International Dairy Journal 90 (2019) 95–97

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Short communication

Non-invasive monitoring of curd syneresis upon renneting of raw and heat-treated cow's and goat's milk

Elena Curti, Alessandra Pardu, Stefania Del Vigo, Roberta Sanna, Roberto Anedda*

Porto Conte Ricerche s.r.l., S.P. 55 Porto Conte-Capo Caccia, Km 8.400 Loc. Tramariglio, Alghero, SS, Italy

ARTICLE INFO

Article history:
Received 18 June 2018
Received in revised form
12 November 2018
Accepted 13 November 2018
Available online 8 December 2018

ABSTRACT

Molecular mobility of water during curd syneresis was investigated in raw and heat-treated cow's and goat's whole milk by Time-Domain ^{1}H Nuclear Magnetic Resonance (TD-NMR). Rennet was added to raw and heat-treated (72 °C) milk inside the NMR magnet at 40 °C, and curd evolution was monitored non-invasively over time. Distributions of ^{1}H NMR spin–spin relaxation time constants (T_2) were obtained at time zero (just after rennet addition), after 30 min (complete curd coagulation) and during serum expulsion (syneresis), every 10 min up to 70 min. Relaxation times and abundances of detected ^{1}H populations were calculated at each time point. Although further statistical validation would require a larger sample number, raw and heat-treated milk and the corresponding curd samples showed different NMR behaviour. It can be concluded that TD-NMR is able to identify differences between the molecular dynamics characterising raw milk and curd, and their heat-treated counterparts.

1. Introduction

The application of heat treatments to raw milk, apart from reducing or eliminating undesirable spoilage microflora, may result in irreversible physico-chemical modifications of milk components (Fox, Uniacke-Lowe, Mc Sweeney, & O'Mahony, 2015), which influence cheese production process (e.g., rennet coagulation time and syneresis; Montilla, Balcones, Olano, & Calvo, 1995) and affect the final product quality. Time-Domain Nuclear Magnetic Resonance (TD-NMR) has been already applied to investigate the effects of thermal treatments on milk components and their functional properties (Lambelet, Berrocal, & Renevey, 1992), fat crystallisation (Bertram, Wiking, Nielsen, & Andersen, 2005), acidification (reconstituted skimmed milk; Mariette, Tellier, Brule, & Marchal, 1993) and syneresis (Hansen et al., 2009; Tellier, Mariette, Guillement, & Marchal, 1993).

In this work, TD-NMR was applied to establish molecular mobility of water in coagulated raw and heat-treated whole cow's and goat's milk, induced by the addition of rennet and during syneresis.

2. Materials and methods

2.1. Milk preparation and analysis

Fresh raw whole milk samples (cow and goat) were obtained from local farms. Two cow's milk batches were sampled from two different producers and in two different lactation periods (season 1, Producer 1 – April; season 2, Producer 2 – October), to preliminarily assess batch-to-batch variability. Each batch was sampled in a limited period of time (a week) to assure a stable macro-composition. Goat's milk was provided by one producer within two weeks between November and December.

Raw (*R*) milk samples were stored at 6 °C in a laboratory refrigerator and equilibrated at room temperature just before NMR analysis or heat treatment. To obtain heat-treated (*HT*) samples, 500 mL of raw milk were poured into a 1 L beaker and heated to 72 °C (digital thermometer; VWR Collection, Milano, Italy) on a hot-plate magnetic stirrer (Falc Instruments, Treviglio, Italy). When the milk reached 72 °C (~35 min), it was held at 72 °C for 1 min with continuous stirring, quench-cooled in an ice-water basin with manual stirring for 15 min and refrigerated until analysis. *R* and *HT* milk samples (1 mL) were inserted into NMR glass tubes (10 mm) and equilibrated inside the NMR (20 min) to reach the temperature suitable for enzymatic clotting (40 °C, corresponding to the NMR magnet temperature). Then, 10 μL of liquid rennet [Naturen®, 130 International Milk Clotting Units (IMCU) mL^{-1}; Christian Hansen,

