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# DEVELOPMENT OF REDUCE- SODIUM CHEESES MADE FROM LACTOSE-HYDROLYSED COW'S MILK

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**INFORMAN:**

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**List of Abbreviations:**

Cheeses: Cc: control cheese

Cr: Reduced-salt cheese

$C_{0.1}$: Reduced-salt cheese with 0.1% commercial yeast extract

$C_{0.2}$: Reduced-salt cheese with 0.2% commercial yeast extract

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**Abstract:**

High sodium intake, negatively affects consumer health, thus there is active interest in lowering sodium levels in dairy foods. The effect of salt reduction and addition of flavor enhancer on pH, dry matter, and free amino acid levels, microbiological, color, texture and sensorial characteristics were analyzed in cow milk cheese without lactose at 30 and 60 days of ripening. To obtain cheeses reduced in salt, it was necessary to reduce the salting time from 4.30h to 2.15h in brine at 20% of sodium chloride.

The reduction of salt did not affected the pH, dry matter or free amino acid levels of cheeses. The addition of flavor enhancer produced a slight increase in dry matter and amino acid levels at 60 days of ripening. The microbiological counts were higher in cheeses reduced in salt at 30 days, but these differences disappeared with ripening. However, the addition of 0.2% of flavor enhancer produced a decrease in microbial growth. Cheeses with 0.2 % flavor enhancer exhibit higher lightness and lower yellowness, however the reduction of salt has no significant difference regarding the samples color observed by the panelists. Also, no significant difference was reported regarding the texture of the cheese samples. The panelists reported the reduction of salt causes a less sensation of saltiness in cheeses at 30 days of ripening. The addition of flavor enhancer was significantly effect at 60 days of ripening, the cheeses with 0.2% of flavor enhancer were reported as the saltiest and most bitter in taste.

## 1. Introduction

Sodium is an essential nutrient with important functions in the regulation of extracellular fluid volume and the transport of molecules through cell membranes. However, excessive sodium intake is associated with the prevalence of hypertension, prehypertension (Doyle & Glass, 2010) and other adverse health effects, which justifies the recommendation of the World Health Organization of a daily intake of less than 2 g sodium per day (5 g salt per day; WHO, 2012). Therefore, the food industry is continuously looking for alternative ways to reduce salt content while maintaining consumer satisfaction.

Cheese is one of the most ancient forms of food. Consumption of cheese dates back to 7000 years BC. Nowadays, cheese is consumed in a large quantity worldwide; however, the amount consumed varies from country to country. In European countries, the average annual consumption is 20 kg per person (Perry, 2004; Walter, Schimid, Steber, & Wertmuller, 2008). Cheese supplies essential nutrients for human nutrition, especially proteins, bioactive peptides, lipids, vitamins, and minerals (FAO, 2013), essentially calcium, magnesium and phosphorus (Kira & Maihara, 2007). However, the high sodium levels in cheese is of major concern for public health worldwide, as it has been associated with a high risk of hypertension, cardiovascular disease, and other chronic no communicable diseases (Felicio et al., 2013).

Salt reduction in cheese is considered a major technological challenge due to the importance of salt to the characteristics of this product. In addition to providing flavor, salt affects microbial growth, enzyme activity, and syneresis, which in turn affects the cheese composition (Guinee & Fox, 2004). The effect of salt on the proteolysis in cheese is due to its ability to stimulate or inhibit microbial and enzymatic activities (Guinee & Fox, 2004). In general, sensory defects including the development of bitterness and reduction of firmness have been associated with salt reduction and the related changes in the proteolysis behavior (Mistry & Kasperson, 1998; Rulikowska et al., 2013).

Several strategies for reducing salt levels in cheeses have been found (Mistry & Kasperson, 1998; Ganesan, Brown, Irish, Brothersen, & McMahon, 2014; Rulikowska et al., 2013). The simple reduction of sodium chloride is one of them, and can be achieved by reducing brining time (Pavia et al., 2000), using brine with different salt concentration (Madadlou et al., 2007) or by adding less salt to the cheese curd (Ganesan et al., 2014). Another common technique is the use of salt substitutes to reduce the amount of sodium. Grumer et al. (2012) studied different salt substitutes such as potassium chloride (KCl), calcium chloride ($CaCl_2$) and magnesium chloride ($MgCl_2$). The use of $CaCl_2$ and $MgCl_2$ gave rise to taste differences compared with sodium control. Only a mixture of NaCl and KCl achieved acceptable results. Since salt substitutes may affect the taste due to inherent flavors, such as bitter and metallic, there is also a great interest in adding flavor enhancers (Grummer, Bobowski, Karalus, Vickers, & Schoenfuss, 2013). Monosodium glutamate, hydrolyzed vegetable protein, yeast extract, disodium inosinate, and disodium guanylate have been tried to produce low sodium Cheddar cheese with high saltiness intensity without bitter flavor (Grummer et al., 2013). Yeast extract is composed primarily of amino acids, peptides and nucleotides, with high protein content, being rich in B vitamins, selenium and dietary fiber (Halász & Lásztity, 1991).

To be able to name a cheese as reduced in fat, it must have a minimum reduction of 25% fat compared to its counterpart (Regulation EU 1924/2006).

Lactose is a disaccharide occurring in the milk of mammals with important nutritional and prebiotic properties. The lactose concentration in human milk is relatively high (7.0%) while cow's milk contains about 4.6%. (Schaafsma, 2008). Ripened cheeses are naturally lactose-free or contain very low levels of lactose because, during fermentation by starter bacteria, the lactose is transformed to lactic acid in the initial stages of cheese ripening (McSweeney, 2004). However, in many cases, people with lactose malabsorption are reluctant to eat cheeses because the lactose levels of those products are not clearly declared. To remove lactose from milk and other dairy products commercial β-galactosidases for instance Kluyveromyces lactis are available. With these enzymes lactose concentrations in products can be reduced to<0.01% (van Scheppingen et al., 2017).

The objective of this study was to evaluate a reduced-salt ripened cheese made from lactose-hydrolyzed cow's milk during 30 and 60 days of ripening.

