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Partial substitution of 40 g/100 g fresh milk with reconstituted low heat skim milk powder in high-moisture mozzarella cheese production: Rheological and water-related properties

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LWT

Partial substitution of 40 g/100 g fresh milk with reconstituted low heat skim milk powder in high-moisture Mozzarella cheese production: rheological and water-related properties --Manuscript Draft--

Manuscript Number:	LWT-D-20-04471R2						
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	Giorgio Giraffa						
Abstract:	Skim milk powders may be used as a convenient alternative to fresh milk in high moisture Mozzarella cheese manufacturing. The effects of a blend of 40 g/100 g of reconstituted low heat skim milk powder and 60 g/100 g fresh milk on processing and quality of Mozzarella cheese (experimental) were evaluated, in comparison with cheeses produced only with fresh milk (control). The ability of experimental curd to retain fat during stretching was lower than control, as showed by the fat content in stretching water (2.85 \pm 0.45 g/100 g and 2.01 \pm 0.31 g/100 g for experimental and control curds, respectively). However, cheeses showed a similar composition. Cheese rheological properties were affected, as experimental Mozzarella showed a more organized casein network with tan δ and n' values lower than control cheeses. The use of powder milk also increased the fraction of solvation water measured with low field NMR when compared with control cheeses. This study demonstrated the applicability of a blend with 40 g/100 g of reconstituted milk to obtain Mozzarella cheese without major changes in product quality.						

Cover Letter

Marcello Alinovi Ph.D. Department of Food and Drug, University of Parma Parco Area delle Scienze 47/A 43124, Parma, Italy +39 334 328 320 6 marcello.alinovi@studenti.unipr.it

> Prof. Harald Rohm Editor LWT – Food Science and Technology

October 08, 2020

Dear Editor,

On behalf of all authors, I am pleased to re submit the revised article entitled "Partial substitution of 40 g/100 g fresh milk with reconstituted low heat skim milk powder in high-moisture Mozzarella cheese production: rheological and water-related properties" by Flavio Tidona, Marcello Alinovi, Salvatore Francolino, Gianluca Brusa, Roberta Ghiglietti, Francesco Locci, Germano Mucchetti and Giorgio Giraffa, for consideration for publication in *LWT – Food Science and Technology*.

We appreciate the interest that the Editor and Reviewers have taken in our manuscript and the constructive criticism they have given. We hope to have addressed the major observations of the Reviewers and Editor. We feel that these changes have clearly improved our manuscript. We have also included a point-by-point response to the Reviewers in addition to making the changes described above in the manuscript. Changes to the manuscript are marked in red or are formatted as revisions.

In this manuscript, we studied the applicability of reconstituted milk from low-heat, skimmed milk powders on high moisture Mozzarella cheesemaking performances and product's quality. We discovered that the use of reconstituted milk in Mozzarella cheesemaking promoted some differences in the structural organization of the network and in the water molecular distribution. Despite of these differences, we demonstrated the possibility to manufacture Mozzarella cheese from reconstituted milk having similar composition to the one produced with fresh milk.

As at the present, milk powders are largely adopted in cheesemaking, this study is useful to better understand the influence of this ingredient in cheesemaking. Thus, considering the novelty of this study and its industrial relevance, we believe that this manuscript is appropriate for publication by *LWT – Food Science and Technology*.

This manuscript has not been published and is not under consideration for publication elsewhere and we have no conflicts of interest to disclose. Please address all correspondence concerning this manuscript to me at marcello.alinovi@studenti.unipr.it.

Thank you for your consideration.

Sincerely yours,

Marcello Alinovi

Malle Aland



Response to reviewers

Manuscript LWT-D-20-04471

entitled

Impact of reconstituted skim milk powders on high moisture Mozzarella cheese: rheological and waterrelated properties

We would like to thank the Reviewers and Editor for their time and efforts in reviewing this manuscript, and for the constructive feedback which they were able to provide. We feel these suggestions have helped to substantially improve the quality of this manuscript. The comment of Reviewer #3 has been carefully considered. Our response is below (responses to Reviewers comments in red) and changes made in the manuscript are highlighted using "Tracked Changes" option.

Reviewer #3: As Reviewer No. 2, I am satisfied with the responses provided by the authors to my suggestions. However, I would like to comment on one response provided by the authors to a query posed by Reviewer No. 2 to the matter of solvation as follows:

P12/261-262: How can solvation water be improved? (Reviewer 2)

To my mind the authors have placed an overdependence on the role of lactose and lactose crystallisation phenomena in trying to explain solvation changes in this case. The recent paper by Alinovi et al (2020), referred to below, points to the effects of high moisture Mozzarella cheese storage on casein dehydration (as shown by NMR relaxometry).

Alinovi et al. (2020) used NMR relaxometry to observe dehydration of caseins in high moisture Mozzarella during refrigerated and frozen storage [Marcello Alinovia Milena CorredigbcGermano MucchettiaEleonora Carinia (2020) Food Research Intl. 137, 109415. Another reference on a model study shows the effect of lactic acid on water removal around lactose

Wijayasinghe et al., (2015) showed that there is a greater energy requirement for water removal around lactose in the presence of lactic acid [Rangani Wijayasinghe TodorVasiljevic JayaniChandrapala (2015) Water-lactose behavior as a function of concentration and presence of lactic acid in lactose model systems J. Dairy Sci. 12, 8505-8514].

We acknowledge the Reviewer for this comment. As we stated in the manuscript we hypothesized two possible reasons for the different water status of the control and experimental cheeses: the different lactose content (as highlighted by the Reviewer, discussed in L332-340 of the R1 manuscript) and the higher presence of denatured proteins in the experimental cheese (discussed in L340-343 of the R1 manuscript). Both hypothesized reasons can offer a motivation for the higher amount of bound water of the experimental cheese. Nonetheless, as reported in the reference kindly suggested by the Reviewer, (Rangani Wijayasinghe TodorVasiljevic JayaniChandrapala (2015) Water-lactose behavior as a function of concentration and presence of lactic acid in lactose model systems J. Dairy Sci. 12, 8505-8514) at increasing lactose concentrations (in the 15-50% wt/wt range) there is a reduction of the water bound to lactose, as it is limited to only one hydration

layer. Moreover, higher lactose contents are responsible for the increase in attractive forces between lactose and water molecules. It should be important to note that in our study the lactose contents of the milks (and then, of the cheeses) is lower than the lower concentration evaluated by Wijayasinghe et al., and that the difference in lactose content of the control and reconstituted milk is slight. Accordingly we agree with the Reviewer that the role of lactose in determining the water status of the cheese (in terms of bound water) may have a lower relevance than the one that we discussed in the last version of the manuscript. On the contrary, the presence of denatured proteins can play a greater role. Following this reasoning we decided to swap the discussion of lactose and denatured proteins role in the revised manuscript, in order to give more visibility to the denatured proteins' role.

Still, as in these lines we are drawing hypotheses that can justify the difference in the bound water of the cheeses, we feel that it is important also to discuss the possible role of lactose. Thus, we integrated the suggested reference and to slightly modify the discussion (L302-320 of the R2 manuscript).

Please note, that the cheeses of this study were manufactured by citric acid injection. Thus, the potential role of lactic acid was not discussed as it was not present in the cheese.

