Food Hydrocolloids 27 (2012) 456–463

Contents lists available at SciVerse ScienceDirect

Food Hydrocolloids

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

The rennet coagulation mechanisms of a concentrated casein suspension as observed by PFG-NMR diffusion measurements

Steven Le Feunteun[a,b,*], Minale Ouethrani[a,b], François Mariette[a,b]

[a] Cemagref, UR TERE, 17 Avenue de Cucillé, CS 64427, F-35044 Rennes, France
[b] Université Européenne de Bretagne, France

ARTICLE INFO

Article history:
Received 28 March 2011
Accepted 16 September 2011

Keywords:
Diffusion
NMR
Milk
Rennet
Casein micelles
Gelation
DOSY

ABSTRACT

Pulsed field gradient-nuclear magnetic resonance (PFG-NMR) was used to monitor the diffusion of caseins throughout the rennet coagulation of a highly concentrated casein suspension. Two types of casein species were distinguished according to their diffusing properties and attributed to the dissolved casein fraction, i.e. dissociated caseins and caseinomacropeptides (CMP), and casein particles, respectively. The NMR signal intensity coming from the dissolved casein fraction, which is related to the number of protons it contains, increased all along the experiment whereas the opposite tendency was observed for casein particles. This was explained by the increasing amount of CMP in the whey and the reciprocal loss of protons contained by casein particles, and used to quantitatively estimate the kinetics of the chymosin action. The diffusion of the dissolved casein fraction remained nearly constant during the coagulation process whereas, as revealed by rheometry measurements, the diffusion of casein particles was very sensitive to the structural reorganization of the sample. It decreased by about 35% during the sol–gel transition and increased in similar proportions during the succeeding rise in gel porosity. Our results also provide different types of information on the respective behaviors of dissociated caseins and casein particles during the course of the process, the most remarkable one being that all the casein particles did not aggregate during the sol–gel transition of our sample. This strongly suggests that the rennet gelation of a concentrated dairy solution may be better visualized by the formation of a network backbone during the sol–gel transition which is thereafter reinforced upon further incorporation of casein particles.

1. Introduction

The pulsed field gradient (PFG)-NMR technique enables measuring the self-diffusion coefficients of one or several molecular constituents of a sample at a micrometer scale. Diffusion coefficients are sensitive to the size of the molecular species under study, to the chemical interactions they may have with their surrounding as well as to the structure they probe during a measurement. This technique is extensively used, especially in polymer and colloidal sciences, to investigate droplet sizes in emulsions (Gabriele et al., 2009; Romoscanu et al., 2010), molecular interactions between different sample constituents (Price, Elwinger, Vigouroux, & Stilbs, 2002; Tran Le et al., 2011) and/or the three-dimensional microstructure of heterogeneous matrices (Colsenet, Soderman, & Mariette, 2006; Masaro, Ousalem, Baille, Lessard, & Zhu, 1999). Some researchers have also proven the efficiency of the PFG-NMR technique in characterizing sol–gel transitions (Brand, Nolis, Richter, & Berger, 2008; Brand, Richter, & Berger, 2006; Hansen, Norby, Roots, & Wu, 2007; Hansen, Olafsen, & Kvernberg, 1998). Nevertheless, up to now, there have been very few studies in which a gelation process is continuously monitored by PFG-NMR measurements. In this article, we will attempt to illustrate the great potentialities of using such an approach to investigate and gain further knowledge on a complex gelation process: the rennet coagulation of a highly concentrated casein suspension.

Caseins represent 80% of the protein content of milk and are of four main types: α_{S1}, α_{S2}, β, and κ-caseins. Most, but not all of them, exist in the form of colloidal particles, known as casein micelles. These particles contain several thousands of individual casein molecules and have diameters ranging from about 50 to 300 nm. The presence of κ-casein molecules protruding from their surface prevents their aggregation by steric and electrostatic repulsions,

* Corresponding author. Present address: INRA, UMR782 Génie et Microbiologie des Procédés Alimentaires, F-78850 Thiverval Grignon, France. Tel.: +33 (0) 130814596; fax: +33 (0)130815597.
E-mail address: steven.le-feunteun@grignon.inra.fr (S. Le Feunteun).

0268-005X/$ – see front matter © 2011 Published by Elsevier Ltd.
doi:10.1016/j.foodhyd.2011.09.008

but this "hairy layer" can be removed by the action of the chymosin enzyme contained in rennet. In such a case, the casein micelle suspension becomes unstable and the system gels. The fresh casein network then undergoes structural modifications which modify the macroscopic properties of the gel during the aging phase and may even lead to syneresis, i.e. whey expulsion. This so-called rennet coagulation constitutes the critical first step in the manufacture of most cheese varieties.

