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# Textural and cooking properties and viscoelastic changes on heating and cooling of Balkan cheeses

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## ABSTRACT

The growth in food service and prepared consumer foods has led to increasing demand for cheese with customized textural and cooking characteristics. The current study evaluated Kačkavalj, Kačkavalj Krstaš, and Trappist cheeses procured from manufacturing plants in Serbia for texture profile characteristics, flow and extensibility of the heated cheese, and changes in viscoelasticity characteristics during heating and cooling. Measured viscoelastic parameters included elastic modulus, $G'$, loss modulus, $G''$, and loss tangent, LT ($G''/G'$). The melting temperature and congealing temperature were defined as the temperature at which LT = 1 during heating from 25 to 90°C and on cooling from 90 to 25°C. The maximum LT during heating was as an index of the maximum fluidity of the molten cheese. Significant variation was noted for the extent of flow and extensibility of the heated cheeses, with no trend of cheese type. As a group, the Kačkavalj cheeses had relatively high levels of salt-in-moisture and pH 4.6–soluble N and low protein-to-fat ratio and levels of $\alpha_{s1}$-CN (f24–199). They fractured during compression to 75%; had relatively low values of cohesiveness, chewiness, and springiness; melted at $\sim$70 to 90°C; reached maximum LT at 90°C; and congealed at 58 to 63°C. Conversely, the Kačkavalj Krstaš and Trappist cheeses had low levels of primary proteolysis and salt-in-moisture content and a high protein-to-fat ratio. They did not fracture during compression, had high values for cohesiveness and chewiness, melted at lower temperatures (56–62°C), attained maximum fluidity at a lower temperature (72–78°C), and congealed at 54 to 69°C. There was a hysteretic dependence of $G'$ and LT on temperature for all cheeses, with the LT during cooling being higher than that during heating, and $G'$ during cooling being lower or higher than the equivalent values during heating depending on the cheese type. Monitoring the dynamic changes in viscoelasticity during heating and cooling of the cheese in the temperature range 25 to 90°C provides a potentially useful means of designing ingredient cheeses, with the desired attributes when heated and cooled under customized specification.

**Key words:** Kashkaval, Trappist, texture, viscoelasticity, heating and cooling

## INTRODUCTION

Most cheeses were traditionally consumed as table cheeses, which may be arbitrarily defined as cheese eaten on its own or as an accompaniment to bread or crackers during meals. However, the rapid growth of the food-service and prepared-consumer-food sectors has led to many of the well-established cheeses being used extensively as ingredients in the preparation of culinary dishes, fast foods, snacks, and ready-prepared meals. Notable examples of natural cheeses used in ingredient applications include Cheddar, Mozzarella, and Emmental. In food-service applications, pertinent functionalities of unheated cheese include texture characteristics of the unheated cheese, and those of the heated cheese include overall appearance, flavor, and extent of flow, stringiness, fluidity, oiling-off, heat stability, and cooling behavior (e.g., how rapidly the cooked cheese congeals).

Kashkaval is a hard stretched-curd or *pasta filata* cheese produced across eastern Europe, central Asia, and north Africa. The cheese was traditionally made from ovine milk but today is made mostly from bovine milk or mixtures of bovine and ovine milks. Kashkaval and its variants include Kačkavalj (former Yugoslavia), Caşcaval Dobrogen (Rumania), Kashkaval Vitosha (Bulgaria), Kasar (Albania and Turkey), Kasseri (Greece), and Romy (Egypt) (Davis, 1976; Kindstedt et al., 2004). Kashkaval comes in the form of wheels varying in weight and minimum levels of DM and fat-in-dry matter (**FDM**) according to national legislation. According to the national standards requirement for Kačkavalj cheese, the Institute for Standardization of Serbia (1997) defines 2 types: Kačkavalj with a weight of 5 to 10 kg and minimum levels of DM and FDM of 56

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and 45% after 8 wk ripening, and Kačkavalj Krstaš with a weight up to 3 kg and minimum DM and FDM contents of 54 and 45%. The cheeses are described as having a high salt content (2.5–3.5%), a salty piquant flavor, and additionally as being sharp and aromatic when made from ovine milk. The production of Kashkaval cheese has been described by Kindstedt et al. (2004). Trappist is a semi-hard, soft, elastic, mild-flavored cheese that was first produced in 1882 at the monastery of "Marija Zvijezda, Banja Luka (Bosnia and Herzegovina) (Kirin, 2003; Budimir, 2014). It is wheel-shaped (1.6–2.0 kg), has a natural yellow smooth rind, a semi-hard smooth elastic consistency, and a clean, milky, slightly sweetish taste (Budimir, 2014). The above cheeses, in addition to being used as table cheeses, are increasingly used in cooking, notable applications such as Kashkaval pane, bouikous con Kashkaval, cheese soufflé, and pizza.

Yet, compared with other varieties such as Cheddar, Mozzarella, Gouda, or Emmental, relatively little is published on Kashkaval or Trappist cheese, especially in relation to their textural or cooking properties. Andiç et al. (2011) reported that the levels of water-soluble N, 12% trichloroacetic acid–soluble N, and 5% phosphotungstic acid–soluble N increased significantly in Kashkar (Turkish equivalent of Kashkaval) over a 180-d ripening period at 4°C to an extent depending on whether cheese was vacuum packaged or not. Simov et al. (2005) studied the effect of increasing milk casein content from 2.29 to 2.6% with micellar casein on Kashkaval cheese; fortification increased the percentage of milk fat and protein recovered to cheese and cheese yield, while giving cheese with typical texture. El-Safti and Khalil (2012) reported that the production of good-quality Trappist from water-buffalo milk necessitated the addition of ~0.1 to 0.15% sodium citrate to the cheese milk, readjustment of the milk pH to original value by addition of 5% lactic acid solution, and the use of *Lactobacillus helveticus* as an adjunct culture.

The objective of the current study was to evaluate the properties of 2 separate batches of factory-produced Kačkavalj, Kačkavalj Krstaš, and Trappist cheeses from Serbia, with special emphasis on the rheological properties of the uncooked cheese, flow and extensibility of the cooked cheese, and viscoelastic changes during heating and cooling.

## MATERIALS AND METHODS

### Cheese Samples

Six different cheeses (Table 1) varying in age were procured from different manufacturing sites in Serbia. Samples C1 to C3 were Kačkavalj cheeses in the form of 6- to 8-kg wheels from small producers using a traditional-style method of manufacture involving the use of whole (unstandardized) milk, manual kneading and stretching of the curd in hot water, and the addition and mixing of dry salt with plasticized curd before molding and dry salting of the cheeses 2 or 3 times during the first 4 to 6 wk of ripening. Samples C4 and C5 were wheel-shaped Kačkavalj Krstaš (~2.3 kg) cheeses produced on a large commercial scale from standardized milk using mechanical kneading–stretching followed by brine salting and vacuum wrapping. Sample C6 was industrially produced Trappist cheese. Two cheese loaves, 1 from each of 2 separate batches produced on the same day, were obtained for each cheese type, giving a total of 12 cheese samples that were analyzed as described below.

### Composition

A large wedge (~1 kg) was cut from each cheese wheel and shredded in a Hallde RG-350 machine (AB Hallde Maskiner, Kista, Sweden) using the raw food grating disc (K) to yield shreds ~25 mm long and ~4 mm in diameter. These shreds were then grated to particle size of <1 mm (Food Processor Russell Hobbs, Spectrum Brands Europe GmbH, Sulzbach, Germany). The grated cheese was analyzed in triplicate for moisture, fat, total protein, and salt using International Dairy Federation standard methods (Guinee et al., 2000a).

**Table 1.** Details of commercial Balkan cheeses

| Cheese | Type | Nominal weight (kg) | Milk | Production | Age (mo) | Packaging |
|---|---|---|---|---|---|---|
| C1 | Kačkavalj | 6.5 | Bovine | Traditional, small scale | 1 | Natural ripening and then vacuum wrapped |
| C2 | Kačkavalj | 6.5 | Bovine | Traditional, small scale | 6 | Natural ripening and then vacuum wrapped |
| C3 | Kačkavalj | 6.5 | Bovine–ovine milk mixture at weight ratio 65:35 | Traditional, small scale | 6 | Natural ripening and then vacuum wrapped |
| C4 | Kačkavalj Krstaš | 2.1 | Bovine | Industrial scale | 1 | Vacuum wrapped |
| C5 | Kačkavalj Krstaš | 2.1 | Bovine | Industrial scale | 3 | Vacuum wrapped |
| C6 | Trappist | 2.3 | Bovine | Industrial scale | 4 | Wax |

The pH was measured on cheese slurry prepared by macerating a blend of the grated cheese (20 g) and heated (40°C) distilled water (12 g).