- 40%-<u>g/100 g</u> recombined <u>skim</u> milk <u>powders</u> and fresh milk were used to manufacture Mozzarella
- Cheese made with recombined<u>and fresh</u> milk showed <u>similar comparable</u> composition and yield<u>to fresh one</u>
- RRecombined milk <u>cheese</u> showed <u>promoted</u> a more organized protein network than fresh <u>milkones</u>
- Recombined milk increased the fraction of solvation bound water if compared to fresh one in Mozzarella cheese

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3	
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14 Abstract

Skim milk powders may be used as a convenient alternative to fresh milk in high moisture 15 Mozzarella cheese manufacturing. The effects of a blend of 40 g/100 g of reconstituted low heat 16 skim milk powder and 60 g/100 g fresh milk on processing and quality of Mozzarella cheese 17 (experimental) were evaluated, in comparison with cheeses produced only with fresh milk (control). 18 The ability of experimental curd to retain fat during stretching was lower than control, as showed by 19 the fat content in stretching water (2.85 \pm 0.45 g/100 g and 2.01 \pm 0.31 g/100 g for experimental 20 and control curds, respectively). However, cheeses showed a similar composition. Cheese 21 rheological properties were affected, as experimental Mozzarella showed a more organized casein 22 network with $tan\delta$ and n' values lower than control cheeses. The use of powder milk also increased 23 the fraction of solvation water measured with low field NMR when compared with control cheeses. 24 This study demonstrated the applicability of a blend with 40 g/100 g of reconstituted milk to obtain 25 26 Mozzarella cheese without major changes in product quality.

27

28 Keywords:

29 Skim milk powder; Recombined milk; Cheesemaking; ¹H NMR; Food rheology

30

31 **1. Introduction**

Reconstituted milk from skimmed milk powder (SMP) may be conveniently used to partially or totally replace fresh milk in cheese processing (Moiseev, Suchkova, & Iakovchenko, 2017; Tidona et al., 2020), especially when periodic fluctuations in market price determine a lower cost of SMP than fluid milk.

The use of recombined milk in Mozzarella cheese processing could impact on some specific 36 37 technological properties such as the curd extensibility and meltability (Lelievre, Shaker and Taylor, 1990). Mozzarella curd stretching is typically carried out by immersion in hot water; this step 38 reduces the fat content of the cheese, due to the unavoidable fat losses in the stretching water 39 40 (Francolino, Locci, Ghiglietti, Iezzi and Mucchetti, 2010), and leads to a peculiar organization of the moisture fraction incorporated in the casein network (Alinovi, Corredig, Mucchetti, & Carini, 41 2020; McMahon, Fife, & Oberg, 1999). These properties can be severely modified during the 42 43 typical storage into the covering liquid (Mucchetti, Pugliese, & Paciulli, 2017).

The quality of SMP (from low to high-heat) influences the successful application of recombined milk for Mozzarella cheese manufacture. Specifically, the level of aggregation of β -lactoglobulin to α_{S2} and k-caseins, and the colloidal calcium content (Martin, Williams, & Dunstan, 2007), can interfere with the typical elastic and stretching behavior of the cheese (Thompson, Flanagan, Brower, & Gyuricsek, 1978). The application of a milk powder with a reduced whey protein content through MF and UF combined processes improved the milk coagulability and the cheese yield of low-moisture Mozzarella (Garem, Schuck, & Maubois, 2000).

The use of SMP implies the addition of fresh cream or anhydrous milk fat to standardize the fat content of recombined milk. This may result in a different surface-to-volume ratio of the oil-inwater emulsion as a consequence of the average fat globules size of the cream, which is higher than milk (Truong, Palmer, Bansal, & Bhandari, 2016). Moreover, the different interaction of fat with the casein network can modify the coagulation process and the texture of the cheese curd (Logan et al., 2014; Michalsky et al., 2003).

Despite the effect of recombined milk on Mozzarella cheese has been evaluated in terms of 57 technological aspects, some functional characteristics (e.g. meltability, stretchability), composition 58 and some basic chemical characteristics of the cheese (Garem et al., 2000; Moiseev et al., 2017; 59 Thompson et al., 1978), poor information is available about the effect of a partial replacement of 60 fresh milk by addition of recombined milk on physico-chemical (with a special regard to water 61 status and dynamics) and rheological properties of high-moisture (HM) Mozzarella cheese. In this 62 study, the dissolution of low-heat SMP was employed to obtain a fixed ratio of 40 g/100 g of fresh 63 milk replacement. The effect of a 40:60 (g/100 g) combined/fresh milk blend on cheese 64 composition, yield, rheological and water-related properties (i.e. water holding capacity and the 65 proton relaxometry by low resolution ¹H NMR) were evaluated after 1 and 10 d of refrigerated 66 storage of the cheese in covering liquid and compared to cheeses made only with fresh milk. 67

68

69 2. Material and methods

70 **2.1 Experimental design**

71 Experimental trials were carried out according to a complete block design. HM Mozzarella were manufactured at the pilot dairy of CREA-ZA (Centro di Ricerca Zootecnia e Acquacoltura, Lodi, 72 Italy). Two different HM Mozzarella were produced using: a 40:60 (g/100 g) mixture of 73 recombined: fresh milk (experimental cheeses); 100 g/100 g fresh milk (control cheese). 74 Recombined milk was prepared from SMP. The 40:60 g/100 g mixture was selected because from 75 the results of a previous study on Crescenza cheese, where other combinations were tested, as it 76 allowed to obtain experimental cheeses having characteristics similar to the fresh ones (Tidona et 77 al., 2020). 78

Experimental and control cheeses were manufactured four times (4 batches/each) in different
weeks. For each batch more than 200 cheese balls were manufactured. To also test the effect of
storage, cheeses were analyzed after one and 10 d of refrigerated storage at 4°C.

82

4

83 **2.2 Cheese making technology**

Fresh milk used for cheesemaking trials was provided by CREA-ZA farm, while low-heat SMP (whey protein nitrogen index of 6.68 mg/g) was supplied by EPI Ingredients (Ancenis, France).

SMP was reconstituted by dissolution in tap water using a tri-blender (S.C.A. Srl, Fiorenzuola d'Arda, Italy) for 30 min. SMP-to-water ratio was established to obtain a protein concentration equivalent to that of fresh milk. About 4 kg of raw milk cream (37 g/100 g of fat) were added to standardize the fat content of reconstituted skim milk to the level measured for fresh milk. Then, 40 kg of fat-standardized, reconstituted milk were mixed to 60 kg of fresh milk (fixed ratio 40 g/100 g :60 g/100 g). Hydration of SMP was completed by overnight storage at 4°C and cream rising was prevented by a 5-min gentle agitation performed every hour.

93 Mozzarella was manufactured according to Francolino et al. (2010), as summartized in Figure 1.

Milk, total drained whey, curd and cheese masses were weighted with a scale (IND690, Mettler
Toledo, Greinfensee, Switzerland) to calculate the cheese yield and component recovery or loss.
Actual cheese yield (Ya) was calculated by dividing the weight of cheese by the weight of milk x
100.

