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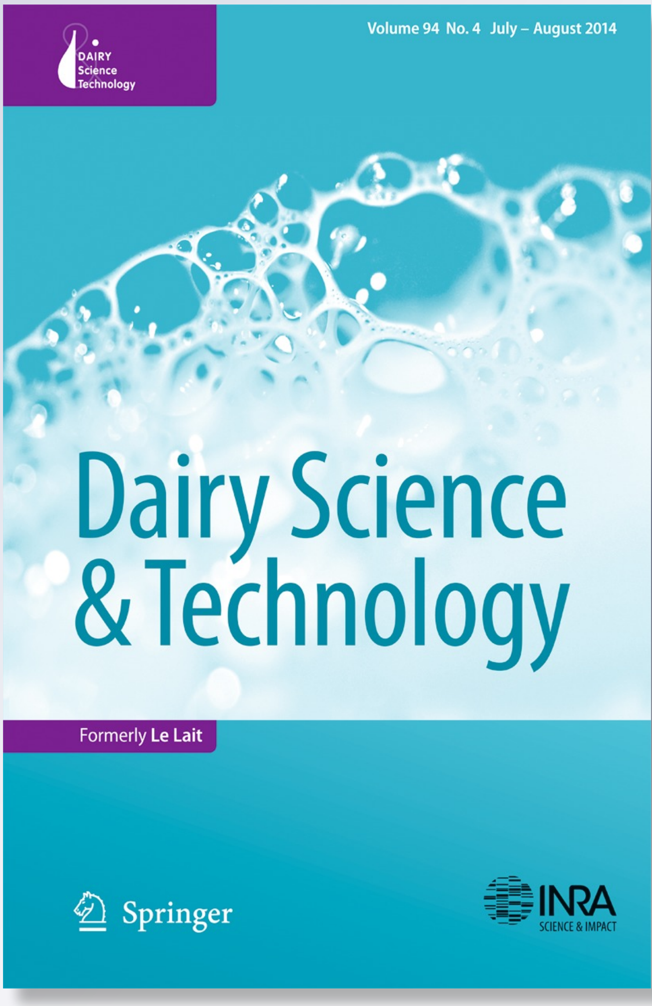

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ORIGINAL PAPER

The influence of sodium chloride reduction on physicochemical, biochemical, rheological and sensory characteristics of Mozzarella cheese

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Abstract Excessive intake of sodium has been associated with harmful effects on human health. Therefore, salt reduction in manufactured products is been targeted as a way to reduce dietary sodium intake. Sodium chloride (NaCl) plays an important role in cheese, and reducing the NaCl level in cheese may adversely affect its characteristics. Our objective was to evaluate the influence of different levels of salt reduction on the physicochemical, biochemical, rheological, and sensory characteristics of Mozzarella cheese. Samples were brine-salted for different periods to obtain cheeses with different levels of salt reduction (C: control cheese; S1: cheese with 60% salt reduction, S2: cheese with 35% salt reduction). Samples were analysed during 43 days of ripening. As expected, salt flavour intensity decreased with a decrease in NaCl levels. Small differences between control and experimental cheeses due to salting condition were

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observed in moisture content, water activity, maturation index, α_{s1}-casein, α_{s1}-I-casein, crossover temperature (when the elastic component equals the viscous component) and in some sensory attributes (aroma, colour, global flavour and creaminess). No significant differences due to salting condition were observed in pH, γ-casein, β-casein degradation, activation energy (parameter that quantifies the matrix degradation with heating), residual flavour, acidity and bitter taste. Taking into account the minor differences observed after the significant salt reduction analysed, it is considered that decreasing NaCl may not jeopardise the standard Mozzarella cheese quality.

Keywords Salt reduction · Mozzarella cheese · Characteristics

1 Introduction

The use of salt (NaCl) in food technology can be explained through three general categories: processing, sensory (taste), and preservation (Hutton 2002). In processing, salt has an important role during the manufacture of bread, biscuits, meat products, fish products and cheeses (e.g. salt has a direct effect on water content of cheeses). Salt confers its own specific flavour and modifies the flavour of other ingredients. Salt reduces water activity (main preservative mechanism of salt) and, therefore, reduces water availability for microorganisms and has a preservation effect.

However, an excessive consumption of sodium has been related to hypertension and consequently to a major risk of stroke and premature death from cardiovascular diseases. The main source of sodium in the diet is NaCl (Ruusunen and Puolanne 2005). Therefore, the World Health Organization (WHO) recommended to achieve a salt intake level less than 5 g/day/person for 2020 (Campbell et al. 2009). In most developed countries, approximately 80% of the ingested salt is added to foods during manufacturing. Therefore, it is crucial that the food industry reduces the amount of salt added to foods to achieve a reduction in population salt intake (He and McGregor 2010).

