Effects of reducing milk pH to 6.2 by CO_2 injection or by addition of lactic acid on the biochemical and functional properties of commercial low-moisture part-skim Mozzarella

Chak Ming To[a,b,c], Barbara Kerkaert[a], Stijn Bossier[a], Dirk Van Gaver[a], Paul Van der Meeren[b] and Timothy P. Guinee[c]

[a]Milcobel CV, DPI: Dairy Products and Ingredients, Kallo, Belgium

[b]Particle and Interfacial Technology Group, Department of Green Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

[c]Department of Food Chemistry and Technology, Teagasc Food Research Centre Moorepark, Fermoy, Co. Cork, Ireland

Corresponding author: Chak Ming To

Mailing address: Particle and Interfacial Technology Group, Department of Green Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium

E-mail: ChakMing.To@UGent.be

Phone: +32 9 260 99 43

Abstract

The effects of adjusting milk pH to 6.2 by CO_2 injection or by addition of lactic acid solution on the properties of commercial low-moisture part-skim Mozzarella stored for 15 or 30 d at 4 °C were investigated. Relative to the control, cheese from milk adjusted to pH 6.2 using CO_2 had a slightly-reduced total Ca content, a lower melting temperature, and required less work to stretch (extend) to 38 cm following heating to 95 °C. Reducing milk pH to 6.2 using lactic acid resulted in a greater decrease in total Ca content, higher moisture content and reductions in cheese firmness, melting temperature and work to stretch. The results indicate that CO_2 injection of milk to 6.2 may be used to promote subtle changes in functionality of Mozzarella without altering gross composition.

1. Introduction

Low-moisture part-skim (LMPS) Mozzarella is renowned for its use as a cheese topping in pizza (Kindstedt, 1999; Thybo, Lillevang, Skibsted & Arhné, 2020). It is expected that the demand for LPMS Mozzarella and other cheeses will continue to increase owing to the increases in urban population, disposable income, and the growing demand for ready-to-eat meals and home-deliveries (Bloomberg, 2019). In pizza, attributes of importance to the consumer include the ability of the cheese shreds to soften, flow and coalesce into a uniform molten mass which expresses some free oil, is moderately fluid, and extends to form strings when eating. In particular, the extensibility characteristics of the heated cheese, which are attained by thermomechanical kneading of the fermented curd in a mixture of hot water or dilute salt solution, or in steam, are of primary interest to the consumer (Fox, Guinee, Cogan & McSweeney, 2017; McMahon & Oberg, 2017). Understanding the relationships between cheese composition and storage-related changes in biochemical parameters facilitates the development of cheese with the desired functional attributes after a given storage period (Smith, Hindmarsh, Carr, Golding & Reid, 2017; To et al., 2020a).

A vast body of research has highlighted significant correlations between the thermophysical properties of LMPS Mozzarella and the degree of protein hydration (Fife & Oberg, 1999; Guo, Gilmore & Kindstedt, 1997; McMahon, Smith et al., 2017), casein hydrolysis (Dave, McMahon, Oberg & Broadbent, 2003; Feeney, Fox & Guinee, 2001), and solubilization of colloidal Ca (Banville, Morin, Pouliot & Britten, 2013; Guinee, Feeney & Fox, 2002; Joshi, Muthukumarappan & Dave, 2004). Casein-bound Ca, in the form of Ca attached directly to casein (e.g., by electrostatic interaction with dissociated carboxyl side change residues of acidic amino acids) or colloidal calcium phosphate nanoclusters (attached electrostatically to phosphoserine residues), is solubilized increasingly as the pH of the milk is reduced from ~ pH 6.6 (native pH) to 4.6 (Le Graet & Brulé, 1993; van Hooydonk, Hagedoorn & Boerrigter, 1986). Conventional methods applied to increase the solubilization of colloidal calcium phosphate (CCP) and release of soluble Ca during cheese manufacture include lowering pH of the milk (by direct acidification with organic acids or glucono-δ-lactone prior to coagulant addition and curd formation), lowering scald temperature and reducing the pH at whey drainage (Fox et al., 2017; Johnson & Lucey, 2006; McMahon & Oberg, 2017). LMPS Mozzarella cheese with reduced total Ca content has been produced by pre-acidifying the milk with acetic acid, citric acid, lactic acid or glucono-δ-lactone to pH values ranging from 6.2 to 5.6 (Guinee et al., 2002; Joshi et al., 2004; Metzger, Barbano, Kindstedt & Guo, 2001; Rehman & Farkye, 2006). Calcium depletion has been found to enhance the capacity of the calcium phosphate para-casein matrix to bind/immobilize water, rearrange, surround and integrate fat-serum channels during storage of Mozzarella cheese (McMahon & Oberg, 2017; McMahon et al., 1999). In addition, reducing the number of CCP clusters lowers the gel-sol transition temperature of semi-hard model cheeses during heating from 20 to 80 °C, and alters the shear behavior of the cheese under large strain (Kern, Weiss, & Hinrichs, 2018). Apart from its effects on casein hydration and cheese thermo-rheological properties, pre-acidification of milk to pH values in the range 6.2 – 5.8 promotes a greater degree of α_{s1}-casein hydrolysis in cheese in which moisture content and pH are normalized relative to the control cheese (with no pre-acidification) (McCarthy, Wilkinson & Guinee, 2017; Metzger et al., 2001); this effect has been attributed to the higher retention of chymosin (Banks, Stewart, Muir & West, 1987; Holmes, Duersch & Ernstrom, 1977). Owing to these effects, low-moisture Mozzarella made from pre-acidified milk spreads (flows) more rapidly on baking or grilling (Joshi et al., 2004; McCarthy et al., 2017; Metzger et al, 2001).

The pH of milk may also be reduced by dosing with CO_2 (carbonation), a method which has been primarily applied to depress the growth of spoilage bacteria in milk and enhance the quality of dairy products and ingredients (Hotchkiss, 2006). By extension, studies have evaluated the effects of milk pH adjustment using CO_2 on the extent of proteolysis during the ripening in Cheddar or Iberico cheeses (Montilla, Calvo & Olano, 1955; Nelson, Lynch & Barbano, 2004; St-Gelais, Champagne & Bélanger, 1997). To our knowledge, no detailed investigation has been conducted on the effects of milk pH adjustment using CO_2 on the functional quality of LMPS Mozzarella. The use of CO_2 is a promising alternative to direct acidification of the milk as it limits the contents of organic acids and salts in the whey (e.g., lactic acid, Ca, P) which are known to impair whey processability (Chandrapala et al., 2015), and simultaneously reduces coagulant consumption (Montilla et al., 1995; St-Gelais et al., 1997).