### *Proteolysis*

The level of pH 4.6–soluble N was measured on a water-soluble extract of each cheese sample prepared from a slurry of grated cheese and water at a weight ratio of 1:2. The extract was adjusted to pH 4.6 using 1 *N* HCl, centrifuged at 3,000 × *g* for 30 min at 4°C, filtered through glass wool, and analyzed in duplicate for N using macro-Kjeldahl (Rynne et al., 2004).

Urea-PAGE was performed on the 2 batches of 6 cheeses on a ProteanS II xi cell vertical slab gel unit (Bio-Rad Laboratories Ltd., Hemel Hempstead, UK), using a separating and stacking gel system, as described by Rynne et al. (2004). The sample buffer (pH 8.7) was prepared by dissolving 0.75 g of Tris (hydroxymethyl) methylamine, 49 g of urea, 0.7 mL of mercaptoethanol, and 0.15 g of bromophenol blue in deionized water to a final volume of 100 mL. Cheeses were dissolved on a protein basis (4.75 mg/mL of sample buffer) and incubated at 55°C for 30 min. Cheese samples were then filtered through glass wool to remove fat. The gels (1 mm thick) were prerun at 280 V for 35 min before sample loading.

Gel electrophoretograms were scanned using a dual lens Epson Perfection V700 Photo Model J221A with Epson Scan software (Epson Deutschland GmbH, Meerbusch, Germany). The area of bands β-CN, $\alpha_{S1}$-CN, and $\alpha_{S1}$-CN (f24–199) for each cheese sample were expressed as a percentage of the total band area.

### *Texture Profile Analysis*

A slice of cheese (3–4 cm thick) along the center diameter was taken from each cheese wheel, and the outer portion (~1 cm) was removed. Three cube samples (2.5 cm) were cut from each cheese (Cheese Blocker; Bos Kaasgereedschap, Boven graven, Postbus, the Netherlands), wrapped securely in tin foil, and stored at 4°C overnight. Cheese cubes were individually taken from the fridge and compressed to 75% of original height in 2 consecutive bites at room temperature at a rate of 1 mm/s on a TAHDi texture analyzer (Stable Micro Systems, Goldalming, UK). The following parameters were defined from the resultant force–time curve (Gunasekaran and Ak, 2003): fracture stress, defined as the force at fracture in bite 1 per unit surface area of sample; hardness, defined as the maximum compression force recorded in bite 1; cohesiveness, defined as the ratio of integral of force by distance (work) to compress sample in bite 2 relative to bite 1, an index of the work required to chew the sample during 2 compressive bites; springiness, defined as the ratio of sample compression distance in bite 2 to sample compression distance in bite 1, an index of the recovery of sample height between bite 1 and bite 2; chewiness, defined as the product of cohesiveness × hardness × springiness, an index of the work required to masticate the sample to a state ready for swallowing; and resilience, defined as the ratio of integral of work during decompression in bite 1 to work during compression in bite 1, an index of the work exerted by the sample on the cross-head during withdrawal in bite 1, or the energy applied by the sample in regaining its original dimensions.

### *Cooking Properties*

A wedge was taken from each cheese wheel and cut into 5-mm-thick slices (MS250 SG slicer, Argenta Catering Equipment Ltd., Dundalk, Co. Louth, Ireland), from which 3 discs (5 mm thick, 47.5 mm in diameter) were cut using a stainless steel ring with a sharpened edge. Heat-induced flowability was defined as the percentage increase in diameter of the disc on heating at 280°C for 4 min according to the principle of the Schreiber test (Guinee and O'Callaghan, 2013). The flow is indicative of the melting behavior of cheese when exposed as a topping (e.g., on pizza) during oven heating.

The extensibility characteristics of molten cheese were measured using uniaxial extension on a texture-profile analyzer at a velocity of 10 mm/s. Grated cheese (60 g) was filled into a plastic container (9 cm × 5.5 cm × 4 cm) containing a comb and placed in a microwave oven (Whirlpool MW201, Fonthill Industrial Estate, Dublin, Ireland) set at 750 W and heated for 55 s to 90 to 95°C. The plastic container with molten cheese was placed in the measuring cell of a TAHDi texture analyzer. The comb was attached to the cross-head, which was programmed to pull the comb and, thereby, extend the molten cheese, to a maximum distance of 380 mm at a rate 10 mm/s. The analysis was undertaken in duplicate. Several parameters were calculated from the resultant force–distance curve (Figure 1): the maximum peak force (**PF**), which represents the maximum resistance to the initial movement of the comb through the molten cheese mass; the force at full extension (**FF**); and work required to extend the cheese by 380 mm (**EW**), as calculated from the integral of force by distance, or area under the curve. The surface temperature, measured using a noncontact infrared thermometer (Optex Thermo-Hunter PT-3S; Graham and White Instruments Ltd., St Albans, UK),

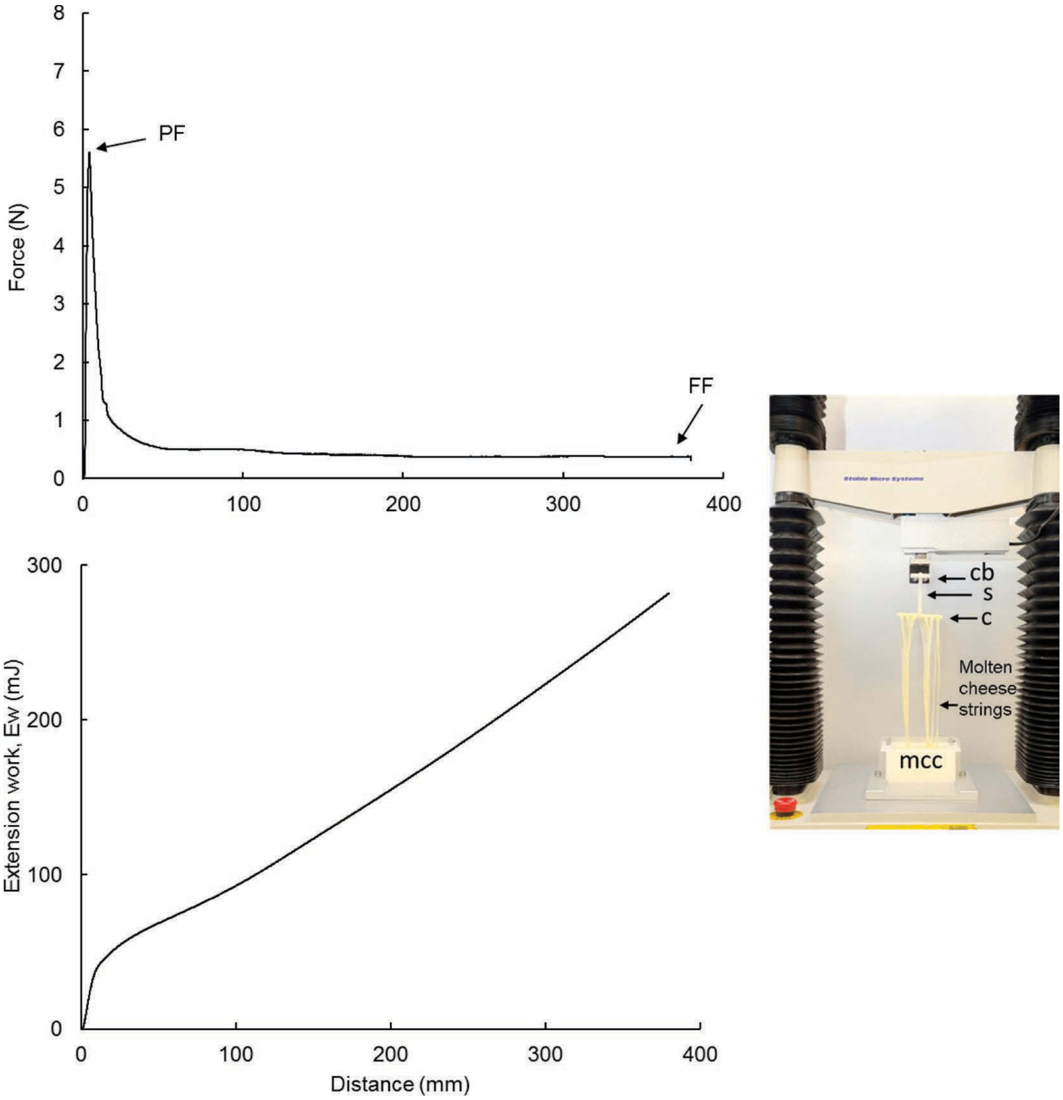

**Figure 1.** Typical force–displacement curve and resultant extension work–distance curves on extending molten Kačkavalj cheese on the texture analyzer. Calculated parameters include peak force (PF), final force at an extension of 380 mm (FF), and the work for full extension (EW), calculated from the integral of force by distance. Inset shows the hot molten cheese (94°C) being extended at 10 mm/s on the TAHDi Texture Analyzer (Stable Micro Systems, Godalming, UK). The measurement apparatus consisted of a molten cheese container (mmc) and an extension device composed of a comb (c), a shaft (s) attached to the comb, and a cross-beam (cb) that connects to the cross-head of the texture analyzer. Color version available online.

decreased from ~94°C for the molten cheese mass on removal from the oven to ~35 to 45°C for the extended strings at maximum extension.

