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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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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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 ± 0.45 g/100 g and 2.01 ± 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 tanδ 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.

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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

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DEGLI ALIMENTI E DEL FARMACO

Response to reviewers

Manuscript LWT-D-20-04471

entitled

Impact of reconstituted skim milk powders on high moisture Mozzarella cheese: rheological and water-related 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~~%~~ g/100 g recombined ~~skim~~ milk ~~powders~~ and fresh milk were used to manufacture Mozzarella
- Cheese made with recombined and fresh milk showed ~~similar~~ comparable composition and yield ~~to fresh one~~
- ~~R~~Recombined milk cheese showed ~~promoted~~ a more organized protein network than fresh ~~milk~~ones
- Recombined milk increased the fraction of ~~solvation~~ bound water ~~if compared to fresh one~~ in Mozzarella cheese

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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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 ± 0.45 g/100 g and 2.01 ± 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 tanδ 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.

Keywords:

Skim milk powder; Recombined milk; Cheesemaking; ^{1}H NMR; Food rheology

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 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 reduces the fat content of the cheese, due to the unavoidable fat losses in the stretching water (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, 2020; McMahon, Fife, & Oberg, 1999). These properties can be severely modified during the 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-in-water 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 technological aspects, some functional characteristics (e.g. meltability, stretchability), composition and some basic chemical characteristics of the cheese (Garem et al., 2000; Moiseev et al., 2017; Thompson et al., 1978), poor information is available about the effect of a partial replacement of fresh milk by addition of recombined milk on physico-chemical (with a special regard to water status and dynamics) and rheological properties of high-moisture (HM) Mozzarella cheese. In this study, the dissolution of low-heat SMP was employed to obtain a fixed ratio of 40 g/100 g of fresh milk replacement. The effect of a 40:60 (g/100 g) combined/fresh milk blend on cheese composition, yield, rheological and water-related properties (i.e. water holding capacity and the proton relaxometry by low resolution ^{1}H NMR) were evaluated after 1 and 10 d of refrigerated storage of the cheese in covering liquid and compared to cheeses made only with fresh milk.

2. Material and methods

2.1 Experimental design

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, Italy). Two different HM Mozzarella were produced using: a 40:60 (g/100 g) mixture of recombined:fresh milk (**experimental cheeses**); 100 g/100 g fresh milk (**control cheese**). Recombined milk was prepared from SMP. The 40:60 g/100 g mixture was selected because from the results of a previous study on Crescenza cheese, where other combinations were tested, as it allowed to obtain experimental cheeses having characteristics similar to the fresh ones (Tidona et al., 2020).

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.

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.

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.

2.3 Compositional analyses

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

2.4 Physico-chemical analyses

pH of the cheese was measured using the Portavo 907 pH meter. Expressible serum (**$\mathbf{ES_{CT}}$**) 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):

$$\mathrm{ES}_{CT}(g/100g) = \frac{ES_{CT\,app}}{MC} \times 100 \qquad (2)$$

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).

2.5 Rheological analyses

Rheological measurements were performed at a controlled temperature of 25.0 ± 0.1°C using an MCR 102 rheometer (Anton Paar, Gratz, Austria); the instrument was equipped with a 25-mm 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):

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

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

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.

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

2.6 ^{1}H low field NMR analyses

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

^{1}H T_2 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 ^{1}H T_1), an interpulse spacing (τ) of 40 μs and 16,000 data points.

^{1}H T_2 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. T_1

and T_2 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:

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

Where $A_2(t)$ is T_2 amplitude exponential function, $T_{2(i)}$ is the spin–spin relaxation time of component i, $A_{2(i)}$ is the spin–spin signal intensity of component i, and the constant, L_2 is the intersect of the polynomial function and represent the instrumental noise of the measurements. Each sample was analyzed in quadruplicate.