98

99 **2.3 Compositional analyses**

100 Moisture, fat, protein lactose and ashes of milk and drained whey were determined by FT-IR using a MilkoScan FT2 (Foss, Hillerød, Denmark). Moisture and ash content of cheese and stretching 101 water were determined gravimetrically by weighing the residual weight after oven drying at 102 °C 102 (FIL-IDF, 2004) and by dry incineration at 550 °C (FIL-IDF, 1964), respectively. Fat and protein 103 content of cheeses were determined by means of MilkoScan analyses: 25 g of cheese were 104 dissolved into a solution of 125 mL NaOH 0.2 N with 1 g/100 mL of Triton X-100 (Sigma-Aldrich, 105 Saint Louis, USA) at 55 °C for 3 h. Before analyses, the solutions contained into 250 mL flasks 106 were homogenized with an Ultra-Turrax (IKA Werke, Staufen, Germany) at 20,000 rpm per 90 s. 107 Spectra acquisition for each sample was made at 40 ± 1 °C in duplicate. Chemometric models based 108

on Partial Least Squares Regression were built using commercial and pilot-scale produced
Mozzarella cheeses. Residual fat and proteins in stretching water were determined by Gerber (FILIDF, 2018) and Kjeldahl methods (FIL-IDF, 2014), respectively whereas lactose was determined by
HPLC (Bouzas, Kantt, Bodyfelt, & Torres, 1991). Compositional analyses were performed in
triplicate.

114

115 **2.4 Physico-chemical analyses**

pH of the cheese was measured using the Portavo 907 pH meter. Expressible serum (**ES**_{CT}) was measured in triplicate applying a centrifugation method reported by Alinovi & Mucchetti (2020). ES_{CT} (g /100 g) was calculated as the ratio of apparent expressible serum (ES_{CTapp}) weighed using an analytical scale (mod. AR 2140, Ohaus Corporation, New Jersey, USA) to the moisture content (MC), according to the following equation (1):

121
$$ES_{CT}(g/100g) = \frac{ES_{CT_{app}}}{MC} \times 100$$
 (2)

The electrical conductivity of the expressible serum was measured with a Portamess conductometer
(mod. 913, Knick Elektronische, Berlin, Germany) and a TetraCon 325 probe (WTW Xylem
Analytics, Weilheim, Germany).

125

126 **2.5 Rheological analyses**

127 Rheological measurements were performed at a controlled temperature of $25.0 \pm 0.1^{\circ}$ C using an 128 MCR 102 rheometer (Anton Paar, Gratz, Austria); the instrument was equipped with a 25-mm 129 parallel plate sandblasted geometry.

Analyses were performed in quadruplicate as previously reported (Alinovi & Mucchetti, 2020).
Disk-shape samples (thickness 4 mm, diameter 30 mm) were gently portioned from the central part
of Mozzarella cheese using a slicer and a borer. Frequency sweep tests were performed within the
linear viscoelastic region, using a 0.05% constant strain. The frequency-dependence of storage (G')

and loss modulus (G'') were evaluated using power-law equations (Sharma, Munro, Dessev, &Wiles, 2016):

136
$$G' = k'(f)^{n'}$$
 (3)

137
$$G'' = k''(f)^{n''}$$
 (4)

Where k' and k'' coefficients represent the magnitude of G' and G" at a frequency of 1 rad/s and are indicative of the viscoelastic behavior of the material, while the n' and n'' values reflects the dependency of viscoelastic properties on the frequency variation.

141 The ratio between the loss and the storage moduli (G''/G', defined as tan δ) was also calculated.

142

143 **2.6** ¹H low field NMR analyses

144 NMR analyses were performed using a low resolution ¹H NMR spectrometer (the Minispec, 145 Bruker, Massachusetts, USA) with a frequency of 20 MHz and a magnetic field strength of 0.47 T. 146 Temperature during the analyses was set at 25.0 ± 0.1 °C using an external thermostatic bath

147 (Julabo F30, Julabo Labortechnik GmbH, Seelbach, Germany).

Mozzarella cheese samples were cut from the central part of the cheese and transferred into NMR tubes (outer diameter of 10 mm) that were filled up to 10 mm height; to avoid moisture loss during the analysis, the tube was sealed with parafilm®.

¹H T₂ spin–spin relaxation curves were measured with a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence (Meiboom & Gill, 1958), by performing sixteen scans for each replication, with a RD of 3 s (> 5 ¹H T₁), an interpulse spacing (τ) of 40 µs and 16,000 data points.

¹H T₂ curves were analyzed as quasi-continuous distributions of relaxation times using UPENWin
 software (Alma Mater Studiorum, Bologna, Italy, Borgia, Brown, & Fantazzini, 1998, 2000).
 Default values for all UPEN settings parameters were used except for the LoXtrap parameter that
 was set to 1 to avoid extrapolation of relaxation times shorter than the first experimental point. T₁

and T₂ relaxation curves were also fitted with multiexponential models using Sigmaplot, v.10
(Systat Software Inc., USA) according to Gianferri, Maioli, Delfini, & Brosio (2007), as follows:

160
$$A_2(t) = L_2 + \sum_i A_{2(i)} \cdot e^{-\tau/T_{2(i)}}$$
 (5)

161 Where $A_2(t)$ is T₂ amplitude exponential function, $T_{2(i)}$ is the spin–spin relaxation time of 162 component *i*, $A_{2(i)}$ is the spin–spin signal intensity of component *i*, and the constant, L_2 is the 163 intersect of the polynomial function and represent the instrumental noise of the measurements. Each 164 sample was analyzed in quadruplicate.

165

166 **2.7 Statistical analyses**

167 To test the effect of the type of milk used for Mozzarella manufacture on milk, whey, and stretching168 water a one-way ANOVA was performed.

169 To evaluate the main effect of the type of milk used (fresh or recombined milk) on Mozzarella

170 cheesemaking, the storage time at 4°C (1, 10 days), and the significance of their interactions, split-

171 plot ANOVA models were created for all the parameters evaluated according to Alinovi, Rinaldi, &

172 Mucchetti (2018). The batch of cheese was used as the blocking factor of the models.

173 Post hoc tests were performed by Tukey's honest significant differences test ($\alpha = 0.05$) when 174 significant main effects and interactions were found.

Pearson's correlation coefficients (r) were also calculated to find relationships among the evaluated
variables. The univariate ANOVA analyses were performed using PRC GLM of SAS (SAS Inst.
Inc., NC, USA), while the correlations among variables were performed using SPSS v.26 (IBM,
Armonk, USA).

179

180 **3. Results and discussion**

181 **3.1** Compositional and physico-chemical analysis

182 The composition of milk, whey, stretching water, and curd is shown in **Table 1**. The protein, fat, 183 and moisture content of the two types of milk used for cheesemaking were not significantly

different (P>0.05). To obtain recombined milk with the same protein content of fresh milk (37 184 g/100 g protein on defatted dry matter), a higher amount of SMP (34 g/100 g protein on dry matter) 185 had to be dissolved, leading to a significant (P<0.001) increase of lactose content compared to fresh 186 milk. Consequently, the lactose content in drained whey and in stretching water of experimental 187 cheeses was significantly (P<0.001 and P<0.05, respectively) higher than control cheeses (Table 1). 188 Fat loss in the drained whey during cheesemaking was significantly (P<0.05) higher ($\sim +0.7\%$) in 189 control than experimental cheeses (Table 1). As the average fat globules size of fresh cream used 190 for recombined milk standardization is generally higher than that of fresh milk (Truong et al., 191 2016), it may be hypothesized that bigger fat globules possibly derived from cream were more 192 easily retained in the coagulum network of experimental cheeses. The moisture content of 193 experimental whey was significantly (P<0.001) lower than that of the control. 194

Fat loss in stretching water in experimental cheeses was significantly (P<0.05) higher than in control cheeses (**Table 1**). This difference could be due to a weaker structure of experimental curd that may be less efficient in entrapping the fat globules during stretching.

