Attempt to produce a "lactose-free" mozzarella by using different technological solutions: a chemical and sensory study

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Abstract

Lactose intolerance is a pivotal issue for dairy-consumers due to their malabsorption leading to clinical problems. Then, many lactose-free products were produced using commercial enzymes, leading to products with different characteristics (i.e. more intense sugar taste due to the presence of free glucose and galactose). Thus, the aim of the present study was to obtain a lactose-free mozzarella without using addition of enzymes and using some practices useful to remove lactose, i.e. curd washing or curd pressing. Results shows that it is possible when using curd washing practice, since lactose was suddenly reduced, reaching 0.1% after 5 days of storage. The organic acids content is reduced when applied double curd washing processing. No differences were observed on VOC profile among C-C and C-W, except for some compounds which result absent in the latter or lower compared to C-C; whereas C-W2s samples were deeply diluted, containing lowest amount of VOC, in both fresh and stored samples. On the sensory point of view, fresh C-C and C-W were similar, on the other hand, C-W2s were very poor of aroma, but preserved their mild aroma until 1 week of storage, differently from C-C, C-W and C-P which gained higher score of sour milk odor, or acid and bitter taste.

Keywords: curd washing, curd pressing, mozzarella, lactose-free, sensory analysis, chemical analysis.

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1. Introduction

The lactose intolerance is a problem whose scientists and food industries had to face during last decades, currently the prevalence of confirmed cases worldwide is about 57%, the estimated cases overcome 65% (Catanzato et al., 2021). The inability to digest lactose cause many clinical problems i.e. diarrhea, abdominal pain, flatulence or bloating (Suchy et al., 2010). For this reason, the lactose-free products fragment is the fastest growing segment of the dairy industries, it has been estimated to reach 9-billion-euro turnover by 2022 (Dekker et al., 2019). In general, lactose-free products are gaining interest not only for clinical reasons but also for health appeal, in fact they are consumed even from lactose-tolerance consumers, thereby reducing calorie addition, having lactose content below 0.1% (McCain et al., 2018; Gille et al., 2018). Among dairy products, many of them have naturally very little content of lactose, such as aged cheeses (McSweeney, 2004). On the other hand, fresh cheeses could have high amount of the disaccharide (Dickel et al., 2016; Gille et al., 2018). Depending on the type of cheese, during cheesemaking, most part of the lactose is lost in the whey during whey drainage, (Huffman & Kristoffersen, 1984). Usually, the way to reduce lactose in a lactose-free dairy product involves the use of the enzyme lactase (Dekker et al., 2016) or naturally consumption by lactic acid bacteria, when is possible; otherwise, many studies aimed to treat curd by washing or pressing it, to obtain a product with different characteristics, such as lower level of lactose or aimed to lowering organic acids content, obtain different yield, reduced oiling-off, to evaluate the influence on starters and NSLAB, proteolysis, texture, volatile compounds and sensory characteristics (da Silva et al.,2020; Richoux et al., 2008; Everard et al., 2011; Michalski et al., 2003; Hou et al., 2014a; Hou et al., 2014b; Hou et al., 2012; Moynihan et al., 2016; Batty et al.,2019; Hynes et al., 2000; Osaili et al., 2010). The curd washing processing is normally used for Colby, Monterey, and Gouda cheese (Fox and McSweeney, 2004; Lee et al., 2011), but all these studies focused on different kind of cheese, such as Swiss cheese, Camembert, Cheddar and Saint-Paulin cheese, having different characteristics. Only few focused on low-moisture mozzarella, but we think high-moisture mozzarella is worth of studying too, due to its large consume worldwide. Thus, the aim of the present study was to obtain a “lactose-free” mozzarella without using enzymes, which could have negative aspects: i) its cost; ii) difficulties in process management (are delicate and need specific time and temperature to obtain the right results); iii) could alterate product taste (releasing glucose and galactose from lactose breackdown); iiii) could be considered as a “not natural” ingredient by consumers. To do so, different “natural” technological solutions such as curd washing and curd pressing were performed aiming to reduce lactose, and evaluate if these practices could affects the chemical and sensory properties of high-moisture mozzarella.

2. Materials and Methods

2.2. Cheesemaking trial

Raw milk was collected from a dairy located in Apulia region (South of Italy) and suddenly transferred to the University of Bari Aldo Moro at 4°C for the trials. The cheesemaking process was done according to Natrella et al. (2020a) following the industrial procedure (direct acidification of milk) with slight modifications, in brief: raw milk was splitted in 6 aliquot and each of them was added with a solution of citric acid 10% until pH of 5.7; then heated to 38°C and added of 0.18ml L^{-1} of calf rennet useful for milk clotting. Once obtained the coagulum each aliquot was treated differently, as reported in figure 1: the first portion was cutted into 1x1cm pieces and settled for 15 minutes, then the curd was extracted from whey, stretched with hot water and suddenly cooled, thus obtaining control mozzarella (C-C); the second aliquot was cutted as for C-C and whey was drained , then the curd was placed in a plastic basket and pressed by using a bottle of 1.5Kg for 10 minutes, finally curd was stretched and the mozzarella cooled (C-P); the third aliquot of coagulum was cutted into 10x10 cm pieces and settled for 15 minutes, then whey was gently drained and the curd washing process was done, adding twice the volume of drained whey. Then, curd was cutted into 1x1 cm pieces, after 15 minutes the curd was extracted, stretched and cooled (C-W). The fourth aliquot was a mix of C-W and C-P, because the coagulum was cutted as done for C-W, the curd washed and cutted until 1x1 cm dimension, then before stretching, curd was pressed as C-P sample (C-WP). Finally, the last two treated mozzarella were obtained as C-W but with a double curd washing process, using twice the same aliquot of water at two different temperature, 25°C and 35 °C, obtaining the double washed mozzarella (C-W2-25 and C-W2-35). Samples were analysed at day 0 and after 1 week of storage at 4°C. Each trial was done in triplicate, then, a final trial was done at the end of the experimentation to replicate the cheesemaking process of the samples which had the best results in lactose reduction, to monitor its decreasing trend each day of storage.