2.7 Statistical analyses

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

To evaluate the main effect of the type of milk used (fresh or recombined milk) on Mozzarella cheesemaking, the storage time at 4°C (1, 10 days), and the significance of their interactions, split-plot ANOVA models were created for all the parameters evaluated according to Alinovi, Rinaldi, & Mucchetti (2018). The batch of cheese was used as the blocking factor of the models.

Post hoc tests were performed by Tukey's honest significant differences test ($\alpha = 0.05$) when 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).

3. Results and discussion

3.1 Compositional and physico-chemical analysis

The composition of milk, whey, stretching water, and curd is shown in **Table 1**. The protein, fat, 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 g/100 g protein on defatted dry matter), a higher amount of SMP (34 g/100 g protein on dry matter) had to be dissolved, leading to a significant ($P<0.001$) increase of lactose content compared to fresh milk. Consequently, the lactose content in drained whey and in stretching water of experimental cheeses was significantly ($P<0.001$ and $P<0.05$, respectively) higher than control cheeses (**Table 1**). Fat loss in the drained whey during cheesemaking was significantly ($P<0.05$) higher (~ +0.7%) in control than experimental cheeses (**Table 1**). As the average fat globules size of fresh cream used for recombined milk standardization is generally higher than that of fresh milk (Truong et al., 2016), it may be hypothesized that bigger fat globules possibly derived from cream were more easily retained in the coagulum network of experimental cheeses. The moisture content of experimental whey was significantly ($P<0.001$) lower than that of the control.

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).

Concerning the water holding capacity of the cheeses, no significant differences ($P>0.05$) of the percentage of ES_{CT} were observed. Conversely, electrical conductivity values of expressible serum 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.

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).

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

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-casein and the possible activity of plasmin have also been reported for Italian HM Mozzarella (Alinovi, Wiking, Corredig, & Mucchetti, 2020; Faccia, Gambacorta, Natrella, & Caponio, 2019). Moreover, also the calcium content decrease and the equilibrium 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, Mastromatteo, Conte, & Del Nobile, 2013; Feeney, Guinee, & Fox, 2002; Guinee, Feeney, Auty, & Fox, 2002; Kern, Bähler, Hinrichs, & Nöbel, 2019; Kern, Weiss, & Hinrichs, 2018). On the contrary, the type of milk did not show a significant effect on k' and k'' values ($P>0.05$).

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 ($P<0.05$); in general, the rheological indexes increased at longer storage times, indicating a slight 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' and n'' values ($P<0.05$). At 1 d of storage, n' and n'' in experimental cheeses were also significantly lower than in control cheeses, meaning that the cheese matrix obtained with recombined milk was more structured. Because of the drying process, milk reconstituted from SMP may be characterized by a higher extent of whey proteins aggregation or by possible interaction 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 influence the network structure, as their increase can contribute to the formation of a more 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 $CaCl_2$. 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, tanδ showed differences among samples that were dependent of the considered angular frequency. In particular, tanδ was higher in control cheeses than in experimental cheeses in the low frequency range (0.1-10 rad/s) (**Figure 2C**); as reported in **Table 4**, tanδ 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 cheeses. On the contrary, in the high frequency range (10-100 rad/s), control at 0 d was similar to experimental cheeses. A similar behavior was also observed by Bancalari et al., (2020) in caciotta-type cheese. It is known that a viscoelastic matrix, when subjected to low-frequency deformations, reacts as a liquid-like material, while at high-frequency deformations behaves more like a solid-like 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, & 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 in a semi-poor solvent. According to this theory, slower relaxation frequencies may involve localized shear induced rearrangements (colloquially named “hops”) and eventually cause the particle to get trapped in deeper energy states (Pitkowski, Durand, & Nicolai, 2008). In the high frequency region, according to Zwanzig & Mountain (1965) the dominating term over the 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), the high-frequency rheological moduli are expected to be very similar. Thus, in HM Mozzarella cheese, low-frequency structural transformations dominate since there are many sites where transformations can occur.