In the case of cheeses, salt has different functions, e.g. it regulates the activity of starter culture microorganisms, it modifies enzyme activity, it has a direct effect on water content, it retards the growth of most bacteria and it also has an effect on the final flavour of the product (Hutton 2002). Although the contribution to sodium intake depends on cheese variety (NaCl contents ranging from about 0.7% in swiss to about 6% in pickled cheeses such as domiati) and on consumption levels by population (Guinee and Fox 2004), cheese has been identified as one of the main food vectors contributing to salt intake (Saint-Eve et al. 2009). The production of Mozzarella cheese has been increasing over the last two decades because it is one of the most popular cheeses used for pizza manufacturing. Therefore, the production of low-salt Mozzarella cheese will contribute to worldwide demands of reducing salt in foods (Ayyash and Shah 2011).

Different approaches to reduce NaCl level in cheese include the following (Guinee and Fox 2004): reduction of the level of added salt per se, partial or complete substitution of NaCl by other salts (e.g. KCl, $MgCl_2$ or $CaCl_2$), reduction of salt level in combination with flavour-enhancing substances, use of ultrafiltration and reverse osmosis retentate-supplemented milks to alter the mineral level in the cheese, alterations of cheesemaking procedure (e.g. washing curd at a low temperature and heating moulded curd to a core temperature curd of 85 °C). Probably, the reduction of the salt content in cheese is the first option to be considered to reach the minimum NaCl content that ensures the appropriate final quality of the product. Taking into account that integral studies related to Mozzarella cheese elaborated with reduced salt content are scarce, the influence of salt reduction on physicochemical, biochemical, rheological and sensory characteristics of Mozzarella cheese was evaluated in this work.

2 Materials and methods

2.1 Cheese samples and treatments

Unsalted fresh Mozzarella cheeses ($28\times10\times10$ cm^3 in size and 3.63±0.02 kg weight) were provided by a local factory (SanCor Cooperativas Unidas Limitada, Centeno, Argentina). Cheeses were manufactured according to the specifications of the Food Code of Argentina (CAA 2010). A thermophilic starter culture was used for acidification. Slabs of 2-cm thickness were cut perpendicularly to the principal axis of the cheese block. Salting was carried out at 4 °C using a concentrated immersion brine (23% *w*/*w* NaCl, 0.55% Ca^{+2}, pH 5.2) with a relation of cheese-volume to brine-volume of 1:5.

Twenty-four slabs were used for each treatment. Three salting times were used to obtain different NaCl contents in cheese. Cheeses S1, S2 and C were obtained after 5, 20 and 60 min of immersion, respectively. Cheese C had a salt content similar to commercial cheese. After brining, samples were carefully wiped with a paper towel, packed under vacuum using a Turbovac Serie S packaging machine (Cerveny, 's-Hertogenbosch, Netherlands) and stored at 4 °C during 6 weeks. Six slabs per treatment were randomly selected at 1, 15, 28 and 43 days of ripening for physicochemical, biochemical, rheological and sensory analyses in duplicate.

2.2 Physicochemical analysis

The pH was determined with a pH electrode for solid foods (pH Spear, Oakton Instruments, Vernon Hills, IL, USA). The half of a slab of Mozzarella cheese was completely grated and analysed to determine moisture and chloride contents (Zorrilla and Rubiolo 1994), total nitrogen (TN), water-soluble nitrogen at pH 4.6 (WSN) (Sihufe et al. 2003) and water activity (a_w) using an Aqualab Series 4TE (Decagon Devices, Inc., Pullman, WA, USA) water activity metre at 25 °C. Maturation index (MI) was expressed as a percentage of WSN of the cheese TN (WSN$\times$100/TN). Fat content was determined for initial composition (International Dairy Federation 1969). Determinations were carried out in duplicate on each sample.

2.3 Urea-PAGE

Electrophoretic analysis was performed as described by Sihufe et al. (2010). Cheese fractions were obtained dissolving grated cheese (3 g) in 25 mL of 8.66 mol.L^{-1} urea in a procedure including fat removal by cold filtration and centrifugation. Electrophoretic runs of the fractions were made on vertical discontinuous polyacrylamide gels using anodic buffers. The temperature and current during runs were set at 15 °C and 60 mA, respectively. Coomassie blue R250 was used to stain the gels. Stained gels were scanned, and images were processed to obtain relatives areas for each band of interest using Gel-Pro Analyzer software (Media Cybernetics, Silver Spring, MD, USA). Standards of α_{s1}-casein and β-casein (Sigma Chemical Co., St Louis, MO, USA) were run in two lanes of each gel.

2.4 Rheological analysis

Rheological measurements were performed as described by Olivares et al. (2012). Samples corresponding to 2, 15, 28 and 42 days of ripening were used for rheological measurements. A rheometer Haake RheoStress RS80 (Haake Instrument Inc., Paramus, NJ, USA) with parallel plates (35-mm diameter, 2-mm gap) was used. A frequency of 1 Hz was used for rheological measurements, and sand paper in the upper plate was used to eliminate slippage. The temperature of the lower plate of the measuring system was maintained by circulating water from water bath. The disk-shaped cheese sample was placed on the lower plate and then the upper plate was brought in contact with the sample to attain temperature equilibrium. A thin film of silicone oil (20 cP) covered the edge of samples to avoid evaporation during measurements.