## 2. Materials and methods

### 2.1. Determination of salting condition

#### 2.1.1. Cheese manufacture

Cheese were produced in the Food Technology pilot plant of the university Autònoma de Barcelona (Bellaterra, Spain) using 200 L of whole pasteurized milk (72ºC, 15s) from the farm Can Bado SAT (Santa Agnès de Malanyanes, Barcelona). The day before cheese making, 0.5 % of β-galactosidase (Larbus S.A., Madrid, Spain) was added to hydrolyse lactose. Milk was heated to 32ºC, and additioned with 2% of starter (Lactococos *lactis ssp*. *lactis*, Lactococos *lactis ssp*. *cremoris*, DuPont NHIB Iberica S.L., Barcelona, Spain), 0.01% of calcium chloride (35%, Laboratory Arroyo, Santander, Spain) and 0.03% of calf rennet (Laboratory Arroyo). After 45 min, the curd was cut with cheese knives in to small pieces about 1 cm and heated to 37°C for 10 min. After heating, all the whey was drained off and curd was transferred to moulds (~1, 2 Kg) with cotton cloth. Curd was pressed at 0.5 kg/cm³ for 1 h, 1 kg/cm³ for 1 h, 2 kg/cm³ for 3 h and 3 kg/cm³ for 2 h. Cheeses were divided into four lots for salting with the different procedures described below. After salting, cheeses were ripened in a room at 14ºC and 85% relative humidity for 30 and 60 days.

#### 2.1.2. Salting condition

All cheeses were salted in a brine (20 % of sodium chloride) at different time: 4:30 h (as a control) and 3:30 h, 2:30 h, 2 h and 1:30 h in order to know the time necessary to achieved a minimum of the 25% of salt reduction to comply with the denomination of reduced cheeses according to European regulations.

#### 2.1.3. Determination of salt content

Salt content of cheeses was determined using a chloride analyzer (Sherwood Model 926, Laboratory equipment supplier in Cambridge, England) and results were expressed as g NaCl for 100 g of cheese.

### 2.2. Study of cheeses reduced in salt

### 2.2.1. Cheese manufacture

Four different types of cheeses were elaborated as described in 2.1.1. A group of cheeses were salted in brine (20 % NaCl) during 4.30 h, to be used as a control cheese (Cc). The rest of the cheese samples, were salted during 2.15 h (Cr), to comply with the legislation of reduced cheeses. In order to improve the low salty taste in these cheese samples, some of them were added with 0.1% and 0, 2 % of commercial flavor enhancer (C0, 1 and C0, 2, respectively) before salting (Table1). The flavor enhancer was composed by yeast extract comercial (Carinsa, Barcelona, Spain).

Table 1. Denomination of cheeses uses in this study

| | | Brine conditions | |
|---|---|---|---|
| Cheeses | Flavor enhancer | Composition | Time |
| $C_c$ | - | 20% NaCl | 4.30 h |
| $C_r$ | - | 20% NaCl | 2.15 h |
| $C_{0.1}$ | 0.10% | 20% NaCl | 2.15 h |
| $C_{0.2}$ | 0.20% | 20% NaCl | 2.15 h |

### 2.2.2. Physicochemical analysis

To determine the cheese pH, 10 g of grated cheese were homogenized with 10 mL of distilled water to create a cheese slurry for each cheese sample and analyzed by pH meter (Crison 2001, Alella, Spain). Dry matter content was determined by total drying (IDF, 1982). Salt content of cheese samples was determined using a chloride analyzer (Sherwood Model 926, Cambridge, England) and results were expressed as g NaCl for 100 g of cheese. Free amino acids were determined on the water-soluble nitrogen obtained by the method of Kunchroo and Fox (1982) by the cadmium-ninhydrin method (Folkerstma and Fox, 1992), and expressed as mg Leu for 100 g of cheese.

### 2.2.3. Microbiological analysis

Ten grams of cheese were diluted in 90 ml of peptone water (Oxoid, Hampshire, UK) and homogenized in a Stomacher (Laboratory Blender, Stomacher® 400 Circulator, UK) for 3 min followed with 1 min in a homogenizer (Pulsifier II®, UK). Total aerobic mesophilic bacteria were counted on Plate Count Agar (Oxoid) at 30 °C for 48 h. Lactococci was cultured on M17 added with 10% of lactose solution (Oxoid, Hampshire, UK) and lactobacilli was counted on MRS Agar (Oxoid); both incubated at 30 ºC for 48 h. Enterobacteriaceae were counted on Violet Red Bile Glucose Agar (VRBGA, Oxoid) for 24 h at 37 °C. Result were expressed as logarithm of colony forming units per gram of sample (log cfu g-1).

### 2.2.4. Color analysis

Color measurements were performed in a colorimeter Hunter Lab (Konica Minolta CR-400 laboratory Inc Japan), using D65 illuminant and 10º observer. Measurements were taken at four points of the cheese interior to obtain the color space parameters: L* (lightness which ranges between 0 and 100), a* value measures from the redness to greenness (positive to negative values, respectively) and b* value from the yellowness to blueness (positive to negative values, respectively). Measurements were made at day 30 and 60 of ripening.

### 2.2.5. Texture profile analysis

Five cheese samples were cut into 1 × 1 cm2 pieces and maintained at 20 ºC during the evaluation. A texture profile analysis (TPA) test was applied using a Texture Analyser (TA-TX2 State Microsytem, Survey, UK) with a 245N load cell, using p/50 mm diameter cylindrical probe. Force (g) and time (s) deformation was applied respectively for the first and second compression with and a crosshead speed of 5.00 mm/sec. The measured parameters were fracturability, hardness, adhesiveness, springiness, cohesiveness and chewiness. TPA measurements were determined after at 30 and 60 days of ripening.

### 2.2.6. Sensory evaluation

Sensory evaluation was performed by ten panelists of the Universitat Autònoma de Barcelona who were experienced in the sensory assessment of cheeses. Each kind of cheese were compare with the control, using a 7-point negative to positive scale (0: no differences with control; +/- 1: minimal differences respect to the control; +/- 2: moderate differences respect to the control; +/- 3: big differences respect to the control, i.e., negative or positive, indicates lower or greater perception. The samples were evaluated for color, aroma, flavor (bitter, salty, acid and after taste) texture (hardness, elasticity, adhesiveness) at 30 and 60 days of ripening (Annex 1). A preference test also was made in 9-pont scale from 9 (like extremely) to 1 (dislike extremely)

(Annex 1). The order of presentation was randomized, and cracker biscuits and mineral water at room temperature were served to assessors for palate cleansing between samples.

### 2.2.7. Statistical analysis

Analysis of variance (ANOVA) was performed by Statistica 7 (Hilbe, 2007) using salt as a factor at each ripening stage. Mean comparisons were carried out using the Duncan test. Evaluations were based on a significance level of $P < 0.05$. The processing was repeated two times.

## 3. Results and discussion

### 3.1. Results of salting condition

Fig 1 shows the reduction of salt content in cheeses brined at different time, compared to control cheese (brined during 4.30 h). As can be observed from the figure, it was necessary a time between 2 and 2:30 h to obtain the necessary reduction (≥25 %).