3.4 ^{1}H 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 T_2 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 it peaked at very short relaxation times (~0.2 ms), can be attributed to the ^{1}H of water strongly bound to the casein structure (Gianferri, Maioli, et al., 2007) and/or to other non-fat solids (i.e. lactose) as solvation water; in facts, the relative abundance of ^{1}H T_2 component A was found to be correlated to the protein content of the cheeses (r=0.633). The relative abundance of this component in experimental was significantly higher ($P<0.05$) than in control cheese, despite the difference was slight (**Table 5**). This difference can be due to the whey proteins denaturation and/or aggregation 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 water protons (Goetz & Koehler, 2005). This observation can be in accordance with rheological findings. Interestingly, relative abundance of ^{1}H T_2 component A was strongly correlated with *k'* and *k''* (r=0.762, 0.764, respectively), and negatively correlated with tanδ (r=-0.721).

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 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 g/100 g range) reduced the amount of water bound to lactose. Conversely, higher lactose contents 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 this study is small if compared to that evaluated by Wijayasinghe et al. (2015) and may not explain 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 recombined milk, can improve the structuration of the final gel network and thus reduce the molecular mobility of water protons~~ . ~~This observation can be in accordance with rheological findings. Interestingly, relative abundance of ^{1}H T_2 component A was strongly correlated with *k'* and *k''* (r=0.762, 0.764, respectively), and negatively correlated with tanδ (r=-0.721).~~

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).

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 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 with ^{1}H low-field NMR were detected in the final products, as Mozzarella obtained with reconstituted milk showed a stronger gel structure and higher percentage of bound water than Mozzarella produced with fresh milk. Despite these differences, the study demonstrated the applicability of a blend with 40 g/100 g of reconstituted milk from low-heat skim milk powders to obtain Mozzarella cheese without major changes in product quality.

Conflicts of interest

The authors declare no conflicts of interest.

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Figure Captions

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

Figure 2. Storage modulus (G’) (**A**), loss modulus (G’’) (**B**) and tangent of the phase angle (tanδ) (**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.

Figure 3. ^{1}H T_2 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

Table 1. Composition of milk, whey, ~~curd ~~and stretching water used/obtained in HM Mozzarella cheese manufacturing, expressed as a function of the type of milk used: fresh milk (~~MFM~~control cheese) or a blend of 40~~% (w/w)~~ g/100 g reconstituted milk (~~MRM~~experimental cheese) and fresh milk. Results are expressed as means ± standard deviations (n=4).

		Treatment		
Substrate	Component (g/100 g~~% /w/w~~)	~~MFM~~Control cheese	~~MRM~~Experimental cheese	Sign.
milk	moisture	87.09 ± 0.11	87.03 ± 0.23	
	fat	3.84 ± 0.16	3.64 ± 0.14	
	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	
whey	moisture	92.57 ± 0.03	92.21 ± 0.11	***
	fat	0.45 ± 0.02	0.38 ± 0.02	*
	protein	0.92 ± 0.02	0.93 ± 0.02	
	lactose	4.71 ± 0.01	5.10 ± 0.05	***
	ash	n.d.	n.d.	
stretching water	moisture	94.27 ± 0.22	92.91 ± 1.96	
	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