No significant differences (P>0.05) in moisture, fat and protein content between experimental and control cheeses were observed (**Table 2**). The ash content of experimental cheeses at 1 d storage was significantly (P<0.05) higher than that of control cheeses at 10 d. As Italian-style Mozzarella was stored in covering liquid with 0.4 g/100 g of NaCl (**Figure 1**), a migration of solutes (e.g. salts, organic acids) during storage (Ghiglietti et al., 2004) may be responsible for the encountered ash content variation.

Also an increase of 0.1 pH units was observable during the storage time of both control and experimental cheeses, due to the migration of the citric acid from the cheese to the covering liquid (Ghiglietti et al., 2004).

207 Concerning the water holding capacity of the cheeses, no significant differences (P>0.05) of the 208 percentage of ES_{CT} were observed. Conversely, electrical conductivity values of expressible serum 209 in experimental cheeses were significantly (P<0.05) higher than in control cheeses (**Table 2**). Based on the opinion and perception of the authors, no sensory differences were perceivable between the cheeses, in terms of texture (e.g. 'powdery or sandy' defects), taste, flavor and color. In particular, the experimental cheese was not characterized by the presence of off-flavors or off-tastes (e.g. oxidized tastes and flavors) that may derive from the utilization of skim milk powders.

214

215 **3.2** Cheesemaking yield and recovery of milk constituents

The cheese yield and the recoveries of total solids, fat and proteins of Mozzarella cheeses are reported in **Table 3.** The use of recombined or fresh milk did not cause a significant variation of the actual yield of the cheese (P>0.05). The actual yield and component recoveries were found to be in accordance with previous data on HM Mozzarella cheese (Francolino, Locci, Ghiglietti, Iezzi, & Mucchetti, 2010).

221 Despite the loss of proteins in the stretching water was higher in control than in experimental (P 222 <0.05) cheese, control cheeses showed a significantly (P<0.05) higher recovery of total solids than 223 experimental cheeses. Accordingly, the loss of total solids in whey (P<0.01) and fat in stretching 224 water (P<0.05) during cheesemaking of experimental cheeses were significantly higher than control 225 cheeses.

226

227 **3.3 Rheological properties**

Rheological curves can be observed in **Figures 2A-C**. It can be observed that the different type of milk (recombined or fresh milk) and the storage times influenced the measured rheological parameters.

An increase of storage time (from 1 to 10 d of storage) caused a significant decrease of k' and k''(P<0.05) in control cheeses (**Table 4**). The decrease of the G' and G'' for control cheeses can be due to proteolytic phenomena mainly determined by plasmin activity; Lamichhane, Sharma, Kennedy, Kelly, & Sheehan, (2019) highlighted the breakdown of β -casein in low-moisture Mozzarella stored for 20 d in refrigerated conditions period at 4°C, probably caused by plasmin

activity. The appearance of γ -case in and the possible activity of plasmin have also been reported for 236 237 Italian HM Mozzarella (Alinovi, Wiking, Corredig, & Mucchetti, 2020; Faccia, Gambacorta, Natrella, & Caponio, 2019). Moreover, also the calcium content decrease and the equilibrium 238 239 variation from its colloidal to soluble form are usually associated to a lowering of the para-casein network and a reduction of cheese firmness or rheological elasticity (Faccia, Angiolillo, 240 Mastromatteo, Conte, & Del Nobile, 2013; Feeney, Guinee, & Fox, 2002; Guinee, Feeney, Auty, & 241 Fox, 2002; Kern, Bähler, Hinrichs, & Nöbel, 2019; Kern, Weiss, & Hinrichs, 2018). On the 242 contrary, the type of milk did not show a significant effect on k' and k'' values (P>0.05). 243

The frequency dependence of rheological parameters that can be estimated from n' and n'' values reported in equations (3) and (4) can be useful to describe the type of bonding of the structural elements present in the matrix (Sharma et al., 2016). The samples were characterized by a relatively low frequency dependence (as n' and n'' were always lower than 0.20) that was in accordance with previous results reported for Italian Mozzarella commercial cheeses (Alinovi & Mucchetti, 2020; Alinovi, Wiking, et al., 2020), indicating the presence of strong and cross-linked gels with permanent covalent bonds (Sharma et al., 2016).

For the experimental cheese, n' and n'' showed significant differences related to the storage time 251 (P<0.05); in general, the rheological indexes increased at longer storage times, indicating a slight 252 253 increase of the frequency dependence of rheological moduli and a consequently decrease of the strength of the protein network. This increase was significant in experimental cheeses for both n' 254 and n'' values (P<0.05). At 1 d of storage, n' and n'' in experimental cheeses were also 255 significantly lower than in control cheeses, meaning that the cheese matrix obtained with 256 recombined milk was more structured. Because of the drying process, milk reconstituted from SMP 257 may be characterized by a higher extent of whey proteins aggregation or by possible interaction 258 259 between whey protein and casein, that could cause an increase in the gel structural strength of the cheese (Sołowiej, Cheung, & Li-Chan, 2014). Also, the total and colloidal calcium contents can 260 influence the network structure, as their increase can contribute to the formation of a more 261

organized and stronger gel structure (Ong, Dagastine, Kentish, & Gras, 2013). Bähler, Kunz, & Hinrichs (2016) observed a significant increase of textural hardness in Mozzarella cheese stretched with a hot brine containing 0.02 g/100 g CaCl₂. However, in our work a different calcium content of the cheeses cannot be hypothesized, as the ash content of the cheeses was not significantly different (P>0.05). However, *n*' and *n*'' were negatively correlated with the ash content of the cheeses (r = -0.709 and -0.732, respectively).

Because of differences in k', k'' and n', n'' parameters, tand showed differences among samples 268 that were dependent of the considered angular frequency. In particular, $tan\delta$ was higher in control 269 cheeses than in experimental cheeses in the low frequency range (0.1-10 rad/s) (Figure 2C); as 270 271 reported in **Table 4**, $\tan \delta$ at 5.06 rad/s showed a significant difference (P<0.05) between control and experimental cheeses, as experimental exhibited a higher elastic-to-viscous response than control 272 273 cheeses. On the contrary, in the high frequency range (10-100 rad/s), control at 0 d was similar to 274 experimental cheeses. A similar behavior was also observed by Bancalari et al., (2020) in caciottatype cheese. It is known that a viscoelastic matrix, when subjected to low-frequency deformations, 275 276 reacts as a liquid-like material, while at high-frequency deformations behaves more like a solid-like 277 material (Bähler, Nägele, Weiss, & Hinrichs, 2016). From a physical point of view, the casein micellar system has been frequently modelled as a nano-material or colloidal gel (Bähler, Back, & 278 279 Hinrichs, 2015; Nöbel, Weidendorfer, & Hinrichs, 2012). Gillies (2019) theorized the cheese rheological system as a series of core-shell hard nano particles assembled from calcium and protein 280 in a semi-poor solvent. According to this theory, slower relaxation frequencies may involve 281 localized shear induced rearrangements (colloquially named "hops") and eventually cause the 282 particle to get trapped in deeper energy states (Pitkowski, Durand, & Nicolai, 2008). In the high 283 frequency region, according to Zwanzig & Mountain (1965) the dominating term over the 284 285 rheological moduli is the volume fraction occupied by the proteins. As in the present study the protein volume fraction of the cheeses is expected to be constant and relatively low (Gillies, 2019), 286 the high-frequency rheological moduli are expected to be very similar. Thus, in HM Mozzarella 287

cheese, low-frequency structural transformations dominate since there are many sites wheretransformations can occur.