The objectives of the current study were twofold, firstly to evaluate the effects of reducing the pH of milk to 6.2 prior to coagulant addition using CO_2 injection (carbonation) on the composition, biochemical (pH, water-soluble Ca, pH 4.6 soluble N) and functional (firmness of the unheated cheese, heat-induced changes in viscoelastic properties, extensibility and flow of the heated cheese) properties of commercial LMPS Mozzarella cheese, and secondly to determine the comparative effects of reducing milk pH prior to coagulant addition by carbonation or by addition of dilute lactic acid solution.

2. Material and methods

2.1 Cheese production

2.1.1 Control cheese

Control cheese (CTL) was manufactured based on the procedure described by To et al. (2020a). In brief, standardized milk (milk protein content = ~ 3.7 g 100 g^{-1} milk, protein-to-fat ratio = 1.25) was pasteurized, cooled to 35.5 °C, pumped to the cheese vat (24.000 L), inoculated with a freeze-dried culture (*Streptococcus thermophilus*), and incubated for ~ 55 min; during this time, there was no reduction in the milk pH from its native value (~ 6.6). Chymosin (EC 3.4.23.4; 200 IMCU ml^-

[1]) was dosed at a level of ~ 0.52 IMCU g^{-1} milk protein in accordance with the supplier's specifications. After gel setting (~ 30 min), the curd was cut, stirred and cooked at 39 °C for ~ 40 min. The curd-whey mixture was drained, and the curd grains were held at 39 °C to promote curd dehydration and acidification to pH ~ 5.20. The fermented curd was continuously milled, heated (~ 60 ± 2.5 °C) and kneaded mechanically using a water-steam mixture, after which the hot curd was dry-salted (0.9 % w/w), molded into blocks (~ 2.5 kg; 28 cm × 10 cm × 8 cm), and cooled in brine (4 °C; 20%, w/w NaCl) for ~ 6 to 8 h. After brining, the cheeses were drip-dried and vacuum-packed.

2.1.2 Experimental cheese

The manufacturing process of the experimental cheeses followed the same procedure as that for the CTL cheese with the exception that the pH of the cheese milk was altered from its natural value (~ 6.6) to 6.2 at the start of vat filling by CO_2 injection (DA1) or by dosing with a 5 % (w/w) lactic acid solution (DA2). The adjustment of the milk pH was carried out while agitating the milk at 35.5 °C and was completed before coagulant addition. The amount of coagulant added during the manufacturing of DA1 and DA2 cheese was reduced to half the quantity used during the manufacturing of the CTL cheese, so as to normalize curd firmness at cutting.

Four cheese blocks were sampled at the end of the line, and designated equally and randomly to either 15 or 30 d of storage at 4 °C. The manufacturing of CTL and DA1 cheese was conducted in quadruplicate, and that of DA2 cheese in duplicate. Hence, 16 CTL, 16 DA1 and 8 DA2 cheese blocks were sampled. The experimental cheese and their corresponding CTL cheese were manufactured within 2 consecutive days.

2.2 Experimental analysis

2.2.1 Development of pH during cheese manufacture

For each cheese vat, samples of the milk prior to coagulant addition, of the curd-whey mixture after vat drainage, and of the curd at milling were directly analyzed for pH (Knick, 765 Laboratory pH meter, SE 503 pH sensor, Berlin, Germany).

2.2.2 Cheese composition

Grated LMPS Mozzarella was analyzed for moisture, total N, salt, and total Ca using standard International Dairy Federation methods (IDF 2004, 2006, 2007, 2014). Fat was determined by nuclear magnetic resonance (Smart Turbo, CEM Corporation, Mathews, NC, USA). Moisture, fat, salt and total Ca contents were determined in triplicate per cheese block after 15 d of storage. Total N was determined in duplicate per cheese block after 15 d of storage.

2.2.3 Cheese biochemical properties

2.2.3.1 Cheese pH

The pH of each cheese block was measured in duplicate after each storage time on a slurry of cheese (20 g) and distilled water (12 g) using the method described by the British Standards Institution (1976).

2.2.3.2 Water-soluble Ca and pH 4.6 soluble N

After each storage time, water-soluble cheese extracts were prepared in triplicate by stomaching a blend of grated cheese with distilled water (50 °C) at a weight ratio of 1:2 for 5 min using a Stomacher (Lab-Blender 400; Seward Medical, London, UK) and holding the mixture for 1 h in a water bath set at 50 °C. Then, the mixture was centrifuged at 3000 *g* for 20 min at 4 °C, and the supernatant was filtered through glass wool to yield the water-soluble extract (WSE). An aliquot (4 mL) of the WSE was digested at 550°C, and the ash was analyzed for Ca (IDF 2007) to determine the water-soluble Ca content in the cheese (WSCa). The remaining portion of the WSE was pH-adjusted to 4.6 using 10%, w/w HCl (Honeywell Fluka™ Chemicals, Offenbach, Germany), centrifuged at 3000 *g* for 20 min at 4 °C, and filtered through glass wool to obtain the pH 4.6 WSE.

The extract was analyzed for total N (IDF 2014) to determine the level of pH 4.6 soluble N (pH4.6SN). WSCa was expressed as a percentage of total cheese Ca, and pH 4.6 soluble N, an index of primary proteolysis, was expressed as a percentage of total cheese N.

2.2.4 Cheese functional properties

The functional properties of both the unheated and heated cheese were determined as described previously (To et al., 2020b). A brief overview is given below.

2.2.4.1 Texture profile analysis

Individual cheese cubes (25 mm × 25 mm × 25 mm) were compressed to 60% of their original height on a TAHDi texture analyzer fitted with a 100-kg load cell (Stable Micro Systems, Godalming, UK). The firmness was defined as the maximum force recorded during compression, and measurements were undertaken in sextuplicate per cheese block.

2.2.4.2 Extensibility of the heated cheese

Shredded cheese was heated to 95 °C and the molten curd (85 - 95 °C) was uniaxially extended to a height of 380 mm a rate of 10 mm s^{-1} on a TAHDi texture analyzer (Stable Micro Systems, Godalming, UK). The cumulative work (force by distance) required to extend the hot molten cheese to 380 mm directly after heating and after holding for 5 min at room temperature was measured, and expressed as EW_0 and EW_5, respectively. Measurements of EW_0 and EW_5 were conducted in triplicate and duplicate, respectively, per cheese block and per storage time.

