Practical Implications of the Phase-Compositional Assessment of Lipid-Based Food Products by Time-Domain NMR

Franck P. Duval, John P.M. van Duynhoven, and Arjen Bot*

Unilever Research and Development Vlaardingen, NL-3133 AT Vlaardingen, The Netherlands

ABSTRACT: The objective of this work is to determine the solid fat content (SFC) of the fat-oil phase in oil-in-water (O/W) emulsions, i.e., the droplet SFC, using transverse relaxation decay deconvolution (TRDD) analysis. The TRDD NMR experiment classifies protein protons as mobile, semimobile, and nonmobile. Hence, protein contributes more or less to the solid content detected by TRDD as a function of pH, protein content, and protein denaturation. By taking into account the protein contribution to the overall solid content, one can estimate the droplet solid content in O/W emulsions. The SFC can then be deduced if one converts the ingredients' mass fraction into ingredients' proton fraction using the ingredients' proton densities.

Paper no. J11392 in *JAOCS 83*, 905–912 (November 2006).

KEY WORDS: O/W emulsions, SFC, solid content, transverse relaxation decay deconvolution (TRDD), whey proteins.

The key to understanding many functional properties of food products and materials is in the physical state of their ingredients (1). Texture (hardness) and melting behavior of margarine and chocolate, for example, are determined by the phase behavior of their lipid component, i.e., the balance between the solid/liquid state and the effects of crystal polymorphism (1,2). For many cereal materials, the phase composition of the carbohydrate components determines processing and textural properties. The various phases comprise different crystalline starch polymorphs, gel states, mesomorphic phases, and liquid-like dissolved phases (3,4).

For the assessment of crystal polymorphism of food materials, one traditionally deploys X-ray diffraction and thermal analysis (mostly DSC) (5). These techniques are unsurpassed in addressing specific phase-compositional details of lipid and carbohydrate materials, such as polymorph identification. However, they mostly fall short when the phase composition of complex foods products needs to be assessed in a quantitative manner. For this purpose, NMR relaxometry currently presents the most powerful option. Strong advantages of NMR over other methods are that it probes molecular mobility of the different phases, and that the obtained signals directly relate to corresponding proton densities. For this purpose, dedicated low-field NMR relaxometers have been designed that are relatively cheap and easily operated by nonexperts. The earliest example of a widespread application within the foods community is the determination of solid fat content (SFC) of lipids by NMR (6). This methodology uses rather crude acquisition and data treatments of the free induction decay (FID). More recent sophisticated NMR line-shape analysis procedures (7–9) were introduced to derive detailed quantitative phase-compositional information on polysaccharides (10,11) and on lipids (12). Even thermal denaturation of proteins (13) was investigated. This type of analysis also can be applied to complex food products (8,9,14,15). In our implementation for lipid-based products, we denoted this approach as transverse relaxation decay deconvolution (TRDD) (8,9).

In this study we address practical aspects of determining the phase composition of protein-stabilized oil-in-water (O/W) food emulsions. These can be considered as examples of complex food products, where functional properties such as texture and water-holding capacity are determined by the phase behavior of the lipid component. We consider the effect of proteins on the phase composition of the emulsion as determined by TRDD NMR. We also provide guidelines on how to make an assessment of the phase composition of the lipid component within the emulsion. We demonstrate that such data can be used to study supercooling of lipids during crystallization within small oil droplets.

EXPERIMENTAL PROCEDURES

Ingredients. Demineralized water and other ingredients were used without further treatment. Citric acid (Jungbunzlauer, Basel, Switzerland) and sodium hydroxide (Merck, Darmstadt, Germany) were used to adjust the pH of the products. Sodium sorbate (Daicel Chemical Industries, Tokyo, Japan) was used as preservative in acidified O/W emulsions (0.1% by wt). As a result of processing, it was also present in neutral O/W emulsions.

Whey protein concentrate (WPC) (Nutrilac QU7560; Arla Foods, Wageningen, The Netherlands) was used as protein source. According to the supplier, the WPC composition is the following: 75% whey protein, 7% fat, 6% water, 4% lactose, and 7% others (minerals, etc.), and the degree of denaturation of the whey proteins is 12%. Whey proteins are on average composed of 60% β-lactoglobulin, 12% α-lactalbumin, 12% BSA, 5% immunoglobulin G, and 11% proteose-peptones. The

*To whom correspondence should be addressed at Unilever Research Vlaardingen, Olivier van Noortlaan 120, NL-3133 AT Vlaardingen, The Netherlands. E-mail: arjen.bot@unilever.com

Copyright © 2006 by AOCS Press

isoelectric points of the first three proteins are, respectively, 5.1–5.3, 4.2–4.5, and 4.7–5.1 (16).

