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# Effect of sodium chloride reduction on physicochemical, biochemical, rheological, structural and sensory characteristics of Tybo cheese

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ABSTRACT

Tybo cheeses with different salt contents (0.46, 0.91, 1.28 g NaCl 100 $g^{-1}$ cheese) at different ripening times (1, 14, 26, 40 days of ripening) were evaluated for the effect of salt reduction on physicochemical, biochemical, rheological, structural and sensory characteristics. Reducing salt (from 1.28 g NaCl 100 $g^{-1}$ cheese; cheese C3) by 30% (cheese C2) or 65% (cheese C1) resulted in a significant increase in moisture content, maturation index, $\alpha_{S1}$-I-casein degradation, chromatographic area of water-soluble fraction at pH 4.6; the level of total intact caseins decreased significantly. Rheological parameters significantly decreased with salt reduction; cheeses C3 had smaller voids than those of samples C1 and C2. Generally, flavour and texture attributes showed similar profiles for the different salting conditions; salty, acid and overall persistence significantly decreased with salt content. Overall, the reduction of 30% salt content resulted in a cheese similar to a typical Tybo cheese in most respects.



## 1. Introduction

A diet high in salt (NaCl) is harmful for human health. Hypertension, cardiovascular and kidney diseases are among the most significant undesirable effects (Campbell, Correa-Rotter, Neal, & Cappuccio, 2011; He, Campbell, & MacGregor, 2012). Although the recommended intake of salt is less than 5 g $d^{-1}$ per person (WHO, 2012), the intake can be several times over that level. In most developed countries, approximately 80% of the ingested salt is added during food manufacturing. Therefore, it is critical that the food industry reduces the amount of salt added to foods to decrease population salt intake (He & McGregor, 2010).

The use of salt in food technology can be explained through three main categories, i.e., processing, sensory, and preservation (Hutton, 2002). In cheeses, salt has many direct and indirect effects. It affects the water activity (salt effect on microbial growth, enzyme activity, and biochemical changes during cheese ripening) and the development of flavour and aroma. Moreover, salt, pH and the calcium level affect *para*-casein hydration or aggregation (salt effect on water-binding capacity of casein matrix, casein matrix syneresis, and rheological and textural characteristics of cheese) (Guinee & Fox, 2004). Although, the contribution to sodium intake depends on cheese variety (i.e., ~0.7% NaCl in Swiss to ~6% in Domiati cheese) and on consumption levels by population (Guinee & Fox, 2004), cheese has been identified as one of the food sources contributing to salt intake (Saint-Eve, Lauverjat, Magnan, Déléris, & Souchon, 2009).

Reduction in salt level during cheese manufacturing is an effective and simple technological alternative to reduce the intake of sodium through cheeses, but special care should be taken to ensure the characteristic final quality of the product (Cruz et al., 2011).

Tybo cheese is a semi-hard pressed cheese, maximum 45.9% moisture with 44.9% fat in dry matter and it is ripened in plastic bags at least 25 days and at a temperature lower than 12 °C, according to the Food Code of Argentina (CAA, 2014). It is a Danish cheese type, salted by immersion in brine solutions. In 2016, semi-hard cheese production in Argentina was 188,987 tons, Tybo being among the most important semi-hard cheeses (Ministerio de Agroindustria de Argentina, 2017). Taking into account that studies related to Tybo cheese production with reduced salt content are scarce, the influence of salt reduction on physicochemical,

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biochemical, rheological, structural and sensory characteristics of Tybo cheese was evaluated in this work.

## 2. Materials and methods

### 2.1. Cheese samples and treatments

Unsalted fresh Tybo cheeses (loaf form) were provided by a local factory (Ricolact SRL, San Martín de las Escobas, Argentina). Cheeses were manufactured according to the specifications of CAA (2014). A thermophilic frozen starter culture was used for acidification. Slabs of 2 cm thickness (13.3 cm height, 8.5 cm length) were cut perpendicularly to the principal axis of the cheese block. Salting was carried out at 6 °C using an immersion brine (21.3%, w/w, NaCl; 0.65%, w/w, $Ca^{2+}$; pH 5.2).

Three salting times of 5, 30 and 80 min giving treatments C1, C2, and C3, respectively, were used to obtain different NaCl contents in cheese. Cheeses C3 had a salt content similar to that of commercial cheeses (Minetti, Zannier, Sbodio, & Tercero, 2002). After brining, samples were carefully wiped with a paper towel, packed under vacuum and stored at 10 °C for 6 weeks. Six slabs per treatment were randomly selected at 1, 14, 26 and 40 d of ripening to perform the physicochemical, biochemical, rheological, structural and sensory analysis in duplicate.

### 2.2. Physicochemical analysis

The pH was determined with a pH electrode for solid foods, HANNA FC200B (HANNA instruments, Limena, Italy). Grated cheese samples were analysed to determine (in g 100 $g^{-1}$ cheese) moisture, NaCl (Zorrilla & Rubiolo, 1994), total nitrogen (TN) and water-soluble nitrogen at pH 4.6 (WSN) (ISO, 2011; ISO, 2014). Maturation index (MI) was expressed as a percentage of WSN of the cheese TN (WSN × 100 × $TN^{-1}$). Fat content (g 100 $g^{-1}$ cheese) was determined for initial composition (ISO, 2004). Determinations were carried out in duplicate.