290

291 **3.4** ¹H NMR results

Spin–spin relaxation curves can be observed in **Figure 3**. HM Mozzarella cheese samples were characterized by the presence of four 1 H T₂ populations (A, B, C, D) in accordance with previous observations of Mozzarella cheeses made with buffalo and cow milk (Alinovi, Corredig, et al., 2020; Gianferri, D'Aiuto, Curini, Delfini, & Brosio, 2007; Gianferri, Maioli, et al., 2007).

The shortest relaxing component A (Figure 3), that relaxed in the range between 0.1 and 5.0 ms and 296 it peaked at very short relaxation times (~0.2 ms), can be attributed to the 1 H of water strongly 297 bound to the casein structure (Gianferri, Maioli, et al., 2007) and/or to other non-fat solids (i.e. 298 lactose) as solvation water; in facts, the relative abundance of ¹H T₂ component A was found to be 299 correlated to the protein content of the cheeses (r=0.633). The relative abundance of this component 300 in experimental was significantly higher (P<0.05) than in control cheese, despite the difference was 301 302 slight (Table 5). This difference can be due to the whey proteins denaturation and/or aggregation 303 that may occur during SMP processing and continue during heat treatments of recombined milk, can improve the structuration of the final gel network and thus reduce the molecular mobility of 304 water protons (Goetz & Koehler, 2005). This observation can be in accordance with rheological 305 306 findings. Interestingly, relative abundance of ¹H T₂ component A was strongly correlated with k'and k'' (r=0.762, 0.764, respectively), and negatively correlated with tan δ (r=-0.721). 307

Moreover, also the higher lactose content of recombined milk compared to the fresh one can play a role in determining the amount of bound water; although not measured, this difference may lead also to a different lactose content in the cheeses. When it is present in an aqueous media, lactose is hydrated; solvation water is bond to lactose by means of hydrogen bonds (Vilén & Sandström, 2013). Higher lactose contents have been reported to improve the fast diffusive exchange of protons in reconstituted milk proteins systems (Le Dean, Mariette, Lucas, & Marin, 2001), and could be the 314 cause of the increase in the solvation water measured in the experimental cheeses. Wijayasinghe, Vasiljevic, & Chandrapala (2015) reported that increasing lactose concentrations (in the 15-50 315 g/100 g range) reduced the amount of water bound to lactose. Conversely, higher lactose contents 316 317 were responsible for the increase in attractive forces between lactose and water molecules (Wijayasinghe et al., 2015). It is important to note that the difference of lactose contents observed in 318 319 this study is small if compared to that evaluated by Wijayasinghe et al. (2015) and may not explain 320 alone the difference in the water status of the cheeses. Also, whey proteins denaturation and/or aggregation that may occur during SMP processing and continue during heat treatments of 321 recombined milk, can improve the structuration of the final gel network and thus reduce the 322 molecular mobility of water protons . This observation can be in accordance with rheological 323 findings. Interestingly, relative abundance of ${}^{4}H$ T₂ component A was strongly correlated with k'324 and k'' (r=0.762, 0.764, respectively), and negatively correlated with tan δ (r=-0.721). 325

On the contrary, relaxation times and relative abundances of populations B, C and D, attributed to protons of water trapped in the protein meshes and to the lipids, and to a quote of expressible serum water of buffalo Mozzarella cheese, respectively (Gianferri, Maioli, et al., 2007), showed no significant differences (P>0.05) between the cheeses (**Table 5**). These populations were largely overlapped (**Figure 3**), in accordance with previous observations on cow Mozzarella (Alinovi, Corredig, et al., 2020).

Interestingly, relaxation times of components B, C and D were strongly negatively correlated with k' and k'' (r ~ -0.80, -0.60, respectively).

Experimental cheeses were characterized by a higher relative percentage of population B than control cheeses (**Table 5**); this difference was found to be at the limit of significance (P=0.08).

336

337 4. Conclusions

A blend of 40 g/100 g of reconstituted milk from low-heat skim milk powders and 60 g/100 g of fresh milk enabled to produce Mozzarella cheeses with comparable composition and actual yield

with respect to those obtained with only fresh milk, despite some differences in cheesemaking 340 341 performances and cheese properties were observed. The use of reconstituted milk in cheesemaking caused a higher fat loss during curd stretching. Rheological and water-status differences measured 342 with ¹H low-field NMR were detected in the final products, as Mozzarella obtained with 343 reconstituted milk showed a stronger gel structure and higher percentage of bound water than 344 Mozzarella produced with fresh milk. Despite these differences, the study demonstrated the 345 applicability of a blend with 40 g/100 g of reconstituted milk from low-heat skim milk powders to 346 obtain Mozzarella cheese without major changes in product quality. 347

348

349 **Conflicts of interest**

350 The authors declare no conflicts of interest.

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355 **References**

Alinovi, M., Corredig, M., Mucchetti, G., & Carini, E. (2020). Water status and dynamics of high-

357 moisture Mozzarella cheese as affected by frozen and refrigerated storage. *Food Research*

358 *International*, *137*, 109415.

Alinovi, M., & Mucchetti, G. (2020). Effect of freezing and thawing processes on high-moisture
 Mozzarella cheese rheological and physical properties. *LWT - Food Science and Technology*,
 124, 109137.

Alinovi, M., Rinaldi, M., & Mucchetti, G. (2018). Spatiotemporal characterization of texture of
Crescenza cheese, a soft fresh Italian cheese. *Journal of Food Quality*, 2018, 1–8.

Alinovi, M., Wiking, L., Corredig, M., & Mucchetti, G. (2020). Effect of frozen and refrigerated

storage on proteolysis and physico-chemical properties of high-moisture citric Mozzarella
cheese. *Journal of Dairy Science*, *103*(9), 7775–7790.

Bähler, B., Back, R., & Hinrichs, J. (2015). Evaluation of oscillatory and shear strain behaviour for
 thermo-rheological plasticisation of non-ripened cheese curd: Effect of water, protein, and fat.
 International Dairy Journal, 46, 63–70.

- Bähler, B., Kunz, A., & Hinrichs, J. (2016). Hot brining of pasta filata cheese: effect of sodium and
 calcium chloride on composition, yield, and hardness. *Dairy Science and Technology*, *96*(5),
 703–714.
- Bähler, B., Nägele, M., Weiss, J., & Hinrichs, J. (2016). Temperature and Time-Dependent

Relaxation of Compressed Cheese Curd Cubes: Effect on Structuring of Pasta-Filata Cheese.

Journal of Texture Studies, 47(1), 58–67.