2.2.4.3 Heat-induced changes in viscoelastic behavior of the heated cheese

Cheese discs (50 mm diameter; 2 mm thickness) were placed between parallel cross-hatched plates (PP50/P2-SN27902; INSET I-PP50/SS/P2) on a strain-controlled rheometer (MCR501, Anton Paar GmbH, Graz, Austria), subjected to a low amplitude shear strain (γ = 0.0063) at an angular frequency of 1 Hz, and heated from 25 to 90 °C at a rate of 3.25 °C min^{-1}. The cross-over temperature (COT), corresponding to the point at which the cheese transitioned from solid to a viscoelastic liquid, and the maximum value of the loss tangent (LT_{max}), an index for the fluidity of the cheese during heating, were reported. Measurements were conducted in duplicate per cheese block and per storage time.

2.2.4.4 Flow of the heated cheese

Cheese discs (47.5 mm diameter; 4 mm thickness) were heated at 280 °C for 4 minutes in a convection oven (Binder FD 35, Binder GmbH, Tuttlingen, Germany), removed, and allowed to cool down to room temperature. Flow was defined as the percentage increase in mean diameter during heating. Measurements were conducted in quadruplicate per cheese block and per storage time.

2.2.5 Baking test

A baking test was conducted using a previous procedure (To et al., 2020b) but with a slight modification. Shredded cheese (150 g) was sprinkled on top of a pizza base containing tomato paste (Bladerdeeg Van Marcke, Belgium, ø = 25 cm) and baked at 244 °C for 5.25 min in a conveyor oven (Lincoln Impinger 1100 series, Fort Wayne, IN, USA). Following baking, the pizza was allowed to cool down for 5 min at room temperature, after which the cheese was evaluated by trained laboratory personnel at Milcobel (n = 3). The main sensory attributes reported in this study are ‘blister color’, ‘blister coverage’, ‘oiling off’, ‘meltability’, ‘first chew’ and ‘chewiness’ (definitions are described by To et al., 2020b). Panelists awarded each of these sensory attributes a score ranging from 0 to 4. A scoring of 2 was awarded if the characteristic was ‘just right’, whereas scorings ranging from 0 to < 2 or from > 2 to 4 indicated that the characteristic was subpar or above par, respectively. In addition to these attributes, the ‘stretch’ of the cheese was evaluated manually using a fork by lifting the cheese from the pizza surface and extending it to a height of 30 cm. A scoring of 0 was awarded if the cheese did not stretch, whereas a scoring of 2 was awarded if the cheese strings remained intact at a height of 30 cm. Scorings ranging from 0 to 2 were given depending on how quickly the cheese strings broke before reaching the aforementioned distance. The baking test was conducted on one cheese block of CTL, DA1 and DA2 cheese only, stored after 15 and 30 d at 4 °C. Hence, these results were included as an observation only.

2.3 Statistical analysis

JMP 15 (SAS Institute Inc., Cary, NC, United States) was the statistical software package used to treat the data at $\alpha = 0.05$ throughout.

One-way ANOVA and Tukey's HSD post-hoc test were used to determine significant differences between mean compositional, biochemical (pH, WSCa and pH4.6SN) and functional (firmness, COT, LT_{max}, EW_0, EW_5 and flow) values of the different milk pre-treatments, whereas a multiple linear regression analysis was used to investigate the effects of milk pre-treatment, storage time, and/or their interaction effect on the biochemical and functional properties of the cheese.

3. Results and discussion

3.1 In-line process analysis

Adjusting the pH of milk may affect the microbial metabolism of lactic acid bacteria (Broadbent, Larsen, Deibel & Steele, 2010; Ma, Barbano & Santos, 2003; Vianna et al., 2012; Wee, Kim & Ryu, 2006). A profile of the pH development for all treatments at different stages of manufacture (Fig. 1) shows that between coagulant addition and whey drainage (whey pH), the pH increased (~ 0.1 unit) for DA1, and decreased for CTL (~ 0.2 unit) and DA2 (~ 0.04 unit). The decrease in pH, which was more rapid for CTL than DA2, is consistent with the metabolism of lactose to lactic acid by the starter culture, whereas the slight increase for DA1 suggests that some of the carbonic acid formed on the addition of CO_2 gradually became volatile over time with stirring and the increase in temperature of the curd particle-whey mixture from ~35 to ~39 °C (Champagne, St-Gelais & de Candolle, 1998; Hotchkiss et al, 2006). The partial reversal of pH after CO_2 addition to milk has been considered advantageous for cheese-making as it may retain the whey quality (e.g., limit increases in of Ca and P) for further processing, while simultaneously enhancing milk gelation and cheese properties (Nelson et al., 2004). The relatively low reduction of pH in DA2 between starter addition and whey drainage compared to CTL likely reflects the logarithmic nature of the pH scale, with quantity of acid required to induce a given pH change increasing as the pH is reduced; hence, the quantity of lactic acid excreted by the starter culture was sufficient to affect a pH reduction of ~ 0.2 units in CTL at whey drainage, but only ~ 0.04 units in DA1.

After whey drainage, the pH of all curds decreased rapidly (commensurate with continued lactose metabolism and the concentration of lactic acid with dehydration of the curd) and attained a similar value (~ 5.10 to 5.20) by curd milling (at ~ 4h post starter culture addition). Hence, while the dynamics of pH development changed with carbonation and pre-acidification prior to coagulant addition, the overall time-related pH reduction between starter addition and curd milling was unaffected. This finding agrees with the critical range of pH (pH 5.0 to pH 5.5) at which the cell viability of *S. thermophilus* has been found to be impaired (Hutkins & Nannen, 1993; Nannen & Hutkins, 1991); hence, gradual pH adjustment of milk to 6.2 was not expected to affect the performance of the culture during cheese manufacture. Similarly, carbonation of milk to pH 6.2 had no effect on the curd pH at milling. The inhibiting effects of CO_2 on micro-organisms are mainly reported for gram-negative psychrotrophs, particularly *Pseudomonas spp.* (Hotchkiss, 2006).

3.2 Effects of reducing milk pH by CO_2 injection or addition of lactic acid solution

3.2.1 Cheese composition

All cheeses conformed to the specifications of dry matter (DM) and fat-in-dry matter (FDM) for low-moisture part-skim Mozzarella, as defined by the Code of Federal Regulations (FDA, 2020) (Table 1).