Two lipid sources were used: sunflower oil (SF), which is a fully liquid oil, and a partly crystalline vegetable fat (PC-VF) that is a 1:1 mixture of fully hardened coconut oil and dry fractionated palm oil.

Solution processing. WPC powder was diluted in water at five concentrations—4, 8, 12, 16, and 20% (w g/g)—which correspond to a whey protein concentration ranging from 3 to 15% by weight. A Turrax (Janke & Kunkel, IKA-Labortechnik, Staufen, Germany) was used to disperse the powder in distilled water. Some of the solutions were kept neutral (pH 6.8) whereas the pH of others were adjusted to one of five values using sodium hydroxide and citric acid: 12, 9, 5.5, 4.5, 3.5 (±0.1). A minimum of 24 h elapsed before the solutions were transferred to NMR tubes for measurements at 5°C. Samples identified as "heated samples" were warmed in a water bath for 20 min at 85°C. This time and temperature correspond to the heating conditions in the emulsion processing.

Emulsion processing. All emulsions were prepared the same way by using WPC. Water and a fraction of the fat blend (FB), both at 60°C, and WPC were mixed in a 5-L jacketed mixing bowl (Stephan UMC5; Stephan Machinery GmbH & Co., Hameln, Germany). The temperature of the jacketed bowl was initially set to 60°C, and the mixing knives turned at 300 rpm. The temperature was then raised to 85°C, after which the rest of the FB was added. The FB composition was fixed at 30% by weight, and the protein content was varied (2–20%). The temperature is held during 15 min to allow for protein denaturation. During that time the knives' speed is set to 600 rpm. Then the emulsion was dispersed with a Turrax mixer (Ultra-Turrax T50; Janke & Kunkel) at 8,000 rpm for 5 min. To obtain a well-defined droplet size (D_{33} around 1.5 mm), the emulsion was homogenized at a defined pressure (300 bar first stage, 0 bar second stage) using an APV Lab1000 homogenizer (Invensys APV, Hendrik Ido Ambracht, The Netherlands) (17,18). Some of the emulsion was kept at pH 6.8 (neutral) whereas some of the emulsion was acidified to a pH of 4.6 in the Stephan pan with addition of citric acid. After filling into 200-mL tubs and sealing, the products were stored at 5°C for at least a week before being measured at 5, 10, 15, and 25°C.

NMR data acquisition and processing. Measurements were performed on a 20 MHz NMR spectrometer (Bruker MQ20). The sequence used is a combination of a FID and a Carr–Purcell–Meiboom–Gill (CPMG) sequence (19). Both are designed to measure spin-spin relaxation. The FID gives information on the relaxation of the fast-relaxing populations, typically protons with extremely slow motion (nonmobile protons). The CPMG gives information on the relaxation of the mobile protons. This combination is mandatory to have access to the full proton population's spectrum (8,9). The echo time between the 90° pulse and the first 180° pulse was set at 100 ms. The FID dead time was around 11 ms. For the FID, 230 points were recorded between the dead time and the echo time at a sampling rate of 2.55 MHz. For the CPMG, 5,000 consecutive echoes were recorded spaced by 200 ms. The recuperation delay was set at 15 s to avoid T_1 weighting, and the number of scans was either 32 or 64. WPC solutions at pH 6.8, heated or nonheated, were measured twice and gave the same results. Consequently, most of the other samples were only measured once.

Two NMR SFC methods are currently used on a daily basis: the direct method and the indirect method (6,20–22). Recently, a new method was proposed that combines the measurement of SFC and the assignment of the fat crystal polymorphism (23). The approach is based on the full deconvolution of the NMR relaxation curves, unlike the previous methods that use only two-point comparison. Relaxation behavior of pure crystal polymorphs can be modeled by well-defined mathematical functions. Using control mixtures of specific polymorphs, it was shown that a quantitative assignment of β and β' crystals vs. α crystal is possible.