- Bancalari, E., Montanari, C., Levante, A., Alinovi, M., Neviani, E., Gardini, F., & Gatti, M. (2020).
 Lactobacillus paracasei 4341 as adjunct culture to enhance flavor in short ripened Caciottatype cheese. *Food Research International*, *135*, 109284.
- Borgia, G. C., Brown, R. J. S., & Fantazzini, P. (1998). Uniform-Penalty Inversion of
 Multiexponential Decay Data. *Journal of Magnetic Resonance*, *132*(1), 65–77.
- Borgia, G. C., Brown, R. J. S., & Fantazzini, P. (2000). Uniform-Penalty Inversion of
- Multiexponential Decay Data: II. Data Spacing, T2 Data, Systematic Data Errors, and
 Diagnostics. *Journal of Magnetic Resonance*, *147*(2), 273–285.
- Bouzas, J., Kantt, C. A., Bodyfelt, F., & Torres, J. A. (1991). Simultaneous determination of sugars
 and organic acids in Cheddar cheese by High- Performance Liquid Chromatography. *Journal of Food Science*, *56*(1), 276–278.
- Faccia, M., Angiolillo, L., Mastromatteo, M., Conte, A., & Del Nobile, M. A. (2013). The effect of
- incorporating calcium lactate in the saline solution on improving the shelf life of Fiordilatte
 cheese. *International Journal of Dairy Technology*, 66(3), 373–381.
- 390 Faccia, M., Gambacorta, G., Natrella, G., & Caponio, F. (2019). Shelf life extension of Italian

- 391 mozzarella by use of calcium lactate buffered brine. *Food Control*, *100*, 287–291.
- Feeney, E. P., Guinee, T. P., & Fox, P. F. (2002). Effect of pH and Calcium Concentration on
 Proteolysis in Mozzarella Cheese. *Journal of Dairy Science*, 85(7), 1646–1654.
- 394 FIL-IDF. (1964). Determination of the ash content of processed cheese products Standard FIL-IDF
- 395 27. Brussels, Belgium: International Dairy Federation.
- FIL-IDF. (2004). Cheese and processed cheese: Determination of the total solids content, IDF
 Standard 4a. Brussels, Belgium: International Dairy Federation.
- 398 FIL-IDF. (2014). Milk and milk products–Determination of nitrogen content–Part 1: Kjeldahl
- 399 principle and crude protein calculation. Brussels, Belgium: International Dairy Federation.
- 400 FIL-IDF. (2018). Milk. Determination of Fat Content. Gerber Butyrometers. Brussels, Belgium:
 401 International Dairy Federation.
- 402 Francolino, S., Locci, F., Ghiglietti, R., Iezzi, R., & Mucchetti, G. (2010). Use of milk protein
- 403 concentrate to standardize milk composition in Italian citric Mozzarella cheese making. *LWT* 404 *Food Science and Technology*, *43*(2), 310–314.
- Garem, A., Schuck, P., & Maubois, J. L. (2000). Cheesemaking properties of a new dairy-based
- 406 powder made by a combination of microfiltration and ultrafiltration. *Lait*, 80(1), 25–32.
- 407 Ghiglietti, R., Santarelli, S., Francolino, S., Perrone, A., Locci, F., & Mucchetti, G. (2004).
- 408 Evoluzione del contenuto di zuccheri, acidi lattico, citrico e calcico durante la produzione e la 409 conservazione di mozzarella: confronto tra Mozzarella di Bufala Campana e mozzarella di
- 410 vacca. *Scienza e Tecnica Lattiero Casearia*, 55(4), 227–249.
- 411 Gianferri, R., D'Aiuto, V., Curini, R., Delfini, M., & Brosio, E. (2007). Proton NMR transverse
- relaxation measurements to study water dynamic states and age-related changes in Mozzarella
- di Bufala Campana cheese. *Food Chemistry*, *105*(2), 720–726.
- 414 Gianferri, R., Maioli, M., Delfini, M., & Brosio, E. (2007). A low-resolution and high-resolution
- 415 nuclear magnetic resonance integrated approach to investigate the physical structure and
- 416 metabolic profile of Mozzarella di Bufala Campana cheese. *International Dairy Journal*,

417 *17*(2), 167–176.

- Gillies, G. (2019). Predictions of the shear modulus of cheese, a soft matter approach. *Applied Rheology*, *29*(1), 58–68.
- 420 Goetz, J., & Koehler, P. (2005). Study of the thermal denaturation of selected proteins of whey and
- 421 egg by low resolution NMR. *LWT Food Science and Technology*, *38*(5), 501–512.
- 422 Guinee, T. P., Feeney, E. P., Auty, M. A. E., & Fox, P. F. (2002). Effect of pH and Calcium
- 423 Concentration on Some Textural and Functional Properties of Mozzarella Cheese. *Journal of*424 *Dairy Science*, 85(7), 1655–1669.
- 425 Kern, C., Bähler, B., Hinrichs, J., & Nöbel, S. (2019). Waterless single screw extrusion of pasta-
- filata cheese: Process design based on thermo-rheological material properties. *Journal of Food Engineering*, 260, 58–69.
- Kern, C., Weiss, J., & Hinrichs, J. (2018). Additive layer manufacturing of semi-hard model cheese:
 Effect of calcium levels on thermo-rheological properties and shear behavior. *Journal of Food Engineering*, 235, 89–97.
- 431 Lamichhane, P., Sharma, P., Kennedy, D., Kelly, A. L., & Sheehan, J. J. (2019). Microstructure and
- 432 fracture properties of semi-hard cheese: Differentiating the effects of primary proteolysis and
 433 calcium solubilization. *Food Research International*, *125*, 108525.
- 434 Le Dean, A., Mariette, F., Lucas, T., & Marin, M. (2001). Assessment of the State of Water in
- 435 Reconstituted Milk Protein Dispersions by Nuclear Magnetic Resonance (NMR) and
- 436 Differential Scanning Calorimetry (DSC). *LWT Food Science and Technology*, *34*(5), 299–
- 437 305.
- 438 Logan, A., Day, L., Pin, A., Auldist, M., Leis, A., Puvanenthiran, A., & Augustin, M. A. (2014).
- 439 Interactive Effects of Milk Fat Globule and Casein Micelle Size on the Renneting Properties of
 440 Milk. *Food and Bioprocess Technology*, 7(11), 3175–3185.
- 441 Martin, G. J. O., Williams, R. P. W., & Dunstan, D. E. (2007). Comparison of casein micelles in
- raw and reconstituted skim milk. *Journal of Dairy Science*, *90*(10), 4543–4551.