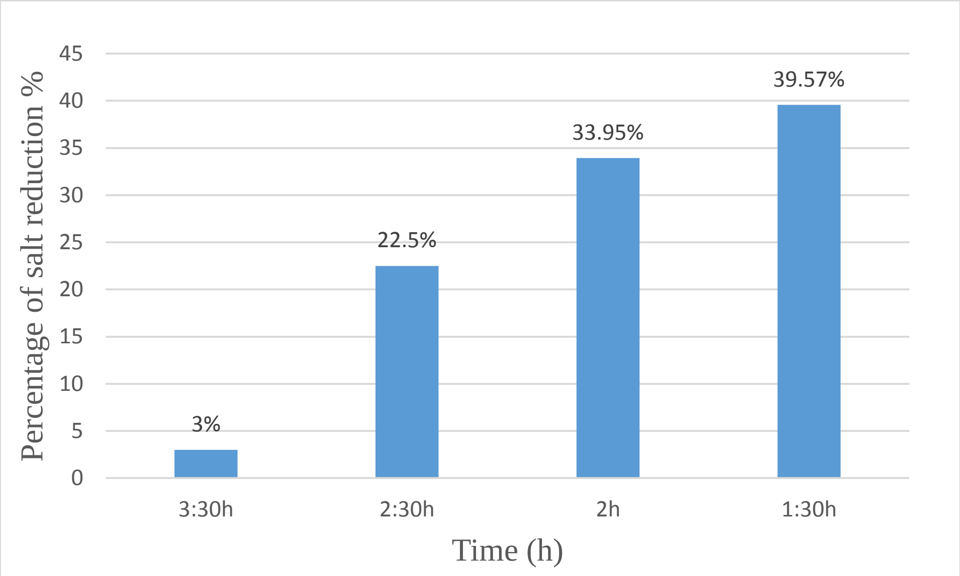


Fig. 1. Percentage of salt reduction of cheeses compared to control cheese.

### 3.2. Results of cheeses reduced in salt

#### 3.2.1. Physicochemical results

All the cheeses reduced in salt presented a reduction of 30.80 ± 1.50% respect to control cheese, complying with the denomination of reduced cheeses according to European regulations (results not shown).

The reduction of salt not affected the pH of cheeses (Table 2). The same result was reported in Tybo cheese reduced in salt. (Sihufe et al., 2018).

The pH was reduced by ripening time due to lactic fermentation (Karimi, Mortazavian, & Cruz, 2011). At 60 days of ripening cheeses with 0.2 % of flavor enhancer had the low pH maybe due to the increased proteolysis or because of increase the content of flavor enhancer.

Table 2. Mean ± standard deviation of pH values of cheeses at 30 and 60 days of ripening.

| | [1]Cheeses | Ripening time (day) 30 | 60 |
|---|---|---|---|
| pH | $C_c$ | 4.87 ± 0.04 Ab | 4.75 ± 0.03 Aa |
| | $C_r$ | 4.81 ± 0.02 Ab | 4.74 ± 0.04 Aa |
| | $C_{o.1}$ | 4.71 ± 0.27 Aa | 4.77 ± 0.02 Aa |
| | $C_{0.2}$ | 4.84 ± 0.01 Ab | 4.68 ± 0.03 Ba |

A,B,C Means for the same column by different letters are significantly ($P \leq 0.05$) different.
a,b Means for the same row by different letters are significantly ($P \leq 0.05$) different.
[1]Cheeses: $C_c$: control cheese, $C_r$: Reduced-salt cheese, $C_{0.1}$: Reduced-salt cheese with 0.1% of flavor enhancer, $C_{0.2}$: Reduced-salt cheese with 0.2% of flavor enhancer.

Dry matter values of all cheeses throughout 30 and 60 days of ripening are presented in Table 3. Salt reduction had no significant effect on the moisture of cheeses at 30 days of ripening. However, at 60 days of ripening reduced-salt cheeses presented high dry matter than control cheeses, however in cheddar cheese, the reduction in salt produced an increase in their moisture content due to lower sodium leads to reduced curd syneresis. (Guinee and Fox, 2004). On the other hand the inclusion of flavor enhancer (0.2%) produced an increase of dry matter, expected result are due to the addition of this solid particle. Dry matter value increases with ripening time due to evaporation of water. These result are in agreement with Van den Berg & Exterkate (1993) in Gouda cheese.

Table 3. Mean ± standard deviation of dry matter values of cheeses at 30 and 60 days of ripening.

| | [1]Cheeses | Ripening time (day) 30 | 60 |
|---|---|---|---|
| Dry matter | $C_c$ | 53.84 ±0.67 Aa | 58.27 ± 0.83 Ab |
| | $C_r$ | 53.85 ±0.67 Aa | 58.91 ± 0.28 ABb |
| | $C_{o.1}$ | 54.33 ±1.01 Aa | 59.48 ± 0.52 BCb |
| | $C_{0.2}$ | 53.65 ±0.25 Aa | 60.05 ± 0.85 Cb |

A,B,C Means for the same column by different letters are significantly ($P \leq 0.05$) different.
a,b Means for the same row by different letters are significantly ($P \leq 0.05$) different.
[1]Cheeses: $C_c$: control cheese, $C_r$: Reduced-salt cheese, $C_{0.1}$: Reduced-salt cheese with 0.1% of flavor enhancer, $C_{0.2}$: Reduced-salt cheese with 0.2% of flavor enhancer.

Table 4 shows the amino acid content of cheeses during 30 and 60 days of ripening. The reduction of salt not affected the values of amino acids in cheeses. On the contrary, Silva et al. (2017) reported an increase in proteolysis of reduced-sodium Prato cheese when compared to the regular cheese, and was related to more favorable environment due to the increased moisture content found in reduced cheeses. The addition of flavor enhancer increased the amino acid levels at 60 days of ripening due to flavor enhancer made by Peptides. A significant increase in amino acid content was observed with ripening time due to the action of proteases and amino peptidases in cheese.

Table 4. Mean ± standard deviation of amino acid values (mg Leu 100 $g^{-1}$ cheese) of cheeses at 30 and 60 days of ripening.

| | | Ripening time (day) | |
|---|---|---|---|
| | [1]Cheeses | 30 | 60 |
| Amino acid | $C_c$ | 2.06 ± 1.00 Aa | 3.46 ± 0.21 Ab |
| | $C_r$ | 2.27 ± 1.60 Aa | 3.31 ± 0.46 Ab |
| | $C_{o.1}$ | 2.60 ± 1.56 Aa | 4.20 ± 0.34 Bb |
| | $C_{0.2}$ | 1.65 ± 1.20 Aa | 4.19 ± 0.28 Bb |

A,B,C Means for the same column by different letters are significantly ($P \leq 0.05$) different.
a,b Means for the same row by different letters are significantly ($P \leq 0.05$) different.
[1]Cheeses: $C_c$: control cheese, $C_r$: Reduced-salt cheese, $C_{0.1}$: Reduced-salt cheese with 0.1% of flavor enhancer, $C_{0.2}$: Reduced-salt cheese with 0.2% of flavor enhancer.