Table 2. **C**omposition ~~(% w/w)~~(g/100 g) and pH values of HM Mozzarella cheeses manufactured using fresh milk (~~MFM~~control 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 (g/100 g~~% w/w~~)	Fat (g/100 g ~~% w/w~~)	Protein ~~(% w/w~~ g/100 g)	Ash (g/100 g~~% w/w~~)	ES_{CT} (g/100 g~~% w/w~~)	Electrical conductivity (mS)	pH
~~MFM~~Control cheese	1	63.3^{a} ± 2.5	17.9^{a} ± 1.3	16.4^{a} ± 1.4	1.61^{ab} ± 0.01	41.5^{a} ± 13.9	11.5^{c} ± 1.3	6.15^{b} ± 0.02
	10	64.8^{a} ± 3.2	17.7^{a} ± 1.5	15.6^{a} ± 1.7	1.54^{b} ± 0.08	43.8^{a} ± 10.9	12.2^{bc} ± 3.0	6.24^{a} ± 0.05
~~MRM~~Experimental cheese	1	63.8^{a} ± 1.7	17.1a ± 1.2	16.5^{a} ± 0.7	1.96^{a} ± 0.26	41.5^{a} ± 11.2	14.4^{a} ± 3.2	6.14^{b} ± 0.03
	10	64.1^{a} ± 2.3	17.4^{a} ± 1.5	16.3^{a} ± 1.2	1.66^{ab} ± 0.10	41.4^{a} ± 6.3	14.3^{ab} ± 2.8	6.23^{a} ± 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/loss of milk components of HM Mozzarella cheeses manufactured using fresh milk (~~MFM~~control cheese) or a blend of 40~~% (w/w)~~ g/100 g reconstituted milk (~~MRM~~experimental cheese) and fresh milk. Results are expressed as means ± standard deviations (n=4).

Parameter		Treatment ~~MFM~~Control cheese	 ~~MRM~~Experimental cheese	Sign.
Actual yield		15.1 ± 0.6	14.6 ± 0.4	
Recovery/loss of total solids	whey	47.4 ± 0.5	49.5 ± 0.6	**
	stretching water	8.3 ± 0.3	10.2 ± 2.8	
	cheese	42.6 ± 1.3	40.3 ± 1.0	*
Recovery/loss of fat	whey	9.7 ± 0.7	9.0 ± 0.6	**
	stretching water	9.7 ± 0.9	14.7 ± 2.7	*
	cheese	70.6 ± 5.3	68.7 ± 2.1	
Recovery/loss of proteins	whey	22.5 ± 0.6	23.0 ± 0.2	
	stretching 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

Table 4. Power law rheological parameters (*k'*, *n'*, *k''*, *n''*) calculated by equation 3 and 4, and tanδ reported at 5.06 rad/s of HM Mozzarella cheeses manufactured using fresh milk (~~MFM~~control cheese) and a blend of 40~~% (w/w)~~ g/100 g reconstituted skim milk and fresh milk (~~MRM~~experimental 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$_{(5.06\ \mathrm{rad/s})}$
~~MFM~~Control cheese	1	9208^{a} ± 4692	0.196^{a} ± 0.007	3249^{a} ± 1593	0.149^{ab} ± 0.010	0.306^{b} ± 0.009
	10	6120^{b} ± 3155	0.199^{a} ± 0.007	2165^{b} ± 1058	0.163^{a} ± 0.013	0.313^{a} ± 0.011
~~MRM~~Experimental cheese	1	8386^{ab} ± 2724	0.183^{b} ± 0.006	2897^{ab} ± 909	0.138^{b} ± 0.005	0.295^{c} ± 0.007
	10	7746^{ab} ± 2976	0.196^{a} ± 0.006	2650^{ab} ± 930	0.157^{a} ± 0.009	0.300^{c} ± 0.007

Table 5. Percentages and relaxation times estimated from ^{1}H T_2 relaxation curves of HM Mozzarella cheeses manufactured with fresh milk (~~FM~~control cheese) and a blend of 40~~% (w/w)~~ g/100 g reconstituted skim milk and fresh milk (~~RM~~experimental cheese) at 1 and 10 d of refrigerated storage (4°C).

