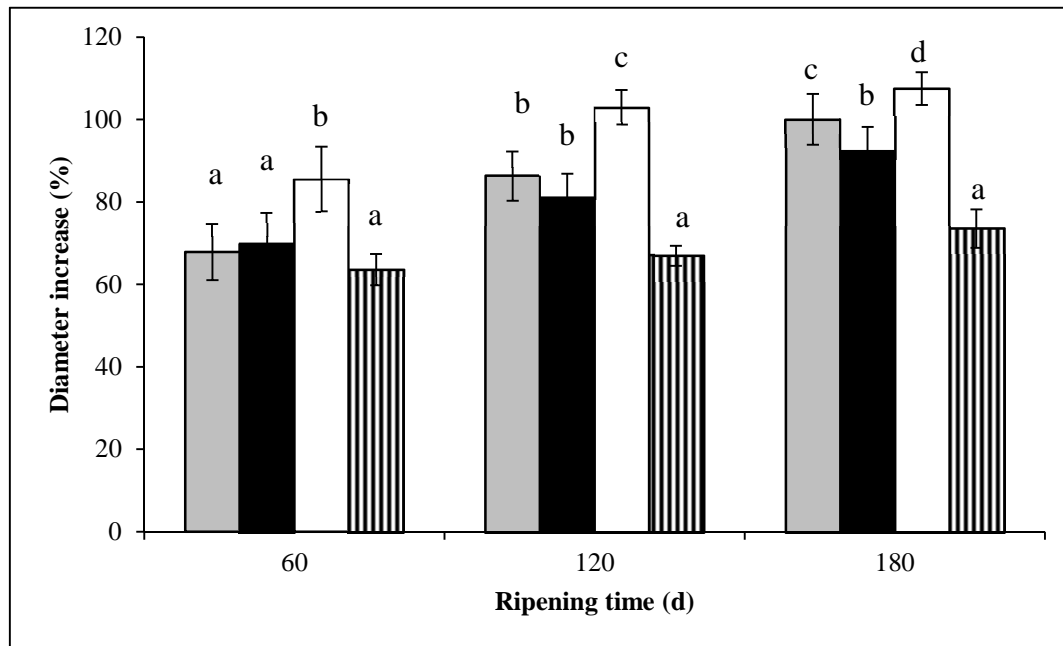


Figure 6