* Corresponding author. Tel.: +39 079 998 578.
E-mail address: anedda@portocontericerche.it (R. Anedda).

https://doi.org/10.1016/j.idairyj.2018.11.003
0958-6946/

Parma, Italy] were added to milk to monitor curd evolution in the NMR tubes. After 30 min (T_{3C}), the renneted milk was cross-shaped cut with a stainless steel spatula inside the tube, to promote whey expulsion (syneresis). Samples were analysed at T_0, T_{3C}, and every 10 min until 70 min (T_4, T_5, T_6 and T_7). Two triplicates of *R* and two of *HT*, milked on different days, were analysed for each milk batch.

2.2. ^{1}H NMR molecular mobility analysis

A Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence (recycle delay: 6 s; interpulse spacing 0.05 ms; 8 scans; 8000 data points) [Bruker the miniSpec (20 MHz, 0.47 T), Germany] was applied to investigate ^{1}H T_2 mobility and T_2 quasi-continuous distributions (400 points; range: 1–3000 ms) were obtained (CONTIN Bruker software). Characteristics ^{1}H T_2 relaxation times and populations abundances (% of protons) were also calculated (PeakFit 4.12 trial version, Systat Software, CA, USA).

One-way-analysis of variance ($p \leq 0.05$; ANOVA, Tukey post-hoc test) was used to assess significant differences in ^{1}H T_2 relaxation times and populations' abundances for each sample during syneresis. Significant differences between *R* and *HT* at each syneresis time point were evaluated with an independent Student's t-test ($p \leq 0.05$; GraphPad Prism v.5, CA, USA).

3. Results and discussion

Representative proton (^{1}H) T_2 quasi-continuous distributions at T_0 are shown in Fig. 1a.

TD-NMR distributions give an overall representation of proton's molecular mobility in the sample. Since water is the main component of the milk system, its protons are mainly contributing to this distribution and any of its changes.

The interplay of water diffusion, morphology of the system and relaxation rates, leads to a multi-exponential distribution, with two proton populations relaxing in the ranges 50–200 ms (Population P) and 250–750 ms (Population F, characterised by a wide range of T_2). Population P was assigned to labile protons, mainly affected by exchange phenomena in the protein gel network (Hansen et al., 2009; Le Dean, Mariette, & Marin, 2004) and Population F to fat protons or protons largely interacting with fat globules. Whole milk usually had more complex T_2 patterns and fat-related protons are often dispersed in dairy products (due to a relevant scattering of the relaxation) (Bertram et al., 2005; Hills, Takas, & Belton, 1990; Mariette, 2018; Song, 2009), making the assignment of Population F quite debatable. In fact, a double exponential decay in a CPMG signal was previously observed in defatted dairy systems, and associated to exchangeable and non-exchangeable protons (Le Dean et al., 2004; Mariette, 2018). However, our assignment of Population F to fat-related protons was based on its disappearance in skimmed (by centrifugation) cow's milk T_2 distributions (data not shown), and was supported by previously reported mono-exponential behaviour in fat-free dairy systems (Mariette et al., 1993; Song, 2009; Tellier et al., 1993).

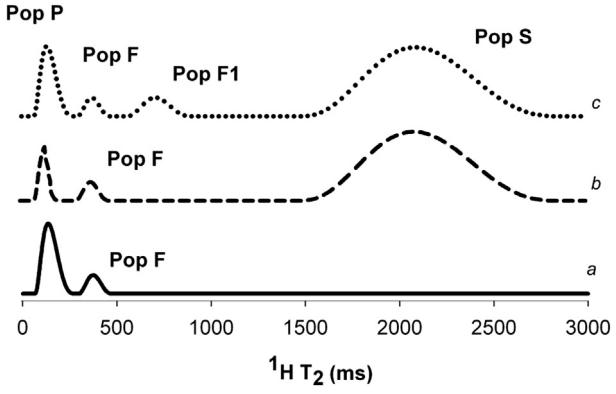

Fig. 1. Pictorial representations of quasi-continuous distributions of ^{1}H T_2 just after rennet addition (T_0) in both cow's and goat's milk (solid line; a), and after 70 min of syneresis (T_7) for cow's milk (dashed line; b) and goat's milk (dotted line; c).