This method, TRDD, can also be deployed to measure solid content of various kinds of products including O/W emulsions (8). Hence, it allows the determination of the ratio of the number of nonmobile protons to the total number of protons. In the following, the protons that have the relaxation behavior of protons associated with solids or molecules with extremely low mobility will be called nonmobile protons.

The FID-CPMG experiment monitors the relaxation of all individual protons (^{1}H) to their equilibrium state after excitation. This macroscopic signal can be deconvoluted as follows:

$$I\,(t,n_i) = \sum n_i f_i\,(t) \quad [1]$$

where n_i is the number of protons corresponding to a given population i and $f_i\,(t)$ is the characteristic relaxation function corresponding to each population i. In this approach, populations are dependent on the data analysis procedure.

In the literature concerning NMR relaxation in complex systems (such as food emulsions), protons are classified in a number of pools of protons (i.e., protons belonging to molecules having the same mobility) to give a physical meaning to the detected populations. The protons in the water molecules can be divided into three pools: (i) a pool with protons retaining their bulk water-like properties, (ii) a pool with protons trapped within a macromolecular network, and (iii) a pool with protons interacting strongly with the network molecules. For the last two pools, one can expect that their mobility will be decreased compared with the mobility in bulk water.

The protons of the macromolecules can be divided in two pools: (i) labile protons, which can chemically exchange with the water protons, and (ii) nonlabile protons. Labile protons belong to carboxyl groups, –COOH; hydroxyl groups, –OH; and amino groups, –NH, $–NH_2$, $–NH_3$ (24–26). Protons from –NH amino groups that belong to the protein backbone exchange slowly with water protons and will later in this paper be considered as nonlabile protons. The exchange rates and the lability are pH dependent, but we will neglect this aspect as a first approximation for the calculation of WPC proton densities. Nonlabile protein protons have the mobility of the protein.

Oil protons are mobile. As for water, there could be more

than one pool of them. α and β–β′ crystals give rise to two separate pools of nonmobile protons.

During an NMR experiment, some physical phenomena may occur, such as diffusion of molecules carrying the protons and proton exchange affecting the magnetization of the protons. Molecular diffusion, chemical exchange, and magnetization transfer can mix different pools of protons. Thus, a population (Eq. 1) does not always correspond to a single pool of protons (27). A single population is often found in an NMR relaxation experiment involving macromolecular solutions, representing the average relaxation of the three water pools and the labile protons from the macromolecules (24,25,28,29). A second population with much shorter relaxation time is sometime detected and is associated with the nonlabile protons of the macromolecules.

Some protons can be part of semisolid structures. In this case, their relaxation is neither the one of purely mobile nor the one of purely nonmobile protons (23).

Three basic functions were used to deconvolute the NMR relaxation decays. For a given population the functions $f_i(t)$ (Eq. 1) will be a combination of them (Table 1): exponential: $\exp(-t/T_{2i})$; Gaussian: $\exp[-0.5\,(t/T_{2i})^2]$; and Pake: $\exp(-A^2 \times t^2/2) \times \sin(B \times t)/(B \times t)$.

Relaxation of mobile protons is well described by exponentials with a relaxation time T_{2i} greater than 2 ms. Relaxation of nonmobile protons is usually described in the literature by Gaussian and exponential expressions with relaxation time T_{2i} in the microsecond range (24,25,28). Recent works have shown that when protons are in strong dipole-dipole interaction, Pake functions are more suitable than Gaussian (23,30).

As our aim is to quantify SFC of the fat-oil phase of O/W emulsions, we have used the constraints and definitions that were proposed for measurement on oil-continuous emulsions (8). The relaxation time for the Gaussian function was set at 0.01189 ms, and the parameter B (Pake function) was free to vary from 150 to 188 ms^{-1}. The parameter A (Pake function) was fixed at 66.5 ms^{-1}. Relaxation times between 0.01 and 0.045 ms were attributed to nonmobile protons with additional degrees of freedom compared with other nonmobile protons. Relaxation times between 0.05 and 2 ms were attributed to semimobile protons. Relaxation times above 2 ms were attributed to mobile protons (Table 1).

In the deconvolution analysis, the number of populations is not fixed. The exact identification of the populations with the ingredients of the emulsions or the solutions is not possible with the current method. For SFC quantification analysis, knowledge of the emulsion composition is then required.