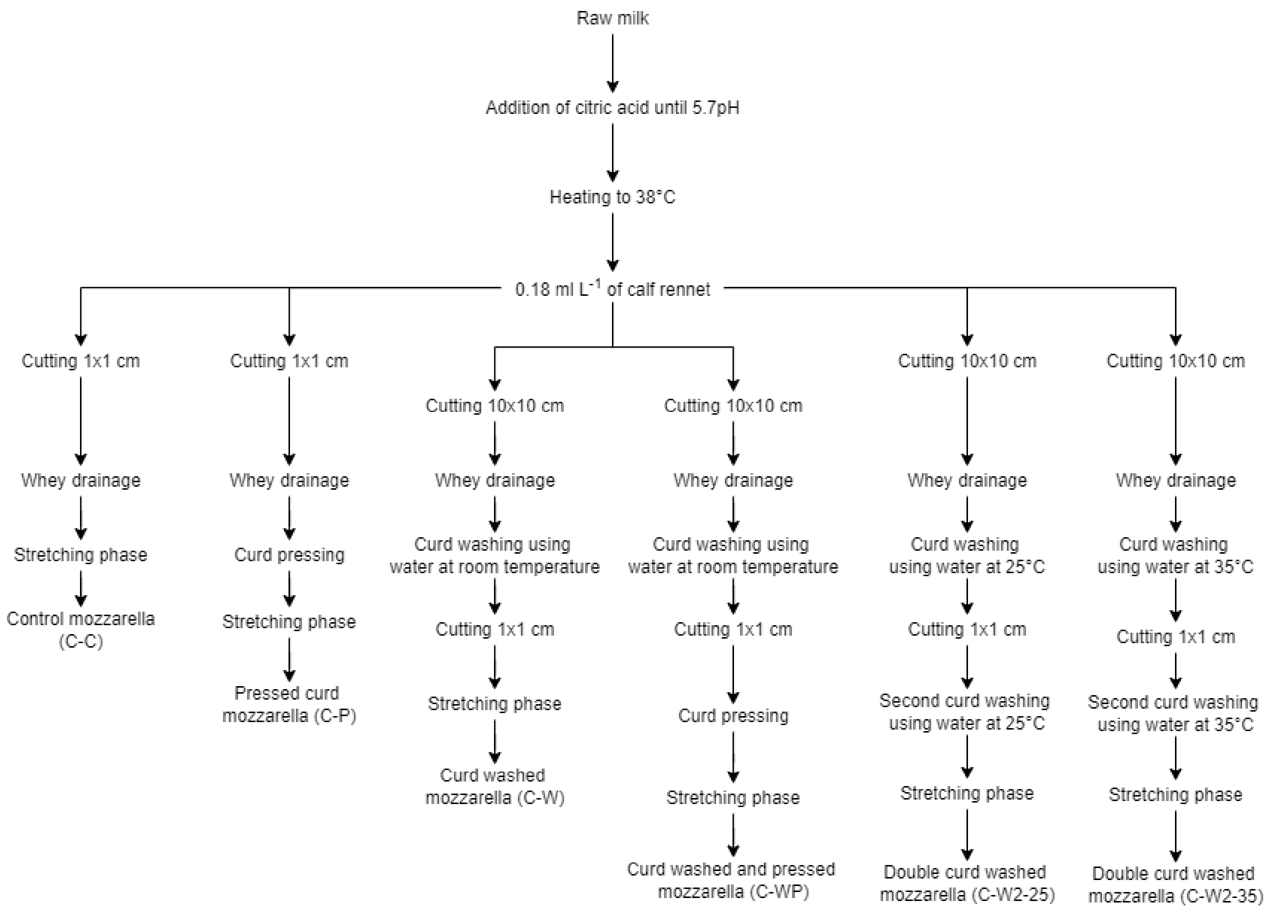

Figure 1. Technological scheme of mozzarella cheesemaking.

2.3. Chemical and sensory analysis

Sugars content of mozzarella cheese were obtained according to Faccia et al. (2021a), in brief: 10g of minced mozzarella was added of 20ml of pot water and left to stir for 1 hour, then centrifuged and the supernatant filtered with syringe filter of 0.2 μm. Ten μL was injected into the HPLC-RID system (Agilent, Palo Alto, CA, US) in isocratic condition using milli-Q water (Millipore Corp., Bedford, MA, USA) as mobile phase. Sugars were separated on a Rezex RCM-monosaccharide 300 x 7.8 mm column (Phenomenex, Torrance, CA, USA) heated to 80°C. Sugars were quantified by external calibration curves method, by using pure standard of lactose, glucose and galactose (Sigma Aldrich, Milano, Italy).

Organic acids were determined according to Natrella et al. (2020b). Five grams of minced sample was added of 20 ml of orthophosphoric acid 0.1% and shaked for 30 min, then centrifuged and the supernatant filtered at 0.2 μm. Organic acids separation was carried out on a Synergi Hydro RP Column 80 Å, 4μm, 250mm× 4.6mm (Phenomenex, Torrance, CA, USA) equipped on a Waters HPLC-DAD system. The mobile phases used were 0.1% orthophosphoric acid in water (eluent A) and acetonitrile (eluent B). The initial flow was 1 ml min^{-1}, whereas the gradient was 0–18 min 100% A, then 18–18.3 min from 100% to 20% A; 18.3–19.5 min increasing flow rate to 1.4 mL min^{-1}, then 19.5–22.5 isocratic and 22.5–23 min from 20% to 100% A and 23–43 min final isocratic. Detection was done at λ = 214 nm, and the quantitative analysis was done using the external calibration curve methods, by using pure organic acids standard solutions (Sigma Aldrich, Milano, Italy).

The primary proteolysis was evaluated by urea polyacrylamide gel electrophoresis (Urea-PAGE), as reported by Faccia et al. (2021b). Cheese sample was dissolved in 9M urea and then added of sample buffer (Tris-HCl buffer solution) and β-mercaptoethanol. Once the samples were loaded into the gel system, electrophoresis was performed at constant amperage (20 mA) for 1.5 hour, to obtain the protein fractions separation. Then, the electrophoretic gels were stained with Brilliant Blue Coomassie G250 overnight, and destained with double distilled water to remove the excess of dye. Finally, gels were scanned by using an Image scanner II (Amersham Biosciences, Buckinghamshire, UK).

Cheese moisture was determined according to the IDF method (IDF,1986), and pH was measured by pHmeter equipped with a penetration probe (HANNA Instruments, Woonsocket, RI, USA).