The dynamic rheological data obtained included the two components of complex shear modulus (G^*): the storage modulus or elastic component (G') and the loss modulus or viscous component (G'') (Gunasekaran and Ak 2003). Temperature sweep tests were carried out from 25 to 60 °C (1.33 °C.min^{-1}) at a strain amplitude of $0.01 \pm 5 \times 10^{-4}$. The linear viscoelastic region was determined by performing strain sweep tests from 0.001 to 0.1 at 25 and 60 °C at the beginning and at the end of the ripening period studied. From temperature sweeps, the crossover temperature (T_c) of moduli ($G' = G''$) was determined, which may be used for identifying the transition to the melted state as cheese is heated. Moreover, the effect of temperature on complex viscosity ($|\eta^*| = |G^*|/\omega$, ω: frequency of oscillation) was studied by an Arrhenius-type equation:

$$|\eta*| = A_{\mathrm{VISC}} \exp(E_a/RT) \tag{1}$$

where A_{VISC} is the pre-exponential factor, E_a is the activation energy (cal.mol^{-1}), R is the gas constant (1.9872 cal.mol^{-1}.K^{-1}) and T is the temperature (K) (Rao 1999; Tunick 2000, 2010).

2.5 Sensory analysis

Sensory evaluation was carried out the same day that cheese samples were obtained. For evaluation, cheese samples were cut in 1 cm × 1 cm × 10 cm pieces. A trained eight-assessor panel, using a quantitative descriptive sensory analysis, evaluated cheese samples taking into account eight sensory attributes: colour (1=natural white; 9=yellow), aroma, global flavour, creaminess, saltiness, bitterness, acidity and residual flavour (1=very slight; 9=pronounced). The vocabulary used for sensory analysis is in agreement with ISO (2008). Panel members were trained with samples of Mozzarella cheese from the local market. Each sensory attribute was evaluated twice by each assessor. Scores of the sensory attributes were quantified marking on an unstructured scale of 10 cm anchored in the extremes. Sensory assessment was conducted in individual booths in a sensory laboratory, which complies with the international standards for the design of test rooms (IRAM 2012). Free access to water and unsalted crackers were provided to each assessor for palate cleansing between samples. Scores for each sample were averaged over all assessors and replicates.

2.6 Statistical analysis

For statistical analysis, ripening time and salting conditions were selected as main factors for ANOVA performed using Minitab (Minitab Inc., State College, PA, USA). For significant differences ($P<0.05$) between treatment effects, a multiple comparison of means was performed by least significant differences (LSD) test using Statgraphics (Statgraphics Inc., Rockville, MD, USA). Principal component analysis (PCA) was performed taking into account information coming from sensory analysis and using a covariance matrix. PCA was carried out using Minitab (Minitab Inc., State college, PA, USA).

3 Results and discussion

3.1 Physicochemical characteristics

The initial composition of the cheeses was 47.64±0.14 g moisture/100 g cheese, 21.13±0.21 g fat/100 g cheese and 27.06±0.55 g protein/100 g cheese, while chloride was not detected. Table 1 shows the average values, and ANOVA results corresponding to the physicochemical parameters evaluated in the samples of Mozzarella cheese studied. NaCl

Table 1 Average values and standard deviation of the physicochemical parameters analysed in the samples of Mozzarella cheese studied (n=2)

Cheese	Time (days)	NaCl (g/100 g moisture)	Moisture (g/100 g cheese)	a_w	MI	pH
C	1	2.45 ± 0.02^{d}	46.46 ± 0.19^{ab}	0.9848 ± 0.0003^{c}	2.26 ± 0.14^{a}	5.63 ± 0.02^{bc}
	15	2.55 ± 0.16^{de}	46.34 ± 0.22^{ab}	0.9836 ± 0.0005^{b}	2.87 ± 0.01^{cd}	5.42 ± 0.01^{a}
	28	2.80 ± 0.22^{ef}	46.25 ± 0.25^{ab}	0.9800 ± 0.0011^{a}	3.34 ± 0.12^{de}	5.48 ± 0.14^{ab}
	43	2.92 ± 0.20^{f}	46.17 ± 0.01^{a}	0.9809 ± 0.0005^{a}	3.87 ± 0.47^{fg}	5.75 ± 0.02^{c}
S1	1	1.03 ± 0.04^{a}	47.15 ± 0.27^{c}	0.9912 ± 0.0001^{e}	2.36 ± 0.10^{ab}	5.63 ± 0.21^{bc}
	15	1.10 ± 0.03^{a}	47.68 ± 0.28^{def}	0.9908 ± 0.0003^{e}	3.11 ± 0.12^{cde}	5.32 ± 0.11^{a}
	28	1.10 ± 0.10^{a}	48.01 ± 0.19^{f}	0.9902 ± 0.0003^{e}	3.59 ± 0.04^{efg}	5.77 ± 0.08^{c}
	43	1.03 ± 0.07^{a}	47.84 ± 0.32^{ef}	0.9909 ± 0.0002^{e}	4.56 ± 0.24^{h}	6.05 ± 0.05^{d}
S2	1	1.59 ± 0.05^{b}	46.67 ± 0.01^{b}	0.9878 ± 0.0002^{d}	2.17 ± 0.00^{a}	5.70 ± 0.02^{c}
	15	1.60 ± 0.12^{bc}	47.31 ± 0.04^{cd}	0.9872 ± 0.0003^{d}	2.76 ± 0.18^{bc}	5.31 ± 0.10^{a}
	28	1.81 ± 0.15^{bc}	47.22 ± 0.14^{c}	0.9857 ± 0.0005^{c}	3.46 ± 0.16^{ef}	5.74 ± 0.10^{c}
	43	1.87 ± 0.10^{c}	47.51 ± 0.04^{cde}	0.9859 ± 0.0010^{c}	4.05 ± 0.47^{g}	5.81 ± 0.04^{c}
Ripening time		*	*	*	*	*
Salting condition		*	*	*	*	NS
Interaction		NS	*	*	NS	*