### 3.2.2. Microbiological results

The reduction of salt slightly increased the microbial counts in cheese (Table 5). Parente & Cogan (2004) and Sheibani (2016) in Cheddar-type Cheeses also reported an increase in the growth of bacteria by the reduction in salt concentration. On the contrary, Hystead et al. (2013) not reported differences in aerobic bacteria based on salt treatment in cheddar cheese. The addition of flavor enhancer significantly decrease the microorganisms in cheeses, becoming $C_{0.2}$ the cheeses with the low microorganisms counts at 60 days of ripening. A possible explanation will be the reduction of moisture content in these cheeses (Table 3). Hayaloglu (2016) reported that the growth rate will be more rapid in high moisture content cheeses than those with low moisture. These results are not consistent with Sivla et al. (2017) who reported that sodium reduction and addition of flavor enhancers did not constitute inhibitory factors for the survival of microorganisms after 30 days in Prato cheese. No Enterobacteriaceae was observed in the cheese samples.

Table 5. Mean ± standard deviation of microbial counts (log cfu $g^{-1}$) of cheeses at 30 and 60 days of ripening.

| | | Ripening time (day) | |
|---|---|---|---|
| | [1]Cheeses | 30 | 60 |
| Areobic Mesophlic Total | $C_c$ | 8.60 ± 0.11 BCb | 6.95 ± 0.07 Aba |
| | $C_r$ | 9.01 ± 0.51 Cb | 7.09 ± 0.12 Ba |
| | $C_{o.1}$ | 8.26 ± 0.39 ABb | 6.81 ± 0.05 Aa |
| | $C_{0.2}$ | 7.74 ± 0.06 Ab | 6.00 ± 0.00 Ca |
| Lactococcus | $C_c$ | 8.55 ± 0.03 ABb | 7.76 ± 0.59 Aa |
| | $C_r$ | 9.04 ± 0.39 Bb | 7.82 ± 0.01 Aa |
| | $C_{o.1}$ | 8.37 ± 0.47 Ab | 6.99 ± 0.12 ABa |
| | $C_{0.2}$ | 8.04 ± 0.06Ab | 6.75 ± 0.21 Ba |
| Latobacillus | $C_c$ | 8.63 ± 0.23 Bb | 7.26 ± 0.21 Aa |
| | $C_r$ | 9.11 ± 0.37 Cb | 7.23 ± 0.07 Aa |
| | $C_{o.1}$ | 8.16 ± 0.12 Ab | 7.07 ± 0.10 Aa |
| | $C_{0.2}$ | 8.00 ± 0.00 Ab | 6.59 ± 0.16 Ba |

A,B,C Means for the same column by different letters are significantly ($P \leq 0.0\ 5$) different.
a,b Means for the same row by different letters are significantly ($P \leq 0.05$) different. [1]Cheeses: $C_c$: control cheese, $C_r$: Reduced-salt cheese, $C_{0.1}$: Reduced-salt cheese with 0.1% of flavor enhancer, $C_{0.2}$: Reduced-salt cheese with 0.2% of flavor enhancer.

### 3.2.3. Color result

Table 6 shows color parameters for cheese samples at 30 and 60 days of ripening. At 30 days of ripening the reduction of salt did not effected $L*$, $b*$, $a*$ values of cheeses. Cheeses with 0.2 % flavor enhancer exhibit higher lightness and lower $b*$, indicating cheeses with less yellowness. The color of flavor enhancer could modified the color of cheeses. At 60 days of ripening differences were disappearing, with only statistical differences in $L*$ value. These differences were not detected by the panelists (Table 8).

During ripening the $L*$ value decreased and $a*$ and $b*$ increased. Similar results have been described for Emmental cheese by Rohm and Jaros (1996a) and goats' milk and Valdeón cheese (Buffa et al., 2001; Diezhandino et al., 2016). Lightness values decreased at 60 days of ripening due to dehydration and proteolysis.

Table 6. Mean ± standard deviation for color parameters of cheeses at 30 and 60 days of ripening.

| | | Ripening time (day) | |
|---|---|---|---|
| | [1]Cheeses | 30 | 60 |
| L* | $C_c$ | 91.47 ± 0.62 Ab | 89.85 ± 1.27 Aa |
| | $C_r$ | 91.72 ± 0.58 ABb | 89.98 ± 0.31 ABa |
| | $C_{o.1}$ | 91.20 ± 0.73 Ab | 89.58 ± 1.06 Aa |
| | $C_{0.2}$ | 92.26 ± 0.35 Bb | 90.86 ± 0.43 Ba |
| b* | $C_c$ | 15.57 ± 0.44 Aa | 16.93 ± 0.41 ABb |
| | $C_r$ | 15.39 ± 0.41 Aa | 17.12 ± 0.63 Bb |
| | $C_{o.1}$ | 15.68 ± 0.85 Aa | 16.50 ± 0.64 ABb |
| | $C_{0.2}$ | 14.36 ± 0.39 Aa | 16.27 ± 1.02 Ab |
| a* | $C_c$ | -2.10 ± 0.28 Aa | -1.74 ± 0.19 Ab |
| | $C_r$ | -2.00 ± 0.10 Aa | -1.70 ± 0.14 Ab |
| | $C_{o.1}$ | -2.16 ± 0.32 Aa | -1.23 ± 1.06 Ab |
| | $C_{0.2}$ | -2.15 ± 0.02 Aa | -1.68 ± 0.34 Ab |

A,B,C Means for the same column by different letters are significantly ($P \leq 0.0\ 5$) different.
a,b Means for the same row by different letters are significantly ($P \leq 0.05$) different.
[1]Cheeses: $C_c$: control cheese, $C_r$: Reduced-salt cheese, $C_{0.1}$: Reduced-salt cheese with 0.1% of flavor enhancer, $C_{0.2}$: Reduced-salt cheese with 0.2% of flavor enhancer

### 3.2.4. Texture result

Texture is an important parameter affecting the quality of cheese. This parameter is highly dependent on different factors such as the degree of proteolysis and breakdown of the protein network, water content, and pH (McSweeney & Sousa 2000). The composition of cheese affects the texture profile and microstructure of cheese through the Salt-in-moisture (S/M), fat and solid content (Dimitreli & Thomareis 2007). Storage temperature is another important factor affecting cheese quality (Zheng et al.2016). Table 7 presents the texture profile results at 30 and 60 days of ripening. The reduction of salt did not seem to affect greatly the texture parameters of cheeses reduced in salt compared to the control. At 30 days of ripening only were observed slightly higher values of hardness, fracturability, gumminess and chewiness of conventional cheeses compared to the others. Rulikowska et al (2013) reported hardness decreased with the decreasing NaCl content and ripening time. At 60 days of ripening, values of hardness increased in all cheeses as consequence of water loss in the cheeses. Those reduced in salt were more fracturable. There weren't any different on cohesiveness and springiness. At 60 days of ripening the control cheese and reduced-salt cheese were more adhesive. The gumminess increased by ripening time. Gumminess is directly affected by hardness because it results from hardness × cohesiveness (Fox et al. 2000a; Gunasekaran & Ak 2003). The adhesiveness increased by ripening time, according to (Diezhandion et al.2016) for Spanish blue cheese the chewiness, fracturability and hardness increased by ripening time, as was also observed by (Floury et al., 2009) which is the consequence of a decrease of the salt concentration is an of the hardness in hard-type cheese.