The analysis of volatile organic compounds (VOC) profile was done according to Natrella et al. (2021). Mozzarella samples were minced, and 1 gram was inserted in a glass vial, added with internal standard (3-pentanone) and closed by a silicone/PTFE septum and an aluminium cap. After VOC extraction at 37°C for 15 min, a SPME fiber (divinylbenzene/carboxen/polydimethylsiloxane 50/30 mm) was inserted to adsorb VOC into the vial headspace. Then, VOC were desorbed at 220°C for 2 minutes in the injection port. Molecules were separated in a VFWAX-MS thermo capillary column (60 m, 0.25 mm, 0.25 mm, Agilent Technologies, Palo Alto, CA, USA) installed on a GC-MS system (ThermoFisher Scientific). The analysis was done under the following condition: oven temperature, 40 °C for 0.1 min then 4 °C min^{-1} to 140 °C, 10°C min^{-1} to 220 °C and a final isothermal for 7.5 min. The mass detector was set at the following conditions: detector voltage, 1700 V; source temperature, 250 °C; ionisation energy, 70 eV; scan range 33e200 amu. The tentative identification of molecules was done according to VOC standard retention time and by matching their spectra with the reference mass spectra of the NIST library (National Institute of Standards and Technology).

Sensory analyses were performed by a trained panel belonging to the Italian Association of Cheese Tasters (ONAF). A quantitative analysis was done, assessors were asked to give a score on a scale from 0 to 4 on odor, taste and texture descriptors. All the results collected were used to perform the Product Characterization analysis to obtain a clear description of characterizing mozzarella descriptors.

2.4. Statistical analysis

Statistical analyses were done considering the complete dataset from all the cheesemaking trial. The analysis of variance (ANOVA) was performed to the complete dataset, whereas Product Characterization was performed only on sensory dataset results. All these analyses were computed by Xlstat-sensory software (Addinsoft, France).

3. Results and discussion

According to Hou et al. (2012) pH value was affected by curd washing (data not show), in fact control mozzarella had the lowest value (5.8), followed by C-P (5.85), probably due to the presence of some aliquot of whey trapped inside the curd (Hynes et al.,2003), whereas washed samples had value ranging from 5.9 to 6. In agreement with the findings of other researchers (Hou et al., 2012; Huffman & Kristoffersen, 1984; Shakeel-Ur-Rehman et al., 2004) the moisture content is not affected by curd washing. The sugars and organic acids content on fresh mozzarella cheese are reported in table 1. Since lactose is soluble in water, whey removal process led to a lactose content reduction in the final product (Huffman & Kristoffersen, 1984). The highest amount of the disaccharide was found in C-P and C-C (1.36 and 1.15%, respectively). C-P had high lactose content probably because some aliquot of whey was stuck and remained inside the curd, leading to a lactose content similar to control mozzarella as for the pH value. On the other hand, the curd washing process led to a reduced lactose content in all curd washed samples. In fact, C-W and C-WP had lower lactose amount compared to control mozzarella (0.72 and 0.85% respectively); even if C-WP curd was pressed as C-P, the previous curd washing process managed to remove more whey prior to pressing process. Furthermore, samples subjected to double curd washing process (C-W2s) reached the lowest lactose concentration due to the process applied (with 0.42 and 0.40% for C-W2-35 and C-W2-25, respectively), obtaining approximately 64% less lactose than control mozzarella. Between these latter two samples, the one washed with water at 25°C obtained slightly higher lactose remotion. Among the two monosaccharides, only glucose showed few differences among samples: all samples but C-W2s had lower amount of glucose, meaning that it could be metabolized by microorganisms, and C-W2 samples delayed this process. Hou et al. (2012;2014) said that curd washing does not influence microbial count, but in the present study, it seems that could affect their activity. If considering the organic acids content, no differences were found among C-C, C-W and C-WP, but when the curd was washed twice something changes, as demonstrated for lactose content. In fact, no lactic and acetic acid was found in these samples, meaning that these compounds are removed with water or that the microbial activity is delayed. On the other hand, citric acid showed a reduction of about 60% if compared to control mozzarella (0.05 vs 0.14% for C-W2-25 and C-C respectively), this finding suggests a curd leaching as well.

%	C-C	C-W	C-P	C-WP	C-W2-35	C-W2-25
Lactose	1.15 a	0.72 b	1.36 a	0.85 d	0.42 e	0.40 f
Glucose	0.01 b	0.01 b	0.01 b	0.01 b	0.03 a	0.03 a
Galactose	0.01 a	0.01 a	0.01 a	0.01 a	0.01 a	0.01 a
Lactic acid	0.02 a	0.02 a	0.02 a	0.02 a	0.00 b	0.00 b
Acetic acid	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
Citric acid	0.14 ab	0.08 b	0.16 a	0.11 b	0.06 c	0.05 c

Table 1. Sugars and organic acids content on fresh mozzarella cheeses. $P<0.05$

Table 2 shows the total amount and single VOC chemical classes found in fresh mozzarella cheese. If considering the total VOC amount and the statistical results is possible to distinguish samples in three different group: i) C-C and C-W; ii) C-P and C-WP; iii) C-W2-25 and C-W2-35. Within the first group, no differences were found between C-C and C-W, suggesting a scarce influence of curd washing on the mozzarella total VOC profile. C-P and C-WP had a similar total VOC content and statistically lower than samples of the first group (467.7 and 391.1 μg/kg for C-WP and C-P vs 1314.8 and 1447.9 μg/kg for C-C and C-W, respectively). The differences found among single chemical classes of the second group samples are upon acids content, which was higher in C-WP than C-P; whereas, acids along with ketones and aldehydes were statistically less abundant than first group samples. Then, the cheeses belonging to the third group had the lowest value of VOC total amount, having 65.4 and 67.6 μg/kg for C-W2-25 and C-W2-35 respectively, thus undergoing a reduction of about 95% compared to the control. This reduction is charged to many chemical classes such as: acids, alcohols, ketones, sulphur compounds and aldehydes. According to Hou et al. (2014) the curd washing process led to a VOC reduction, transferring molecules from the most concentrate matrix (curd) to the less concentrate ones (water), in the present study this effect was mostly observed in conjunction with the second curd washing, being total VOC amount of C-W similar to C-C mozzarella.