Relative to the CTL, DA1 cheese had similar levels of moisture, FDM, crude protein and S/M ($P > 0.05$), and a slightly, but significantly, lower total Ca content ($P < 0.001$), whereas DA2 cheese had the highest moisture content ($P < 0.001$) and lowest levels of crude protein ($P < 0.01$) and total Ca ($P < 0.001$). Nelson et al. (2004) reported that reducing the pH of milk to 5.93, using carbonation, enhanced calcium solubilization during Cheddar cheese manufacture, reduced total Ca content (~ 25%) of the resultant cheese, and altered the arrangement of the para-casein calcium-phosphate cheese matrix and its ability to retain fat or salt. As the pH of the carbonated milk in the current study (pH 6.20) was higher than that (pH 5.93) in the study of Nelson et al. (2004), it is likely that the degree of Ca reduction in our study (~ 4%) was sufficiently low (van Hooydonk et al., 1986) to prevent structural attenuation of the para-casein network and its fat- or salt- retention capacity.

The lower total Ca and higher moisture content of DA2 cheese concurs with the results of previous studies, i.e., higher moisture content in reduced-fat Cheddar and Mozzarella cheeses made from milk pre-acidified to pH 6.2 with lactic acid in the absence of pertinent process interventions (Banks, 1988; Banks et al., 1987; Guinee et al., 2002), and reduced total Ca content with pH reduction to 6.2 prior to coagulant addition (Banville et al., 2013; Guinee et al., 2002; Joshi et al., 2004). These trends are consistent with the higher solubilization of CCP in cheese curd as the pH of milk is reduced prior to gelation (van Hooydonk et al., 1986), and the inverse relationship between casein hydration and concentration of casein-bound Ca (Rüegg & Moor, 1984; Sood, Gaind & Dewan, 1979). Hence, the higher total Ca and lower moisture of DA1 compared to DA2 cheese is attributed to the increase in pH (~ 0.1 unit) during the starter incubation and gelation stages of DA1 manufacture (Fig. 1), which favors the reverse-transfer of serum-soluble Ca and P to CCP (Sinaga et al., 2016).

3.2.2 Cheese biochemical and functional properties

The biochemical and functional properties of LMPS mozzarella were significantly affected by milk pre-treatment (CTL, DA1 vs DA2) and storage time, but not by an interaction effect between milk pre-treatment and storage time (Table 2). The absence of an interactive effect suggests that the effects of milk pre-treatment or storage time could be discussed separately.

3.2.2.1 Effects of storage time

Prolonging storage time from 15 to 30 d led to an increase in pH4.6SN ($P < 0.001$) and reductions in COT ($P < 0.01$), EW_0 ($P < 0.001$) and EW_5 ($P < 0.001$) (Table 2), but had no effect on pH, WSCa, firmness, LT_{max} or flow ($P > 0.05$). The former effects were in accordance with the results of previous studies on commercial LMPS Mozzarella from the same production plant (To et al., 2020a,b). The absence of an effect of storage time on pH, WSCa, firmness, LT_{max}, and flow most likely reflects the storage times at which the cheeses were assayed, i.e., 15 and 30 d. It has been previously found that the biochemical and functional characteristics of the cheese develop most rapidly during the first two weeks of storage at 4 °C, and more slowly thereafter (Smith et al., 2017; To et al., 2020a).

3.2.2.2 Effects of milk pre-treatment on cheese biochemical properties

The effects of milk pre-treatment (CTL, DA1 vs DA2) on the biochemical properties of LMPS mozzarella over the 30-d storage period are illustrated in Figure 2. Relative to the control, reducing the pH of milk from 6.6 to 6.2 using CO_2 did not affect the mean values of pH ($P > 0.05$), WSCa ($P > 0.05$) or pH4.6SN ($P > 0.05$) over the 30-d storage period at 4 °C (Figure 2). The similar rate of proteolysis (pH4.6SN) between CTL and DA1 cheeses despite the lower coagulant-to-casein ratio used during the manufacture of the latter most likely reflects the high degree of inactivation of residual chymosin activity (~ 66 to 76%) at the high curd temperature (60 ± 2.5 °C) reached during curd plasticization (Feeney et al., 2001; Metzger, 2001; Yun, Kiely, Barbano & Kindstedt, 1993), and the overall low levels of pH4.6SN attained during 1 month of storage (< 5% total N; Figure 2). Likewise, the thermomechanical treatment of the curd during cheese manufacturing may have contributed to loss of residual CO_2 from the curd (Champagne et al., 1998; Lamichhane et al., 2021), and thereby yielded a cheese with a slightly reduced total Ca content (30.3 vs 31.5 mg g^{-1} casein, $P < 0.001$) but with similar pH and proportion of WSCa relative to CTL (Figure 2). Nelson et al. (2004) reported that carbonation of milk to pH 5.93 with CO_2 resulted in Cheddar cheeses with higher mean values of pH (~ 0.1 to 0.2 pH units) and pH4.6SN, and lower concentration of serum Ca than the CTL cheese over the 30-d storage period. The inter-study discrepancy on the impact of milk carbonation on cheese pH, WSCa and pH4.6SN may be attributed to the differences in cheese-making procedure, whereby (1) the pH of milk at renneting was 6.2 in the current study compared to 5.93 in the study of Nelson et al. (2004), (2) the plasticization treatment (kneading of the curd in hot water on heating to 55 to 65 °C), as applied in the current study, would dilute the cheese serum (and concentrations of Ca, lactic acid) and inactivate most of the residual coagulant activity in the curd (Feeney et al., 2001), and (3) the immersion of cheeses into brine may have reduced potential differences in pH or WSCa between CTL and DA1 owing to diffusion between the cheese serum and the brine.

Cheeses made from milk adjusted to pH 6.2 by addition of lactic acid (DA2) had the lowest mean pH ($P < 0.01$), the highest mean WSCa ($P < 0.05$), and a slightly higher, though non-significant, mean value of pH4.6SN ($P > 0.05$) over the 30-d storage period (Fig. 2). These changes most likely reflect the greater solubilization of colloidal Ca during curd manufacture, the consequent reduction in the buffering capacity of the calcium-phosphate para-casein network (Upreti, Bühlmann & Metzger, 2006), and the higher retention of chymosin in the curd matrix as the pH of the milk at set and curd at whey drainage is reduced (Bansal, Fox & McSweeney, 2007). These findings corroborate those of previous studies where milk was pre-acidified using lactic acid at a constant coagulant-to-casein ratio (McCarthy et al., 2017; Metzger et al., 2001).