### *Changes in Viscoelasticity During Heating and Cooling*

Dynamic changes in viscoelastic parameters (storage modulus, G′; loss modulus, G″; loss tangent, G″/G′) during heating of cheese discs (40 mm in diameter, 2 mm thick) were measured using low-amplitude strain oscillation rheometry (Anton Paar Rheometer MCR50, Anton Paar GmbH, Graz, Austria), as described previously (Guinee and O'Callaghan, 2013). Cheese discs were placed between 2 parallel, serrated plates (40 mm in diameter) of the rheometer cell, tempered at 25°C for 15 min, and subjected to a low amplitude shear strain ($\gamma$) of 0.0063 at an angular frequency of 1 Hz. The temperature was increased from 25 to 90°C over 20 min and then reduced to 25°C over 20 min. The following parameters were calculated from the changes in G′ and G″ with temperature during heating (h): G′ at 25°C, an index of the elasticity on the unheated cheese at 25°C; the value of loss tangent at 25°C, which equals G″ at

25°C/G′ at 25°C; the crossover temperature, where G′ during heating = G″ during heating, or loss tangent during heating (**LTh** = G″ during heating/G′ during heating) = 1, and which marks the transition from a viscoelastic solid to a viscoelastic fluid, an index of the melting point; the maximum value of loss tangent (**LThmax**), an index of the maximum fluidity the sample attains during heating; the temperature at LThmax (**TLThmax**), an index of temperature at which the melting cheese mass is most fluid; loss tangent at 90°C; difference between LThmax and loss tangent at 90°C, an index of the loss of fluidity on heating to temperatures >TLThmax; and G′ at 90°C, an index of elasticity at 90°C. Similarly, various indices were obtained from the changes in G′ and G″ during cooling (c) of the heated cheese to 25°C: the crossover temperature during cooling (**COTc**), where G′ during cooling = G″ during cooling, or loss tangent during cooling (**LTc**) = 1, an index of the congealing temperature; loss tangent after cooling heated cheese back to 25°C, an index of fluidity of cooled cheese; and G′ after cooling to 25°C, an index of the elasticity of the cooled cheese.

### Statistical Analysis

The data were analyzed using a randomized complete block design that incorporated 6 treatment levels (cheeses) and 2 blocks (replicate batches of each cheese). An ANOVA was carried out using a SAS procedure (SAS Institute Inc., 2003) where the effect of treatment and replicates were estimated for all response variables. Tukey's multiple-comparison test was used for paired comparison of treatment means, and the level of significance was determined at $P < 0.05$. The data for some response variables were also analyzed by linear regression to establish possible correlations between the response variables [e.g., salt-in-moisture (**S/M**) and cohesiveness]. The significance of correlations was determined by applying Student's *t*-test to correlation coefficients, where n is the actual number of data points, and DF is the degrees of freedom (n-2).

## RESULTS AND DISCUSSION

### Cheese Composition

The compositions of the cheeses are shown in Table 2. Cheeses C1 to C3 complied with the minimum levels of DM (56%) and FDM (45%) specified for Kačkavalj (Institute for Standardization of Serbia, 1997). Nevertheless, significant intercheese compositional variation was evident for these cheeses, especially for moisture, fat, salt, and S/M content. This undoubtedly reflects the small-scale production and the traditional method of manufacture, which involves the use of whole (unstandardized) milk, manual kneading and stretching of the curd in hot water, mixing of dry salt with the plasticized curd before molding, and additional dry salting of the cheeses 2 or 3 times during the first 4 to 6 wk of ripening, and ripening without wrapping. The relatively high salt and S/M levels reflect the traditional-style manufacture of Kačkavalj-type cheeses, where high salt content was employed as a means of enhancing the keeping quality of cheese from milks of poor microbiological status and high lactic acid content in a hot climate. Such a process is conducive to interbatch variation in protein-to-fat ratio of the cheese milk and moisture loss during ripening and, hence, to inconsistency in composition. Similarly, analysis of the published data shows significant variation between Kashkaval cheeses depending on milk used and region of manufacture (Davis, 1976; Kindstedt et al., 2004).

The DM and FDM levels of cheese C5 conformed to the levels (54 and 45%, respectively) specified for Kačkavalj Krstaš (Institute for Standardization of Serbia, 1997), whereas the DM of C4 was lower than specified. Despite significant differences in DM, salt, S/M, and moisture in nonfat substances of the Kačkavalj Krstaš cheeses, the magnitude of the differences was comparatively small relative to the differences between the Kačkavalj cheeses. The greater compositional uniformity reflects the use of standardized, industrial-scale manufacture of the Kačkavalj Krstaš cheeses, which involves milk standardization, mechanical kneading, stretching and salting of the curd in hot-brine solution with a defined salt level, and ripening of vacuum-wrapped cheese in laminated plastic bags.

Comparison of the Kačkavalj and Kačkavalj Krstaš cheeses showed that the former had significantly lower levels of moisture and protein-to-fat ratio and higher levels of salt and S/M.

The Trappist cheese had higher levels of moisture, fat, moisture in nonfat substances, salt, and S/M and lower levels of protein than those reported in the literature (Popoević-Vranjaneš et al., 2005; Budimir, 2014).

### Proteolysis

***Urea-PAGE.*** The urea-PAGE gel electrophoretogram patterns of both batches of cheese are shown in Figure 2. Although the replicate batches differed in the intensity of different proteins and peptides (Table 3), the overall pattern was generally similar. In agreement with the electrophoretic for most hard-cheese varieties including Cheddar, Mozzarella, and Gouda, the concentration of intact β-CN was notably higher than that of $\alpha_{s1}$-CN in all cheeses. Nevertheless, all cheeses revealed the presence of β-CN degradation products,

**Table 2.** Composition of commercial Balkan cheeses[1]

| | Cheese code[3] | | | | | |
|---|---|---|---|---|---|---|
| Item[2] | C1 | C2 | C3 | C4 | C5 | C6 |
| Moisture (% wt/wt) | 43.5$^{c}$ | 40.8$^{e}$ | 34.9$^{f}$ | 47.6$^{a}$ | 45.8$^{b}$ | 42.6$^{d}$ |
| | (0.42) | (0.49) | (0.57) | (0.71) | (0.35) | (0.67) |
| Fat (% wt/wt) | 33.1$^{a}$ | 31.8$^{a}$ | 33.8$^{a}$ | 27.9$^{a}$ | 29.2$^{a}$ | 30.3$^{a}$ |
| | (2.97) | (1.06) | (1.73) | (2.67) | (1.69) | (1.12) |
| Protein (% wt/wt) | 20.6$^{c}$ | 23.3$^{b}$ | 24.1$^{ab}$ | 23.4$^{b}$ | 25.5$^{a}$ | 24.6$^{ab}$ |
| | (0.93) | (0.11) | (0.03) | (0.06) | (0.3) | (0.15) |
| Salt (% wt/wt) | 4.01$^{a}$ | 2.37$^{c}$ | 3.17$^{b}$ | 1.18$^{d}$ | 0.92$^{e}$ | 2.31$^{c}$ |
| | (0.29) | (0.38) | (0.17) | (0.11) | (0.04) | (0.23) |
| S/M (% wt/wt) | 9.22$^{a}$ | 5.81$^{b}$ | 9.09$^{a}$ | 2.48$^{d}$ | 2.01$^{e}$ | 5.43$^{c}$ |
| | (0.58) | (0.87) | (0.34) | (0.20) | (0.08) | (0.45) |
| DM (% wt/wt) | 56.6$^{d}$ | 59.2$^{b}$ | 65.1$^{a}$ | 52.4$^{f}$ | 54.2$^{e}$ | 57.4$^{c}$ |
| | (0.42) | (0.49) | (0.57) | (0.71) | (0.35) | (0.67) |
| MNFS (% wt/wt) | 63.0$^{ab}$ | 59.6$^{b}$ | 54.1$^{c}$ | 61.8$^{a}$ | 61.0$^{ab}$ | 58.3$^{ab}$ |
| | (3.52) | (1.65) | (0.52) | (3.43) | (2.04) | (1.95) |
| FDM (% wt/wt) | 58.6$^{a}$ | 53.7$^{a}$ | 51.9$^{a}$ | 53.2$^{a}$ | 53.8$^{a}$ | 52.9$^{a}$ |
| | (5.7) | (2.27) | (2.2) | (5.82) | (3.47) | (2.58) |
| PF (-) | 0.67$^{b}$ | 0.73$^{ab}$ | 0.72$^{ab}$ | 0.84$^{a}$ | 0.88$^{a}$ | 0.81$^{a}$ |
| | (0.03) | (0.03) | (0.04) | (0.08) | (0.06) | (0.06) |
| pH[4] | 5.20 | 5.17 | 5.20 | 5.13 | 5.24 | 5.45 |

[a–f] Values within a row not sharing a common superscript differ significantly ($P < 0.05$).