### 2.3. Urea-PAGE electrophoresis

Electrophoretic analysis was performed as described by Sihufe, Zorrilla, and Rubiolo (2010). Briefly, 3 g of grated cheese were dissolved in 25 mL of 8.66 mol $L^{-1}$ urea; fat was removed by cold filtration with qualitative filter paper. Electrophoretic runs of the fractions obtained were made on vertical discontinuous polyacrylamide gels using anodic buffers. During runs, the temperature was set to 15 °C and current to 60 mA. Coomassie blue R250 was used to stain the gels. Stained gels were scanned and images processed to obtain relative areas for each band of interest using GelPro Analyzer software (Media Cybernetics, Silver Spring, MD, USA).

### 2.4. Reverse phase-high performance liquid chromatography analysis of the water soluble fraction

The water-soluble fraction at pH 4.6 (WSF) was obtained as described by Sihufe, Zorrilla, and Rubiolo (2003) and analysed by reverse phase-high performance liquid chromatography (RP-HPLC). The WSF (100 μL) was filtered through a disposable 0.2 μm filter prior to injection onto the chromatographic system. Equipment and chromatographic conditions for peptide analysis by RP-HPLC were as described by Sihufe et al. (2010). Separation was performed on a Microsorb-MV (250 × 4.6 mm) C18, 300 Å column (Varian Inc., Palo Alto, CA, USA).

### 2.5. Rheological analysis

Cheese cubes (15 mm each side) were used for the uniaxial compression measurements. Samples were compressed to 70% of their original height at room temperature and at a rate of 1 mm $s^{-1}$ on a universal testing system Instron 3344 (Instron, Norwood, MA, USA), using a plate of 30 mm diameter and a 1000 N load cell. Determinations were carried out in at least quadruplicate.

The measured force F (N) and the sample height h (m) were transformed to displacement Δh (m), stress σ (Pa) and deformation ε (−) by (Gunasekaran & Ak, 2003):

$$\Delta h = h_0 - h \tag{1}$$

$$\sigma = \frac{F}{A_0}\frac{h}{h_0} \tag{2}$$

$$\varepsilon = \ln\left(\frac{h_0}{h}\right) \tag{3}$$

where $h_0$ (m) is the initial height of cheese sample and $A_0$ ($m^2$) is the initial cross-sectional area.

From a force–displacement curve, the required energy W (J) to compress the sample to 70% of the original height can be calculated as the area under the curve. The maximum stress $\sigma_{max}$ (Pa) at 70% compression of the original height (McCarthy, Wilkinson, Kelly, & Guinee, 2016) and the modulus of deformability E (Pa) were obtained from the stress–deformation curve. E is defined as (Gunasekaran & Ak, 2003):

$$E = \frac{\sigma}{\varepsilon} \tag{4}$$

Due to small imperfections on the cheese surfaces, the modulus of deformability was calculated as the tangent to the linear stress curve in the deformation range of 0.05–0.10 (Vandenberghe, Choucharina, De Ketelaere, De Baerdemaeker, & Claes, 2014).

### 2.6. Microstructure analysis

The microstructure of the cheese samples was examined with scanning electron microscopy (SEM). Samples were prepared according to Kuo and Gunasekaran (2003) and Ribero, Rubiolo, and Zorrilla (2009). Briefly, strips of 20 × 2 × 2 $mm^3$ were fixed in 2.8% glutaraldehyde in a 0.05 M sodium phosphate buffer (pH 6) for 48 h at 4 °C. Then, the strips were dehydrated in a graded ethanol series for 30 min in each of 25, 50, 70, 80, 95, 100, 100, and 100% (v/v) ethanol solution. The strips were defatted three times in chloroform for 30 min. Finally, the defatted strips were dehydrated three times with absolute ethanol for 30 min. Strips were then frozen in liquid air and fractured. The cryofractured specimens were mounted on SEM stubs with silver conducting paint, and dried and coated with gold in argon atmosphere using a laboratory evaporator Veeco VE-300 (Veeco Instruments Inc., Long Island, NY, USA). The specimens were examined in a JEOL JSM-35C scanning electron microscope (JEOL Ltd., Tokyo, Japan) operated at an accelerating voltage of 20 kV. Images were obtained at three different magnifications: 120×, 400×, and 1200×.

### 2.7. Sensory analysis

Sensory evaluation was carried out on the cheese samples corresponding at 26 and 40 days of ripening. A trained panel (7 assessors), using a quantitative descriptive sensory analysis, evaluated the cheese samples using 13 sensory attributes: odour intensity, aroma

intensity, sweet, salty, acid, overall persistence, residual flavour, rubberiness, firmness, adhesiveness, solubility, moist, and creamy according to Bérodier et al. (1997) and Lavanchy et al. (1993). Panel members were trained according to international standards (IRAM, 1998, 2001). Scores for the sensory attributes were quantified by marking on a scale from 1 to 7. Each sensory attribute was evaluated in duplicate by each assessor. Scores for each sample were averaged over all assessors and replicates.

### 2.8. Statistical analysis

For statistical analysis, ripening time and salting conditions were selected as main factors for two-way analysis of variance (ANOVA) with test for interaction, 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).

## 3. Results and discussion

### 3.1. Physicochemical characteristics

The composition of the cheeses before brining was (in g 100 $g^{-1}$ cheese): moisture, 44.82; fat, 28.95; protein, 23.88; NaCl, 0.11. The average values and ANOVA results corresponding to the physicochemical parameters studied in the Tybo cheese samples after brining are shown in Table 1.