3.2.2.3 Effects of milk pre-treatment on cheese functional properties

Figure 3 illustrates the effects of milk pre-treatment (CTL, DA1 vs DA2) on the mean values of the different functional properties of the cheese over the 30-d storage period. Compared to CTL, DA1 cheese had significantly lower mean values of COT and flow and numerically lower, though non-significant, EW_0 ($P = 0.08$). The results indicate that pH adjustment to pH 6.2 using CO_2 resulted in slightly faster-melting cheeses which required less work to extend (stretch) directly after melting but had a lower spread after heating. The lower values of COT and EW_0 of DA1 relative to the control is consistent with the lower total Ca content ($P < 0.001$; Table 1) and hence degree of calcium-induced cross linking between the caseins molecules constituting the structural scaffolding of the cheese matrix, i.e., calcium phosphate para-casein network (Kern, Weiss, & Hinrichs, 2018); however, the lower flow was somewhat unexpected. Despite numerical differences in the magnitude of the various parameters, the CTL and DA1 cheeses did not significantly differ with respect to the firmness of the unheated cheese ($P > 0.05$), or LT_{max} ($P > 0.05$) or EW_5 ($P > 0.05$) of the heated cheese (Fig. 3). Hence, the overall functionality of DA1 was quite comparable to that of CTL, which accords with their overall similar composition and biochemical characteristics (Table 1, Fig. 2). The fact that only some functional parameters (i.e., COT, EW_0 and flow) were influenced by CO_2 carbonation, and others not, suggests different controlling mechanisms of, or differences in the weighted contributions of the various compositional (e.g., moisture, FDM, total Ca) and biochemical (e.g., pH, WSCa, pH4.6SN) characteristics to the different aspects of functionality.

Relative to DA1 cheese, DA2 cheese had lower firmness ($P < 0.01$), COT ($P < 0.05$) and higher flow ($P < 0.001$). These findings indicated that the former cheese was less firm, more conducive to melting at a lower temperature, and had greater spread after heating. In addition, DA2 had numerically lower values for EW_0 and EW_5 than DA1 (Fig. 3) which coincides with the increases in moisture (Table 1, $P < 0.001$) and WSCa (Fig. 2, $P < 0.05$), and decreases in pH (Fig. 2 , $P < 0.05$) and total Ca (Table 1, $P < 0.001$). In practice, a lower EW_5 reflects a lower tendency of the pizza cheese topping to congealing and becoming stodgy with time after serving (Guinee, Pudja, Miočinović, Wiley & Mullins, 2015), which in effect extends the consumer experience of hot-off-the-oven pizza, and is of increasing relevance corresponding with the growth of home deliveries.

3.2.3 Baking test

Figure 4 presents an overview of the scorings of the various sensory attributes after baking the cheese on a pizza.

After 15 d storage, DA1 was awarded slightly more acceptable scores (closer to target value

2) than CTL for ‘blister color’, ‘blister coverage’, ‘stretch’ and ‘first chew’ and slightly poorer scores for ‘oiling off’ and ‘chewiness’. A similar trend was found at 30 d except that DA1 was awarded a slightly better score on ‘chewiness’. Overall, the sensory panel succeeded in identifying slight but noticeable differences between CTL and DA1 cheese, with DA1 receiving better scores for the majority of the evaluated sensory attributes, probably reflecting its numerically lower Ca content (Table 1) and values for EW_0 (Fig. 3).

After 15 d of storage, DA2 was overall awarded more desirable scores than CTL or DA1 for most attributes including ‘blister color’, ‘blister coverage’, ‘oiling off’, ‘stretch’, ‘first chew’ and ‘chewiness’. A similar trend was found at 30 d except that DA2 received higher scorings for ‘oiling off’ and ‘meltability’ and a low scoring on ‘first chew’, indicating that it was less prone to scorching after baking, underwent a higher degree of shred fusion, and had a softer mouthfeel and more desirable stretch (requiring less effort to extend when eating on pizza). The sensory results are consistent with the instrumental methods, which showed that DA2 had numerically higher values of flow and lower values of EW_0 and EW_5 than CTL or DA1 (Fig. 3).

4. Conclusions

The current research examined the effects of reducing the pH of milk at renneting from 6.6 to 6.2 using CO_2 injection on the biochemical and functional properties of commercial LMPS Mozzarella cheese, and compared these effects with those obtained on reducing milk pH to 6.2 using lactic acid.

Relative to the control, the adjustment of milk pH using CO_2 resulted in a slight, though significant, reduction in total Ca content of the cheese (~ 1 to 2 mg g^{-1} protein), but otherwise did not affect the gross composition or mean pH of the cheese. Nevertheless, cheeses from the CO_2-treated milk had improved functionality on heating when compared to the control, as evidenced by the lower melting temperature (COT), lower work required to stretch (EW_0), and better scores for the majority of evaluated sensory attributes. Adjusting the pH of the milk using CO_2 thus enables commercial manufacturers to produce cheeses with similar gross composition and cheese firmness, but with altered functional properties, such as its melting or extensibility characteristics, and thus offers new perspectives in designing customized cheese recipes.

Altering the pH of the milk to 6.2 using lactic acid instead of CO_2 had a greater impact on the properties of LMPS Mozzarella (lower firmness, COT, and higher flow), which likely reflects the greater decalcification of the cheese, lower pH, higher water-soluble Ca content and higher moisture. The weighted contributions of the lower calcium and higher moisture contents of cheeses made from milk pH adjusted to pH 6.2 with lactic acid on specific functional characteristics remains uncertain. Further studies involving the comparative effects of pH adjustment of milk to different values (e.g., 5.8 to 6.4, as encountered across the range of drain pH values found for different cheese varieties) using carbonation or pre-acidification with lactic acid, while systematically controlling cheesemaking conditions to normalize gross composition (e.g., moisture, total protein) of the cheese, would be of interest in revealing the full potential of these approaches in differentiating cheese functional attributes.

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Declaration of interests

There are no conflicts of interest.

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LIST OF CAPTIONS

Fig. 1 pH at different stages during the manufacturing of control commercial low-moisture part-skim Mozzarella cheese produced from standardized milk with a pH value of 6.6 and inoculated with a thermophilic starter culture (CTL; ○), and corresponding experimental cheeses from standardized milk inoculated with a thermophilic starter culture and pH-adjusted to 6.2 by injecting CO_2 (DA1; □) or by dosing with 5% (w/w) lactic acid solution (DA2, ■).

Fig. 2 Comparison of the biochemical properties [pH, water-soluble Ca (WSCa) and pH 4.6 soluble N (pH4.6SN)] of commercial low-moisture part-skim Mozzarella cheese produced from standardized milk with a pH value of 6.6 and inoculated with a thermophilic starter culture (CTL), and corresponding experimental cheeses from standardized milk inoculated with a thermophilic starter culture and pH-adjusted to 6.2 by injecting CO_2 (DA1) or by dosing 5% (w/w) lactic acid solution (DA2). Presented data are the mean values for cheeses produced in replicate trials (4 for CTL and DA1, 2 for DA2) and analyzed after 15 and 30 d storage at 4 °C; error bars represent standard deviations, and different superscripted letters within a graph denote a difference between treatment means at $P < 0.05$.