μg/kg	Aldehydes	Sulphur compounds	Ketones	Lactones	Esters	Alcohols	Phenols	Acids	Aromatic Hydrocarbons	Total
C-C	575.25 a	6.65 a	528.13 a	0.00 b	1.77 a	150.5 a	0.00 b	51.81 a	0.73 b	1314.8 a
C-W	701.97 a	6.04 a	497.16 a	0.00 b	1.39 ab	161.9 a	0.00 b	78.65 a	0.81 ab	1448.0 a

C-P	178.30 b	3.46 a	136.73 b	0.00 b	0.47 b	33.77 b	0.00 b	36.77 b	1.61 ab	391.1 b
C-WP	248.18 b	1.40 a	115.18 b	0.00 b	0.14 b	46.67 b	0.00 b	54.3 a	1.81 ab	467.7 b
C-W2-25	13.56 c	0.77 b	25.23 c	0.37 a	1.75 a	14.33 c	0.41 a	5.86 b	3.10 a	65.4 c
C-W2-35	6.96 c	0.76 b	27.83 c	0.00 b	1.89 a	14.44 c	0.00 b	12.93 b	2.79 ab	67.6 c

Table 2. Total amount and single VOC chemical classes found in fresh mozzarella cheese. P<0.05

A total of 36 VOC was found among all samples, all of them are reported in table S1 of supplementary material. Deepening the VOC content of C-C and C-W profiles, some differences were found: control mozzarella had higher content of 2-butanone and 2,3-butanedione than C-W sample, other compounds were absent in C-W such as dimethyl sulfide, 1-pentanol, 1-hexanol, phenylethyl alcohol and nonanoic acid. On the other hand, some compounds were higher in C-W than C-C, i.e. dimethyl sulfone, acetic acid and 3-methylbutanoic acid. C-P and C-WP had lower amount of many molecules found in C-C, among aldehydes 3-methylbutanal is the compound found at very low concentration If compared to control cheese (156.7 μg/kg for C-P vs 545 μg/kg for C-C); as well as acetoin among ketones (57.18 μg/kg for C-P vs 329.96 μg/kg for C-C). When considering C-W2s, the double curd washing process impoverished the mozzarella VOC profile, in fact, 26 molecules were found compared to 36 found in C-C. Besides, the VOC in common with control cheese were found at very low concentration, such as: nonanal, 3-methylbutanal, acetone, acetoin, ethanol, 3-methylbutanol, acetic acid and many others. All these compounds could arise from microbial activity, which is a pivotal factor in VOC production and product characterization. Although Hou et al. (2012) found no influence of curd stretching process on microbial population (both starters and NSLAB), in this case the double treatment could have a possible effect in delaying their activities.

μg/kg	C-C	C-W	C-P	C-WP	C-W2-25	C-W2-35
Heptanal	2.99 a	3.42 a	2.46 a	2.36 a	1.27 b	0.84 b
Nonanal	16.63 a	17.06 a	10.89 b	12.10 b	4.81 c	3.66 c
Furfural	0.00 b	0.00 b	0.21 a	0.14 a	0.00 b	0.00 b
Butanal, 2-methyl	5.12 a	7.00 a	3.28 a	2.45 a	0.00 b	0.00 b
Butanal, 3-methyl	545.0 a	670.1 a	156.7 b	227.8 b	5.02 c	1.69 c
Hexanal	4.87 a	3.91 a	2.98 a	3.36 a	1.83 b	0.77 b
Benzenacetaldehyde	0.00 b	0.00 b	1.33 a	0.00 b	0.00 b	0.00 b
Benzaldehyde	0.67 a	0.45 ab	0.40 ab	0.00 b	0.62 a	0.00 b
Dimethyl sulfide	4.44 a	0.00 c	1.23 b	0.82 b	0.77 b	0.76 b
Dimethyl sulfone	2.21 b	6.04 a	2.24 b	0.59 c	0.00 d	0.00 d
Acetone	77.94 a	67.15 a	58.26 b	33.56 bc	13.56 c	13.36 c
2-Butanone	20.56 a	10.97 b	19.85 a	7.37 b	7.81 b	11.06 b
2,3-Butanedione	97.52 a	30.11 b	0.00 c	0.00 c	0.00 c	0.00 c
2-Heptanone	1.52 a	1.37 a	0.99 a	0.88 a	0.56 a	0.48 a
5-Hepten-2-one, 6-methyl	0.64 a	1.51 a	0.36 a	0.84 a	0.83 a	0.45 a
Acetoin	329.96 a	386.0 a	57.18 b	72.53 b	2.47 c	2.49 c

Butyrolactone	0.00 b	0.00 b	0.00 b	0.00 b	0.37 a	0.00 b
Ethyl Acetate	1.45 a	1.26 a	0.47 a	0.14 a	1.25 a	1.07 a
Hexanoic acid, ethyl ester	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.34 a
Octanoic acid, ethyl ester	0.09 ab	0.05 b	0.00 b	0.00 b	0.20 a	0.29 a
Butanedioic acid, diethyl ester	0.11 ab	0.07 ab	0.00 b	0.00 b	0.30 a	0.20 ab
Acetic acid, 2-phenylethyl ester	0.12 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
2-Butanol	0.69 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
Ethanol	41.71 a	55.74 a	11.37 b	12.14 b	3.21 c	2.74 c
1-Butanol, 3-methyl	100.87 a	102.8 a	20.71 b	31.34 b	5.57 c	8.21 c
1-Pentanol	0.32 a	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
1-Hexanol	0.42 a	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
2-Octanol	0.13 b	0.00 b	0.00 b	0.00 b	0.73 a	0.00 b
1-Hexanol, 2-ethyl	0.16 a	1.71 a	0.00 a	1.03 a	0.48 a	0.00 a
Ethanol, 2-(2-butoxyethoxy)	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.39 a
Phenylethyl Alcohol	5.26 a	0.00 c	1.69 b	2.16 a	3.78 a	2.51 a
Ethanol, 2,2'-oxybis	0.89 a	1.58 a	0.00 b	0.00 b	0.56 a	0.59 a
Phenol	0.00 b	0.00 b	0.00 b	0.00 b	0.41 a	0.00 b
Acetic acid	14.90 b	37.01 a	12.58 b	14.61 b	1.59 c	0.80 c
Propanoic acid, 2-methyl	0.00 a	0.63 a	0.00 a	0.19 a	0.00 a	0.00 a
Butanoic acid	10.18 a	10.20 a	6.89 a	11.24 a	2.62 b	1.85 b
Butanoic acid, 3-methyl	3.47 b	9.57 a	1.71 b	2.15 b	0.00 c	0.00 c
Pentanoic acid	0.00 a	0.00 a	0.00 a	0.24 a	0.00 a	0.00 a
Hexanoic acid	13.23 b	13.22 b	10.62 b	18.63 a	0.00 d	5.21 c
Heptanoic acid	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	2.07 a
Octanoic acid	7.77 a	7.29 ab	4.09 ab	6.68 ab	1.65 b	2.31 ab
Nonanoic acid	1.53 a	0.00 a	0.57 a	0.00 a	0.00 a	0.69 a
n-Decanoic acid	0.73 a	0.74 a	0.31 a	0.58 a	0.00 a	0.00 a
Toluene	0.58 bc	0.00 c	1.43 b	1.62 b	3.14 a	2.79 a
Limonene	0.00 a	0.64 a	0.00 a	0.00 a	0.00 a	0.00 a
Styrene	0.15 a	0.18 a	0.00 a	0.20 a	0.00 a	0.00 a
Totale	1314.8 a	1448 a	391.1 b	467.7 b	65.4 c	67.6 c