- McMahon, D. J., Fife, R. L., & Oberg, C. J. (1999). Water Partitioning in Mozzarella Cheese and
 Its Relationship to Cheese Meltability. *Journal of Dairy Science*, 82(7), 1361–1369.
- Meiboom, S., & Gill, D. (1958). Modified Spin- Echo Method for Measuring Nuclear Relaxation
 Times. *Review of Scientific Instruments*, 29(8), 688–691.
- 447 Michalsky, M.-C., Gassi, J.-Y., Famelart, M.-H., Leconte, N., Camier, B., Michel, F., & Briard, V.
- (2003). The size of native milk fat globules affects physico-chemical and sensory properties. *Lait*, 83, 131–143.
- 450 Moiseev, N., Suchkova, E., & Iakovchenko, N. (2017). Possibility of using reconstituted milk in
- 451 manufacture of cheese with cheddaring and cheese curd stretching. *Agronomy Research*, *15*(2),
 452 1358–1368.
- 453 Mucchetti, G., Pugliese, A., & Paciulli, M. (2017). Characteristics of Some Important Italian
- 454 Cheeses. In R. M. S. da Cruz & M. M. C. Vieira (Eds.), *Mediterranean Foods: Composition*455 *and Processing* (pp. 1–34). New York, USA: CRC Press.
- Nöbel, S., Weidendorfer, K., & Hinrichs, J. (2012). Apparent voluminosity of casein micelles
 determined by rheometry. *Journal of Colloid and Interface Science*, *386*(1), 174–180.
- 458 Ong, L., Dagastine, R. R., Kentish, S. E., & Gras, S. L. (2013). The effect of calcium chloride
- addition on the microstructure and composition of Cheddar cheese. *International Dairy Journal*, *33*(2), 135–141.
- Pitkowski, A., Durand, D., & Nicolai, T. (2008). Structure and dynamical mechanical properties of
 suspensions of sodium caseinate. *Journal of Colloid and Interface Science*, *326*(1), 96–102.
- Sharma, P., Munro, P. A., Dessev, T. T., & Wiles, P. G. (2016). Shear work induced changes in the
 viscoelastic properties of model Mozzarella cheese. *International Dairy Journal*, *56*, 108–118.
- 465 Sołowiej, B., Cheung, I. W. Y., & Li-Chan, E. C. Y. (2014). Texture, rheology and meltability of
- processed cheese analogues prepared using rennet or acid casein with or without added whey
 proteins. *International Dairy Journal*, *37*(2), 87–94.
- 468 Thompson, M., Flanagan, J., Brower, D., & Gyuricsek, D. (1978). The manufacture of Mozzarella

469 cheese from reconstituted nonfat dry milk solids. In *Proceedings from the 15th Annual*

470 *Marschall Invitational Italian Cheese Seminar 1977.* Madison, WI, USA.

- 471 Tidona, F., Francolino, S., Ghiglietti, R., Locci, F., Brusa, G., Alinovi, M., ... Giraffa, G. (2020).
- 472 Application of recombined milk to produce Crescenza cheese in laboratory mini-
- 473 cheesemaking : implications on technology and sensory properties. *Foods*, 9(7), 928.
- 474 Truong, T., Palmer, M., Bansal, N., & Bhandari, B. (2016). Effect of Milk Fat Globule Size on the
- 475 *Physical Functionality of Dairy Products*. (T. Truong, M. Palmer, N. Bansal, & B. Bhandari,
 476 Eds.). Berlin, Germany: Springer.
- Vilén, E. M., & Sandström, C. (2013). NMR study on the interaction of trehalose with lactose and
 its effect on the hydrogen bond interaction in lactose. *Molecules*, *18*(8), 9735–9754.
- Wijayasinghe, R., Vasiljevic, T., & Chandrapala, J. (2015). Water-lactose behavior as a function of
 concentration and presence of lactic acid in lactose model systems. *Journal of Dairy Science*,
 98(12), 8505–8514.
- 482 Zwanzig, R., & Mountain, R. D. (1965). High-frequency elastic moduli of simple fluids. The
- 483 *Journal of Chemical Physics*, *43*(12), 4464–4471.
- 484

485 **Figure Captions**

486 **Figure 1.** HM Mozzarella cheese making process applied in this study.

487

Figure 2. Storage modulus (G') (**A**), loss modulus (G'') (**B**) and tangent of the phase angle (tanð) (**C**) frequency-dependent rheological curves measured at 25°C (n=4) of HM Mozzarella cheeses manufactured with fresh milk (control cheese) and a blend of 40 g/100 g reconstituted skim milk and fresh milk (experimental cheese) at 1 and 10 d of refrigerated storage (4°C). (•) control at 1 d of storage; (**□**) control at 10 d of storage; (•) experimental at 1 d of storage; (**□**) experimental at 10 d of storage.

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Figure 3. ¹H T₂ relaxation curves of HM Mozzarella cheeses manufactured with fresh milk (control cheese) and a blend of 40 g/100 g reconstituted skim milk and fresh milk (experimental cheese) at 1 and 10 d of refrigerated storage (4°C). (____) control at 1 d of storage; (____) control at 10 d of storage; (____) experimental at 1 d of storage; (____) experimental at 10 d of storage. **Table 1.** Composition of milk, whey, <u>eurd</u> and stretching water used/obtained in HM Mozzarella cheese manufacturing, expressed as a function of the type of milk used: fresh milk (<u>MFMcontrol</u> <u>cheese</u>) or a blend of 40% (<u>w/w) g/100 g</u> reconstituted milk (<u>MRMexperimental cheese</u>) and fresh milk. Results are expressed as means ± standard deviations (n=4).

		Treatment		
		MFMControl	MRMExperimental	
Substrate	Component ($g/100 g \% / w/w$)	cheese	cheese	Sign.
	moisture	87.09 ± 0.11	87.03 ± 0.23	
milk	fat	3.84 ± 0.16	3.64 ± 0.14	
IIIIIK	protein	3.36 ± 0.06	3.34 ± 0.06	
	lactose	4.70 ± 0.06	5.05 ± 0.06	***
	ash	2.32 ± 0.21	2.38 ± 0.22	
	moisture	92.57 ± 0.03	92.21 ± 0.11	***
whow	fat	0.45 ± 0.02	0.38 ± 0.02	*
wney	protein	0.92 ± 0.02	0.93 ± 0.02	
	lactose	4.71 ± 0.01	5.10 ± 0.05	***
	ash	n.d.	n.d.	
	moisture	94.27 ± 0.22	92.91 ± 1.96	
stretching water	fat	2.01 ± 0.31	2.85 ± 0.45	*
	protein	0.46 ± 0.13	0.21 ± 0.15	
	lactose	0.84 ± 0.02	0.94 ± 0.05	*
	ash	2.36 ± 0.15	2.10 ± 0.41	

* P<0.05, ** P<0.01, *** P<0.001

Table 2. Composition (% w/w)(g/100 g) and pH values of HM Mozzarella cheeses manufactured using fresh milk (MFMcontrol cheese) or a blend of 40% (w/w) g/100 g reconstituted milk (MRM) and fresh milk (experimental cheese), at 1 and 10 d of refrigerated storage at 4°C. Results are expressed as means ± standard deviations (n=4).