Fig. 3 Comparison of the functional properties [firmness, cross-over temperature (COT), maximum value of the loss tangent (LT_{max}), extension work at 0 or 5 min after melting (EW_0 or EW_5) and flow] of commercial low-moisture part-skim Mozzarella cheese produced from standardized milk with a pH value of 6.6 and inoculated with a thermophilic starter culture (CTL), and corresponding experimental cheeses from standardized milk inoculated with a thermophilic starter culture and pH-adjusted to 6.2 by injecting CO_2 (DA1) or by dosing 5% (w/w) lactic acid solution (DA2). Presented data are the mean values for cheeses produced in replicate trials (4 for CTL and DA1, 2 for DA2) and analyzed after 15 and 30 d storage at 4 °C; error bars represent standard deviations, and different superscripted letters within a graph denote a difference between treatment means at $P < 0.05$.

Fig. 4 Scores awarded by trained laboratory personnel (n = 3) for different attributes of baked pizza with commercial low-moisture part-skim Mozzarella cheese produced from standardized milk with a pH value of ~ 6.6 and inoculated with a thermophilic starter culture (CTL), and corresponding experimental cheeses from standardized milk inoculated with a thermophilic starter culture and pH-adjusted to 6.2 by injecting CO_2 (DA1) or by dosing 5% (w/w) lactic acid solution (DA2). The scale of scores ranged from 0 to 4, whereby a given score of 2 implied that the intensity of the attribute was 'just right', and scores 0 to < 2 or > 2 to 4 signifying that the intensity was either too little or too high, respectively. The baking test was conducted on one block of CTL, DA1 and DA2 cheese after 15 and 30 d of storage at 4 °C; the data are included as an observation.

Table 1. Effect of reducing milk pH to 6.2 using CO_2 or 5% (w/w) lactic acid solution prior to coagulant addition on the gross composition of commercial low-moisture part-skim Mozzarella cheese.[a,b,c]

Gross composition	Cheese treatment		
	CTL	DA1	DA2
Moisture (%, w/w)	47.5^b	47.8^b	50.0^a
FDM (%, w/w)	41.9^a	42.1^a	42.1^a
Crude protein (%, w/w)	25.2^a	24.9^a	24.0^b
S/M (%, w/w)	2.5^a	2.4^a	2.6^a
Total Ca (mg g^{-1} protein)	31.5^a	30.3^b	28.1^c

[a]Abbreviations: FDM = fat-in-dry matter; S/M = salt-in-moisture.
[b]Cheese treatments: Control cheese (CTL) was produced from standardized milk (pH of 6.6) inoculated with starter culture; experimental cheeses DA1 and DA2 were produced from standardized milk inoculated with starter culture and with pH reduced from 6.6 to 6.2 by injecting CO_2 or by dosing 5% (w/w) lactic acid solution, respectively, prior to coagulant addition.
[c]Presented data are the mean values for cheeses produced in replicate trials (4 for CTL and DA1, 2 for DA2). Values within a row with different superscripted letters differ statistically at $P < 0.05$.

Table 2. Effect of reducing milk pH to 6.2 using CO_2 or 5% (w/w) lactic acid solution, storage time and their interaction on the biochemical and functional properties of commercial low-moisture part-skim Mozzarella cheese.[a,b,c]

	Treatment	Storage time	Treatment x Storage time
Biochemical properties			
pH (-)	***	-	-
WSCa (% Ca)	**	-	-
pH4.6SN (%TN)	-	***	-
Functional properties			
Firmness (N)	***	-	-
COT (°C)	***	**	-
LT_{max} (-)	-	-	-
EW_0 (mJ)	***	***	-
EW_5 (mJ)	**	***	-
Flow (%)	***	-	-

[a]Abbreviations: WSCa = water-soluble Ca, pH4.6SN = pH 4.6 soluble N, %TN = % total N, COT = cross-over temperature, LT_{max} = maximum value of the loss tangent, EW_0 = extension work at 0 min after melting, EW_5 = extension work at 5 min after melting.

[b]A multiple linear regression model was used to evaluate the effects of treatment (adjusting the pH of the cheese milk from 6.6 to 6.2 by injecting CO_2 or by dosing 5% (w/w) lactic acid solution prior to coagulant addition, relative to the control where the pH was not adjusted) and storage time (15 or 30 d) at 4 °C on the biochemical and functional properties of commercial low-moisture part-skim mozzarella. The mean values for the different properties were from cheeses produced in replicate trials (4 for CTL and DA1, 2 for DA2) and analyzed after 15 and 30 d storage at 4 °C.

[c]The statistical significance (*P*) is given where $P > 0.05$, $P < 0.01$ and $P < 0.001$ are denoted by -, ** and ***, respectively.