Last rows show the ANOVA result for the different factors analysed

NS nonsignificant effect ($P>0.05$), *MI* maturation index

*$P<0.05$, significant effect

[a–h] Average values in the same column with different letters are significantly different ($P<0.05$)

content, moisture content, a_w and MI were significantly affected by ripening time and salting condition.

Levels of NaCl obtained for cheese C were similar to the NaCl content of commercial Mozzarella cheeses. Important levels of salt reduction were obtained for cheeses S1 and S2 (approximately 60 and 35%, respectively). Higher levels of moisture were observed in cheese S1 than in cheeses C and S2, which can be explained by the usual behaviour observed in cheeses salted by brine immersion where NaCl content increased with salting time, while moisture decreased with salting time (Simal et al. 2001). Similar results were obtained by Rowney et al. (2004), who studied Mozzarella cheeses manufactured to different salt levels using combination of dry and hot brine salting and cold brine salting. The amount of salt added through cold brining to the Mozzarella cheese had a significant effect on some of the compositional parameters. Cheeses with higher salt levels had lower moisture content due to moisture losses during salt uptake.

The preservative action of NaCl is due to its effect on the water activity of the medium. In general, a_w of most cheese varieties is not low enough to prevent the growth of yeasts and moulds and many bacteria but is quite effective in controlling microbial growth in combination with low pH and low temperature (Guinee and Fox 2004). In this study, although the differences were small, it can clearly be observed that values of a_w in cheese S1 were higher than in cheeses C and S2 (Table 1). These results are in agreement with other studies. Rulikowska et al. (2013) evaluated the impact of reduced sodium chloride content on quality of Cheddar cheese. The authors manufactured cheeses with different added levels of NaCl (% *w/w*): 0.5, 1.25, 1.80, 2.25, 2.50 and 3.00 and observed that decreasing salt resulted in an increase in water activity.

The a_w of foods depends on its moisture content and the concentration of low molecular mass solutes. Particularly, in the case of young cheeses, a_w is determined almost entirely by the concentration of NaCl in the aqueous phase (Marcos et al. 1981; Guinee and Fox 2004):

$$a_w = 1 - 0.00565(\mathrm{NaCl}) \tag{2}$$

where (NaCl) is the concentration of NaCl as g/100 g cheese moisture. Equation (2) is the result of the best fit of experimental data corresponding to cheese varieties with moisture contents >40%. Hickey et al. (2013) also found a significant correlation between a_w and the salt-in-moisture content in Cheddar cheeses at ripening times <30 days. Interestingly, Eq. (2) is quite close to the model of depression of water activity in ideal solutions that allows calculating a_w directly through the mole fraction of water (Karel and Lund 2003) (i.e. the insoluble components of the cheese matrix do not contribute to the water activity depression). Accordingly, when Eq. (2) was used to calculate a_w, it was observed that the difference between experimental and theoretical values was less than 0.004 unit of a_w, which is in the order of the accuracy of the water activity metre used (±0.003).

The values of MI increased with the ripening time for all samples analysed; salting condition showed a clear effect at the end of the ripening period studied (Table 1). The MI value at 43 days of ripening for cheese S1 was higher than for cheeses C and S2. Similar behaviour was observed by Rulikowska et al. (2013).

Finally, no significant effect of salting condition on pH was observed (Table 1). Values of pH were similar to those reported for this type of cheese (Ribero et al. 2009;

Olivares et al. 2012). Moreover, values of pH were in a range considered safe in relation to potential spoilage with unusual microorganisms such as moulds.

3.2 Urea-PAGE

Four fractions with different electrophoretic mobility (γ-, β-, α_{s1}- and α_{s1}-I-casein) were identified. The most important caseins (α_{s1}- and β-casein) were identified by comparison with standards, while the other fractions (γ- and α_{s1}-I-casein) were identified taking into account the results published in previous works (Olivares et al. 2012). Table 2 shows the values of integrated optical density (IOD) and the ANOVA results for the different treatments studied.