Average NaCl contents of 0.46, 0.91, 1.28 g NaCl 100 $g^{-1}$ cheese were obtained for cheeses C1, C2, and C3, respectively. The NaCl content for the salting condition C3 is in the order of the values expected for a traditional Tybo cheese (Bertola, Bevilacqua, & Zaritzky, 1992). The salting conditions C1 and C2, in comparison with condition C3, represent a reduction of salt of 65% and 30%, respectively.

Average moisture contents of 42.40, 42.72, 41.74 g moisture 100 $g^{-1}$ cheese were obtained for cheeses C1, C2, and C3, respectively. These values are comparable with those recommended in the Protocol of Quality for Tybo and Dutch cheeses of Argentina (MAGyP, 2009). In the case of the cheeses salted by immersion in brines, the uptake of NaCl occurs with a concomitant water loss (Guinee & Fox, 2004). The moisture content of cheeses C3 was significantly lower than those of cheeses C1 and C2, which is in agreement with a higher NaCl uptake.

No significant effect of the salting condition on the pH was observed. The values of pH were in the range of 5.40–5.64, which are expected values for this type of cheese (Bertola et al., 1992; MAGyP, 2009).

Finally, the ripening time and the salting condition affected significantly the MI. The values of MI increased with the ripening time from approximately 7% for 1 d of ripening to 13% for the end of the ripening time studied (40 d). It can be observed that at 40 d of ripening, the MI for cheeses C3 was significantly smaller than for cheeses C1 and C2. However, taking into account that the reduction of salt can affect the enzyme activity in cheeses (increasing the proteolysis), the values of MI in cheeses C1 and C2 were not greatly higher than those corresponding to cheeses C3 at the end of the ripening time studied.

### 3.2. Urea-PAGE electrophoresis

Four fractions with different electrophoretic mobility (γ-, β-, $\alpha_{S1}$- and $\alpha_{S1}$-I-casein) were identified (Table 2). The salting condition affected significantly the four fraction levels while the ripening time did not affect the level of γ-casein. The major fractions β- and $\alpha_{S1}$-casein showed a clear decreasing trend with ripening time. The $\alpha_{S1}$-I-casein ($\alpha_{S1}$-CN f24-199), which is a product of the degradation of $\alpha_{S1}$-casein due to the residual coagulant action on the $\alpha_{S1}$-casein primary site $Phe_{23}$-$Phe_{24}$ (McSweeney, Pochet, Fox, & Healy, 1994), had an increasing trend. The behaviour of the casein levels with ripening time was similar for all the salting conditions studied. Only a slight difference in the $\alpha_{S1}$-I-casein formation was observed in the case of cheeses C1, which showed lower values than in the case of cheeses C2 and C3. It is possible that at lower salt content, a more rapid degradation of that fraction may occur. Indeed, this behaviour is in agreement with the behaviour of the WSF observed through RP-HPLC as described below. This behaviour may be explained taking into account the inhibitory effect of NaCl on proteolysis (Guinee & Fox, 2004); if NaCl content is lowered, proteolysis might increase due to the water activity is higher and microbial activity is increased (Akkerman et al., 2017). However, it is also worth mentioning that some discrepancies on the effect of salt on cheese proteolysis may occur because many other factors must be considered including the type of coagulant, pH, moisture content, etc (McCarthy et al., 2016).

It is interesting to note that if the sum of the integrated optical density values of the 4 fractions involved is considered, a decreasing trend with ripening time can be observed associated

**Table 1**
Salt and moisture content, pH, and maturation index (MI) of C1, C2 and C3 Tybo cheeses stored for up to 40 days.[a]

| Cheese code | Time (days) | NaCl (g 100 $g^{-1}$ cheese) | Moisture (g 100 $g^{-1}$ cheese) | pH | MI (%) |
|---|---|---|---|---|---|
| C1 | 1 | $0.43 \pm 0.03^{a}$ | $42.62 \pm 0.22^{b}$ | $5.40 \pm 0.03^{ab}$ | $6.98 \pm 0.01^{a}$ |
| | 14 | $0.50 \pm 0.02^{a}$ | $42.84 \pm 0.23^{bc}$ | $5.54 \pm 0.16^{bc}$ | $7.44 \pm 0.03^{b}$ |
| | 26 | $0.49 \pm 0.04^{a}$ | $41.90 \pm 0.52^{ab}$ | $5.53 \pm 0.07^{abc}$ | $11.20 \pm 0.08^{f}$ |
| | 40 | $0.44 \pm 0.06^{a}$ | $42.24 \pm 0.70^{b}$ | $5.64 \pm 0.01^{c}$ | $13.43 \pm 0.24^{i}$ |
| C2 | 1 | $0.92 \pm 0.01^{b}$ | $41.87 \pm 0.41^{ab}$ | $5.43 \pm 0.01^{ab}$ | $7.40 \pm 0.18^{b}$ |
| | 14 | $0.89 \pm 0.03^{b}$ | $42.84 \pm 0.13^{bc}$ | $5.59 \pm 0.08^{c}$ | $9.85 \pm 0.02^{d}$ |
| | 26 | $0.91 \pm 0.05^{b}$ | $42.46 \pm 0.55^{b}$ | $5.55 \pm 0.08^{bc}$ | $11.62 \pm 0.09^{g}$ |
| | 40 | $0.92 \pm 0.06^{b}$ | $43.71 \pm 0.22^{c}$ | $5.60 \pm 0.00^{c}$ | $13.65 \pm 0.03^{i}$ |
| C3 | 1 | $1.18 \pm 0.01^{c}$ | $41.97 \pm 0.27^{ab}$ | $5.40 \pm 0.01^{a}$ | $7.63 \pm 0.17^{b}$ |
| | 14 | $1.34 \pm 0.03^{d}$ | $41.13 \pm 1.11^{a}$ | $5.53 \pm 0.00^{abc}$ | $9.32 \pm 0.14^{c}$ |
| | 26 | $1.24 \pm 0.01^{c}$ | $41.12 \pm 0.07^{a}$ | $5.50 \pm 0.00^{abc}$ | $10.48 \pm 0.04^{e}$ |
| | 40 | $1.35 \pm 0.05^{d}$ | $42.74 \pm 0.50^{bc}$ | $5.56 \pm 0.00^{bc}$ | $12.89 \pm 0.02^{h}$ |
| Salting condition | | * | * | NS | * |
| Ripening time | | * | * | * | * |
| Interaction | | * | * | NS | * |