[1] Values presented are the means of 2 replicate batches for each cheese; SD of the mean is in parentheses.

[2] S/M = salt in moisture; MNFS = moisture in nonfat substances; FDM = fat in DM; PF = protein-to-fat ratio.

[3] Cheese code (as defined in Table 1): C1 = Kačkavalj; C2 = Kačkavalj; C3 = Kačkavalj; C4 = Kačkavalj Krstaš; C5 = Kačkavalj Krstaš; C6 = Trappist.

[4] Data for one batch of the cheeses only; data included as an observation.

β-CN (f29–209) ($\gamma_1$), β-CN (f106–209) ($\gamma_2$), and β-CN (f108–209) ($\gamma_3$). The presence of γ-CN in the Kačkavalj and Kačkavalj Krstaš cheeses, C1 to C5, suggests the activity of plasmin (Farkye and Fox, 1992), which survives the high temperature (~60°C) to which the curd is subject during the plasticization process. Farkye and Fox (1990) reported that plasmin activity in rennet curd cooked and held for 45 min increased significantly as the cook temperature was increased from 30 to 60°C, probably due to the increasing conversion of plasminogen to plasmin.

$\alpha_{s1}$-Casein was hydrolyzed in all cheeses to fragments of higher electrophoretic mobility including $\alpha_{s1}$-CN (f102–199), $\alpha_{s1}$-CN (f24–199), $\alpha_{s1}$-CN (f33), $\alpha_{s1}$-CN (f60-), and $\alpha_{s1}$-CN (f24-), as identified according to McSweeney et al. (1994). The content of intact $\alpha_{s1}$-CN was lowest for C3 and highest in C1. $\alpha_{s1}$-Casein (f24–199), considered the primary degradation product of $\alpha_{s1}$-CN on hydrolysis by chymosin, accumulated to high levels in the Kačkavalj Krstaš and Trappist cheeses but was more extensively degraded in the Kačkavalj cheeses. Cheese C3 made from a mixture of bovine and ovine milk contained 2 distinct bands of slightly lower electrophoretic mobility than $\alpha_{s1}$-CN and which likely correspond to $\alpha_{s2}$/$\alpha_{s3}$-CN, as identified by Richardson and Creamer (1976) in ovine milk.

***pH 4.6–Soluble N.*** The level of pH 4.6–soluble N ranged from ≤5.1% of total N in the Kačkavalj Krstaš (C4, C5) and Trappist (C6) cheeses to ~ ≥12.1% in the Kačkavalj cheeses (C1–C3) (Table 3). The low levels in the Kačkavalj Krstaš and Trappist cheeses are consistent with their relatively high quantities of $\alpha_{s1}$-CN (f24–199). Nevertheless, this trend is somewhat surprising considering that the former cheeses had the lowest levels of S/M (Table 2). However, apart from composition, the level of pH 4.6–soluble N is also likely to be influenced by intercheese differences in rennet activity (as influenced by pH at whey drainage, its thermal stability, and curd temperatures during plasticization), ripening time and temperature, and type of starter culture (e.g., ratio of *Streptococcus thermophilus* to *Lactobacillus* strains; Yun et al., 1993a,b; Feeney et al., 2001).

### Texture Profile Analysis

The texture profile analysis parameters of the different cheeses are shown in Table 4. The most distinguishing feature of the analysis was the shape of the stress–time (displacement) curves. The Kačkavalj cheeses C1 to C3 (Figure 3) were characterized by a distinctive inflection point in bite 1, representing a fracture peak. For these cheeses, the stress increased convex upward, then decreased as fracture occurred, and eventually increased as the fractured cheese mass was further compressed. In contrast, the force increased continuously concave

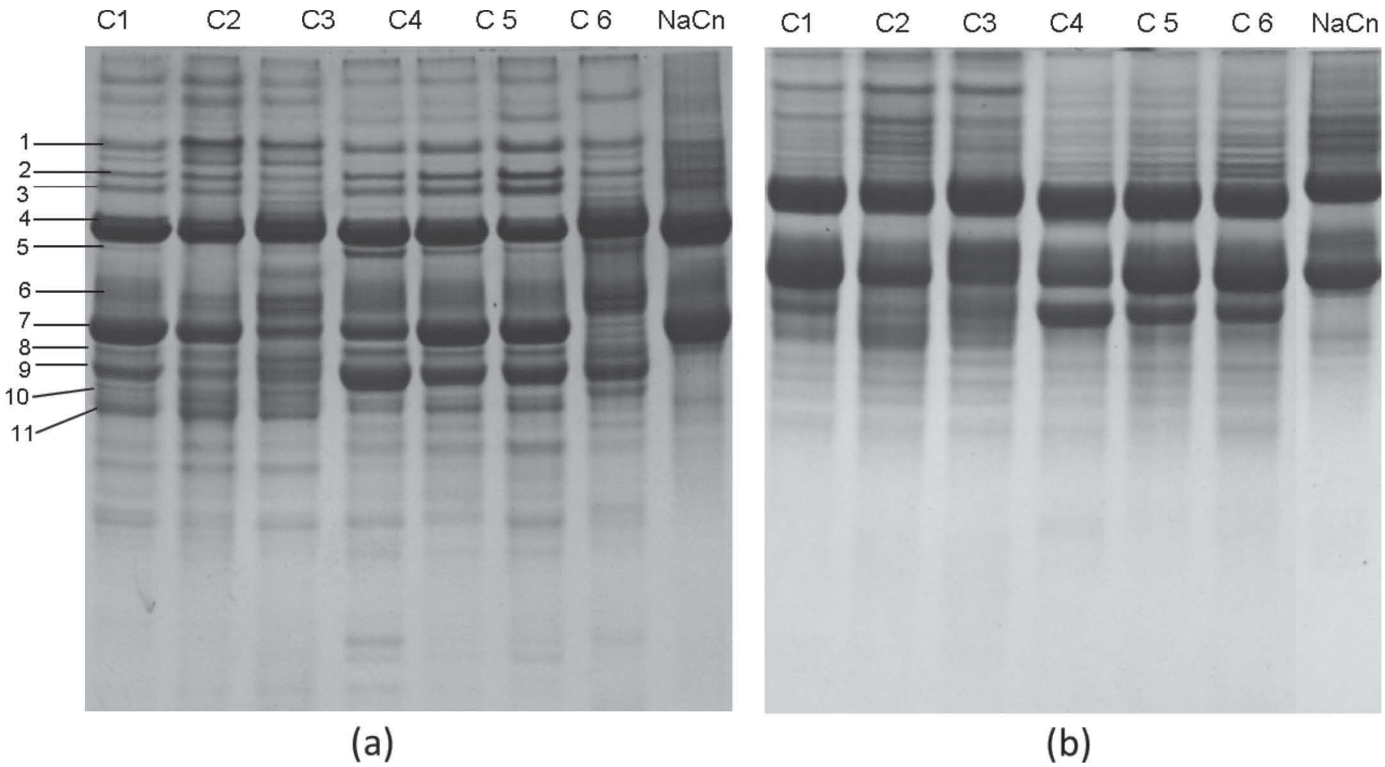

**Figure 2.** Urea-polyacrylamide gel electrophoretograms of replicate batches of commercial cheese (a and b), each batch consisting of Kačkavalj (C1–C3), Kačkavalj Krstaš (C4, C5), and Trappist cheese (C6); sodium caseinate (NaCn) was used as a control. The gel was loaded with a fixed weight of protein (4.25 mg of protein per lane). Proteins and peptides were identified according to Richardson and Creamer (1976) and McSweeney et al. (1994): 1 = β-CN (f106–209) ($\gamma_2$); 2 = β-CN (f29–209) ($\gamma_1$); 3 = β-CN f108–209 ($\gamma_3$); 4 = β-CN; 5 = β-CN (f1–192); 6 = $\alpha_{s2}$/$\alpha_{s3}$-CN; 7 = $\alpha_{s1}$-CN; 8 = $\alpha_{s1}$-CN (f102–199); 9 = $\alpha_{s1}$-CN (f24–199); 10 = $\alpha_{s1}$-CN (f121–199); and 11 = $\alpha_{s1}$-CN (f33-*).

upward without fracture for the Kačkavalj Krstaš and Trappist cheeses.