~~Milk~~Treatment	Storage (d)	pop. A (%)	pop. B (%)	pop. C (%)	pop. D (%)	T_{2A} (ms)	T_{2B} (ms)	T_{2C} (ms)	T_{2D} (ms)
Control cheese~~FM~~	1	$5.71^{ab} \pm 1.12$	$47.01^{a} \pm 1.51$	$30.70^{a} \pm 1.65$	$16.58^{a} \pm 1.43$	$1.48^{a} \pm 0.22$	$23.47^{a} \pm 5.60$	$77.66^{a} \pm 4.36$	$352.17^{a} \pm 31.62$
	10	$5.51^{b} \pm 1.04$	$46.88^{a} \pm 3.24$	$30.84^{a} \pm 1.33$	$16.77^{a} \pm 2.45$	$1.45^{a} \pm 0.28$	$23.39^{a} \pm 4.86$	$77.04^{a} \pm 6.61$	$350.51^{a} \pm 47.32$
~~RM~~Experimental cheese	1	$6.73^{a} \pm 1.27$	$49.16^{a} \pm 2.81$	$28.71^{a} \pm 1.81$	$15.41^{a} \pm 0.89$	$1.73^{a} \pm 0.85$	$19.83^{a} \pm 1.46$	$76.69^{a} \pm 2.88$	$387.48^{a} \pm 23.45$
	10	$5.74^{ab} \pm 0.40$	$47.41^{a} \pm 2.9$	$30.23^{a} \pm 1.89$	$16.62^{a} \pm 1.57$	$1.48^{a} \pm 0.36$	$20.81^{a} \pm 1.59$	$76.34^{a} \pm 3.42$	$374.19^{a} \pm 43.94$