Table S1. Single VOC found in fresh mozzarella cheese. $P<0.05$

The results of Product Characterization analysis on fresh mozzarella are reported in Figure 2. The colored histograms are helpful to define the descriptors characterizing the sample, blue color is associated to coefficient that have significant positive score and red have significant negative value. Control mozzarella and C-W samples were positively related to “odor intensity”, confirming the instrumental VOC results observed. Moreover, C-W was characterized also by soul milk odor and higher aftertaste than control mozzarella. C-P result to be a product with neutral odor and taste, on the other hand C-WP was positively related to aftertaste descriptor (as C-W) and a harder texture. Finally the two samples C-W2s, according to VOC analysis, were negatively related with odor intensity, aftertaste and sour milk odor (only for C-W2-35), being considered poor of aroma and taste. Thus, as reported by literature, when considering a single curd washing process no differences were found between treated and control mozzarella. Nonetheless, if double curd washing is applied some differences was observed, obtaining a mozzarella with a mild aroma and taste, but it is still accepted by panelists.

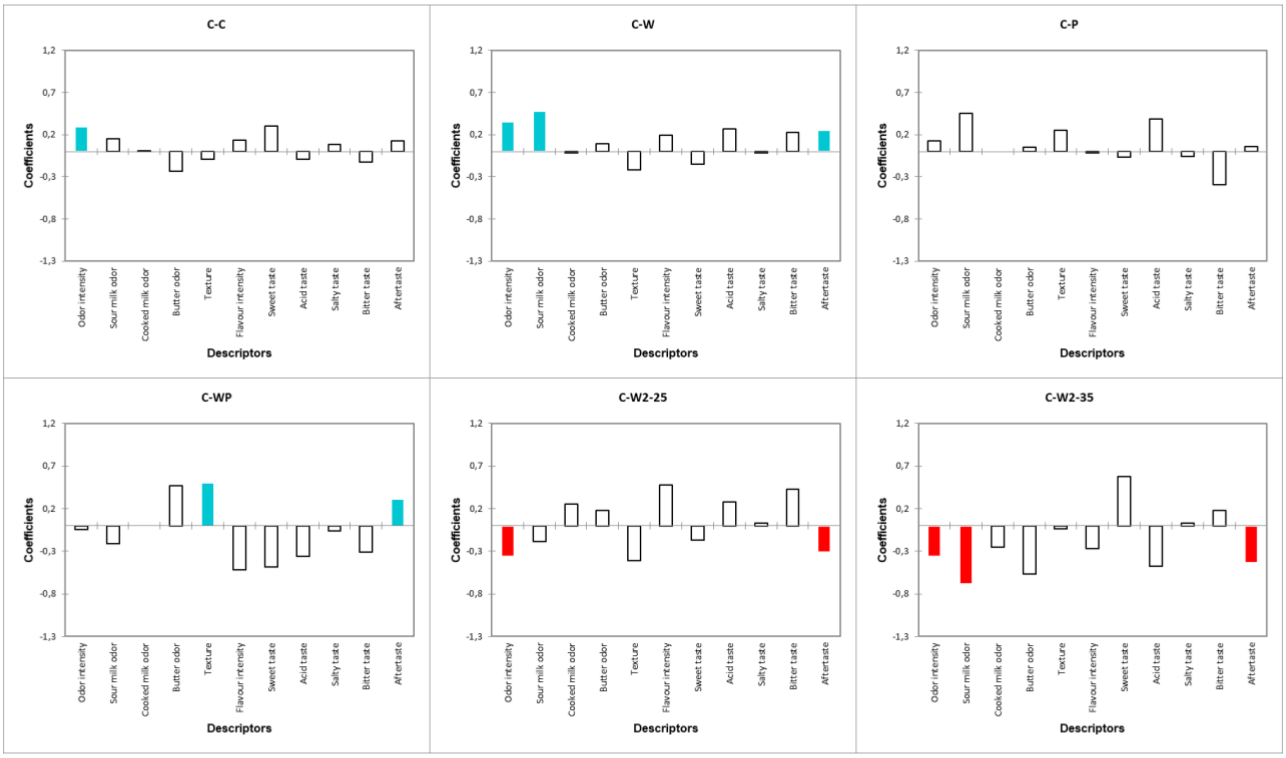

Figure 2. Product characterization analysis results of fresh mozzarella panel test score.

The sugars and organic acids content of stored mozzarella were listed in table 3. After 7 days of storage the lactose content, as expected, was significantly reduced reaching values under 0.1%. Control mozzarella, C-P, C-WP and C-W2-35 were the samples with the highest amount of lactose with 0.09%, 0.03%, 0.06% and 0.02% respectively, whereas no lactose was found in the remaining samples. Glucose content was similar among all samples except for C-W2s, in which there were no sugar. On the organic acids content found, lactic acid was tenfold the amount found in the fresh products in almost all samples, reaching values between 0.15 and 0.32%. The lactic acid content found in C-W2s was subject to a slight increase from 0% (T0) to 0.07-0.09% (T7). C-C, C-P and C-WP had higher amount of acetic acid (0.02%), whereas no acetic acid was found in C-W2 samples. Thus, the raw milk autochthonous microbiota, although limited by storage temperature (4°C), seems to have a role in lactose reduction and organic acids production. Moreover, another possible explanation of the lactose reduction and the absence of production of organic acids in C-W2s could be related to the matter exchanges between mozzarella and its brine.

%	C-C	C-W	C-P	C-WP	C-W2-35	C-W2-25
Lactose	0.09 a	0.00 b	0.03 ab	0.06 ab	0.02 ab	0.00 b
Glucose	0.01 a	0.01 a	0.01 a	0.01 a	0.00 b	0.00 b

Galactose	0.02 a	0.01 a	0.01 a	0.01 a	0.01 a	0.02 a
Lactic acid	0.27 a	0.19 b	0.32 a	0.15 b	0.07 c	0.09 c
Acetic acid	0.02 a	0.01 b	0.02 a	0.02 a	0.0 c	0.00 c
Citric acid	0.03 a	0.03 a	0.02 a	0.01 a	0.01 a	0.01 a

Table 3. Sugars and organic acids content on 7days stored mozzarella cheeses. $p<0.05$

Table 4 reports the total amount and the single VOC chemical classes found in mozzarella samples after 7-days of storage. On the total amount is clear that the concentrations between the fresh and stored products are very different; being mozzarella samples obtained by raw milk, the presence of NSLAB deeply affect the product during storage. The total amount of C-C is almost 13-fold higher if compared to the same fresh product; C-P was the mozzarella with the highest total amount found (with 27594.3 μg/Kg), due to the highest amounts of aldehydes, ketones, alcohols and acids. As saw for fresh products, C-C and C-W still were similar, no differences were found neither on totals nor on single chemical classes. C-W2s samples were the poorest samples found (2536.2 and 1530.6 μg/kg for C-W2-25 and C-W2-35, respectively), having almost 88% fold lower amount of VOC if compared to control sample.