During syneresis, significant changes of ^{1}H T_2 distributions were observed in cow's milk (Fig. 1b): an additional proton Population S, relaxing in the range 1000–2500 ms, was observed from T_{3C}, and assigned to more mobile protons in the diluted aqueous solutions (measured T_2 of pure water = 2500 ms), originating from whey protons expelled from the shrinking curd (Hansen et al., 2009; Tellier et al., 1993). In goat's milk, an additional population (F1) between populations F and S was also observed from T_{3C} (Fig. 1c). ^{1}H populations' abundances (% Population P and S) and T_2 relaxation times (T_{2P} and T_{2S}), obtained from distributions fitting, are shown in Table 1.

Populations F and F1 barely changed as a function of syneresis, and will not be further discussed.

From T_0 to T_7, a decrease in Population P abundance corresponded to an increase in Population S, and characteristic relaxation times, T_{2P} and T_{2S}, decreased and increased, respectively, in all samples (Table 1). This behaviour was previously reported (Hansen et al., 2009; Tellier et al., 1993) and properly reflected the physico-chemical modifications occurring during syneresis. Changes in Population P (decrease in % and T_{2P}) and Population S (increase in % and T_{2S}) are interdependent events, that well represent two phenomena of syneresis: curd protein shrinkage and increasing solute concentration on one hand (Population P), and expulsion of the whey (physically separated phase), with proton transfer to the more mobile environment (Population S) (Hansen et al., 2009; Tellier et al., 1993), on the other. Statistical analysis (ANOVA) of each parameter (Population P, T_{2P}, Population S, T_{2S}) corresponding to T_0, T_{3C}, T_4, T_5, T_6 and T_7 indicated progressive and significant changes both in cow's and goat's milk (Table 1).

Changes in Population P and S were almost complementary (≈86–88% of Population P decrease was compensated by a correspondent increase of Population S), especially in cow's milk, regardless of the lactation season. In goat's milk, the increase in Population S was slightly larger (by approximately 10%) than Population P decrease, suggesting that a fraction of protons, not detectable at T_0, contributes to Population S at later stages of syneresis.

Significant differences between *R* and *HT* milk were observed in T_{2P} at T_0 for all samples. These findings corroborate previous observations on the effect of heat treatment on model systems (Goetz & Koehler, 2005; Lambelet et al., 1992), and expand experimental evidences to real dairy systems. The optimisation of analytical tools to detect the application of heat treatments to milk would represent a relevant diagnostic achievement, especially for non-bovine milk (Anedda, 2015). T_{2P} was generally comparable between *R* and *HT* (Table 1) from T_{3C} to T_7 (a more relevant intra-group variability was observed in season 2). Significant differences between *R* and *HT* were observed in T_{2S} of cow's milk in season 2.

In cow's milk (both season 1 and 2), at the early stages of syneresis (especially at T_4 after cutting the curd), *HT* had a significantly larger Population P and a significantly smaller Population S than *R*, while at later stages (T_5, T_6 and T_7), they became comparable (Table 1).

In goat's milk, statistical analysis suggested a more robust differentiation between *R* and *HT*, with significant differences in T_{2S}, at all syneresis time points, and in Population P and S from T_4 to T_7 (Table 1).