Table 7. Mean ± standard deviation for texture parameters of cheeses at 30 and 60 days of ripening.

| | | Ripening days | |
|---|---|---|---|
| | [1]Cheese | 30 | 60 |
| Hardness (gf) | $C_c$ | 699 ±57 | 1232 ±253 |
| | $C_r$ | 606 ±65 | 1173 ±127 |
| | $C_{0.1}$ | 561 ±70 | 1100 ±151 |
| | $C_{0.2}$ | 559 ±48 | 1350 ±178 |
| Fracturability (g) | $C_c$ | 613 ±58 | 522 ±354 |
| | $C_r$ | 553 ±65 | 605 ±425 |
| | $C_{0.1}$ | 553 ±68 | 656 ±440 |
| | $C_{0.2}$ | 519 ±47 | 865 ±297 |
| Adhesivness (g.sec) | $C_c$ | 0 ±0 | -48 ±20 |
| | $C_r$ | -6 ±9 | -23 ±42 |
| | $C_{0.1}$ | -11 ±14 | -11 ±8 |
| | $C_{0.2}$ | -3 ±4 | -18 ±10 |
| Springness (-) | $C_c$ | 0.46 ±0.04 | 0.44 ±0.11 |
| | $C_r$ | 0.47 ±0.07 | 0.4 ±0.03 |
| | $C_{0.1}$ | 0.46 ±0.06 | 0.52 ±0.09 |
| | $C_{0.2}$ | 0.42 ±0.07 | 0.41 ±0.02 |
| Cohesiveness (-) | $C_c$ | 0.61 ±0.06 | 0.47 ±0.12 |
| | $C_r$ | 0.61 ±0.08 | 0.51 ±0.13 |
| | $C_{0.1}$ | 0.68 ±0.16 | 0.62 ±0.34 |
| | $C_{0.2}$ | 0.56 ±0.10 | 0.44 ±0.1 |
| Gumminess (-) | $C_c$ | 428 ±54 | 556 ±66 |
| | $C_r$ | 369 ±39 | 592 ±136 |
| | $C_{0.1}$ | 375 ±69 | 654 ±281 |
| | $C_{0.2}$ | 317 ±72 | 586 ±63 |
| Chewiness (-) | $C_c$ | 197 ±35 | 244 ±67 |
| | $C_r$ | 174 ±36 | 240 ±72 |
| | $C_{0.1}$ | 171 ±20 | 360 ±225 |
| | $C_{0.2}$ | 135 ±39 | 238 ±18 |

[1] Cheeses: $C_c$: control cheese, $C_r$: Reduced-salt cheese, $C_{0.1}$: Reduced-salt cheese with 0.1% of flavor enhancer, $C_{0.2}$: Reduced-salt cheese with 0.2% of flavor enhancer

### 3.2.5. Sensorial results

Table 8 presents sensorial results of cheeses at 30 and 60 days of ripening. The reduction of salt had no significant difference in color or texture of cheeses. Only cheeses with flavor enhancer were evaluated as more friable at 60 days of ripening, according to instrumental results (Table7). Considering the smell parameter, only the sample with 0.2% of flavor enhancer presented a slight increase, which could be due to the fact that the flavor enhancer has aroma itself, since the difference with control cheese is smaller than 1, which represented a minimal difference. The reduction of salt no significant effect in bitter, however the result for cheddar cheese was reported previously by (Ganesan et al., 2014), where bitter perception increased when salt was lowered. On the other hand, at 60 days of ripening the cheeses with 0.2 % of flavor enhancer was reported to be bitter in taste due to production of peptides. The reduction of salt causes a less sensation of saltiness in cheeses, becoming Cr the least valued, followed by $C_{0.1}$ and $C_{0.2}$. However, at 60 days of ripening the cheeses with 0.2 % of flavor enhancer was reported as the saltiest. Also the cheeses with 0.2% of flavor enhancer reported as the saltiest and the most bitter.

Fig 2 and 3 illustrates the preferences of the panelists at 30 and 60 days of ripening, respectively. In both cases, control cheeses were the preferred by the panelists. At 30 days of ripening, cheese reduced in salt without flavor enhancer were preferred than cheeses with this compound. However, at 60 days of ripening, $C_{0.1}$ were preferred than Cr, showing than the addition of 0.1% of this flavor enhancer could improve the sensorial characteristics of cheeses reduced in salt. In contrast, the addition of 0.2% of flavor enhancer reduced the acceptability of cheeses, possibly due by the increase in salty and bitter taste.

Table 8. Mean ± standard deviation for sensorial results of cheeses at 30 and 60 days of ripening.