A low degradation of casein fractions was observed for Mozzarella cheeses, which is a characteristic for this cheese variety (Olivares et al. 2012). Salting condition significantly affected the IOD profiles for α_{s1} and α_{s1}-I-casein, while ripening time affected IOD profiles for γ- and α_{s1}-I-casein (Table 2). The effect of the salting condition on α_{s1}-casein was related to the slightly high IOD values in cheeses S1 and S2 compared with control cheeses. Clearly, an increase in IOD of α_{s1}-I-casein was observed in cheese S1 compared to cheeses C and S2, although the differences were small. These results are consistent with those observed for MI, which measures the soluble counterpart α_{s1}-casein (f1-23).

Table 2 Average values and standard deviation of the integrated optical density values (IOD/g cheese) for the casein fractions analysed in the samples of the studied Mozzarella cheese (n=2)

Cheese	Time (days)	γ-casein	β-casein	α_{s1}-casein	α_{s1}-I-casein
C	1	0.50 ± 0.07^{a}	6.66±0.39	10.56 ± 0.57^{abc}	0.55 ± 0.01^{a}
	15	0.63 ± 0.02^{ab}	6.83±0.47	10.27 ± 0.27^{a}	0.77 ± 0.60^{bc}
	28	0.71 ± 0.01^{b}	6.90±0.08	10.20 ± 0.42^{a}	0.83 ± 0.09^{cd}
	43	0.91 ± 0.07^{c}	6.77±0.29	10.48 ± 0.57^{ab}	1.08 ± 0.10^{ef}
S1	1	0.61 ± 0.09^{ab}	7.04±0.23	11.55 ± 0.93^{c}	0.51 ± 0.05^{a}
	15	0.69 ± 0.16^{b}	6.80±0.28	10.50 ± 0.18^{ab}	0.81 ± 0.04^{cd}
	28	0.68 ± 0.10^{b}	7.16±0.27	10.86 ± 0.85^{abc}	0.97 ± 0.13^{de}
	43	0.74 ± 0.06^{bc}	7.08±0.03	11.39 ± 0.22^{bc}	1.25 ± 0.03^{f}
S2	1	0.49 ± 0.03^{a}	7.11±0.09	11.32 ± 0.22^{bc}	0.50 ± 0.03^{a}
	15	0.58 ± 0.04^{ab}	7.03±0.19	10.91 ± 0.05^{abc}	0.63 ± 0.03^{ab}
	28	0.70 ± 0.02^{b}	7.20±0.17	10.86 ± 0.39^{abc}	0.77 ± 0.08^{bc}
	43	0.70 ± 0.12^{b}	6.99±0.50	11.07 ± 0.01^{abc}	1.04 ± 0.15^{e}
Ripening time		*	NS	NS	*
Salting condition		NS	NS	*	*
Interaction		NS	NS	NS	NS

Last rows show the ANOVA result for the different factors analysed

NS nonsignificant effect ($P>0.05$)

*$P<0.05$, significant effect

[a–f] Average values in the same column with different letters are significantly different ($P<0.05$)

Different authors reported that proteolysis is considerably more extensive in unsalted than in salted cheese. Schroeder et al. (1988) manufactured Cheddar cheeses containing levels of NaCl ranging from 0.07 to 1.44% and evaluated the effects on different chemical, microbiological and sensory properties. The highest rate of proteolysis was observed in the unsalted cheeses and during the first 30 days of ripening, being more than twice as extensive for the unsalted cheese than the cheese with the highest NaCl concentration. The authors concluded that the higher content of soluble nitrogen and the higher degree of proteolysis with decreased NaCl content of cheese could most likely be attributed to a higher moisture content and the reduced bacteriostatic effect of NaCl. Wisniewska et al. (1990) reported that the salt content in Cheddar, gouda, tilsit, roquefort and camembert was inversely related to the levels of proteolysis. Rulikowska et al. (2013) reported higher proteolysis levels for Cheddar cheeses with lower quantities of NaCl. The authors observed that degradation of α_{s1}-casein increased with decreasing NaCl concentration at the early stages of ripening, while the hydrolysis degree observed for β-casein was lower than the corresponding to α_{s1}-casein. In the case of Mozzarella cheese, the impact of reducing NaCl on proteolysis was not as significant as that found for other cheeses mainly due to the limited proteolysis in Mozzarella cheese (explained by the short ripening period and the coagulant denaturation during cooking/stretching process, Upadhyay et al. 2004).

3.3 Rheological analysis

The influence of temperature on melting properties of Mozzarella cheese was analysed through temperature sweep tests. The rheological parameters analysed for the different conditions studied are shown in Table 3. Samples corresponding to 2 days of ripening could not be analysed because the material probes lost serum and fat as temperature increased during the temperature sweep.