When not stated otherwise, semimobile populations will be counted with the nonmobile populations to make the Solid Content detection (Eq. 2).

$$\text{Solid Content} = [\Sigma n_i\,(\text{nonmobile}) + \Sigma n_i\,(\text{semimobile})]/[\Sigma n_i\,(\text{nonmobile}) + \Sigma n_i\,(\text{semimobile}) + \Sigma n_i\,(\text{mobile})] \quad [2]$$

$$\text{Liquid Content} = \Sigma n_i\,(\text{mobile})/[\Sigma n_i\,(\text{nonmobile}) + \Sigma n_i\,(\text{semimobile}) + \Sigma n_i\,(\text{mobile})] \quad [3]$$

Proton density, P_d. Since NMR is sensitive to proton concentration, one has to convert the mass concentration (w g/g), $C_{\text{ingredient}}$, of each ingredient into a proton fraction, $P_{\text{ingredient}}$, of each ingredient. For this, the proton density, $P_{d\,\text{ingredient}}$, of each ingredient must be calculated. The proton density is defined as the ratio between the number of protons in a molecule, N_p, and its molecular weight, M.

$$P_d = N_p/M \text{ (mol/g)} \quad [4]$$

Water, whey protein (labile protons), whey protein (nonlabile protons), lactose, PC-VF, and SF have proton densities, respectively, of 0.1111, 0.0059 ± 0.0001, 0.0647 ± 0.0002, 0.0643,

TABLE 1
Expected Proton Populations Related to Some Molecules with Their Associated Proton Relaxation Mathematical Functions

Molecules	Mathematical functions	Relaxation parameters
	Mobile populations	
Water and macromolecules (labile protons) Oil Lactose	$\exp(-t/T_2)$	$T_2 > 2$ ms
	Semimobile populations	
Fat-oil Macromolecules	$\exp(-t/T_2)$	$0.045 < T_2 < 2$ ms
	Nonmobile populations	
β and β′ fat crystals Macromolecules (nonlabile)	$\exp(-A^2 * t^2/2) * \sin(B * t)/(B * t)$ and $\exp(-t/T_2)$	$T_2 < 0.045$ ms $A = 66.5\ \text{ms}^{-1}$ $150\ \text{ms}^{-1} < B < 188\ \text{ms}^{-1}$
α fat crystals Macromolecules (nonlabile)	$\exp[-0.5\,(t/T_{2i})^2]$	$T_2 = 0.01189$ ms

0.1147 ± 0.0003, and 0.1176 ± 0.0002 mol/g. Errors due to uncertainties in the chemical compositions of the natural materials were estimated by varying the composition of the main ingredients of the lipid sources and whey protein by ±5%.

$$P_{\text{ingredient}} = (P_{d\,\text{ingredient}} \times C_{\text{ingredient}})/(\Sigma P_{d\,j} \times C_j) \quad [5]$$

The proton fraction of any ingredient or group of ingredients is compared in the Results and Discussion section with the Solid Content detected by TRDD.

RESULTS AND DISCUSSION

In this study we address practical aspects of the determination of the phase composition of protein-stabilized O/W emulsions. In the first part of this study, we will demonstrate that the TRDD method classifies some of the protein protons as nonmobile in WPC solutions. Concentration, pH, and protein denaturation degree were varied. A quantification of the protons in those exchanging systems is proposed based on the populations detected by TRDD. In a second part, we will show that the learning from the first part is still valid when applied to O/W emulsion. In the third part, we will demonstrate that such data can be used to study supercooling of lipids during crystallization in small oil droplets.

The complex NMR relaxation behavior of WPC in solution or in emulsion is similar to that of other protein sources (Duval, F., unpublished results). In the following, we will see that whey protein protons can belong to mobile, semimobile, or nonmobile populations. Their relative amounts are a function of the WPC concentration, the pH, and the degree of denaturation of the protein induced by heating. Knowing the effective contribution of the WPC to the Solid Content detected by TRDD will allow us to determine the SFC of the fat-oil phase dispersed in O/W emulsions.