Figure_1

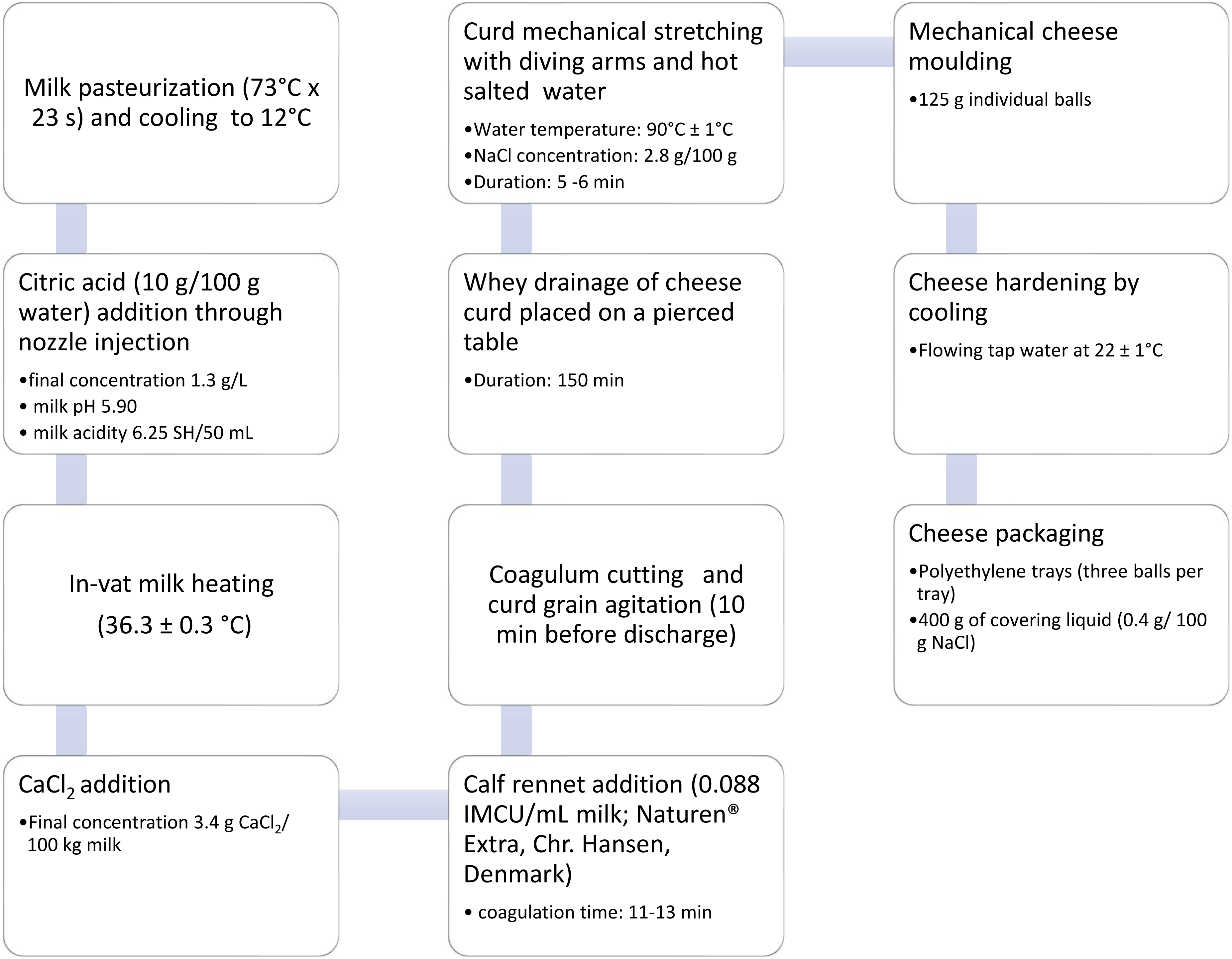

Milk pasteurization (73°C x 23 s) and cooling to 12°C
Citric acid (10 g/100 g water) addition through nozzle injection
•final concentration 1.3 g/L
• milk pH 5.90
• milk acidity 6.25 SH/50 mL
In-vat milk heating
(36.3 ± 0.3 °C)
$CaCl_2$ addition
•Final concentration 3.4 g $CaCl_2$/ 100 kg milk
Calf rennet addition (0.088 IMCU/mL milk; Naturen® Extra, Chr. Hansen, Denmark)
• coagulation time: 11-13 min
Coagulum cutting and curd grain agitation (10 min before discharge)
Whey drainage of cheese curd placed on a pierced table
•Duration: 150 min
Curd mechanical stretching with diving arms and hot salted water
•Water temperature: 90°C ± 1°C
•NaCl concentration: 2.8 g/100 g
•Duration: 5 -6 min
Mechanical cheese moulding
•125 g individual balls
Cheese hardening by cooling
•Flowing tap water at 22 ± 1°C
Cheese packaging
•Polyethylene trays (three balls per tray)
•400 g of covering liquid (0.4 g/ 100 g NaCl)