Among the single chemical classes, aldehydes underwent to a huge increase compared to fresh samples, and C-W had the highest concentration followed by C-P and C-C. The aldehydes amount was very low in C-W2s if compared to the other samples. Sulphur compounds concentrations were absent in pressed samples and C-W2-35, in general they were lower in all samples than fresh products. Esters increased during storage and were higher in all samples except for C-W2, which concentrations were statistically lowest. Ketones content was similar for C-C and C-P, whereas C-W2s were the poorest samples. Acids and alcohols had similar trend, being very low in C-W2s samples and higher in all other samples.

μg/kg	Aldehydes	Sulphur compounds	Ketones	Lactones	Esters	Alcohols	Acids	Aromatic hydrocarbons	Total
C-C	3855,99 b	4,71 a	5561,20 a	0,00 b	12,90 ab	6141,74 ab	1457,68 ab	15,34 a	17049,6 b
C-W	7204,96 a	8,29 a	3744,82 b	0,00 b	14,89 ab	4704,83 ab	1271,11 ab	7,67 ab	16956,6 b
C-P	5188,18 ab	0,00 b	7465,53 a	0,0 b	10,93 b	12246,8 a	2677,21 a	5,68 ab	27594,3 a
C-WP	2370,54 c	0,00 b	3800,46 b	0,00 b	16,55 a	5591,24 ab	1613,8 a	7,59 ab	13400,2 b
C-W2-25	187,32 d	1,58 a	648,36 c	2,15 a	9,98 bc	1474,79 b	202,81 b	9,3 ab	2536,2 c
C-W2-35	110,89 d	0,00 b	576,10 c	0,00 b	7,66 c	770,23 c	62,20 c	3,54 b	1530,6 c

Table 4. Total amount and single VOC chemical classes found in 7 days stored mozzarella cheese. $p<0.05$

If considering the single VOC found (table S2 of supplementary material) after 1 week of storage, as saw in table 4, almost all compounds underwent to a huge increasing trend. The most representative molecules of the mozzarella volatile profiles were mainly originated by microbial activities, i.e.: 2-methylbutanal, 3-metrhylbutanal, 2,3-butanedione, 5-hydroxy-2,7-dimethyl-4-octanone, acetoin, ethanol, 3-methyl-1-butanol, acetic acid and 3-methylbutirric acid (Natrella et al., 2020a). Among all the VOC listed, the lowest concentrations were found on C-W2s samples, as for fresh products. The microbial activity did not flatten the differences originated by the curd washing process (done twice), this could have a role also for the sensory results. In fact, the Product Characterization analysis results on stored mozzarella shows different patterns (Figure 3).

T7	C-C	C-W	C-P	C-WP	C-W2-25	C-W2-35
Heptanal	1.55 b	3.80 a	4.31 a	0.00 d	2.45 ab	0.52 c
Nonanal	17.38 b	29.04 a	35.24 a	16.54 b	11.59 b	3.90 c
Furfural	10.01 ab	0.00 b	4.54 b	14.66 a	0.00 b	0.00 b
Butanal, 2-methyl	66.38 b	103.22 a	53.87 b	19.36 c	3.65 d	3.60 d
Butanal, 3-methyl	3737.37 c	7050.46 a	5063.00 b	2316.83 d	165.94 e	100.28 e
Hexanal	4.14 a	5.99 a	0.00 c	0.00 c	3.68 ab	1.43 b
Benzenacetaldehyde	15.78 ab	10.69 b	21.61 a	3.15 c	0.00 d	0.00 d
Benzaldehyde	3.39 ab	1.75 ab	5.62 a	0.00 b	0.00 b	1.16 ab
Dimethyl sulfide	4.71 a	8.29 a	0.00 c	0.00 c	1.58 b	0.00 c
Acetone	175.30 b	242.12 a	250.19 a	141.24 b	26.76 c	12.70 c
2-Butanone	7.92 ab	13.46 a	0.00 c	9.26 ab	9.57 ab	9.78 ab
2,3-Butanedione	2197.20 ab	1151.63 ab	4303.72 a	654.17 b	223.06 b	188.33 b
2,3-Pentanedione	10.14 ab	4.88 b	20.47 a	4.65 b	2.23 b	0.00 b
2-Heptanone	16.31 a	15.08 a	21.87 a	25.11 a	12.95 a	10.39 a
5-Hepten-2-one, 6-methyl	2.50 a	2.59 a	3.81 a	3.45 a	0.74 a	0.37 a
2-Hydroxy-3-pentanone	2.65 bc	1.87 cd	5.47 b	7.91 a	0.00 d	0.00 d
2-Nonanone	2.932 d	3.18 cd	12.02 b	18.32 a	9.18 bc	0.00 d
4-Octanone, 5-hydroxy-2,7-dimethyl	116.92 a	142.99 a	20.95 b	111.25 a	24.92 b	0.00 c
Acetoin	3027.61 a	2167.01 b	2827.02 a	2825.11 a	338.95 c	354.53 c
Acetyl valeryl	1.74 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
Butyrolactone	0.00 b	0.00 b	0.00 b	0.00 b	2.15 a	0.00 b
Ethyl Acetate	5.53 ab	11.02 a	0.00 b	9.42 a	9.04 a	7.66 a
1-Butanol, 3-methyl-, acetate	5.27 ab	3.87 b	10.93 a	7.14 ab	0.00 c	0.00 c
Butanoic acid, ethyl ester	2.10 a	0.00 b	0.00 b	0.00 b	0.95 a	0.00 b
Ethanol	588.80 a	296.54 b	533.79 a	372.97 ab	567.22 a	221.85 b
1-Propanol, 2-methyl	119.63 b	79.89 bc	203.09 a	92.82 b	44.39 c	24.29 c
1-Butanol, 3-methyl	5401.16 ab	4303.75 ab	11475.55 a	5095.32 ab	856.67 b	519.57 b
3-Penten-2-ol	0.00 a	0.80 a	0.00 a	0.00 a	0.00 a	0.00 a
2-Buten-1-ol, 3-methyl	0.00 a	1.23 a	0.00 a	0.00 a	0.00 a	0.00 a
2-Octanol	0.00 b	7.40 a	6.78 a	7.84 a	0.00 b	0.00 b
1-Hexanol, 2-ethyl	6.12 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
Phenylethyl Alcohol	21.44 ab	13.83 ab	27.59 a	22.29 a	4.41 b	3.37 b
Ethanol, 2,2'-oxybis	4.60 a	1.39 a	0.00 a	0.00 a	2.09 a	1.15 a
Acetic acid	1256.64 ab	846.42 ab	2441.96 a	1308.33 a	151.62 b	42.79 b
Propanoic acid, 2-methyl	9.21 b	24.70 a	12.72 b	12.58 b	1.14 c	0.79 c
Butanoic acid	36.48 a	30.96 a	48.42 a	35.52 a	15.97 b	3.92 b
Butanoic acid, 3-methyl	89.21 c	332.53 a	116.01 c	222.73 b	6.65 d	4.06 d
Hexanoic acid	31.15 ab	27.05 ab	40.75 a	24.01 b	14.11 c	3.84 c
Heptanoic acid	0.00 b	0.00 b	0.00 b	0.00 b	3.99 a	1.30 b
Octanoic acid	16.04 a	7.43 a	17.35 a	10.60 a	4.96 a	3.03 a
Nonanoic acid	13.18 a	2.01 b	0.00 c	0.00 c	4.38 b	2.48 b
n-Decanoic acid	5.78 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
Toluene	9.05 a	6.73 a	5.68 a	5.31 a	7.99 a	3.54 a
Limonene	5.67 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
Styrene	0.61 bc	0.45 bc	0.00 c	2.28 a	1.27 b	0.00 c
o-Cymene	0.00 a	0.31 a	0.00 a	0.00 a	0.00 a	0.00 a