Heated and nonheated whey protein solutions. When fully denatured by the heating step (85°C for 20 min) and when the pH is around 6.8, the WPC Solid Content detected by the TRDD method increases linearly with the amount of WPC in the solutions (Fig. 1). By knowing the WPC composition, Φ_i, and the proton densities of each ingredient, $P_{d\,i}$, the fraction of nonlabile whey protons present in the solutions, $P_{\text{whey nonlabile}}$, can be calculated. Equation 5 can be rewritten as Equation 6,

$$P_{\text{whey_nonlabile}} = \frac{C_{\text{WPC}}(P_{d_\text{whey_nonlabile}}\Phi_{\text{whey}})}{C_{\text{WPC}}(P_{d_\text{whey}}\Phi_{\text{whey}} + P_{d_\text{oil}}\Phi_{\text{fat-oil}} + P_{d_\text{lactose}}\Phi_{\text{lactose}} + P_{d_\text{water}}\Phi_{\text{water}}) + (100 - C_{\text{WPC}})P_{d_\text{water}}} \quad [6]$$

The Solid Content detected by TRDD corresponds to the fraction of nonlabile whey protons present in the solutions. This result is true if one uses 0.067 and 0.004 mol/g, respectively, as nonlabile and labile whey protein proton densities in the calculation of the nonlabile whey protons (see Fig. 1). Different values were tested without any fitting program. Significant changes could be detected by eye for any step of 0.002 mol/g. The value 0.067 mol/g seems acceptable since it is between two

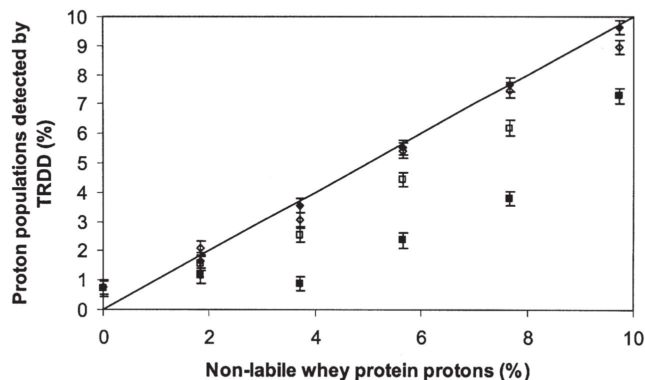

FIG. 1. Group of proton populations detected by transverse relaxation decay deconvolution (TRDD) vs. the amount of nonlabile whey protein protons in neutral solutions. Solid Content detected by TRDD (nonmobile + semimobile populations) for heated solutions (◆) (85°C, 20 min) and for nonheated solutions (■). For nonheated solutions, Solid Content plus some mobile populations defined by a relaxation time range from 2 to 4 ms (□) and by a relaxation time range from 2 to 12 ms (◇). Solid line, is meant as a guide for the eyes. Measurements were done at 5°C.

boundaries that were calculated: 0.071 mol/g, when there are no labile protons, and 0.065 mol/g when all possible labile protons have been taken out. Since lactose is present at concentrations below the saturation concentration (18%), its protons are all mobile and thus one uses the proton density given in the experimental procedures section (0.0643 mol/g).

The percentage of the different populations present in the Solid Content detected by TRDD for the heated neutral WPC solutions is given Fig. 2. This overall distribution indicates that protons do not all have the same mobility. There is even a significant semimobile population, which decreases with increasing protein content. This concentration effect may be attributed to protein aggregation.

The heating step (85°C, 20 min) has an effect on the amount of Solid Content detected by TRDD in the neutral WPC solu-

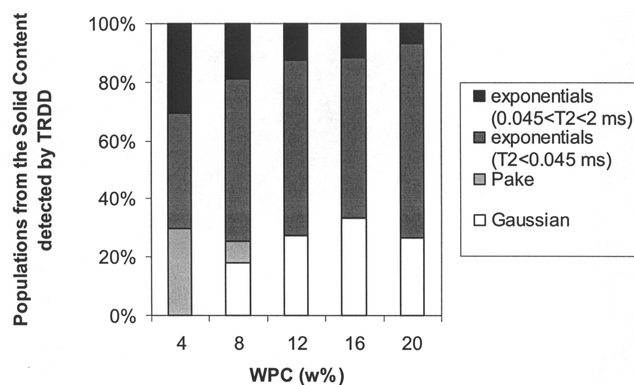

FIG. 2. Solid Content proton populations detected by TRDD of heated (85°C, 20 min) neutral whey protein concentrate (WPC) solutions. Semimobile protons are relaxing exponentially with relaxation times ranging from 0.045 to 2 ms. Nonmobile protons relax according to the other relaxation functions. Measurements were done at 5°C. For other abbreviation see Figure 1.