| | | Repineng time (day) | |
|---|---|---|---|
| | [1]Cheese | 30 | 60 |
| Color | $C_c$ | 0.00 ± 0.00 A | 0.00 ± 0.0 A |
| | $C_r$ | -0.10 ± 0.79 A | 0.35 ± 1.09 A |
| | $C_{0.1}$ | 0.00 ± 0.65 A | 0.25 ± 1.02 A |
| | $C_{0.2}$ | -0.20 ± 0.70 A | 0.30 ± 0.66 A |
| Smell | $C_c$ | 0.00 ± 0.00 AB | 0.00 ± 0.00 A |
| | $C_r$ | -0.75 ± 0.97 A | -0.08± 1.00 A |
| | $C_{0.1}$ | -0.25 ± 1.37 AB | 0.28 ± 0.99 A |
| | $C_{0.2}$ | 0.40 ± 1.27 B | 0.65 ± 1.14 A |
| Hardness | $C_c$ | 0.00 ± 0.00 A | 0.00 ± 0.00 A |
| | $C_r$ | -0.08± 0.86 A | 0.2 ± 0.95 A |
| | $C_{0.1}$ | -0.20 ± 0.83 A | 0.00 ± 0.65 A |
| | $C_{0.2}$ | -0.45 ± 1.00 A | 0.5 ± 1.00 A |
| Elacticity | $C_c$ | 0.00 ± 0.00 A | 0.00 ± 0.00 A |
| | $C_r$ | -0.2 ± 0.70 A | -0.1 ± 0.79 A |
| | $C_{0.1}$ | -0.6 ± 0.99 A | 0.25 ± 1.07 A |
| | $C_{0.2}$ | -0.25 ± 0.85 A | -0.38 ± 1.16 A |
| Adhesiveness | $C_c$ | 0.00 ± 0.00 A | 0.00 ± 0.00 A |
| | $C_r$ | 0.30 ± 0.66 A | 0.48 ± 0.79 A |
| | $C_{0.1}$ | -0.15 ± 0.73 A | 0.00 ± 0.79 A |
| | $C_{0.2}$ | 0.28 ± 1.09 A | -0.13 ± 1.10 A |
| Friability | $C_c$ | 0.00 ± 0.00 A | 0.00 ± 0.00 A |
| | $C_r$ | 0.08 ± 0.86 A | -0.10 ± 0.85 A |
| | $C_{0.1}$ | 0.40 ± 0.88 A | 0.53 ± 0.60 B |
| | $C_{0.2}$ | -0.10 ± 0.79 A | 0.98 ± 0.66 B |
| Bitter | $C_c$ | 0.00 ± 0.00 A | 0.00 ± 0.00 AB |
| | $C_r$ | -0.15 ± 0.67 A | -0.18 ± 0.67 A |
| | $C_{0.1}$ | 0.00 ± 0.73 A | 0.05 ± 0.69 AB |
| | $C_{0.2}$ | 0.25 ± 0.79 A | 0.45 ± 1.15 B |
| Salty | $C_c$ | 0 .00± 0.00 C | 0.00 ± 0.00 BC |
| | $C_r$ | -1.20 ± 0.77 A | -0.60 ± 0.75 A |
| | $C_{0.1}$ | -0.70 ± 0.73 AB | -0.50 ± 0.89 AB |
| | $C_{0.2}$ | -0.55 ± 0.89 B | 0.35 ± 0.88 C |
| Acid | $C_c$ | 0.00 ± 0.00 A | 0.00 ± 0.00 A |
| | $C_r$ | -0.25 ± 1.02 A | 0.10 ± 0.91 A |
| | $C_{0.1}$ | 0.20 ± 1.01 A | 0.13 ± 1.12 A |
| | $C_{0.2}$ | -0.05 ± 1.10 A | 0.35 ± 0.99 A |
| After tase | $C_c$ | 0.00 ± 0.00 A | 0.00 ± 0.00 A |
| | $C_r$ | -0.05± 0.76 A | 0.05 ± 0.60 A |
| | $C_{0.1}$ | 0.28 ± 0.99 A | 0.48 ± 0.64 AB |
| | $C_{0.2}$ | 0.50 ± 1.00 A | 0.70 ± 0.98 B |

A,B,C Means for the same column by different letters are significantly ($P \leq 0.0\ 5$) different.

a,b Means for the same row by different letters are significantly ($P \leq 0.05$) different.

[1]Cheeses: $C_c$: control cheese, $C_r$: Reduced-salt cheese, $C_{0.1}$: Reduced-salt cheese with 0.1% of flavor enhancer, $C_{0.2}$: Reduced-salt cheese with 0.2% of flavor enhancer

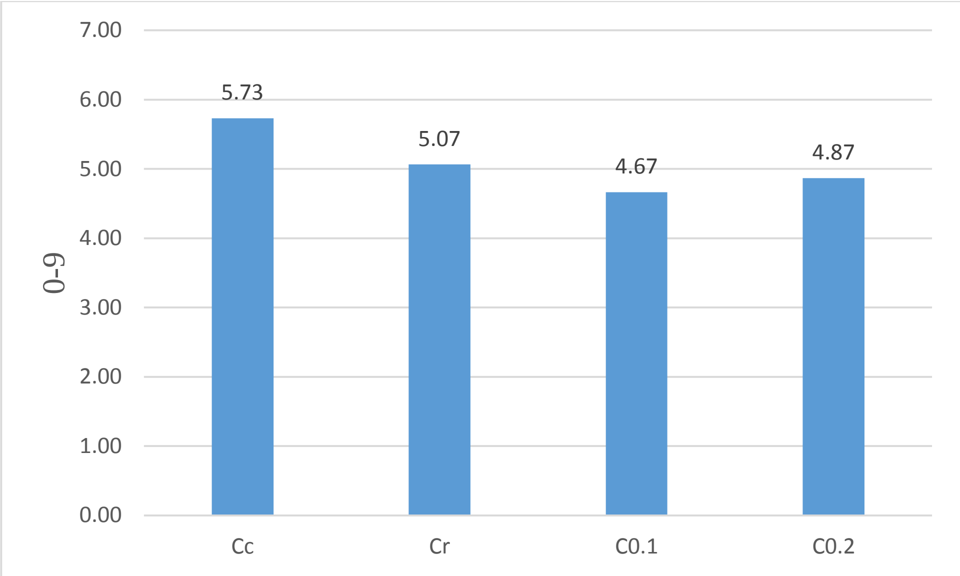


Fig 2. Preference of cheeses compared to control cheeses at 30 days of ripening.

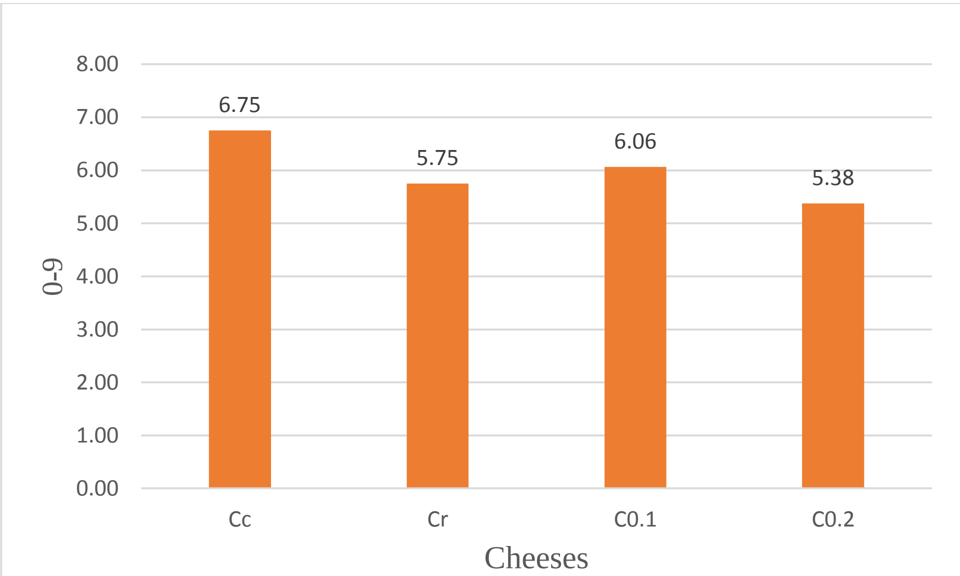

Fig 3. Preference of cheeses compared to control cheeses at 60 days of ripening.