Fig. 1

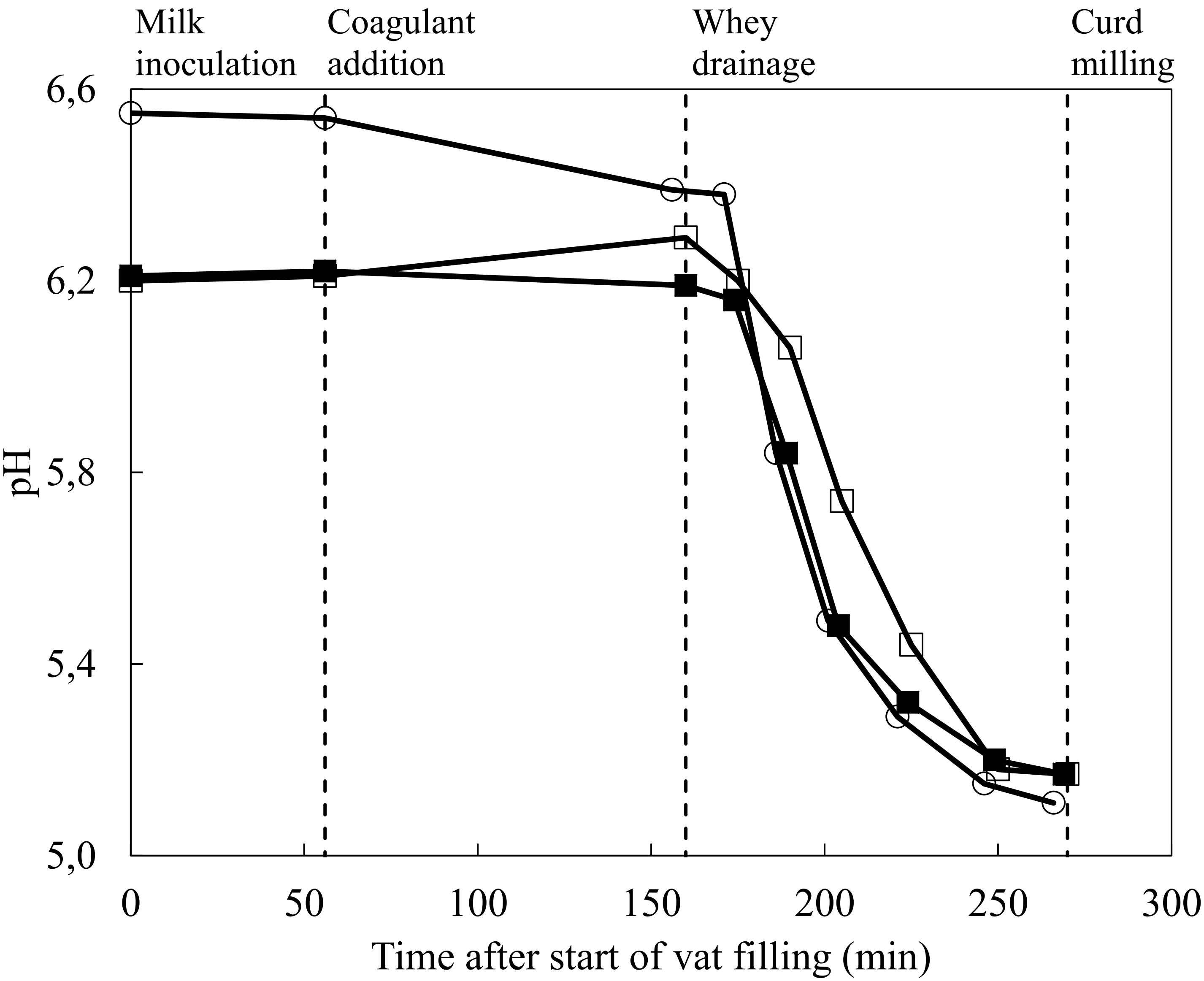
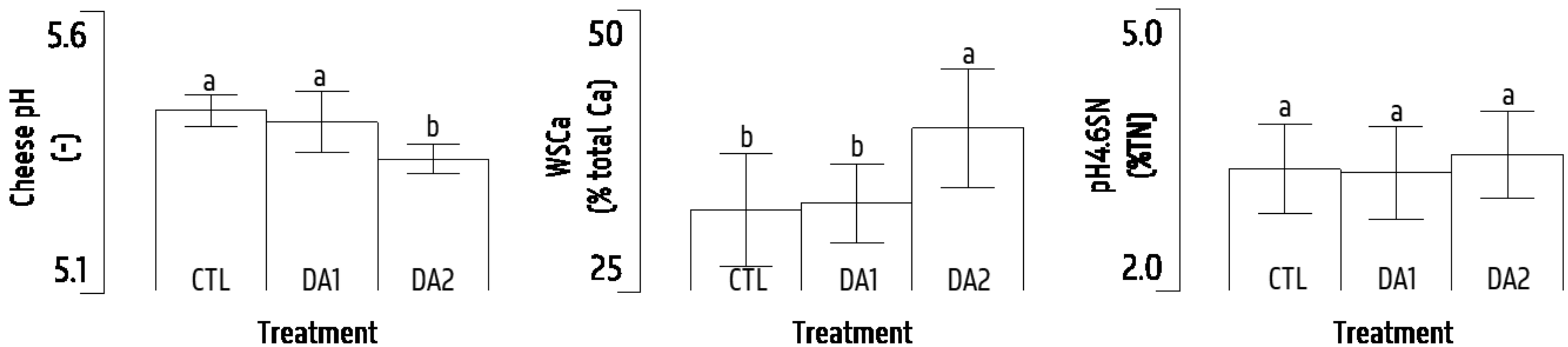

Milk inoculation
Coagulant addition
Whey drainage
Curd milling
6,6
6,2
5,8
5,4
5,0
pH
0
50
100
150
200
250
300
Time after start of vat filling (min)

Fig. 2



Cheese pH
(-)
5.6
5.1
a
a
b
CTL
DA1
DA2
Treatment
WSCa
(% total Ca)
50
25
b
b
a
CTL
DA1
DA2
Treatment
pH4.6SN
(%TN)
5.0
2.0
a
a
a
CTL
DA1
DA2
Treatment