The relatively high level of proteolysis in the Kačkavalj cheeses is likely to be a key contributory factor to their fracture behavior. This suggestion is supported by the reduction in the fracture strain of cheeses such as Cheddar and Gouda during maturation, commensurate with the increase in *para*-casein hydrolysis (Visser, 1991; Fenelon and Guinee, 2000; Costa et al., 2010). However, other factors, including the S/M,

**Table 3.** Proportions of different casein and level of pH 4.6–soluble N (pH4.6SN) in commercial Balkan cheeses[1]

| | Cheese code[2] | | | | | |
|---|---|---|---|---|---|---|
| Item | C1 | C2 | C3 | C4 | C5 | C6 |
| Intact β-CN[3] | 29.4$^{a}$ | 26.6$^{a}$ | 25.2$^{a}$ | 25.8$^{a}$ | 27.4$^{a}$ | 23.5$^{a}$ |
| | (1.3) | (2.2) | (7.3) | (4.7) | (7.7) | (7.6) |
| Intact $\alpha_{S1}$-CN[3] | 18.6$^{a}$ | 12.4$^{abc}$ | 8.2$^{c}$ | 10.2$^{bc}$ | 17.2$^{ab}$ | 13.7$^{abc}$ |
| | (3.0) | (1.3) | (3.6) | (2.3) | (0.1) | (1.7) |
| $\alpha_{S1}$-CN (f24–199)[3] | 4.0$^{bc}$ | 2.2$^{c}$ | 2.7$^{bc}$ | 15.2$^{a}$ | 8.2$^{b}$ | 7.9$^{bc}$ |
| | (1.0) | (0.6) | (0.9) | (2.5) | (2.2) | (2.8) |
| pH4.6SN[4] (% of total N) | 12.1$^{b}$ | 17.2$^{a}$ | 16.4$^{a}$ | 5.1$^{c}$ | 2.9$^{c}$ | 4.4$^{c}$ |
| | (0.35) | (0.64) | (0.10) | (0.66) | (0.09) | (0.71) |

[a–c] Values within a row not sharing a common superscript differ significantly ($P < 0.05$).

[1] Values presented are the means of 2 replicate batches for each cheese; SD of the mean is in parentheses.

[2] Cheese code (as defined in Table 1): C1 = Kačkavalj; C2 = Kačkavalj; C3 = Kačkavalj; C4 = Kačkavalj Krstaš; C5 = Kačkavalj Krstaš; C6 = Trappist.

[3] Proportions of casein expressed as percentage of total protein.

[4] pH4.6SN = pH 4.6 soluble N.

probably also contributed to fracture in these cheeses. A model study, involving the immersion of unsalted Cheddar cheese cylinders in brine solutions of varying S/M content, found that the solubilization of cheese protein increased with S/M in the range 0.1 to 6% but decreased sharply as the S/M content was increased further to 15% (Everett et al., 2014). Hence, the high S/M concentration, especially at the level present in the Kačkavalj cheeses, is likely to contribute to salting out and dehydration of the casein and a less continuous calcium phosphorous *para*-casein network structure. Consequently, cheeses with a high S/M content (>6% S/M) tend to be more brittle (lower fracture strain) than cheeses with lower S/M content (Visser, 1991; Everett et al., 2014). Despite their overall similar fracture behavior, the Kačkavalj cheeses differed; cheese C1 had a significantly lower fracture stress and a higher fracture strain than C2 or C3, indicating that it was more rubbery and less brittle than the latter cheeses. These differences probably reflect variation in age.

The Kačkavalj cheeses were also significantly less cohesive and chewy than the Kačkavalj Krstaš cheese, which was expected because of their higher levels of proteolysis. However, the relatively low protein-to-fat ratio and high S/M probably also contributed to the relatively low cohesiveness and chewiness. Linear regression analysis of the data for all samples indicated that the latter texture profile analysis parameters were positively correlated with protein-to-fat ratio (r = 0.88–0.92) and negatively with S/M content (r = 0.75–0.87).

The values of cohesiveness, chewiness, and springiness of all cheeses were within the range reported for Cheddar cheese ripened over 180 d at 8°C by O'Mahony et al. (2005) and Costa et al. (2010), using measurement conditions similar to those of the current study. In contrast, the values of cohesiveness and springiness were notably lower than those (0.45–0.65 and 0.70–0.85, respectively) reported by Chevanan et al. (2006) for Cheddar cheeses varying in lactose, calcium, and salt level and ripened over 8 mo at 5°C, probably because of the higher degree of compression (75%) compared with that (25%) in the study of Chevanan et al. (2006).

## Cooking Properties

***Flowability.*** The extent of flow on heating at 280°C for 4 min (~28–48%, Table 5) was within the range reported previously for low-moisture part-skim Mozzarella after storage for 20 to 60 d (Yun et al., 1993a; Guinee et al., 2001) and in Cheddar after storage for 30 to 150 d (Guinee et al., 2000a). Surprisingly, the flow of cheese C3, which had a relatively high level of pH 4.6–soluble N and the highest level of $\alpha_{s1}$-CN degradation, was significantly lower than that of all other cheeses, apart from the Trappist, C6, cheese, which had a significantly lower pH 4.6–soluble N. However, previous studies have shown that flow (based on the Schreiber method) increases during ripening concurrently with level of pH 4.6–soluble N to a critical value but then essentially plateaus at a value of 45 to 50% despite further increases in proteolysis (Rynne et al., 2004).

**Table 4.** Texture-profile parameters of commercial Balkan cheeses[1]

| | Cheese code[3] | | | | | |
|---|---|---|---|---|---|---|
| Item[2] | C1 | C2 | C3 | C4 | C5 | C6 |
| $\sigma_{\max}$ (N) | $155.8^{c}$<br>(1.4) | $228.4^{b}$<br>(2.6) | $240.9^{a}$<br>(6.0) | $187.9^{c}$<br>(26.1) | $263.0^{a}$<br>(22.6) | $265.9^{a}$<br>(12.2) |
| $\sigma_{f}$ (kPa) | $129.3^{b}$<br>(9.4) | $217.7^{a}$<br>(17.1) | $220.0^{a}$<br>(3.9) | — | — | — |
| $\varepsilon_{f}$ (-) | $0.55^{a}$<br>(0.04) | $0.31^{b}$<br>(0.02) | $0.26^{b}$<br>(0.02) | — | — | — |
| Springiness (-) | $0.39^{bc}$<br>(0.03) | $0.30^{d}$<br>(0.03) | $0.26^{d}$<br>(0.02) | $0.44^{b}$<br>(0.04) | $0.38^{c}$<br>(0.03) | $0.52^{a}$<br>(0.01) |
| Cohesiveness (-) | $0.21^{bc}$<br>(0.03) | $0.13^{c}$<br>(0.01) | $0.14^{c}$<br>(0.01) | $0.37^{a}$<br>(0.03) | $0.28^{ab}$<br>(0.01) | $0.24^{b}$<br>(0.03) |
| Chewiness (N) | $11.8^{bc}$<br>(1.05) | $8.6^{c}$<br>(0.62) | $8.5^{c}$<br>(1.27) | $30.0^{ab}$<br>(9.87) | $27.7^{ab}$<br>(5.55) | $32.5^{a}$<br>(2.53) |
| Resilience (-) | $0.11^{ab}$<br>(0.014) | $0.08^{b}$<br>(0.004) | $0.08^{b}$<br>(0.002) | $0.15^{a}$<br>(0.025) | $0.08^{b}$<br>(0.010) | $0.08^{b}$<br>(0.00) |

[a–d] Values within a row not sharing a common superscript differ significantly ($P < 0.05$).

[1] Values presented are the means of 2 replicate batches for each cheese; SD of the mean is in parentheses. Samples C4 to C6 did not undergo fracture (—).

[2] $\sigma_{\max}$ = hardness; $\sigma_{f}$ = fracture stress; $\varepsilon_{f}$ = fracture strain.

[3] Cheese code (as defined in Table 1): C1 = Kačkavalj; C2 = Kačkavalj; C3 = Kačkavalj; C4 = Kačkavalj Krstaš; C5 = Kačkavalj Krstaš; C6 = Trappist.

**Table 5.** Flow and extensibility parameters of commercial Balkan cheeses[1]

| | Cheese code[3] | | | | | |
|---|---|---|---|---|---|---|
| Item[2] | C1 | C2 | C3 | C4 | C5 | C6 |
| Flow (%) | 45.3[a] | 47.8[a] | 28.1[b] | 42.8[a] | 41.1[a] | 30.4[b] |
| | (1.3) | (2.1) | (2.8) | (2.4) | (1.6) | (2.1) |
| EW (mJ) | 274[b] | 291[b] | 144[c] | 140[c] | 442[a] | 221[bc] |
| | (35.7) | (37.2) | (18.5) | (14.9) | (37.1) | (0.9) |
| PF (N) | 5.6[a] | 6.5[a] | 3.8[a] | 3.3[a] | 8.5[a] | 6.4[a] |
| | (2.2) | (0.9) | (1.1) | (0.3) | (2.5) | (0.3) |
| FF (N) | 0.70[b] | 0.57[bc] | 0.26[c] | 0.27[c] | 1.21[a] | 0.37[bc] |
| | (0.09) | (0.17) | (0.03) | (0.03) | (0.01) | (0.04) |

[a–c] Values within a row not sharing a common superscript differ significantly ($P < 0.05$).