Although the statistical data here presented are certainly affected by limited sample numerosity, relaxometric differences between *R* and *HT* milk and curds appear evident and possibly attributable to heat treatment-related proteins modifications (e.g., whey proteins denaturation and interactions between β-

Table 1
^{1}H abundances (%) of Population P and Population S, and characteristic ^{1}H T_2 relaxation times (ms) of raw (*R*) and heat-treated (*HT*) milks.*

Time point	Population P		Population S		^{1}H T_{2P}		^{1}H T_{2S}	
	R	*HT*	*R*	*HT*	*R*	*HT*	*R*	*HT*
Cow's milk (season 1)								
T_0	95.0 ± 0.2	96.0 ± 1.4	n.d.	n.d.	179.3 ± 6.2^{aA}	174.3 ± 2.4^{aB}	n.d.	n.d.
T_{3C}	34.9 ± 3.3^{a}	43.3 ± 2.3^{aB}	60.0 ± 3.3^{dA}	50.4 ± 2.2^{dB}	163.5 ± 3.8^{ab}	164.0 ± 0.8^{b}	1766.1 ± 39.8^{d}	1710.2 ± 22.0^{e}
T_4	22.4 ± 2.0^{b}	27.9 ± 1.6^{bB}	73.3 ± 2.1^{cA}	67.0 ± 1.6^{cB}	151.4 ± 3.6^{bc}	153.0 ± 1.5^{c}	1913.7 ± 26.0^{cd}	1878.8 ± 11.4^{d}
T_5	16.3 ± 1.5^{bc}	19.9 ± 1.2^{c}	80.0 ± 1.5^{bc}	76.0 ± 1.3^{c}	141.6 ± 4.5^{c}	142.8 ± 2.0^{d}	1991.0 ± 18.8^{bc}	1974.0 ± 10.1^{c}
T_6	13.2 ± 1.2^{c}	15.7 ± 1.0^{cd}	83.5 ± 1.2^{ab}	80.7 ± 1.0^{b}	134.3 ± 4.5^{cd}	136.4 ± 2.2^{d}	2038.6 ± 16.8^{b}	2027.4 ± 7.8^{b}
T_7	8.9 ± 0.8^{c}	10.7 ± 0.7^{d}	88.4 ± 0.8^{a}	86.4 ± 0.8^{a}	118.6 ± 5.8^{d}	121.2 ± 3.1^{e}	2098.6 ± 13.1^{a}	2082.4 ± 8.4^{a}
Cow's milk (season 2)								
T_0	96.1 ± 2.1	96.4 ± 1.1	n.d.	n.d.	184.0 ± 1.5^{aA}	176.2 ± 1.2^{aB}	n.d.	n.d.
T_{3C}	25.7 ± 10.8^{a}	37.6 ± 8.9^{a}	68.0 ± 9.8^{bA}	55.5 ± 8.7^{cB}	161.0 ± 18.5^{ab}	165.5 ± 2.9^{a}	1845.8 ± 71.4^{d}	1748.2 ± 89.8^{d}
T_4	16.1 ± 5.9^{abA}	23.9 ± 5.7^{bB}	79.1 ± 5.2^{aA}	70.8 ± 5.6^{bB}	145.8 ± 17.9^{bc}	152.2 ± 5.7^{b}	1981.8 ± 34.9^{cA}	1919.5 ± 40.1^{cB}
T_5	12.5 ± 4.2^{b}	17.0 ± 4.6^{bc}	84.0 ± 4.2^{a}	78.8 ± 4.3^{ab}	135.3 ± 21.6^{cd}	142.4 ± 8.1^{bc}	2035.4 ± 33.9^{bcA}	1993.1 ± 24.9^{bcB}
T_6	10.4 ± 3.5^{b}	13.8 ± 4.0^{c}	86.9 ± 3.4^{a}	82.7 ± 3.8^{a}	126.3 ± 20.9^{cd}	134.8 ± 8.3^{c}	2072.1 ± 37.5^{abA}	2028.3 ± 28.1^{abB}
T_7	8.3 ± 2.3^{b}	9.1 ± 3.0^{c}	88.3 ± 3.7^{a}	87.8 ± 2.5^{a}	111.7 ± 21.7^{d}	120.0 ± 12.5^{d}	2120.0 ± 39.4^{a}	2083.8 ± 22.0^{a}
Goat's milk								
T_0	87.8 ± 8.5	89.1 ± 2.7	n.d.	n.d.	131.3 ± 6.6^{aA}	108.2 ± 8.6^{aB}	n.d.	n.d.
T_{3C}	11.4 ± 7.8^{aA}	26.7 ± 5.5^{aB}	83.3 ± 8.6^{cA}	60.9 ± 4.6^{bB}	95.3 ± 11.4^{b}	95.2 ± 6.8^{b}	1883.5 ± 120.8^{cA}	1503.7 ± 108.8^{cB}
T_4	7.5 ± 4.0^{abA}	15.0 ± 2.8^{abB}	87.8 ± 4.5^{bcA}	74.5 ± 2.8^{abB}	87.7 ± 8.2^{bc}	85.8 ± 5.9^{b}	1928.2 ± 77.2^{bcA}	1649.1 ± 85.7^{bcB}
T_5	4.6 ± 2.5^{bA}	10.0 ± 1.9^{bB}	91.5 ± 2.3^{abA}	82.1 ± 2.7^{aB}	79.0 ± 7.0^{cd}	79.0 ± 4.6^{c}	1990.8 ± 62.7^{abA}	1736.8 ± 79.2^{abB}
T_6	3.4 ± 1.7^{bA}	7.6 ± 1.3^{cB}	93.0 ± 1.6^{abA}	85.4 ± 4.9^{aB}	74.4 ± 5.2^{d}	74.9 ± 5.3^{cd}	2031.8 ± 53.8^{abA}	1791.1 ± 79.4^{abB}
T_7	2.1 ± 0.9^{bA}	4.5 ± 0.8^{dB}	95.1 ± 0.3^{aA}	90.3 ± 1.3^{aB}	68.8 ± 2.1^{dA}	64.5 ± 4.6^{dB}	2089.1 ± 44.4^{aA}	1861.7 ± 78.7^{aB}