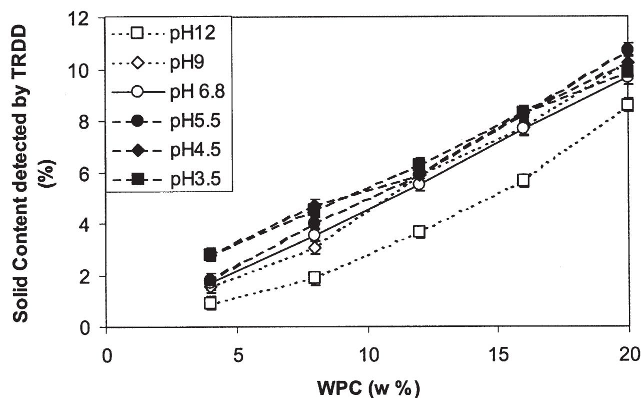

FIG. 3. Solid Content detected by TRDD of heated (85°C, 20 min) WPC solutions at various pH values. Broken or continuous lines are guides for the eyes. For abbreviations see Figures 1 and 2.

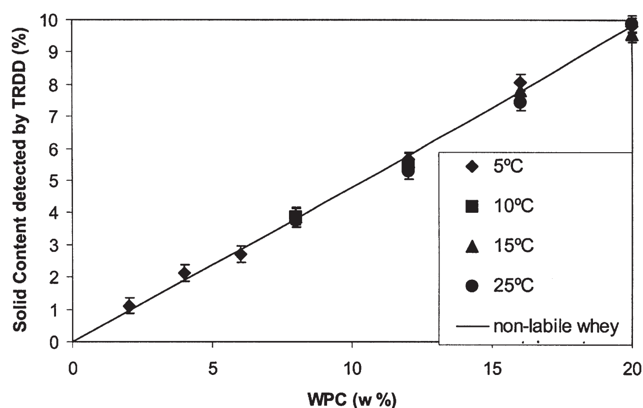

FIG. 4. Solid Content detected by TRDD vs. WPC content at various temperatures for neutral sunflower oil emulsions. Also plotted is the nonlabile whey protein proton content in those emulsions (continuous line). For abbreviations see Figures 1 and 2.

tions (Fig. 1). Contrary to heated solutions, for incompletely denatured protein solutions, the Solid Content did not increase linearly with WPC content. For all concentrations, the detected Solid Content was below the amount of nonlabile whey protein present in the solutions. Below a critical concentration (8% WPC), there was even no significant amount of nonmobile proton populations detected.

As for the heated solution, the detected Solid Content contains contributions of nonmobile and semimobile populations. The mobile protons are composed of populations with various relaxation times. For the neutral nonheated WPC solutions, two of those populations range, respectively, from 2 to 4 ms and from 4 to 12 ms. If one adds those two "mobile" populations to the Solid Content, the results corresponds roughly to the quantity of nonlabile whey protein protons present in the solutions (Fig. 1). One can then identify those two mobile proton populations with nonlabile whey protons. We have thus shown that nondenaturated whey protein has nonmobile, semimobile, and mobile protons.

This experiment shows also that, when not fully denatured, most of the whey proteins are mobile at low concentrations. At higher concentrations, only a fraction of the whey protein remains mobile. The nondenatured whey proteins have a globular structure. With an increase of the protein concentration, some molecular interactions must restrain the mobility of more and more proteins. The denatured proteins have a linear structure. The heating step breaks down the globular structures, unmasking thiol groups (–SH). Strong disulfide bonds can form between the protein molecules. The denatured proteins tend to aggregate even at low concentrations to form small particles (nanometer scale) (31–34).

Heated WPC solutions at various pH values. The Solid Content detected by TRDD for heated protein solutions (85°C, 20 min) is pH dependent (Fig. 3). At pH 9, 6.8, and 5.5, the Solid Content was proportional to the amount of whey protein in the solutions. At pH 12, less Solid Content was detected than at pH 9, and Solid Content did not increase linearly with WPC. At pH 3.5 and 4.5, Solid Content increased with increasing protein content. The direct relation that we observed at pH 6.8 between the nonmobile protons detected by TRDD and the nonlabile whey protein protons present in the solution is not obvious for the other pH values. The overestimation of Solid Content at low pH (3.5–5.5) may be attributed to the formation of whey protein particles that can entrap water that can give a semisolid contribution to the NMR relaxation decay. At pH 12, the Solid Content is underestimated, which may be due to a more dissolved (liquid-like) behavior of the whey protein.