[1] Values presented are the means of 2 replicate batches for each cheese; SD of the mean is in parentheses.

[2] EW = work (integral of force by distance) required to extend the molten cheese by 380 mm; PF = peak force during extension, as indicated in Figure 2a; FF = final force at 380 mm.

[3] Cheese code (as defined in Table 1): C1 = Kačkavalj; C2 = Kačkavalj; C3 = Kačkavalj; C4 = Kačkavalj Krstaš; C5 = Kačkavalj Krstaš; C6 = Trappist.

The critical level of pH 4.6–soluble N at which flow has been found to plateau was ~4 to 8% of total N for low-moisture part-skim Mozzarella (Yun et al., 1993a; Feeney et al., 2001; Guinee et al., 2001) and full-fat Cheddar (Guinee et al., 2000a; Costa et al., 2010).

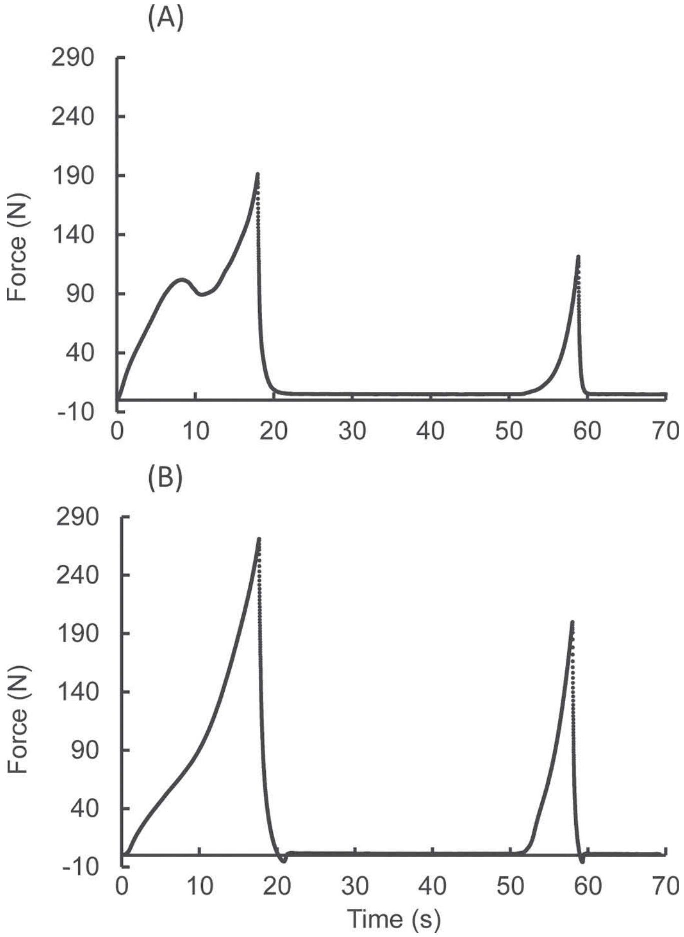

**Figure 3.** Typical texture profile analysis profiles for Kačkavalj cheeses, C1 to C3 (A), and Kačkavalj Krstaš and Trappist cheeses, C4 to C6 (B).

***Extensibility Characteristics.*** A typical force–distance profile on uniaxial extension of the molten cheese is shown in Figure 1. For all cheese, the force increased rapidly to a PF (3–9 N) after 3 to 5 mm. The PF represents the maximum resistance to the initial movement of the comb through the hot molten cheese mass as a result of a combination of forces including gravity (estimated at ~0.6 N based on the sample weight, 60 g), adhesion (of the molten cheese to the insides of the container), viscosity, and elasticity (of the cheese matrix). Once the initial forces are overcome, the force decreased rapidly to a low value (~0.2–0.8 N) after 50 to 60 mm and thereafter decreased gradually to the FF at full extension. The magnitude of the FF probably represents the combined effects of several factors, including the quantity of cheese, the number and dimensions of cheese strings that continue to form a connection between the comb and the residual molten cheese in the container, the extent of interaction between the *para*-casein molecules comprising the cheese network, and the lubrication at surfaces of layers being displaced. A high FF would, therefore, be indicative of a cheese that requires greater force to extend, because of the strings formed on extension of the hot molten cheese remaining intact, stronger interactive forces between the cheese proteins, or a lower degree of surface lubrication.

The various extensibility parameters of the molten cheeses are given in Table 5. The PF varied from ~3 to 9 N but did significantly differ between samples. The values obtained for PF were notably higher than the peak force values (0.5–1.7 N) reported by Ma et al. (2013) on extension of low-moisture Mozzarella cheeses (46–51% moisture) at ~16 mm/min using an analogous principle and device (a 3-prong hook inserted into the molten cheese and connected to a tensile tester). The FF and EW were highest for C5, lowest for C3 and C4, and intermediate for cheeses C1, C2, and C6. The high values for C5, which reflect a high level of toughness of molten extended cheese, are likely to be associated with the combined effects of a low degree of primary proteolysis (Figure 2; Table 3) and the relatively high protein content and protein-to-fat ratio (Guinee et al., 2000a; Richoux et al., 2009). Yet, the statistically different EW or FF values of cheeses C2 and C3, which had similar levels of pH 4.6–soluble N, or the similar EW and FF values for cheeses C3 and C4 despite having notably different levels of pH 4.6 soluble N, indicate the importance of factors other than total level of primary proteolysis on the extensibility characteristics of the cheese. It is likely that extensibility characteristics (EW and FF) are also influenced by factors that promote casein interaction such as high protein content, high calcium-to-casein ratio, high protein-to-fat ratio, reducing S/M in the range 6 to 0.2% or increasing S/M to ≥6%, or increasing the concentration of hydrophobic peptides (for a given level of overall primary proteolysis) through selective use of starter cultures, enhance cross-linking of the casein molecules and thereby increase EW and FF (Guo et al., 1997; Richoux et al., 2009; Everett et al., 2014). Conversely, reduction in the level of free fat in the molten cheese by homogenization of cheese milk significantly reduces the length of strings obtained on extending baked Cheddar cheese at a fixed velocity of 0.066 m/s (Guinee et al., 2000b).

### Changes in Viscoelasticity During Heating and Cooling

The changes in viscoelastic parameters during heating and cooling are shown in Figure 4 for C2 and C6, which are representative of the behavior of cheeses C1 to C3 and C4 to C6, respectively. The various viscoelastic parameters obtained from the curves are presented in Tables 6 and 7.

***Heating.*** Consistent with previous studies on cheese (Guinee et al., 2000b; Guggisberg et al., 2007; Schenkel et al., 2013), heating was accompanied by a decrease in storage modulus, **G′h**, with the decrease being most pronounced in the region 25 to 45°C and more gradual thereafter. Simultaneously, the loss tangent (LTh) increased sigmoidally, with the rate of increase highest in the temperature range 50°C to 70 to 80°C. The LTh of the Kačkavalj cheeses increased continually with temperature reaching a maximum value (LTh-max) at ~90°C, whereas that for the Kačkavalj Krstaš and Trappist cheeses attained its maximum value at a temperature of 73 to 83°C and thereafter decreased on further heating to 90°C.

The reduction in G′h and increase in LTh during heating may be attributed to various physicochemical and microstructural changes in the melting cheese including the liquefaction of fat, which is complete at 42°C (Lopez et al., 2006), fat-globule coalescence and formation of large pools of free fat (Guinee et al., 2000b; Lefevere et al., 2000), heat-induced aggregation of serum-soluble proteins (Metzger et al., 2000), and