In the case of crossover temperatures T_c, ANOVA indicated that main factors—ripening time and salting condition—had significant effect (Table 3). It was observed that T_c decreased as storage time increased. This behaviour can be explained taking into account that as proteolysis takes place, an increase of meltability and a decrease of T_c value occurs (McMahon et al. 1999; Ribero et al. 2007; Olivares et al. 2012). It was also observed that T_c decreased as NaCl content decreased, which implies that when NaCl content decreased, cheese melts at lower temperatures. This behaviour may also be related to the differences found in moisture content and water activity, as NaCl content decreases and moisture content and water activity increase. It is known that water can act as a plasticizer and modify certain properties of food polymers, the primary effect of plasticizers that reduces the stiffness of polymers (Gunasekaran and Ak 2003). Therefore, the lower T_c values obtained in samples S1 and S2 may be partially explained by the higher water contents in those samples.

In addition to its indirect effects on rheology via its influence on gross composition, salt also exerts more direct effects promoting changes in the degree of casein hydration and aggregation which alters the ratio of viscous to elastic character in the cheese (Guinee and Fox 2004). Guo et al. (1997) showed that unsalted part skim Mozzarella cheese had higher levels of expressible serum than brined part skim Mozzarella cheese and concluded that NaCl in the serum phase of Mozzarella cheese promotes microstructural swelling, a concomitant increase in water-holding capacity and the

Table 3 Average values and standard deviation of the rheological parameters determined in the samples of Mozzarella cheese studied (n=2)

Cheese	Time (days)	T_c (°C)	E_a (Kcal.mol^{-1}) range: 25–40 °C	E_a (Kcal.mol^{-1}) range: 40–60 °C
C	15	56.10 ± 0.03^{e}	14.31 ± 0.14^{abc}	12.15 ± 0.89
	28	54.56 ± 0.59^{d}	16.02 ± 0.17^{cd}	12.85 ± 0.14
	42	52.87 ± 0.20^{c}	16.68 ± 1.69^{d}	13.13 ± 0.79
S1	15	54.56 ± 0.10^{d}	13.23 ± 0.74^{a}	12.36 ± 0.05
	28	51.30 ± 0.37^{b}	15.44 ± 0.59^{bcd}	14.36 ± 0.63
	42	49.91 ± 0.12^{a}	14.73 ± 0.27^{abc}	13.49 ± 0.46
S2	15	54.28 ± 1.17^{d}	14.04 ± 1.18^{ab}	12.89 ± 0.06
	28	51.59 ± 0.03^{b}	14.91 ± 0.59^{abcd}	12.27 ± 0.35
	42	50.55 ± 0.54^{ab}	15.66 ± 0.77^{bcd}	13.98 ± 1.30
Ripening time		*	*	NS
Salting condition		*	NS	NS
Interaction		NS	NS	NS

Last rows show the ANOVA result for the different factors analysed

NS nonsignificant effect ($P>0.05$)

*$P<0.05$, significant effect

[a–e] Average values in the same column with different letters are significantly different ($P<0.05$)

solubilisation of intact caseins from the para-casein matrix. Furthermore, Paulson et al. (1998) showed by scanning electron microscopy examination that unsalted cheeses had larger open channels with free serum (whey pockets) than the salted counterparts. As consequence of protein hydration promoted by salt addition, voluminosity of the cheese matrix also increases and distance between proteins in cheese matrix decreases. This would enhance protein-protein interactions and decrease cheese meltability. Therefore, the lower T_c values observed in samples S1 and S2 may be also partially explained by the differences in protein hydration. However, beyond the differences found in melting properties for samples C, S1 and S2, T_c values observed for the samples analysed were in the order of those observed for this type of cheese (Ribero et al. 2007; Olivares et al. 2012).

The values obtained for activation energy E_a (Table 3) are in agreement with values found in the literature for Mozzarella cheese (Ribero et al. 2007). It is worth recalling that the E_a quantifies how quickly the material structure degrades with heating (Tunick 2010). Olivares et al. (2012) proposed to evaluate E_a taking into account different ranges of temperature: T<40 °C (solid-like region) and T>40 °C (liquid-like region). The authors considered 40 °C as a critical temperature because as temperature exceeds 40 °C, cheese begins to melt (fat transition from solid to liquid state and the increase of mobility of the protein occur) and behaves as a structurally different material. In accordance with Olivares et al. (2012), values of E_a in the solid-like region were higher than those in the liquid-like region, indicating a more rapid change in viscosity with temperature in the first case. Also, the cheese matrix degradation with heating was less perceptible in the liquid-like range, where no significant effect of ripening time or salting condition was observed. In the solid-like region, E_a was only significantly affected by the ripening time, showing a slight increment.