**Conclusion:**

A salt reduction of 30.80% comparison to the control cheese was analyzed. The salt reduction and addition flavor enhancer didn't affect the pH, dry matter and amino acid at 30 days of ripening. But during ripening time at 60 days the cheeses with 0.2 % of flavor enhancer had low pH and had more dry matter. The salt reduction had no effect on proteolysis, although during ripening time at 60 days the addition of flavor enhancer increased the amino acid. The reduction of salt slightly increased microbial count. Microbial count in cheeses with a lower salt content ($C_r$) were higher than in the control. During ripening time at 60 days decreased microbial count, and also the addition of flavor enhancer significantly decreased the microbial count. The addition of flavor enhancer exhibit higher lightness. The reduction of salt did not affect the hardness, during ripening time at 60 days the cheeses with flavor enhancer 0.2% was harder and more fracturabule. The hardness and gumminess increased in all cheeses during ripening at 60 days. The sensory results showed that reduced salt cheeses with flavor enhancer 0.2% was reported as the saltiest and the most bitter.

**References:**


- Cheese in nutrition and health | SpringerLink. (n.d.). Retrieved August 24, 2018, from https://link.springer.com/article/10.1051%2Fdst%3A2008012
- Cruz, A. G., Faria, J. A. F., Pollonio, M. A. R., Bolini, H. M. A., Celeghini, R. M. S., Granato, D., & Shah, N. P. (n.d.). Cheeses with reduced sodium content: Effects on functionality, public health benefits and sensory properties. *Trends in Food Science & Technology*, *22*(6), 276–291.
- Determination of major and minor elements in dairy products through inductively coupled plasma optical emission spectrometry after wet partial digestion and neutron activation analysis. (2007). *Food Chemistry*, *100*(1), 390–395. https://doi.org/10.1016/j.foodchem.2005.09.014
- Diezhandino, I., Fernández, D., Sacristán, N., Combarros-Fuertes, P., Prieto, B., & Fresno, J. M. (2016). Rheological, textural, colour and sensory characteristics of a Spanish blue cheese (Valdeón cheese). *LWT - Food Science and Technology*, *65*, 1118–1125. https://doi.org/10.1016/j.lwt.2015.10.003
- Dimitreli, G., & Thomareis, A. S. (2007). Texture evaluation of block-type processed cheese as a function of chemical composition and in relation to its apparent viscosity. *Journal of Food Engineering*, *79*(4), 1364–1373. https://doi.org/10.1016/j.jfoodeng.2006.04.043
- Doyle, M. E., & Glass, K. A. (2010). Sodium Reduction and Its Effect on Food Safety, Food Quality, and Human Health. *Comprehensive Reviews in Food Science and Food Safety*, *9*(1), 44–56. https://doi.org/10.1111/j.1541-4337.2009.00096.x
- Floury, J., Camier, B., Rousseau, F., Lopez, C., Tissier, J.-P., & Famelart, M.-H. (2009). Reducing salt level in food: Part 1. Factors affecting the manufacture of model cheese systems and their structure–texture relationships. *LWT - Food Science and Technology*, *42*(10), 1611–1620. https://doi.org/10.1016/j.lwt.2009.05.026
- Folkertsma, B., & Fox, P. F. (1992). Use of the Cd-ninhydrin reagent to assess proteolysis in cheese during ripening. *Journal of Dairy Research*, *59*(2), 217–224. https://doi.org/10.1017/S0022029900030466
- Fox, P. F., McSweeney, P. L. H., Cogan, T. M., & Guinee, T. P. (2000). *Fundamentals of Cheese Science*. Springer US. Retrieved from //www.springer.com/de/book/9780834212602

- Fundamentals of Cheese Science | P. F. Fox | Springer. (n.d.). Retrieved August 24, 2018, from https://www.springer.com/de/book/9780834212602
- Ganesan, B., Brown, K., Irish, D. A., Brothersen, C., & McMahon, D. J. (2014). Manufacture and sensory analysis of reduced- and low-sodium Cheddar and Mozzarella cheeses. *Journal of Dairy Science*, *97*(4), 1970–1982. https://doi.org/10.3168/jds.2013-7443
- Grummer, J., Bobowski, N., Karalus, M., Vickers, Z., & Schoenfuss, T. (2013). Use of potassium chloride and flavor enhancers in low sodium Cheddar cheese. *Journal of Dairy Science*, *96*(3), 1401–1418. https://doi.org/10.3168/jds.2012-6057
- Grummer, J., Karalus, M., Zhang, K., Vickers, Z., & Schoenfuss, T. C. (2012). Manufacture of reduced-sodium Cheddar-style cheese with mineral salt replacers. *Journal of Dairy Science*, *95*(6), 2830–2839. https://doi.org/10.3168/jds.2011-4851
- Guinee, T. P., & Fox, P. F. (2004). Salt in Cheese: Physical, Chemical and Biological Aspects. In *Cheese: Chemistry, Physics and Microbiology* (Vol. 1, pp. 207–259). Elsevier. https://doi.org/10.1016/S1874-558X(04)80069-1
- Halasz, A., & Lasztity, R. (1990). *Use of Yeast Biomass in Food Production*. CRC Press.
- Hayaloglu, A. A. (2016). Cheese: Microbiology of Cheese. In *Reference Module in Food Science*. Elsevier. https://doi.org/10.1016/B978-0-08-100596-5.00675-2
- Hystead, E., Diez-Gonzalez, F., & Schoenfuss, T. C. (2013). The effect of sodium reduction with and without potassium chloride on the survival of Listeria monocytogenes in Cheddar cheese. *Journal of Dairy Science*, *96*(10), 6172–6185. https://doi.org/10.3168/jds.2013-6675
- Karimi, Mortazavian, A., & Cruz, A. (2011). Viability of probiotic microorganisms in cheese during production and storage: a review. *Dairy Science & Technology*, *91*(3), 283–308. https://doi.org/10.1007/s13594-011-0005-x
- Kuchroo, C. N., & Fox, P. F. (1982). Soluble nitrogen in Cheddar cheese: comparison of extraction procedures. *Milchwissenschaft. = Milk Science International*. Retrieved from http://agris.fao.org/agris-search/search.do?recordID=US201302191633
- McSWEENEY, P. L. H. (2004). CONTRIBUTION Biochemistry of cheese ripening, *57*(2), 19.
- McSweeney, P. L. H., & Sousa, M. J. (2000). Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review. *Le Lait*, *80*(3), 293–324. https://doi.org/10.1051/lait:2000127