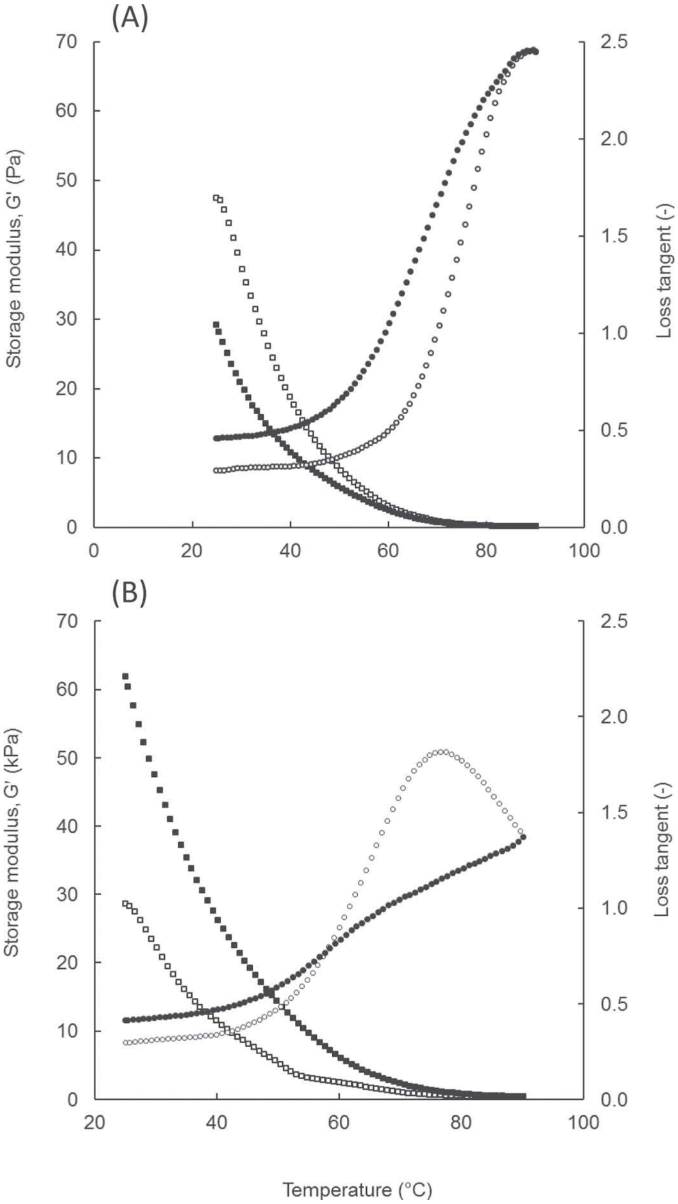

**Figure 4.** Changes in storage modulus (□, ■) and loss tangent (○, ●) of Kačkavalj cheese C1 (A) and Trappist cheese C6 (B) during heating from 25 to 90°C (○, □) and cooling (●, ■).

**Table 6.** Viscoelastic characteristics of commercial Balkan cheeses during heating[1]

| | Cheese code[3] | | | | | |
|---|---|---|---|---|---|---|
| Item[2] | C1 | C2 | C3 | C4 | C5 | C6 |
| G′h25 (kPa) | 50.80$^{abc}$<br>(4.7) | 75.48$^{ab}$<br>(21.6) | 78.79$^{a}$<br>(7.0) | 16.65$^{c}$<br>(0.5) | 32.29$^{bc}$<br>(5.5) | 25.75$^{c}$<br>(4.0) |
| LTh25 (-) | 0.33$^{ab}$<br>(0.00) | 0.33$^{ab}$<br>(0.00) | 0.34$^{a}$<br>(0.01) | 0.31$^{ab}$<br>(0.01) | 0.31$^{ab}$<br>(0.00) | 0.30$^{b}$<br>(0.01) |
| COTh (°C) | 70.3$^{b}$<br>(0.3) | 76.6$^{a}$<br>(0.0) | >90 | 57.7$^{e}$<br>(0.22) | 56.1$^{f}$<br>(0.0) | 61.5$^{d}$<br>(0.3) |
| LThmax (-) | 2.42$^{ab}$<br>(0.05) | 1.95$^{bc}$<br>(0.03) | 0.96$^{d}$<br>(0.04) | 2.74$^{a}$<br>(0.11) | 2.47$^{ab}$<br>(0.31) | 1.81$^{c}$<br>(0.01) |
| TLThmax (°C) | 89.9$^{a}$<br>(0.3) | 90.1$^{a}$<br>(0.0) | 90.1$^{a}$<br>(0.6) | 77.9$^{b}$<br>(2.5) | 72.6$^{b}$<br>(1.2) | 76.8b<br>(0.3) |
| LTh90 (-) | 2.42$^{a}$<br>(0.05) | 1.95$^{b}$<br>(0.03) | 0.96$^{d}$<br>(0.04) | 2.32$^{a}$<br>(0.03) | 1.64$^{c}$<br>(0.09) | 1.43$^{c}$<br>(0.06) |
| LThmax − LTh90 (-) | 0.00$^{b}$ | 0.00$^{b}$ | 0.00$^{b}$ | 0.42$^{ab}$<br>(0.08) | 0.83$^{a}$<br>(0.40) | 0.39$^{ab}$<br>(0.07) |
| G′h90 (Pa) | 118$^{b}$<br>(0.7) | 171$^{b}$<br>(70.7) | 331$^{a}$<br>(62.2) | 106$^{b}$<br>(18.8) | 122$^{b}$<br>(14.1) | 350$^{a}$<br>(0.3) |

[a–f] Values within a row not sharing a common superscript differ significantly ($P < 0.05$).

[1] Values presented are the means of 2 replicate batches for each cheese; SD of the mean is in parentheses.

[2] G′ = storage modulus; G″ = loss modulus; G′h25 = maximum value of G′ at beginning of heating; LTh25 = value of loss tangent (G″h/G′h) at beginning of heating; COTh = crossover temperature, where G′ = G″ (or loss tangent = 1) during heating from 25 to 90°C; LThmax = maximum value of loss tangent (unitless) during heating; TLThmax = temperature at LThmax; LTh90 = loss tangent at 90°C; LThmax − LTh90, difference in LThmax and LTh90 (representing the decrease in loss tangent after LThmax); G′h90 = value of G′ at 90°C.

[3] Cheese code (as defined in Table 1): C1 = Kačkavalj; C2 = Kačkavalj; C3 = Kačkavalj; C4 = Kačkavalj Krstaš; C5 = Kačkavalj Krstaš; C6 = Trappist.

contraction of the calcium phosphate *para*-casein network (Dave et al., 2001). Pastorino et al. (2002) found that heating of nonfat Mozzarella cheese from 10 to 50°C resulted in contraction of the calcium phosphate *para*-casein network, resulting in microphase separation of serum and an increase in the size of serum pockets occurring in the cheese matrix. The heat-induced expression of free moisture and free fat, which act as lubricants on fracture surfaces and confer the melting cheese mass with an increased degree of mobility for a given internal stress, are the likely causative agents for the heat-induced reduction in G′h and increase in LTh (Figure 4A). The decrease in LTh as observed in cheeses C4 to C6 at temperatures of 73 to 83°C has been previously reported for other cheeses (Guinee et al., 2000b; O'Mahony et al., 2006) and processed cheese products (Shirashoji et al., 2006; Guinee and O'Callaghan, 2013); it is likely associated with an increase in hydrophobic-induced protein aggregation or moisture evaporation to a degree dependent on cheese composition (e.g., protein content, protein-to-fat ratio, moisture-to-protein ratio, calcium-to-casein ratio, and pH) and levels of fat coalescence, free fat, and proteolysis. The formation of free fat during heating reduces moisture evaporation and crusting of cheese during heating (Rudan and Barbano, 1998).

**Table 7.** Viscoelastic characteristics of commercial Balkan cheeses during cooling[1]

| | Cheese code[3] | | | | | |
|---|---|---|---|---|---|---|
| Item[2] | C1 | C2 | C3 | C4 | C5 | C6 |
| COTc (°C) | 58.7$^{b}$ | 62.9$^{b}$ | >90 | 53.8$^{b}$ | 60.7$^{b}$ | 68.6$^{ab}$ |
| LTc25 (-) | 0.47$^{bc}$ | 0.54$^{a}$ | 0.51$^{ab}$ | 0.42$^{d}$ | 0.44$^{cd}$ | 0.41$^{d}$ |
| G′c25 (kPa) | 26.75$^{bcd}$ | 29.66$^{bc}$ | 65.73$^{d}$ | 22.85$^{cd}$ | 47.26$^{ab}$ | 60.08$^{ab}$ |

[a–d] Values within a row not sharing a common superscript differ significantly ($P < 0.05$).

[1] Values presented are the means of 2 replicate batches for each cheese.

[2] G′ = storage modulus; G″ = loss modulus; COTc = crossover, or congealing, temperature where G′ = G″ (loss tangent = 1) during cooling the molten cheese from 90 to 25°C; LTc25 = value of loss tangent (unitless; G″h/G′h) at end of cooling; G′c25 = value of G′ at end of cooling.

[3] Cheese code (as defined in Table 1): C1 = Kačkavalj; C2 = Kačkavalj; C3 = Kačkavalj; C4 = Kačkavalj Krstaš; C5 = Kačkavalj Krstaš; C6 = Trappist.