* Values are given as mean ± standard deviation (n.d., not determined); different superscript lowercase letters in each column indicate significant differences for each sample during syneresis ($p \leq 0.05$); different superscript uppercase letters indicate significant differences between raw (*R*) and heat-treated (*HT*) milk at each syneresis time point ($p \leq 0.05$).

lactoglobulins and κ-casein), which could result in an alteration of the syneresis process.

Throughout syneresis, a trend is clearly recognisable. The difference between *R* and *HT* ($\Delta_{R\text{-}HT}$) mean values of all analysed NMR parameters (Populations P, Population S, T_{2P} and T_{2S}) was calculated and provided a better glimpse on this trend.

In cow's milk, a decreasing $\Delta_{R\text{-}HT}$ throughout syneresis (from T_{3C} to T_7) was observed both in Population P (from 8 to 2%, season 1; from 12 to 1%, season 2) and Population S (from 10 to 2%, season 1; from 12 to 1%, season 2). T_{2P} of *HT* was generally higher than that of *R* from T_{3C} to T_7, with slight $\Delta_{R\text{-}HT}$ changes in season 1 and more marked $\Delta_{R\text{-}HT}$ changes in season 2. T_{2S} of *HT* from T_{3C} to T_7 appeared always lower than *R*, with $\Delta_{R\text{-}HT}$ decreasing over syneresis (from 56 to 16 ms, season 1; from 100 to 50 ms, season 2).

Goat's milk data showed more marked $\Delta_{R\text{-}HT}$ values from T_{3C} to T_7 (except for T_{2P} from T_{3C} to T_6): $\Delta_{R\text{-}HT}$ decreased from 15 to 2% in Population P, from 23 to 5% in Population S, from 23 to 4 ms in T_{2P} and from 380 to 330 ms in T_{2S}.