Total	17049.6 ab	16956.6 ab	27594.3 a	13400.2 b	2536.2 c	1530.6 c

Table S2. Single VOC found in 7-days stored mozzarella cheese. $p<0.05$

After 7 days the samples were different compared to fresh cheeses, as expected. C-C was mainly characterized by acid and bitter taste, the same was for C-W and C-P. Moreover, these latter were defined as sample with higher odor intensity, and C-P also by sour milk odor. C-WP obtained highest score for texture, meaning that has more compact texture than the other samples, and low score of acid and bitter taste. C-W2-35 was quite balanced with negative correlation of acid taste descriptor. Finally, C-W2-25 obtained the lowest score for many descriptors, resulting the mozzarella with a mild aroma and taste.

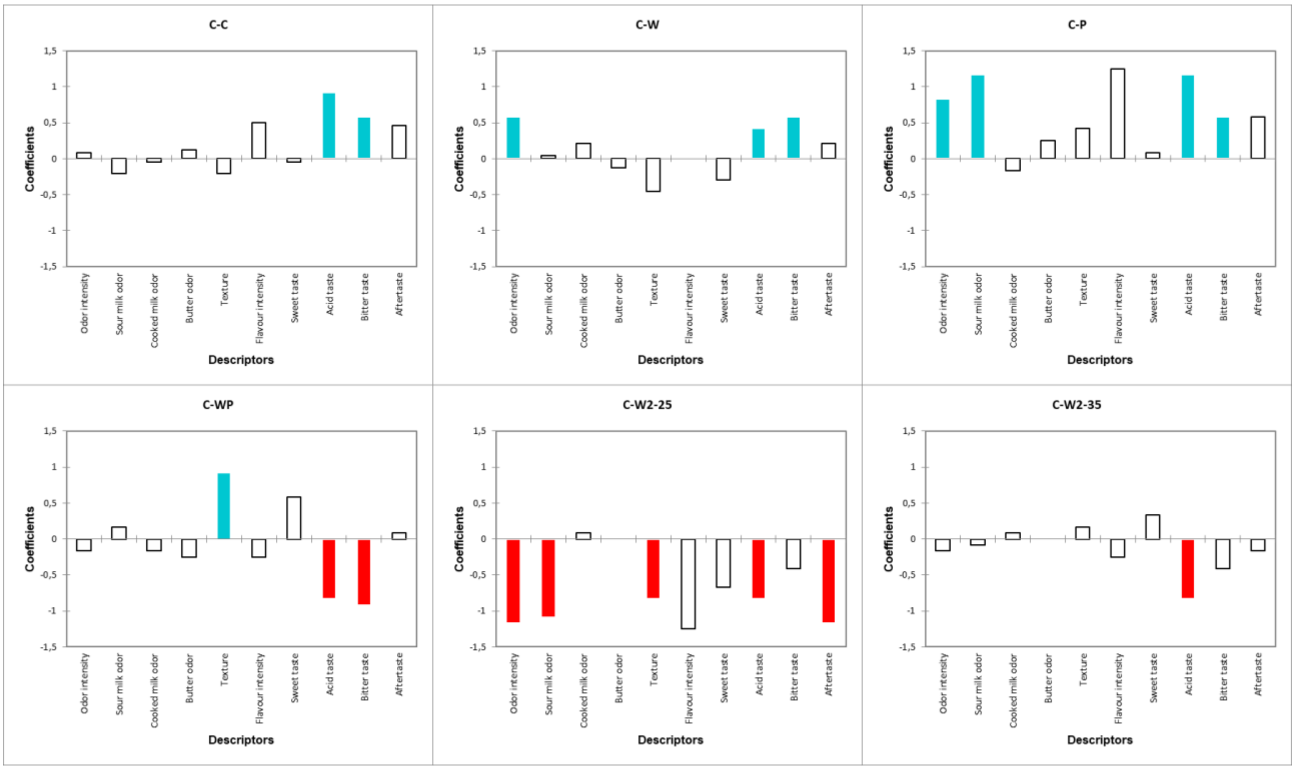

Figure 3. Product characterization analysis results of stored mozzarella panel test score.

The primary proteolysis was evaluated by electrophoretic patterns of the fresh mozzarella samples (Figure 4A), and stored mozzarella samples (Figure 4B). Only the control and curd washed samples were reported, due to the best lactose reduction results of the latter, in line with our purpose. In the electrophoretic gels are clearly showed many protein fractions such as: γ-CN, κ-CN, β-CN and α_{s1}-CN. As reported by many authors (Hou et al., 2014a,2014b; Lee et al., 2011; Moynihan et al., 2016) curd washing does not affect the chemical composition of cheese, thus, in agreement with literature no differences were found among fresh samples, in terms of number and intensity of bands. In the same way, Figure 3B showed no differences among stored samples. Obviously, some differences among fresh and stored samples were observed, i.e. the arise of some band belonging to casein fragment degradation (β-CN-I or αs_1-CN-I), which is a typical decaying process of the product.