***Cooling.*** The changes occurring on heating were reversible on cooling to 25°C, as indicated by the increase in G′c and the decrease in LTc (Figure 4; Table 7). The reswelling of the casein network as affected by the reabsorption of free serum and rehydration of the *para*-casein network is likely to be a key factor responsible for reversibility (Metzger et al., 2000; Dave et al., 2001; Pastorino et al., 2002). The commensurate reswelling and hydration of the *para*-casein is mediated by solubilization of calcium phosphate and weakening of hydrophobic interactions. The contraction and reswelling of the *para*-casein network on heating and cooling is analogous to the observed swelling and deswelling of protein hydrogels as modulated by changes in temperature, pH, or ion concentration of the solvent phase (De et al., 2002; de Kruif et al., 2015). Crystallization of fat is unlikely to contribute to the cooling-induced changes in G′c and LTc because the temperature for the onset of crystallization (~15–16.5°C; Lopez et al., 2006) is below the temperature to which the cheese was cooled, 25°C. Moreover, the increase in viscosity of the liquid fat on cooling is relatively small (~0.4 mPa·s/°C; Walstra and Jenness, 1984). All cheeses congealed on cooling as confirmed by the decrease in LTc to a value <1, which marks the reversion of the molten cheese mass to a viscoelastic solid. Nevertheless, the loss tangent after cooling heated cheese back to 25°C was higher in all cases than the corresponding value before heating, loss tangent at 25°C, indicating that the cooled cheeses had a more viscous than elastic character than the cheeses before heating.

An interesting feature of the heating–cooling process is the hysteretic dependence of G′ and LT on temperature. The values of G′c at any given temperature during cooling were lower than the corresponding values, G′h, during heating for the Kačkavalj cheeses and higher for the Kačkavalj Krstaš and Trappist cheeses C4 to C6; the LTc during cooling was higher than the equivalent LTh during heating at all temperatures <90°C for the Kačkavalj cheeses but only at temperatures ~<50 to 60°C for the Kačkavalj Krstaš and Trappist cheeses. The occurrence of, and extent of, hysteresis of G′ and LT is probably associated with several factors, including the lower temperature required for the onset of fat crystallization during cooling than for the temperature required for completion of melting of fat (~15–16.5°C vs. ~42°C; Lopez et al., 2006) and a time lag in the rate at which *para*-casein rehydrates during cooling compared with the rate at which it dehydrates on heating (Pastorino et al., 2002). This hypothesis is supported by the findings of Metzger et al. (2000), who reported that a protein gel or precipitate formed on heating serum from low-moisture part-skim Mozzarella cheese to 71°C over a 20 min period required several days storage at 7°C to resolubilize into serum-soluble proteins (intact αs- and β-CN and casein-derived peptides at a total level of ~3%, wt/wt). Similarly, the results of Dave et al. (2001), who found that the temperature required for the transition from opacity to translucence (as marked by a reduction in the color coordinate L* to a value ≤85) on cooling nonfat Mozzarella was 10 to 15°C lower than the corresponding temperature required to induce opacity on heating, suggest a delay in protein rehydration and solubilization on cooling following heating. The difference in behavior between Kačkavalj and the Kačkavalj Krstaš and Trappist cheeses may relate to compositional differences, such as S/M and calcium content, which affect protein hydration.

***Comparison of the Different Cheeses.*** The samples differed significantly with respect to several viscoelastic parameters during heating and cooling (Figure 5, Tables 6 and 7). Generally, the Kačkavalj cheeses were characterized by relatively high values for crossover temperature and TLThmax and low difference between LThmax and loss tangent at 90°C during heating. Hence, these cheeses required heating to a high temperature (first) to melt and (second) to reach their maximum fluidity. Conversely, the Kačkavalj Krstaš and Trappist cheeses melted at a lower temperature, reached their maximum fluidity at a significantly lower temperature (~72–83°C vs. ~90°C) and underwent a marked decrease in fluidity on heating to temperatures >TLThmax. Hence from a practical viewpoint, the optimum cooking (assuming that to be where fluidity is highest) for the latter cheeses is achieved by cooking to a lower temperature than for Kačkavalj cheeses, beyond which further heating results in a notable loss of fluidity. The extent of fluidity varied significantly, as reflected by the values for LThmax, which ranged from 0.96 for cheese C3 to ~2.74 for C4 (Table 6). Similarly, Guggisberg et al. (2007) reported a wide spread of LThmax (~1.8–3.0) for Raclette cheese on heating from 20 to 80°C. The occurrence of an LThmax of <1.0 in C3, which also had the lowest Schreiber flow (Table 6), indicated that the cheese did not melt adequately, remaining as a tacky, viscoelastic solid at 90°C. Although the latter cheese would be undesirable for many cooked applications, it would be likely better suited than other cheeses exhibiting much high fluidity (e.g., C1, C4 and C5) and flow to applications requiring softening (melt) but restricted fluidity or flow, such as savory breads. The relatively low flow and fluidity of cheese C3, despite its high level of proteolysis, is probably associated with the mitigating effects of low levels of moisture and moisture in nonfat substances, low protein-to-fat ratio, and high S/M (Everett et al., 2014).

The crossover temperature during cooling, COTc, defined as the temperature at which LTc = 1, marks

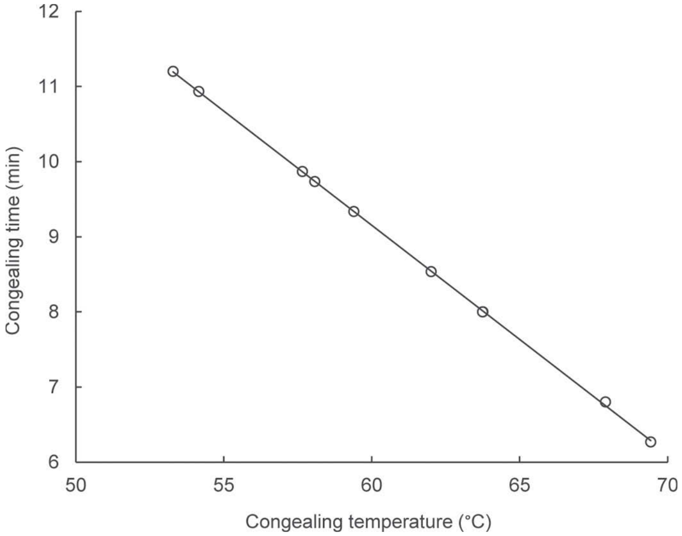

**Figure 5.** Time for heated cheese (90°C) to congeal as a function of congealing temperature, where congealing temperature is the temperature at which the loss tangent (G″/G′) during cooling drops to a value of 1. Data points relate to cheeses C1, C2, C4, C5, and C6; cheese C3, which did not melt (loss tangent <1 at 90°C), was considered congealed before cooling. G″ = loss modulus; G′ = storage modulus.

the temperature at which the cheese congeals. The congealing temperature was ~4 to 14°C lower than the corresponding temperature at which the cheese melted (LT >1) during heating for samples C1, C2, and C4 but was higher (by ~4.5 to 7°C) for samples C5 and C6. Overall, compared with the congealing temperature reported for Raclette cheese, 50 to 64°C (Guggisberg et al., 2007), those of the Kačkavalj cheeses (59–84°C) were higher and those of the Kačkavalj Krstaš and Trappist cheeses, 54 to 69°C, were similar. The practical significance of a reduction in congealing temperature is that the cheese can be held for a longer time before consumption without becoming heavy or stodgy, as for example frequently observed when cheese-based gratins and sauces are held for long times in a bain-marie or when cooked lasagna is held and cooled before reheating. Linear regression analysis of the data for the time elapsed between the start of the cooling (of the heated cheese) to the onset of congealing indicated an inverse relationship between COTc and the time for which the cooked cheese could be held before congealing, with the latter increasing by 0.3 min per 1°C reduction in COTc (Figure 5).

## CONCLUSIONS

Balkan cheeses, comprising Kačkavalj, Kačkavalj Krstaš, and Trappist, exhibited marked differences in composition, proteolysis, texture-profile characteristics, heat-induced flow and work to extend, and viscoelasticity during heating and cooling. The Kačkavalj cheeses underwent fracture during compression and tended to have low levels of cohesiveness and chewiness. In contrast, the Kačkavalj Krstaš and Trappist cheeses were characterized by the absence of fracture during compression and relatively high values for cohesiveness and chewiness. On heating from 25 to 90°C, all cheeses showed a decrease in storage modulus, G′, and increase in loss tangent, LT, from 0.25 to 0.35 in the unheated cheese to 1 to 2.7 in the heated cheese. Although these changes were largely reversible on cooling, a hysteresis dependence of G′ and LT on temperature was observed. Monitoring the dynamic changes in viscoelasticity during heating and cooling of the cheese in the temperature range 25 to 90°C enables the calculation of several parameters that are useful in characterizing the heating and cooling and behavior of cheese, such as the temperature at which the cheese melts, the maximum fluidity it attains during melting, the loss of fluidity of the molten cheese on heating to temperatures higher than the optimum, the congealing temperature, and the time for which the cheese can be held before congealing. Such parameters are potentially useful in designing ingredient cheeses with bespoke characteristics, according to the protocol followed during heating and cooling.

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