[a] C1, C2, and C3 correspond to 5, 30 and 80 min of salting times, respectively. Results are average values and standard deviation (n = 2); values in the same column with different superscript letters are significantly different ($P < 0.05$). The last three rows show the ANOVA results for the different factors analysed: an asterisk indicates a significant effect ($P < 0.05$); NS indicates no significant effect ($P > 0.05$).

**Table 2**
Casein fraction levels in C1, C2 and C3 Tybo cheeses stored for up to 40 days.[a]

| Cheese code | Time (days) | γ-casein | β-casein | $\alpha_{S1}$-casein | $\alpha_{S1}$-I-casein |
|---|---|---|---|---|---|
| C1 | 1 | $2.53 \pm 3.10^{ab}$ | $349.13 \pm 10.82^{f}$ | $409.42 \pm 16.42^{e}$ | $1.16 \pm 0.01^{a}$ |
| | 14 | $3.35 \pm 0.33^{ab}$ | $317.71 \pm 30.57^{def}$ | $343.52 \pm 0.23^{d}$ | $4.37 \pm 1.02^{a}$ |
| | 26 | $7.26 \pm 1.47^{abc}$ | $287.01 \pm 11.73^{cde}$ | $340.31 \pm 6.05^{d}$ | $25.35 \pm 6.51^{bc}$ |
| | 40 | $7.62 \pm 4.71^{abc}$ | $199.99 \pm 53.94^{a}$ | $235.75 \pm 36.10^{ab}$ | $25.93 \pm 1.54^{bc}$ |
| C2 | 1 | $2.34 \pm 0.58^{a}$ | $302.38 \pm 16.14^{def}$ | $417.92 \pm 3.87^{e}$ | $12.09 \pm 11.44^{ab}$ |
| | 14 | $4.86 \pm 0.01^{ab}$ | $244.94 \pm 20.16^{abc}$ | $333.24 \pm 24.99^{d}$ | $33.73 \pm 8.18^{c}$ |
| | 26 | $6.16 \pm 0.21^{abc}$ | $225.24 \pm 3.68^{ab}$ | $300.07 \pm 47.29^{cd}$ | $54.35 \pm 15.85^{d}$ |
| | 40 | $4.59 \pm 1.45^{ab}$ | $234.44 \pm 31.56^{abc}$ | $203.43 \pm 13.27^{a}$ | $106.39 \pm 4.70^{e}$ |
| C3 | 1 | $5.78 \pm 1.51^{ab}$ | $332.53 \pm 29.04^{ef}$ | $394.71 \pm 10.04^{e}$ | $9.19 \pm 0.73^{a}$ |
| | 14 | $12.72 \pm 7.50^{c}$ | $328.84 \pm 17.69^{ef}$ | $407.77 \pm 15.59^{e}$ | $34.54 \pm 0.03^{c}$ |
| | 26 | $7.93 \pm 2.61^{abc}$ | $264.02 \pm 23.17^{bcd}$ | $345.44 \pm 17.75^{d}$ | $66.55 \pm 3.24^{d}$ |
| | 40 | $9.14 \pm 3.67^{bc}$ | $206.75 \pm 23.77^{a}$ | $261.81 \pm 14.14^{bc}$ | $107.91 \pm 9.18^{e}$ |
| Salting condition | | * | * | * | * |
| Ripening time | | NS | * | * | * |
| Interaction | | NS | NS | NS | * |

[a] Cheese codes are defined in Table 1. Values, in integrated optical density $g^{-1}$ cheese, are averages (n = 2) and standard deviation; values in the same column with different superscript letters are significantly different ($P < 0.05$). The last three rows show the ANOVA result for the different factors analysed: an asterisk indicates a significant effect ($P < 0.05$); NS indicates no significant effect ($P > 0.05$).

with the expected primary proteolysis. On the other hand, the level of total intact caseins was smaller for cheeses with low salt content (61.5%, 74.7% and 78.1% for cheeses C1, C2 and C3, respectively). Indeed, McCarthy et al. (2016) studied the effect of salt reduction on proteolysis of Cheddar cheese and observed smaller levels of intact major caseins (particularly $\alpha_{S1}$-casein) as salt content was reduced.

### 3.3. RP-HPLC analysis of WSF

The RP-HPLC chromatograms of the water-soluble fraction are very useful indices to show the characteristic proteolytic pattern of cheese varieties (Fox, Guinee, Cogan, & McSweeney, 2017). In our case, the chromatographic patterns were similar for the 3 salting conditions (Fig. 1).