Table 4 Average values and standard deviation of sensory attributes determined for Mozzarella cheese samples studied (n=2)

Cheese	Time (days)	Aroma	Colour	Global flavour	Creaminess	Saltiness	Bitterness	Acidity	Residual flavour
C	1	4.59 ± 0.10^{f}	4.63 ± 0.19^{e}	4.14 ± 0.05^{g}	3.49 ± 0.01^{d}	3.49 ± 0.02^{f}	1.60 ± 0.08^{a}	1.87 ± 0.34^{a}	2.16 ± 0.02^{bc}
	15	3.69 ± 0.00^{abc}	4.47 ± 0.11^{bcde}	4.14 ± 0.06^{fg}	3.46 ± 0.11^{d}	3.94 ± 0.05^{g}	2.98 ± 0.03^{d}	2.02 ± 0.21^{ab}	1.91 ± 0.18^{ab}
	28	3.59 ± 0.03^{abc}	4.62 ± 0.07^{e}	3.81 ± 0.12^{ef}	3.68 ± 0.10^{d}	3.11 ± 0.11^{e}	1.72 ± 0.07^{a}	1.77 ± 0.01^{a}	2.24 ± 0.07^{cd}
	43	4.32 ± 0.06^{ef}	4.61 ± 0.01^{de}	4.26 ± 0.13^{g}	3.65 ± 0.03^{d}	3.47 ± 0.24^{f}	1.87 ± 0.22^{ab}	2.54 ± 0.24^{c}	1.88 ± 0.01^{ab}
S1	1	3.92 ± 0.13^{cd}	4.31 ± 0.09^{abcde}	3.23 ± 0.09^{bcd}	2.96 ± 0.01^{bc}	1.83 ± 0.08^{a}	2.27 ± 0.20^{c}	1.69 ± 0.03^{a}	1.86 ± 0.03^{ab}
	15	4.13 ± 0.01^{de}	4.22 ± 0.01^{abc}	3.11 ± 0.00^{abc}	2.94 ± 0.27^{bc}	2.85 ± 0.05^{e}	2.02 ± 0.08^{bc}	1.92 ± 0.25^{ab}	2.62 ± 0.02^{e}
	28	3.52 ± 0.10^{ab}	4.12 ± 0.19^{ab}	3.17 ± 0.12^{abc}	2.61 ± 0.14^{a}	2.06 ± 0.11^{ab}	2.28 ± 0.04^{c}	1.89 ± 0.03^{a}	1.76 ± 0.04^{a}
	43	3.46 ± 0.37^{a}	4.08 ± 0.22^{a}	2.89 ± 0.11^{a}	2.75 ± 0.22^{abc}	2.21 ± 0.27^{bc}	2.14 ± 0.14^{bc}	1.92 ± 0.14^{ab}	1.96 ± 0.00^{abc}
S2	1	3.83 ± 0.20^{bcd}	4.34 ± 0.05^{abcde}	2.99 ± 0.12^{ab}	2.91 ± 0.05^{abc}	2.41 ± 0.18^{cd}	2.08 ± 0.16^{bc}	2.09 ± 0.03^{ab}	2.04 ± 0.27^{abc}
	15	4.13 ± 0.06^{de}	4.52 ± 0.31^{cde}	3.51 ± 0.02^{de}	2.80 ± 0.19^{abc}	2.86 ± 0.05^{e}	2.16 ± 0.17^{bc}	1.92 ± 0.07^{ab}	2.55 ± 0.26^{de}
	28	3.45 ± 0.03^{a}	4.24 ± 0.27^{abcd}	3.35 ± 0.02^{cd}	3.04 ± 0.14^{c}	2.86 ± 0.14^{e}	2.20 ± 0.08^{c}	1.91 ± 0.14^{ab}	2.06 ± 0.26^{abc}
	43	3.79 ± 0.27^{abcd}	4.30 ± 0.11^{abcde}	3.37 ± 0.43^{cd}	2.71 ± 0.05^{ab}	2.52 ± 0.11^{d}	2.27 ± 0.17^{c}	2.31 ± 0.33^{bc}	1.89 ± 0.12^{ab}
Ripening time		*	NS	NS	NS	*	*	*	*
Salting condition		*	*	*	*	*	NS	NS	NS
Interaction		*	NS	*	NS	*	*	NS	*

Last rows show the ANOVA result for the different factors analysed

NS nonsignificant effect (P>0.05)

*P<0.05, significant effect

[a–g] Average values in the same column with different letters are significantly different (P<0.05)

3.4 Sensory analysis

According to the Food Code of Argentina (CAA 2010), Mozzarella cheese should have a natural white to pale yellow uniform colour and a slight degree of acid flavour, characteristics of lactic acid. The panel members agreed on the use of the eight attributes and scales to describe Mozzarella cheese. Table 4 shows the values of the scores for the eight attributes analysed in the samples of the studied Mozzarella cheese.