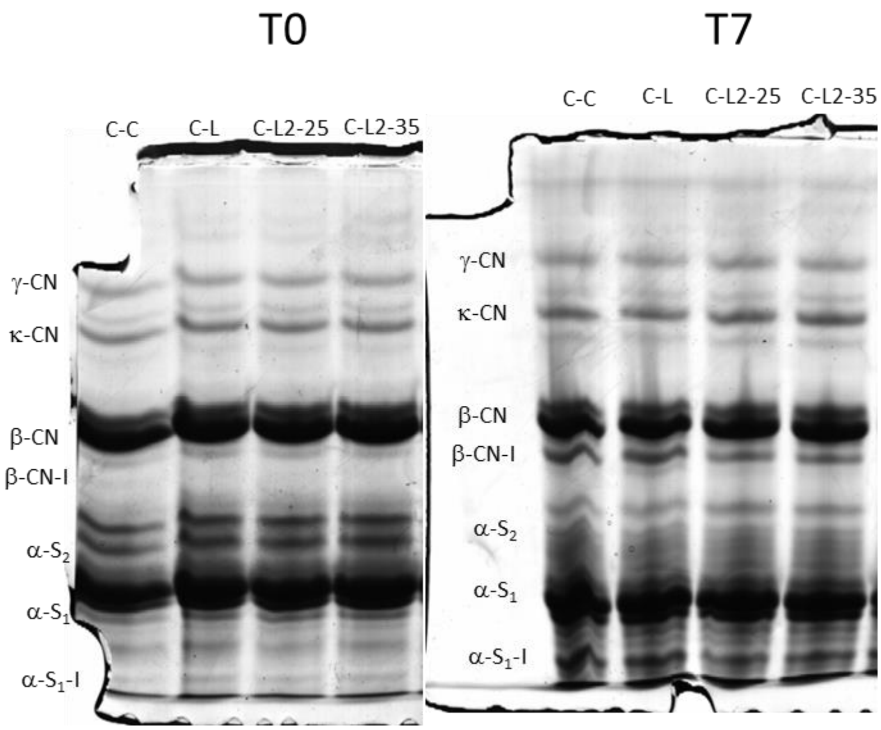

Figure 4. Urea-PAGE pattern of samples of Mozzarella. IMMAGINE DA SOSTITUIRE CON QUELLA IN INGLESE

Figure 5 shows the lactose content of the control sample and the treated sample, which showed the best results, during the 7 days of storage. The figure shows the last trial results and how many days are needed to obtain a “lactose-free” mozzarella made without technological coadjutant. As observed by previous tables, the initial content of lactose in control mozzarella is about 3-fold higher than treated mozzarella. The latter, having lowest concentration of the sugar, reached concentrations below 0.1% earlier (fifth day), specifically 2 days before control cheese, with lactose content of 0.08%; whereas, control mozzarella contains 0.36% of lactose after 5 days of storage. At the end of the monitoring period, control mozzarella reached 0.09% of lactose content, on the other and treated mozzarella had 0.02%.

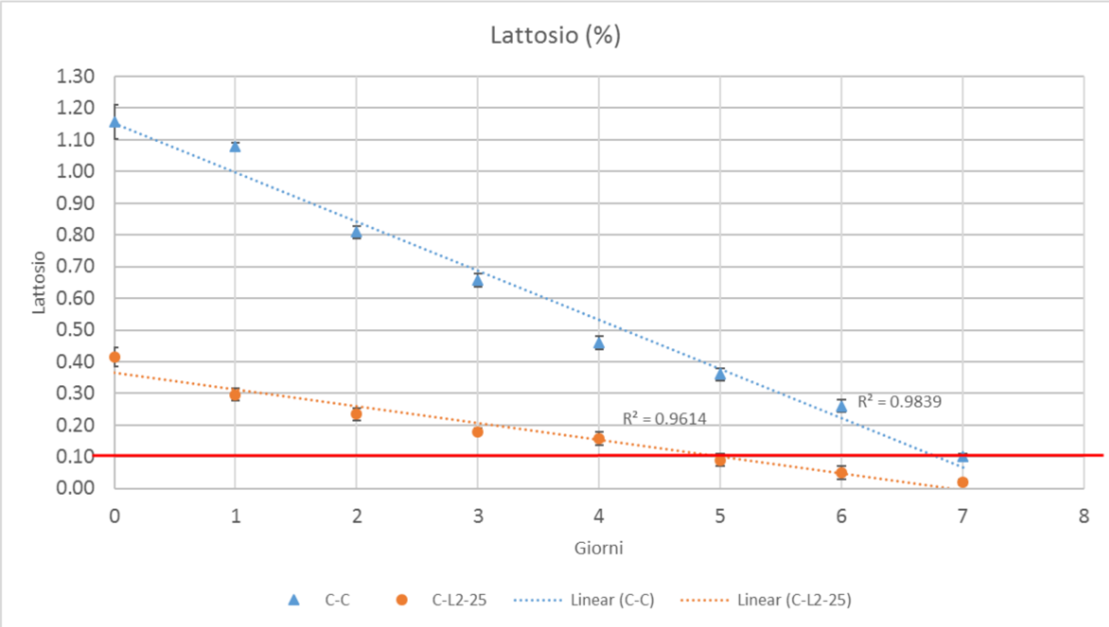

Figure 5. Lactose decreasing trend on control mozzarella and C-W2-25, monitored each day of storage. IMMAGINE DA SOSTITUIRE CON QUELLA IN INGLESE

4. Conclusion

As a conclusion, it is possible to obtain a lactose-free mozzarella without using the enzyme. Curd washing could be a very interesting “natural” way to remove lactose in production of lactose-free mozzarella. In fact, results show how this practice is way better than curd pressing in reducing the disaccharide, since lactose was suddenly reduced (about 64% less for curd washed twice), reaching 0.1% after 5 days of storage. The organic acids content is unaltered between samples, except when double curd washing processing was applied, resulting lower than control cheese. No differences were observed on total VOC profile among C-C and C-W, as reported by literature; on the other hand, some differences were found if considering the single VOC, which result absent in the treated samples or lower compared to C-C; whereas VOC profile of C-W2s samples were deeply influenced containing lowest amount of VOC, in both fresh and stored samples. On the sensory point of view, although fresh C-C and C-W were similar, on the other hand, C-W2s were very poor of aroma, but preserved their mild aroma until 1 week of storage, differently from C-C, C-W and C-P which gained higher score of sour milk odor, or acid and bitter taste.

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