It is worth recalling that in a RP-HPLC column, hydrophilic compounds elute from the column first while the more hydrophobic compounds are retained more strongly and elute last (Snyder, Kirkland, & Glajch, 1997). Therefore, the chromatogram information was evaluated considering the integrated area between 10 and 60 min and between 60 and 100 min of run time to explore the effect on hydrophilic and hydrophobic regions of the chromatogram, respectively (Table 3). The total chromatographic area was also studied. The ripening time and the salting condition had significant effect on the chromatographic areas studied. The chromatographic areas increased with ripening time, the more important difference due to salting condition being observed at 40 d when samples C1 showed the highest area values. This behaviour is in agreement with the behaviour observed through the electrophoresis analysis.

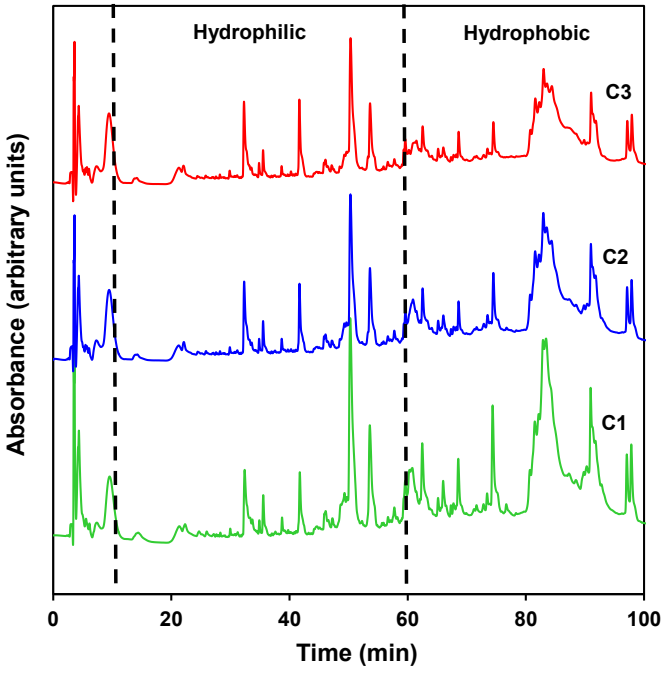


**Fig. 1.** Typical chromatograms of the WSF corresponding to samples of Tybo cheese at 26 days of ripening for salting conditions C1, C2 and C3 (defined in Table 1).

### 3.4. Rheological analysis

The rheological properties of cheese are very important quality attributes, not only for manufacturing or commercialisation, but also for the consumer. These properties are determined as the

**Table 3**
Hydrophobic and hydrophilic regions of the chromatograms of WSF fractions of C1, C2 and C3 Tybo cheeses stored for up to 40 days.[a]

| Cheese code | Time (days) | Hydrophilic region (area 10–60 min) | Hydrophobic region (area 60–100 min) | Total area |
|---|---|---|---|---|
| C1 | 1 | $1.37 \pm 0.06^{a}$ | $2.19 \pm 0.43^{ab}$ | $5.30 \pm 1.97^{ab}$ |
| | 14 | $2.61 \pm 0.21^{bc}$ | $2.80 \pm 0.05^{ab}$ | $5.41 \pm 0.16^{ab}$ |
| | 26 | $3.76 \pm 1.13^{cd}$ | $4.26 \pm 1.79^{b}$ | $8.02 \pm 2.92^{b}$ |
| | 40 | $6.66 \pm 1.44^{e}$ | $16.7 \pm 5.18^{c}$ | $23.40 \pm 6.62^{c}$ |
| C2 | 1 | $1.32 \pm 0.02^{a}$ | $2.22 \pm 0.29^{ab}$ | $3.54 \pm 0.30^{a}$ |
| | 14 | $1.96 \pm 0.18^{ab}$ | $2.27 \pm 0.17^{ab}$ | $4.23 \pm 0.35^{a}$ |
| | 26 | $2.40 \pm 0.14^{bc}$ | $2.44 \pm 0.69^{ab}$ | $4.84 \pm 0.83^{ab}$ |
| | 40 | $3.02 \pm 1.15^{bcd}$ | $2.13 \pm 0.96^{a}$ | $5.15 \pm 2.11^{ab}$ |
| C3 | 1 | $1.34 \pm 0.04^{a}$ | $2.75 \pm 0.03^{ab}$ | $4.09 \pm 0.07^{a}$ |
| | 14 | $2.72 \pm 0.32^{bc}$ | $2.62 \pm 0.05^{ab}$ | $5.34 \pm 0.27^{ab}$ |
| | 26 | $2.27 \pm 0.14^{b}$ | $1.93 \pm 0.33^{a}$ | $4.20 \pm 0.47^{a}$ |
| | 40 | $4.39 \pm 1.66^{d}$ | $3.64 \pm 2.09^{ab}$ | $8.03 \pm 3.75^{b}$ |
| Salting condition | | * | * | * |
| Ripening time | | * | * | * |
| Interaction | | NS | * | * |

[a] Cheese codes are defined in Table 1. Values (chromatographic area $\times 10^{-7}$) are averages (n = 2) and standard deviation; values in the same column with different superscript letters are significantly different ($P < 0.05$). The last three rows show the ANOVA result for the different factors analysed: an asterisk indicates a significant effect ($P < 0.05$); NS indicates no significant effect ($P > 0.05$).