Treatment	Storage (d)	Moisture	Fat	Protein	Ash $(\pi/100,\pi^{0})$	ES_{CT}	Electrical	
		(<u>g/100 g</u> %-	(g/100 g)	$\frac{(\% W/W}{(\% W/W)}$	$(\underline{g}/100 \ \underline{g}^{\text{w}})$	$(g/100 g^{-1})$	conductivity	рн
		₩/₩)	% w/w)	<u>g/100 g</u>)	₩/₩)	₩/₩)	(IIIS)	
MFMControl cheese	1	63.3 <u>ª</u>	17.9 <u>ª</u>	16.4 <u>ª</u>	1.61 ^{ab}	41.5ª	11.5°	6.15 ^b
	1	± 2.5	± 1.3	± 1.4	± 0.01	± 13.9	± 1.3	± 0.02
	10	64.8ª	17.7 <u>ª</u>	15.6 <u>ª</u>	1.54 ^b	43.8ª	12.2 ^{bc}	6.24 ^a
		± 3.2	± 1.5	± 1.7	± 0.08	± 10.9	± 3.0	± 0.05
	1	63.8 <mark>ª</mark>	17.1 <u>a</u>	16.5ª	1.96 ^a	41.5ª	14.4 ^a	6.14 ^b
MRMExperi	1	± 1.7	± 1.2	± 0.7	± 0.26	± 11.2	± 3.2	± 0.03
mental cheese	10	64.1ª	17.4ª	16.3ª	1.66 ^{ab}	41.4 ^ª	14.3 ^{ab}	6.23 ^a
	10	± 2.3	± 1.5	± 1.2	± 0.10	± 6.3	± 2.8	± 0.05

^{a-b} Means with different lowercase letters within the same column indicate a significant difference

(P<0.05)

Table 3. Actual yield and recovery/<u>loss</u> of milk components of HM Mozzarella cheeses manufactured using fresh milk (<u>MFMcontrol cheese</u>) or a blend of 40% (<u>w/w</u>) <u>g/100 g</u> reconstituted milk (<u>MRMexperimental cheese</u>) and fresh milk. Results are expressed as means ± standard deviations (n=4).

		Treatment	Sian	
Parameter		MFMControl	MRMExperimental	Sign
		cheese	cheese	•
Actual yield		15.1 ± 0.6	14.6 ± 0.4	
	whey	47.4 ± 0.5	49.5 ± 0.6	**
Recovery <u>/loss</u> of total	stretching			
solids	water	8.3 ± 0.3	10.2 ± 2.8	
	cheese	42.6 ± 1.3	40.3 ± 1.0	*
	whey	9.7 ± 0.7	9.0 ± 0.6	**
	stretching			
Recovery <u>/loss</u> of fat	water	9.7 ± 0.9	14.7 ± 2.7	*
	cheese	70.6 ± 5.3	68.7 ± 2.1	
	whey	22.5 ± 0.6	23.0 ± 0.2	
	stretching			
Recovery <u>/loss</u> of proteins	water	2.9 ± 0.6	1.2 ± 0.8	*
	cheese	73.3 ± 2.6	72.4 ± 1.6	

* P<0.05; ** P<0.01; *** P<0.001

Table 4. Power law rheological parameters (k', n', k'', n'') calculated by equation 3 and 4, and tan δ reported at 5.06 rad/s of HM Mozzarella cheeses manufactured using fresh milk (MFMcontrol cheese) and a blend of 40% (w/w) g/100 g reconstituted skim milk and fresh milk (MRMexperimental cheese) at 1 and 10 d of refrigerated storage (4°C). Results are expressed as means ± standard deviations (n=4).

Treatment	Storage time (d)	k' (Pa)	n' (-)	k" (Pa)	n" (-)	Tanδ(5.06 rad/s)
MFMCon trol cheese	1	$\begin{array}{l}9208^{a}\\\pm\ 4692\end{array}$	$0.196^{a} \pm 0.007$	$\begin{array}{c} 3249^a \\ \pm \ 1593 \end{array}$	$\begin{array}{c} 0.149^{ab} \\ \pm \ 0.010 \end{array}$	$0.306^{b} \pm 0.009$
	10	6120 ^b ± 3155	$0.199^{a} \pm 0.007$	2165 ^b ± 1058	$0.163^{a} \pm 0.013$	0.313 ^a ± 0.011
MRMExp erimental cheese	1	$\begin{array}{l} 8386^{ab} \\ \pm \ 2724 \end{array}$	$\begin{array}{c} 0.183^b \\ \pm \ 0.006 \end{array}$	$\begin{array}{l} 2897^{ab} \\ \pm \ 909 \end{array}$	$\begin{array}{c} 0.138^b \\ \pm \ 0.005 \end{array}$	$0.295^{c} \pm 0.007$
	10	$7746^{ab} \\ \pm 2976$	$\begin{array}{c} 0.196^{a} \\ \pm \ 0.006 \end{array}$	$\begin{array}{l} 2650^{ab} \\ \pm 930 \end{array}$	$\begin{array}{c} 0.157^a \\ \pm \ 0.009 \end{array}$	0.300 ^c ± 0.007

Table 5. Percentages and relaxation times estimated from ${}^{1}\text{H}$ T₂ relaxation curves of HM Mozzarella cheeses manufactured with fresh milk (FMcontrol cheese) and a blend of 40% (w/w) g/100 g reconstituted skim milk and fresh milk (RMexperimental cheese) at 1 and 10 d of refrigerated storage (4°C).

Milk <u>Treatment</u>	Storage (d)	pop. A (%)	pop. B (%)	pop. C (%)	pop. D (%)	T_{2A} (ms)	$T_{2B}(ms)$	$T_{2C}(ms)$	T_{2D} (ms)
Control cheeseFM	1	$5.71^{ab} \pm 1.12$	$47.01^{\underline{a}} \pm 1.51$	$30.70^{\underline{a}} \pm 1.65$	$16.58^{\underline{a}} \pm 1.43$	$1.48^{\underline{a}} \pm 0.22$	$23.47^{\underline{a}} \pm 5.60$	$77.66^{\underline{a}} \pm 4.36$	$352.17^{\underline{a}} \pm 31.62$
	10	$5.51^{\rm b}\pm1.04$	$46.88^{\underline{a}} \pm 3.24$	$30.84^{\underline{a}} \pm 1.33$	$16.77^{\underline{a}} \pm 2.45$	$1.45^{\underline{a}} \pm 0.28$	$23.39^{\underline{a}} \pm 4.86$	$77.04^{\underline{a}} \pm 6.61$	$350.51^{\underline{a}} \pm 47.32$
RMExperimental cheese	1	$6.73^{\rm a}\pm1.27$	$49.16^{\underline{a}} \pm 2.81$	$28.71^{\underline{a}} \pm 1.81$	$15.41^{\underline{a}} \pm 0.89$	$1.73^{\underline{a}} \pm 0.85$	$19.83^{a} \pm 1.46$	$76.69^{\underline{a}} \pm 2.88$	$387.48^{\underline{a}} \pm 23.45$
	10	$5.74^{ab}\pm0.40$	$47.41^{\underline{a}} \pm 2.9$	$30.23^{\underline{a}} \pm 1.89$	$16.62^{\underline{a}} \pm 1.57$	$1.48^{\underline{a}} \pm 0.36$	$20.81^{\underline{a}} \pm 1.59$	$76.34^{\underline{a}} \pm 3.42$	$374.19^{\underline{a}} \pm 43.94$







1 Credit Author Statement

2 Flavio Tidona: Conceptualization, Methodology, Investigation, Writing - Review & Editing.

- 3 Marcello Alinovi: Methodology, Software, Validation, Formal analysis, Investigation, Data Curation,
- 4 Writing Original Draft, Visualization. Salvatore Francolino: Conceptualization, Methodology,
- 5 Investigation. Gianluca Brusa: Methodology, Investigation. Roberta Ghiglietti: Methodology,
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- 7 Resources, Conceptualization, Supervision, Writing Review & Editing. Giorgio Giraffa: Resources,
- 8 Supervision, Project administration, Funding acquisition, Writing Review & Editing.

AUTHOR DECLARATION

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

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On behalf of my co-authors,

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