Figure_2

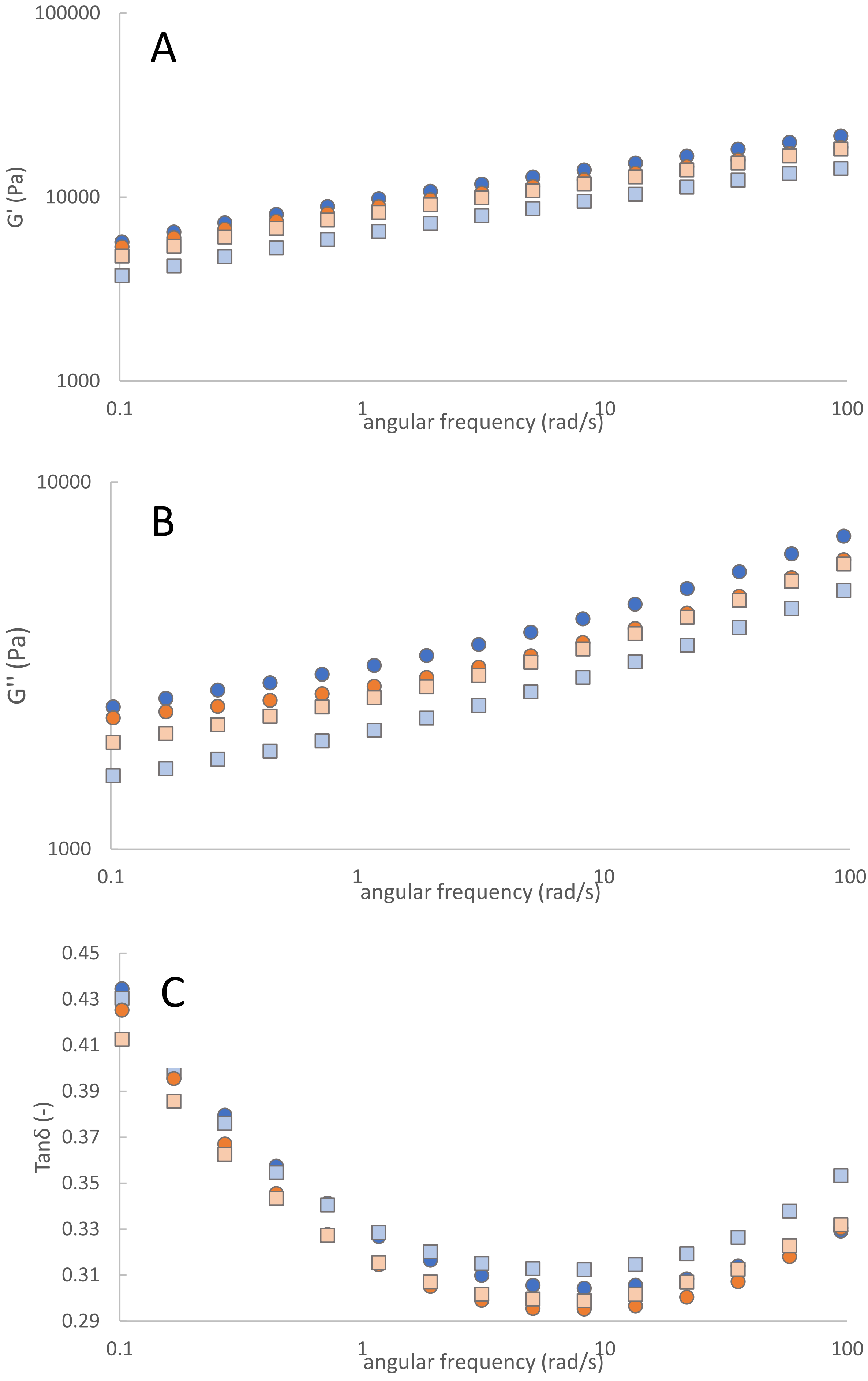
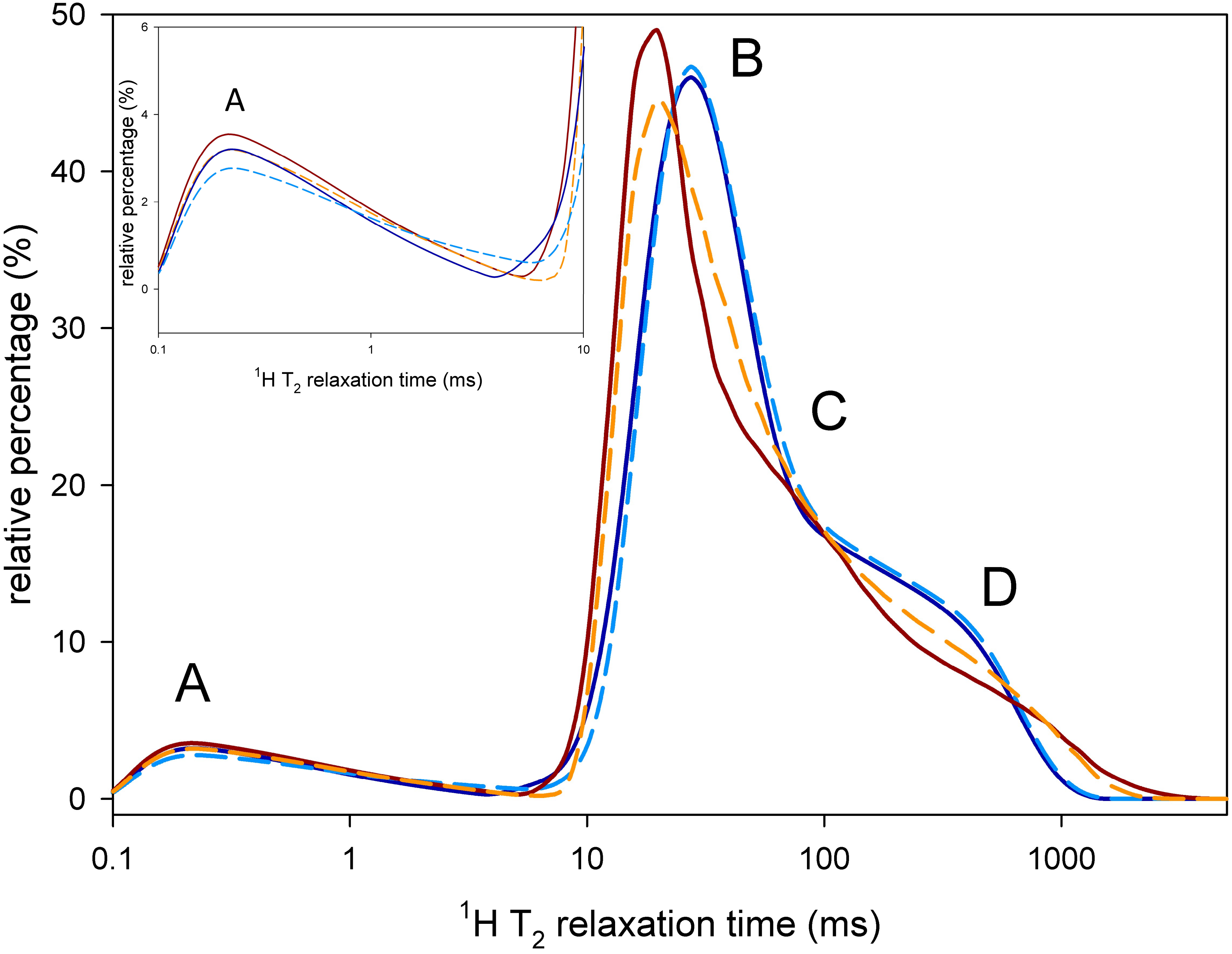