**Table 4**
Rheological analysis of C1, C2 and C3 Tybo cheeses stored for up to 40 days.[a]

| Cheese code | Time (days) | W (J) | $\sigma_{max}$ (kPa) | E (kPa) |
|---|---|---|---|---|
| C1 | 1 | $0.040 \pm 0.006^{d}$ | $61 \pm 9^{f}$ | $247 \pm 48^{e}$ |
| | 14 | $0.035 \pm 0.013^{cd}$ | $41 \pm 14^{cde}$ | $165 \pm 59^{cd}$ |
| | 26 | $0.021 \pm 0.005^{a}$ | $30 \pm 7^{ab}$ | $122 \pm 33^{ab}$ |
| | 40 | $0.020 \pm 0.004^{a}$ | $25 \pm 5^{a}$ | $100 \pm 25^{a}$ |
| C2 | 1 | $0.059 \pm 0.010^{e}$ | $81 \pm 8^{g}$ | $340 \pm 77^{f}$ |
| | 14 | $0.038 \pm 0.006^{d}$ | $47 \pm 5^{e}$ | $193 \pm 16^{d}$ |
| | 26 | $0.029 \pm 0.004^{bc}$ | $39 \pm 6^{cd}$ | $167 \pm 20^{cd}$ |
| | 40 | $0.021 \pm 0.003^{a}$ | $25 \pm 3^{a}$ | $97 \pm 16^{a}$ |
| C3 | 1 | $0.066 \pm 0.004^{f}$ | $91 \pm 4^{h}$ | $403 \pm 31^{g}$ |
| | 14 | $0.056 \pm 0.009^{e}$ | $68 \pm 12^{f}$ | $283 \pm 54^{e}$ |
| | 26 | $0.024 \pm 0.003^{ab}$ | $33 \pm 5^{bc}$ | $143 \pm 29^{bc}$ |
| | 40 | $0.037 \pm 0.003^{d}$ | $43 \pm 3^{de}$ | $170 \pm 5^{cd}$ |
| Salting condition | | * | * | * |
| Ripening time | | * | * | * |
| Interaction | | * | * | * |

[a] Abbreviations are: W, energy to compress the sample to 70% of the original height; $\sigma_{max}$, maximum stress at 70% compression of the original height; E, modulus of deformability. Cheese codes are defined in Table 1. Values are averages (n = 2) and standard deviation; values in the same column with different superscript letters are significantly different ($P < 0.05$). The last three rows show the ANOVA result for the different factors analysed: an asterisk indicates a significant effect ($P < 0.05$); NS indicates no significant effect ($P > 0.05$).

response to an applied stress or strain. In practice, such stress or strain can be related to different processing stages or consumption (i.e., slicing, shredding). The uniaxial compression test is the most popular fundamental method because it is easy to execute and allows evaluating some functional properties of cheese (Gunasekaran & Ak, 2003; O'Callaghan & Guinee, 2004).

The rheological parameters determined are shown in Table 4. According to O'Callaghan and Guinee (2004), the parameter $\sigma_{max}$ can be related to the textural characteristic of firmness while the parameter E can be related to the stiffness of the material to an applied load. The larger the stiffness, the higher the stress needed to cause a given deformation (Gunasekaran & Ak, 2003). In the literature, values of modulus of deformability E or Young's modulus are more frequently found. The values of E found in the present work were comparable with those obtained for Tybo cheese (Bertola et al., 1992), Gouda cheese (Vandenberghe et al., 2014) and other semi-hard cheeses such as Danbo, Edam and Mozzarella cheeses (Gunasekaran & Ak, 2003).

All the parameters were significantly affected by the salting condition, ripening time and interaction (Table 4). In general, the parameters decrease when ripening time increases and salt content decreases. The decrease in the parameter values with the ripening