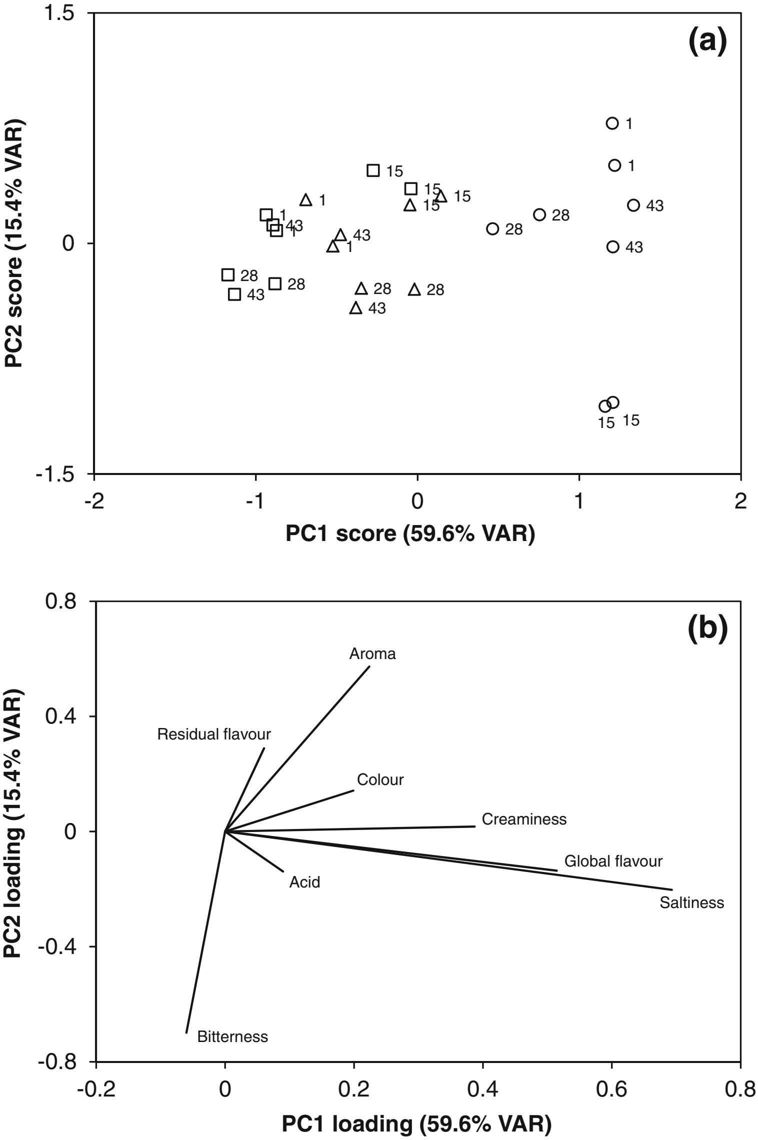

Fig. 1 Plots of the first two principal components corresponding to the sensory analysis of Mozzarella cheese samples. **a** Score plot: (*circle*) control cheese, (*square*) cheese S1, (*triangle*) cheese S2 (*numbers* indicate the ripening time of the samples). **b** Loading plot

The ripening time significantly affected five attributes: aroma, saltiness, bitterness, acidity and residual flavour (Table 4). Although the effect is significant, a clear behaviour of these attributes with ripening time was not definite. The attributes colour, global flavour and creaminess were not significantly affected by the factor ripening time.

The salting condition significantly affected five attributes: aroma, colour, global flavour, creaminess and saltiness (Table 4). In general, the scores observed for cheese C were slightly higher than for cheeses S1 and S2. As expected, salt flavour intensity decreased with a decrease in NaCl levels. The panel members could differentiate the NaCl level successfully grading the samples from the highest to the lowest NaCl content. It is worth mentioning that the differences observed were small.

Mozzarella cheese has a delicate, mild and milky flavour. Therefore, bitterness or acidity can limit cheese acceptability if those attributes are too intense. In this study, the attributes bitterness, acidity and residual flavour were not significantly affected by the salting condition (Table 4).

Principal component analysis was carried out on the basis of the sensory data (Fig. 1). The first two principal components explained 75% of the total variance. The PCA score plot shows that the first component PC1 (59.6% of the variance) differentiates among the experimental and control cheeses. Although there were small differences in the single attributes among the samples, the multivariate map shows three distinct groupings (i.e. from left to right, cheese S1, cheese S2 and cheese C). The loading plot illustrates that PC1 is mainly related to the attributes salty, global flavour and creaminess, which correlated positively with it. The second component PC2 (15.4% of the variance) can be related to the ripening time, the attributes bitterness, aroma and residual flavour being the main contributors to the data variation.

4 Conclusions

The effect of reducing salt content on physicochemical, biochemical, rheological and sensory characteristics of Mozzarella cheese was studied. A reduction of 60 and 35% in the regular content of NaCl in Mozzarella cheese resulted in a slight decrease of crossover temperature, aroma, colour, global flavour and creaminess and in a slight increase of moisture content, water activity, maturation index, degradation of α_{s1}-casein and formation of α_{s1}-I-casein. Salt reduction did not impact pH, γ-casein, β-casein, activation energy, residual flavour, acidity and bitter taste. Taking into account the significant salt reduction analysed in this study and the minor differences observed, it is considered that decreasing NaCl may not jeopardise the standard Mozzarella cheese quality (mostly because Mozzarella has a short ripening period and is mainly used as a pizza ingredient).

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