4. Conclusions

The TD-NMR investigation of curd syneresis in raw and heat-treated whole cow's and goat's milk highlighted its potential in detecting molecular mobility changes (relaxation times and protons abundances). These variations, involving specific milk components, are in agreement with previous studies (mainly carried out on model systems and skimmed milk). Further investigation should be conducted on a larger dataset, to better highlight the impact of heat treatment and seek statistical validation of these preliminary data. Based on this preparatory work, TD-NMR can be suggested as a potential quality and diagnostic control tool in milk and dairy products at an industrial level, thanks to its low cost, robustness and the user friendliness of customised protocols (Anedda, 2015).

Acknowledgements

The authors would like to thank Azienda Agricola Fauro (Alghero, Italy) for supplying cow's milk and Dr. Elia Bonaglini and Azienda Sechi (Siligo, Italy) for providing goat's milk. This work has been funded by Sardinia Regional Government by means of Sardegna Ricerche (art. 26 L.R. 37/98).

References

Anedda, R. (2015). Magnetic resonance analysis of dairy processing suitable tools for the dairy industry. In F. Capozzi, L. Laghi, & P. S. Belton (Eds.), *Magnetic resonance in food science: Defining food by magnetic resonance* (pp. 49–64). London, UK: Royal Society of Chemistry.

Bertram, H. C., Wiking, L., Nielsen, J. H., & Andersen, H. J. (2005). Direct measurement of phase transitions in milk fat during cooling of cream-a low-field NMR approach. *International Dairy Journal, 15*, 1056–1063.

Fox, P. F., Uniacke-Lowe, T., Mc Sweeney, P. L. H., & O'Mahony, J. A. (2015). Heat-induced changes in milk. In P. F. Fox, T. Uniacke-Lowe, P. L. H. McSweeney, & J. A. O'Mahony (Eds.), *Dairy chemistry and biochemistry* (pp. 345–375). Basel, Switzerland: Springer International Publishing.

Goetz, J., & Koehler, P. (2005). Study of the thermal denaturation of selected proteins of whey and egg by low resolution NMR. *LWT-Food Science and Technology, 38*, 501–512.

Hansen, C. L., Rinnan, Å., Engelsen, S. B., Janhøj, T., Micklander, E., Andersen, U., et al. (2009). Effect of gel firmness at cutting time, pH, and temperature on rennet coagulation and syneresis: An in situ ^{1}H NMR relaxation study. *Journal of Agricultural and Food Chemistry, 58*, 513–519.

Hills, B. P., Takacs, S. F., & Belton, P. S. (1990). A new interpretation of proton NMR relaxation time measurements of water in food. *Food Chemistry, 37*, 95–111.

Lambelet, P., Berrocal, R., & Renevey, F. (1992). Low-field nuclear magnetic resonance relaxation study of thermal effects on milk proteins. *Journal of Dairy Research, 59*, 517–526.

Le Dean, A., Mariette, F., & Marin, M. (2004). ^{1}H nuclear magnetic resonance relaxometry study of water state in milk protein mixtures. *Journal of Agricultural and Food Chemistry, 52*, 5449–5455.

Mariette, F. (2018). NMR relaxometry and imaging of dairy products. In G. A. Webb (Ed.), *Modern magnetic resonance* (pp. 1535–1557). Basel, Switzerland: Springer International Publishing.

Mariette, F., Tellier, C., Brule, G., & Marchal, P. (1993). Multinuclear NMR study of the pH dependent water state in skim milk and caseinate solutions. *Journal of Dairy Research, 60*, 175–188.

Montilla, A., Balcones, E., Olano, A., & Calvo, M. M. (1995). Influence of heat treatments on whey protein denaturation and rennet clotting properties of cow's and goat's milk. *Journal of Agricultural and Food Chemistry, 43*, 1908–1911.

Song, Y. Q. (2009). A 2D NMR method to characterize granular structure of dairy products. *Progress in Nuclear Magnetic Resonance Spectroscopy, 55*, 324–334.

Tellier, C., Mariette, F., Guillement, J. P., & Marchal, P. (1993). Evolution of water proton nuclear magnetic relaxation during milk coagulation and syneresis: Structural implications. *Journal of Agricultural and Food Chemistry, 41*, 2259–2266.