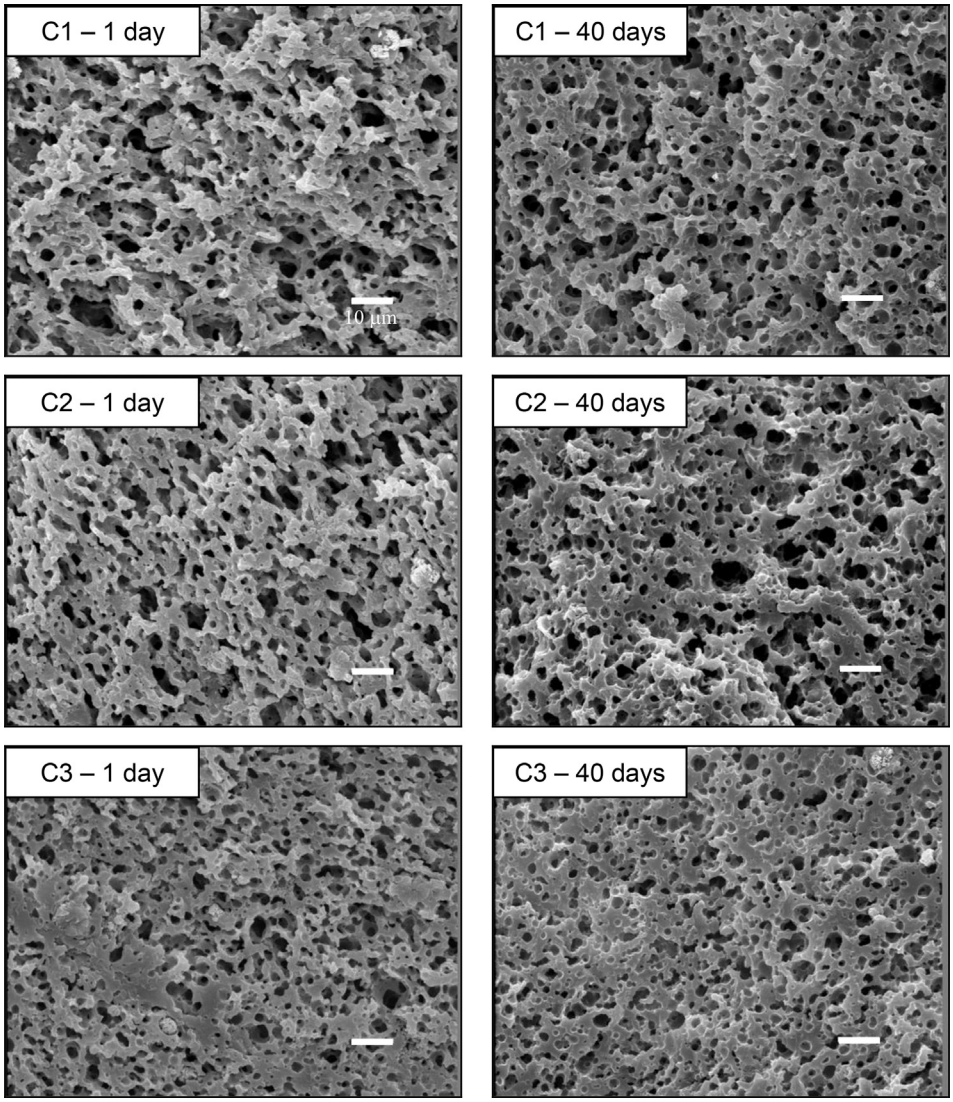


**Fig. 2.** Scanning electron micrographs at 1200 × magnification of samples of Tybo cheese at 1 and 40 days of ripening for salting conditions C1, C2 and C3 (defined in Table 1). White scale bar represents 10 µm.

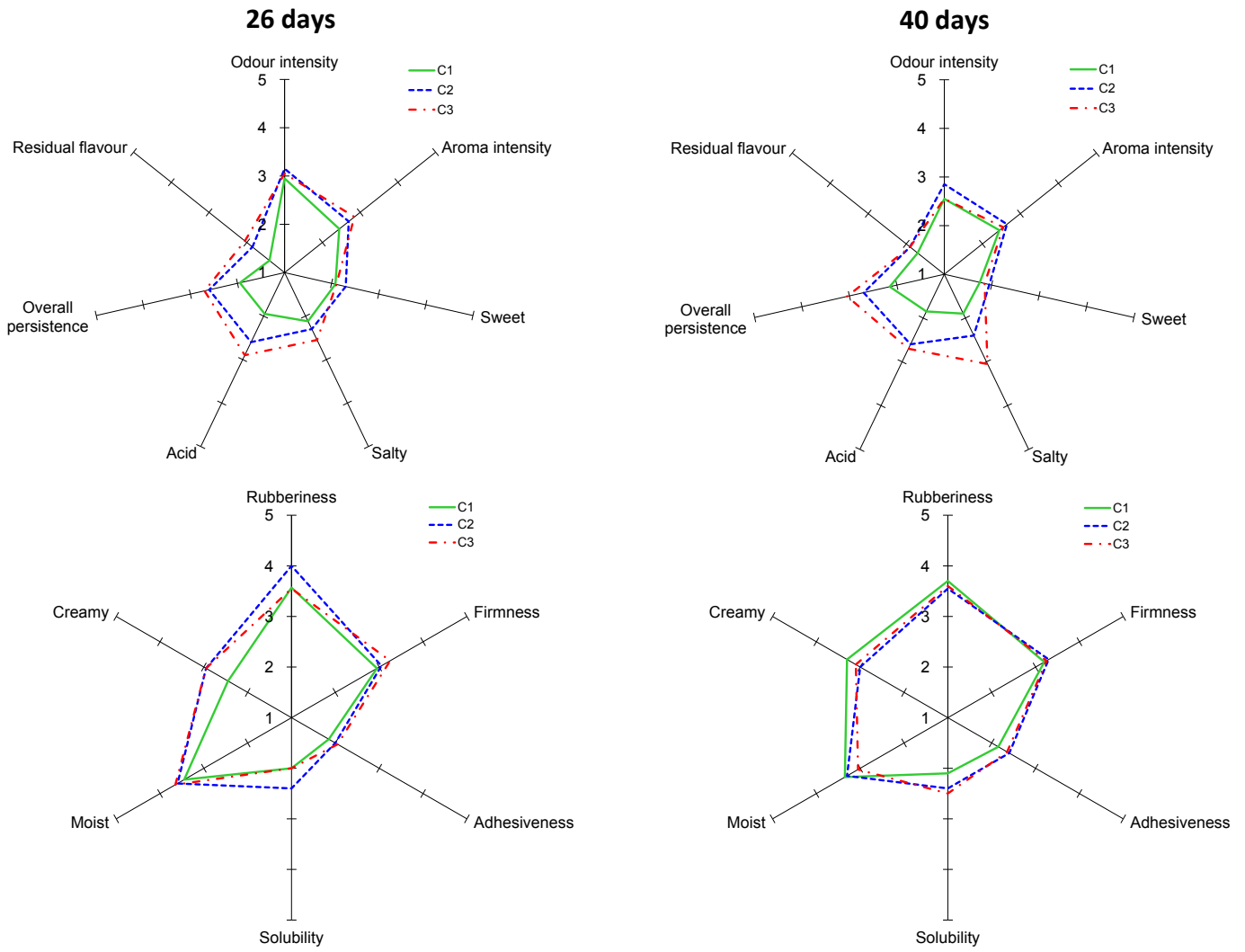


**Fig. 3.** Sensory attributes assessed in samples of C1, C2 and C3 (defined in Table 1) Tybo cheese at 26 and 40 days of ripening. Salty, acid and overall persistence significantly decreased with salt content reduction.