Nevertheless, some details of the rennet coagulation mechanisms remained to be understood, in particular with regards to the effect of the casein concentration. For instance, there is currently no general explanation for the fact that the aggregation phase always starts before the end of the enzymatic reaction (Sandra, Alexander, & Dalgleish, 2007; Walstra & Van Vliet, 1986) and occurs at an even lower degree of κ-casein hydrolysis when the casein concentration is increased (Karlsson, Ipsen, & Ardo, 2007; Sharma, Mittal, & Hill, 1994). Similar questioning also arises when trying to understand why the network structure of casein gels coarsens when the casein concentration is increased (Karlsson et al., 2007). This is partly because most of the conventional methods used to study and/or control this process (viscometry, rheology, microscopy, ultrasonics or light scattering techniques) are either intrusive, efficient in the sol or gel state only, and/or are not adapted to study (highly) concentrated samples. In recent years, great effort has therefore been made to develop and apply analytical methods that are suited to a greater panel of samples and/or that can provide further insights on the phenomena occurring during the course of the coagulation. For instance, diffusing wave scattering (DWS) has emerged as a new tool capable of monitoring the diffusion of casein particles during the renneting of undiluted skim milk (Alexander & Dalgleish, 2004; Dalgleish & Horne, 1991; Gaygadzhiev, Alexander, & Corredig, 2009; Hemar, Singh, & Horne, 2004; Sandra et al., 2007), and has permitted to show that the gelation of milk at its normal concentration cannot be accurately described by a diffusion-limited cluster aggregation, or that particles seem to interact more strongly with each other before they truly aggregate (Sandra et al., 2007). These are important findings because it is difficult to investigate these phenomena in a non-invasive and continuous manner while it is established that the characteristics of the final product depend on the dynamics of the coagulation reactions.

In this context, the present study was thus also motivated by the need of resorting to tools that are well-adapted to study the rennet coagulation of highly concentrated dairy solutions. Indeed, the PFG-NMR technique presents a number of advantages that are of particular interest for such purposes. In contrast with most of the techniques commonly employed to characterize dairy samples, NMR is even more sensitive as the sample is concentrated. The diffusion coefficient of several, if not all, of the matrix constituents can most of the time be measured within the same experiment. Last but not least, measurements are possible whatever the matrix is liquid or solid and may therefore be performed throughout the coagulation process. For instance, Tan and McGrath (2010) recently showed that this technique is very valuable to investigate the interactions of the casein aggregates contained in Na-caseinate dispersions during a concentration-induced sol–gel transition. In our lab, we previously studied the diffusion of a 620 and 96,750 g mol^{-1} polyethylene glycol (PEG), used as non-interacting probes of the sample microstructure, throughout a rennet, an acid-induced and a combined coagulation of a concentrated casein suspension (Le Feunteun & Mariette, 2008a, 2008b). It was found that the diffusion of these molecular probes was closely related to the structural modifications undergone by the casein particles and clusters thereof before and after gelation, respectively.

In the present paper, we investigate the diffusion of casein species using the same rennet coagulation experiment as that described in Le Feunteun and Mariette (2008b) to study the 96,750 g mol^{-1} PEG diffusion. While both articles can be read independently, the previously reported results will be used as comparison to unambiguously interpret the time evolution of the casein diffusion. This study first describes the method for non-NMR experts. Then, it shows that the self-diffusion of both casein particles and soluble caseins can be determined simultaneously, and explains how their evolution can be related to key stages of the coagulation process. Whenever available, the so-recovered findings are confronted to the literature dealing with the rennet coagulation of highly concentrated dairy solutions.

2. Materials and methods

2.1. Sample preparation, chymosin addition and dynamic rheological measurements

Only a brief summary is given below since all these procedures were described in details in Le Feunteun and Mariette (2008b). Native phosphocaseinate powder (INRA STLO, Rennes, France) was rehydrated in a H_2O solution that contained 0.1 M NaCl, 0.1% (w/w) of a 96,750 g mol^{-1} PEG, and 0.2% (w/w) NaN_3 to prevent bacterial growth. The final casein concentration of the suspension was of about 14% (w/w). To start the rennet-induced coagulation process, a dilution of a Chymax-Plus solution (Chr-Hansen, Arpajon, France), having an activity of 2 international milk clotting units (IMCU) mL^{-1} after dilution, was added to the casein suspension at the proportion of 700 μL for 100 g. The viscoelastic properties of the sample was studied with a controlled stress rheometer (Rheostress RS150, Haake, Germany) using a double gap cylinder sensor (DG41). The temperature was maintained at 20 °C and the surface of the sample was covered with silicone oil to prevent evaporation. The storage modulus (G') and the loss modulus (G'') were recorded at a frequency of 1 Hz and the rheometer was programmed to adjust the stress automatically to provide a strain of 0.5%, which was found to be within the linear viscoelastic region of the sample. The phase angle (δ) was calculated according to the equation: $\delta = \tan^{-1}(G''/G')$.

2.2. Principles of the PFG-NMR technique

The aim of this section is to summarize the basis to understand what the data recovered from NMR diffusion measurements are. In-depth descriptions of the PFG-NMR technique can be found elsewhere (Callaghan, 1991; Price, 1997).

A PFG-NMR experiment yields a series of decaying 1D-spectra. The chemical shift resolution of NMR is thus preserved. As an example, Fig. 1 presents a ^{1}H PFG-NMR spectrum of the casein suspension we studied. It shows that the three main constituents of our sample were well-separated by ^{1}H NMR. The signal coming from water molecules, which was suppressed with the PFG-NMR sequence we used, resonate at 4.7 ppm. The sharp peak at 3.6 ppm is the signal coming from the 0.1% (w/w) of a 96,750 g mol^{-1} polyethylene glycol (PEG) we previously investigated in Le Feunteun and Mariette (2008b), a non-interacting probe that will be used as comparison to interpret the casein diffusion results. All the other signals come from the protons of the casein molecules.

The self-diffusion coefficient of one sample constituent is obtained by analyzing the decaying of its resonance(s) in the PFG-NMR spectra. Thanks to the chemical shift resolution, the translational motion dynamics of all the sample constituents can therefore often be measured with one experiment for simple mixtures. In mathematical terms, when the molecular constituent under study shows a single diffusion coefficient, one can demonstrate