Fig. 3

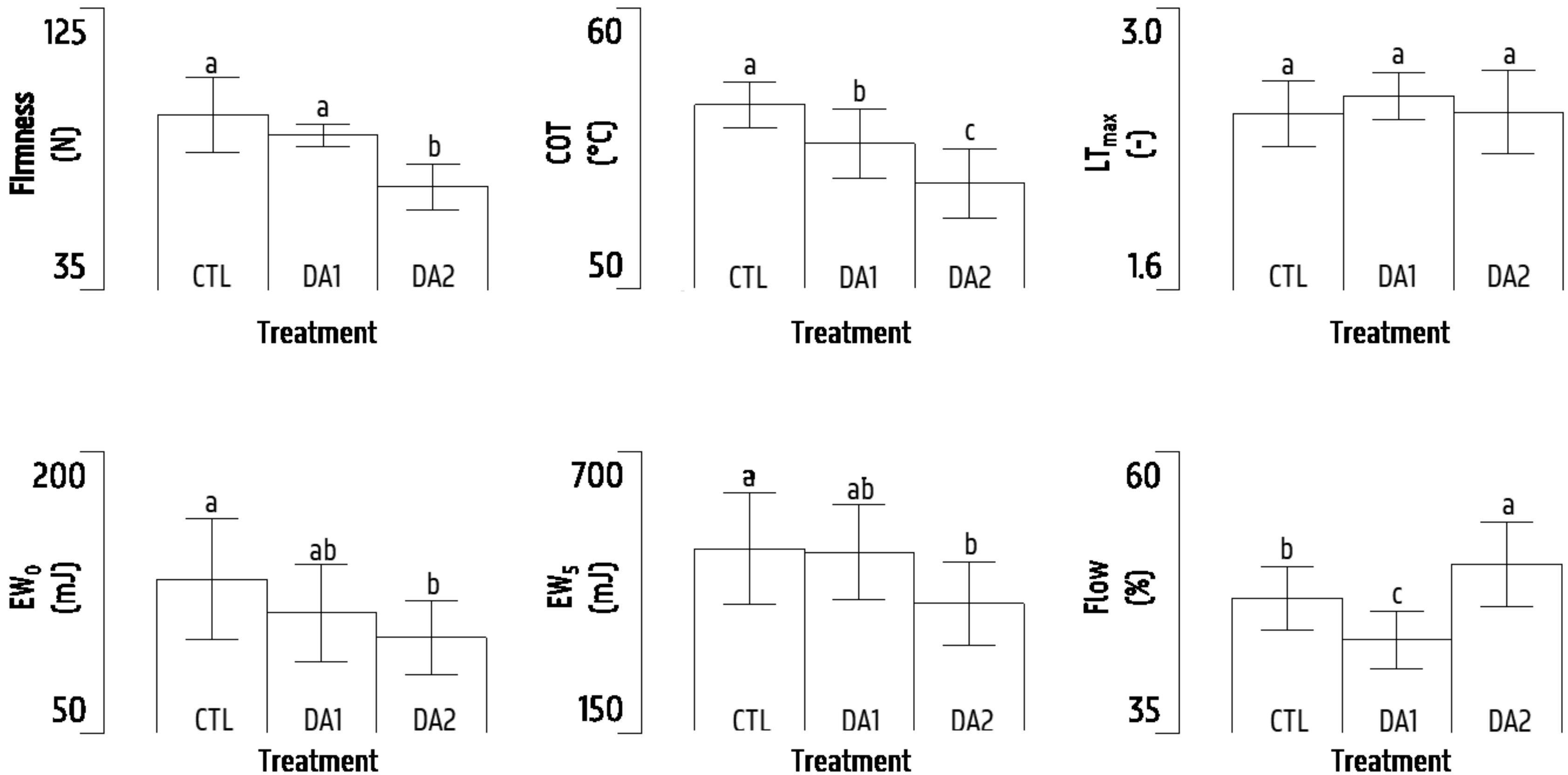
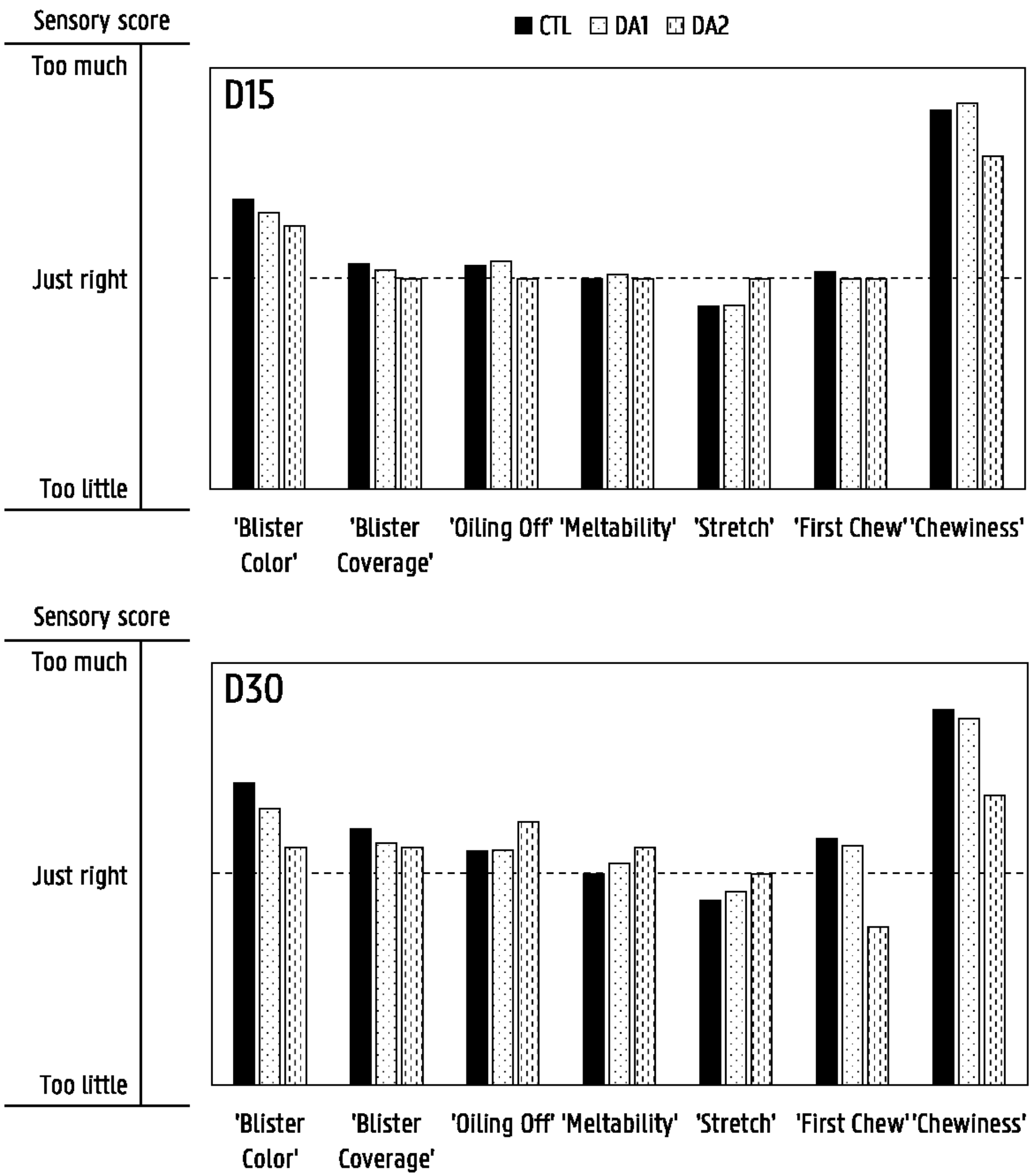

Firmness (N)
125
35
a
a
b
CTL
DA1
DA2
Treatment
COT (°C)
60
50
a
b
c
CTL
DA1
DA2
Treatment
LT_{max} (-)
3.0
1.6
a
a
a
CTL
DA1
DA2
Treatment
EW_0 (mJ)
200
50
a
ab
b
CTL
DA1
DA2
Treatment
EW_5 (mJ)
700
150
a
ab
b
CTL
DA1
DA2
Treatment
Flow (%)
60
35
b
c
a
CTL
DA1
DA2
Treatment

Fig. 4


Sensory score
■ CTL ⊡ DA1 ⊡ DA2
Too much
D15
Just right
Too little
'Blister Color'
'Blister Coverage'
'Oiling Off'
'Meltability'
'Stretch'
'First Chew'
'Chewiness'
Sensory score
Too much
D30
Just right
Too little
'Blister Color'
'Blister Coverage'
'Oiling Off'
'Meltability'
'Stretch'
'First Chew'
'Chewiness'