time is related to the casein hydrolysis and the weakening of the *para*-casein network (Akkerman et al., 2017; McCarthy et al., 2016). The behaviour of MI (Table 1), which is an indirect measurement of the primary proteolysis and the degradation of major casein fractions (Table 2), are in agreement with the trend observed for the rheological parameters. The decrease of the parameters with the decreasing of salt content can be partially related to the increase of moisture content. Water acts as a plasticiser in the protein network. Thus, increasing the moisture content of cheese results in reductions in E and in cheese firmness (Fox et al., 2017). The salt content per se has also an effect on the para-casein hydration and on the volume fraction of the casein network due to reduction in salt content decreases the swelling of the cheese protein matrix (McCarthy et al., 2016; Rulikowska et al., 2013). Finally, the interaction effect can be explained taking into account that the differences between samples due to the salting condition were higher at short ripening times compared with the longest ripening time studied.

### 3.5. Microstructure analysis

A qualitative analysis of the SEM micrographs was carried out. The analysis at 120 × allowed determination of the structural homogeneity of samples and whether or not the cryofracture was correctly carried out. The magnifications 400 × and 1200 × allowed examination of the cheese microstructure. It is important to remark that there is no information in the literature related to Tybo cheese microstructure. Some characteristic micrographs are shown in Fig. 2.

Samples showed a continuous surface (*para*-casein network) with holes of smooth edges that contained the serum and fat globules (with different coalescence level) eliminated during sample preparation (Fox et al., 2017; Ribero et al., 2009). At 40 days of ripening, the depressions were more pronounced due to water migration into the protein matrix, resulting in an increase of the protein matrix volume and in a porosity reduction (Ribero et al., 2009). Some differences can be observed for conditions C1 and C2 compared with C3. In the last case, the size of depressions is smaller, indicating that a higher water migration level into the protein matrix occurred. Indeed, Akkerman et al. (2017), studying the effect of reduced NaCl in a semi-hard Danish cheese, observed by SEM that salted cheeses had a more clearly structured protein matrix with many and smaller voids than non-brined cheeses. The increase in casein hydration with NaCl may be related to the displacement of calcium or calcium phosphate from the *para*-casein by the $Na^{+}$ (Guinee & Fox, 2004). As a result, salted cheeses

may have smaller voids with free serum than cheeses with reduced NaCl content due to higher casein hydration.

### 3.6. Sensory analysis

The effect of the salt reduction on 13 attributes related to the flavour and texture of Tybo cheese was studied. The attributes odour intensity, aroma intensity, sweet, salty, overall persistence, and residual flavour characterised the cheese flavour while rubberiness, firmness, adhesiveness, solubility, moist, and creamy characterised cheese texture.

In general, the flavour and texture attributes for the different salting conditions showed similar profiles and in agreement with the typical profile for mild semi-hard cheeses (Fig. 3) (CAA, 2014). ANOVA allowed the determination that salty, acid and overall persistence were significantly affected by salting condition; those attributes showed a clear trend to decrease with salt content reduction. The texture attributes were not significantly affected by the salting condition, although some differences were detected through the rheological parameters. Instrumental and sensory measurements may show different results because the techniques may have different sensitivity levels, moreover when the differences between samples are small. However, both types of results are equally important and should be considered as complementary (Rosenthal, 1999).

Baptista et al. (2017), who studied the salt reduction effect on sensory acceptance of Prato cheese (a semi-hard cheese variety highly consumed in Brazil), concluded that a reduction of 25% salt content resulted in cheeses with similar sensory acceptance when compared with a control cheese. In our case, it is worth mentioning that the reduction of 30% salt content resulted in a cheese similar to the typical Tybo cheese in most respects.

## 4. Conclusions

The effect of reducing salt content on physicochemical, biochemical, rheological, structural and sensory characteristics of Tybo cheese, was studied. A salt reduction of 65% and 30% in comparison to the regular salt content in Tybo cheese was analysed. The salt reduction significantly affected moisture content, maturation index, profiles of casein fractions (γ-, β-, $\alpha_{S1}$- and $\alpha_{S1}$-I-casein), chromatographic areas of WSF, and rheological parameters (W, $\sigma_{max}$ and E).

In general, moisture content, maturation index, $\alpha_{S1}$-I-casein degradation, and total chromatographic area of WSF increased while total intact caseins and rheological parameters decreased as the salt content increased. These results supported the findings related to microstructure and sensory analysis.

The analytical methods allowed measurement of the expected changes due to the salt reduction, however it is worth mentioning that the results obtained for the different areas analysed are similar to those corresponding to a typical Tybo cheese. Indeed, the salt reduction of 30% could be used as a strategy for the production of Tybo cheese with a lower salt content and an appropriate product quality.

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