- Mistry, V. V., & Kasperson, K. M. (1998). Influence of Salt on the Quality of Reduced Fat Cheddar Cheese. *Journal of Dairy Science*, *81*(5), 1214–1221. https://doi.org/10.3168/jds.S0022-0302(98)75681-4
- Muehlhoff, E., Bennett, A., & McMahon, D. (2013). Milk and dairy products in human nutrition. *Milk and Dairy Products in Human Nutrition.* Retrieved from https://www.cabdirect.org/cabdirect/abstract/20133411376
- Muthukumarappan, K., & Swamy, G. J. (2017). Chapter 10 - Rheology, Microstructure, and Functionality of Cheese. In J. Ahmed, P. Ptaszek, & S. Basu (Eds.), *Advances in Food Rheology and Its Applications* (pp. 245–276). Woodhead Publishing. https://doi.org/10.1016/B978-0-08-100431-9.00010-3
- Pavia, M., Trujillo, A. J., Guamis, B., & Ferragut, V. (2000). Ripening control of salt-reduced Manchego-type cheese obtained by brine vacuum-impregnation. *Food Chemistry*, *70*(2), 155–162. https://doi.org/10.1016/S0308-8146(99)00249-6
- Perry, K. S. P. (2004). Queijos: aspectos químicos, bioquímicos e microbiológicos. *Química Nova*, *27*(2), 293–300. https://doi.org/10.1590/S0100-40422004000200020
- Rohm, H., & Jaros, D. (1996). Colour of hard cheese. *Zeitschrift Für Lebensmittel-Untersuchung Und Forschung*, *203*(3), 241–244. https://doi.org/10.1007/BF01192871
- Rulikowska, A., Kilcawley, K. N., Doolan, I. A., Alonso-Gomez, M., Nongonierma, A. B., Hannon, J. A., & Wilkinson, M. G. (2013). The impact of reduced sodium chloride content on Cheddar cheese quality. *International Dairy Journal*, *28*(2), 45–55. https://doi.org/10.1016/j.idairyj.2012.08.007
- Salt in Cheese: Physical, Chemical and Biological Aspects. (2004). *Cheese: Chemistry, Physics and Microbiology*, *1*, 207–259. https://doi.org/10.1016/S1874-558X(04)80069-1
- Salt in Cheese: Physical, Chemical and Biological Aspects - ScienceDirect. (n.d.). Retrieved August 24, 2018, from https://www.sciencedirect.com/science/article/pii/S1874558X04800691#page=1&zoom=auto,-106,792
- Schaafsma, G. (2008). Lactose and lactose derivatives as bioactive ingredients in human nutrition. *International Dairy Journal*, *18*(5), 458–465. https://doi.org/10.1016/j.idairyj.2007.11.013
- Sheibani, A. (n.d.). The Effect of Salt Reduction, Culture and Process Modifications on the Physicochemical and Microbiological Characteristics of Cheddar-type Cheeses, 196.

- Sihufe, G. A., De Piante Vicín, D. A., Marino, F., Ramos, E. L., Nieto, I. G., Karlen, J. G., & Zorrilla, S. E. (2018). Effect of sodium chloride reduction on physicochemical, biochemical, rheological, structural and sensory characteristics of Tybo cheese. *International Dairy Journal*, *82*, 11–18. https://doi.org/10.1016/j.idairyj.2018.02.006
- Silva, H. L. A., Balthazar, C. F., Esmerino, E. A., Vieira, A. H., Cappato, L. P., Neto, R. P. C., … Cruz, A. G. (2017). Effect of sodium reduction and flavor enhancer addition on probiotic Prato cheese processing. *Food Research International*, *99*, 247–255. https://doi.org/10.1016/j.foodres.2017.05.018
- Smith, R. (2015). Regulation (EC) No 764/2008 of the European Parliament and of the Council. In R. Smith, *Core EU Legislation* (pp. 183–186). London: Macmillan Education UK. https://doi.org/10.1007/978-1-137-54482-7_19
- Van den Berg, G., & Exterkate, F. A. (1993). Technological parameters involved in cheese ripening. *International Dairy Journal*, *3*(4), 485–507. https://doi.org/10.1016/0958-6946(93)90028-X
- Van Scheppingen, W. B., van Hilten, P. H., Vijverberg, M. P., & Duchateau, A. L. L. (2017). Selective and sensitive determination of lactose in low-lactose dairy products with HPAEC-PAD. *Journal of Chromatography B*, *1060*, 395–399. https://doi.org/10.1016/j.jchromb.2017.06.024
- Walther, B., Schmid, A., Sieber, R., & Wehrmüller, K. (2008). Cheese in nutrition and health. *Dairy Science & Technology*, *88*(4), 389–405. https://doi.org/10.1051/dst:2008012
- World Health Organization. (2012). *Guideline.* Retrieved from http://www.ncbi.nlm.nih.gov/books/NBK133309/
- World Health Organization: Guideline: Sodium intake... - Google Académico. (n.d.). Retrieved August 23, 2018, from https://scholar.google.com/scholar_lookup?title=Guideline%3A%20Sodium%20intake%20for%20adults%20and%20children&author=WHO&publication_year=2012

## Annex 1

Sensory Evaluation Panelist Questionnaire

FECHA: NOMBRE DEL CATADOR:

Cátese atentamente las muestras de queso y señálese la valoración que se considere para cada carácter respecto al control. Teniendo en cuenta:
+/- 1: significa una desviación LIGERA del atributo valorado respecto al control
+/- 2: significa una desviación MODERADA del atributo valorado respecto al control
+/- 3: significa una desviación GRANDE del atributo valorado respecto al control

**COLOR**

Blanco — Amarillento

-3 -2 -1 C +1 +2 +3

**OLOR**

Intensidad

-3 -2 -1 C +1 +2 +3

**TEXTURA**

Dureza

-3 -2 -1 C +1 +2 +3

Elasticidad

-3 -2 -1 C +1 +2 +3

Adhesividad

-3 -2 -1 C +1 +2 +3

Friabilidad

-3 -2 -1 C +1 +2 +3

**SABOR**

Amargo

-3 -2 -1 C +1 +2 +3

Salado

-3 -2 -1 C +1 +2 +3

Ácido

-3 -2 -1 C +1 +2 +3

Regusto

-3 -2 -1 C +1 +2 +3

¿A qué?

Preferencia

______ Me gusta muchísimo (9)
______ Me gusta mucho (8)
______ Me gusta moderadamente (7)
______ Me gusta ligeramente (6)
______ Ni me gusta ni disgusta (5)
______ Me desagrada ligeramente (4)
______ Me desagrada moderadamente (3)
______ Me desagrada mucho (2)
______ Me desagrada muchísimo (1)

**Observaciones**