A
G' (Pa)
100000
10000
1000
0.1
1
10
100
angular frequency (rad/s)
B
G'' (Pa)
10000
1000
0.1
1
10
100
angular frequency (rad/s)
C
Tanδ (-)
0.45
0.43
0.41
0.39
0.37
0.35
0.33
0.31
0.29
0.1
1
10
100
angular frequency (rad/s)

Figure_3


50
40
30
20
10
0
relative percentage (%)
0.1
1
10
100
1000
^{1}H T$_2$ relaxation time (ms)
A
B
C
D
6
4
2
0
relative percentage (%)
0.1
1
10
^{1}H T$_2$ relaxation time (ms)
A

Credit Author Statement

Flavio Tidona: Conceptualization, Methodology, Investigation, Writing - Review & Editing. Marcello Alinovi: Methodology, Software, Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Visualization. Salvatore Francolino: Conceptualization, Methodology, Investigation. Gianluca Brusa: Methodology, Investigation. Roberta Ghiglietti: Methodology, Investigation. Francesco Locci: Conceptualization, Formal analysis, Validation. Germano Mucchetti: Resources, Conceptualization, Supervision, Writing - Review & Editing. Giorgio Giraffa: Resources, Supervision, Project administration, Funding acquisition, Writing - Review & Editing.

*Conflict of Interest Form

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,

Marcello Alinovi