Whey protein-stabilized O/W emulsions prepared with SF. SF emulsions are interesting emulsions because, as SF is a liquid oil, nonmobile protons should only be related to the protein content. One can see in Fig. 4 that the Solid Content in neutral emulsions increased as the WPC content increased from 2 to 20% in weight. The Solid Content is not temperature sensitive since measurements done at 5 10, 15, and 25°C gave similar results.

The Solid Content measured at 5°C (◆, Fig. 4) and the amount of nonlabile whey protein protons (continuous line) are in good agreement. The best match was found for proton densities of 0.067 and 0.004 mol/g for the nonlabile and labile whey protons, in line with the values found for the neutral solutions of WPC.

For a given protein concentration, the Solid Content detected by TRDD in acidified emulsions at pH 4.5 is slightly higher (Fig. 5) than for neutral emulsions (Fig. 4). The same effect was seen with the WPC solutions. To have a good match between the amount of nonlabile whey protons present in the emulsions and the Solid Content detected by TRDD, the proton density should be 0.074 mol/g for nonlabile protein protons and 0 mol/g for the labile ones. The value of 0.074 mol/g exceeds the limit value for nonlabile protein protons: 0.071. It was expected that the same values would be obtained as for the WPC solutions at pH 4.5 (0.070 and 0.001 mol/g). Those values can be reduced to 0.070 and 0.001 mol/g if one considers that some protons from water or oil are behaving like nonmobile protons. In this case, their amount is equivalent to 5% of the amount of protein protons. In contrast to solutions at pH

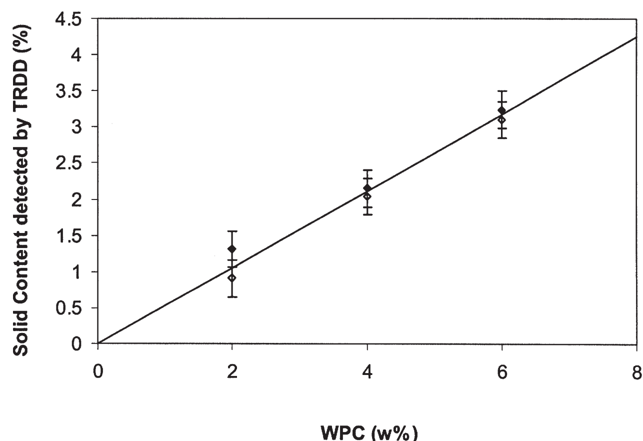

FIG. 5. Acidified sunflower oil emulsions (pH 4.5): Solid Content detected by TRDD vs. WPC content measured at 5 (◆) and 25°C (◇). The continuous line corresponds to nonlabile whey protein present in the emulsions. It was calculated using either a nonlabile proton density of 0.074 mol/g or a nonlabile whey protein of 0.070 mol/g plus nonmobile water-oil protons (5% of the amount of whey protons). For abbreviations see Figures 1 and 2.

4.5, there is no semisolid detected. Hence, it is not obvious to split the contribution of the water/oil protons from the contribution of the whey protons. If this last hypothesis is true, the nonmobile water/oil protons may be even less mobile in emulsions than in solutions.

In a manner similar to the WPC neutral solutions, the corresponding solid-like signal is mainly described by exponentials with short relaxation times and Gaussian functions at 5 or 25°C for acidified or neutral emulsions (Fig. 6). Still, there is almost no semisolid population in the case of emulsions, which indicates that the mobility of the protein is lower in the emulsions than in the solution. This is in agreement with the adsorption of the proteins around the fat globules.

Whey protein-stabilized O/W emulsions prepared with a crystallizing FB. The Emulsion Solid Content, $SC_{emulsion}$, is given by the sum of the Solid Content from the proteins, $SC_{whey\ protein}$, and the crystallized fat in the droplets, $SC_{droplets}$ (FB).

$$SC_{emulsion}\ (FB) = SC_{droplets}\ (FB) + SC_{whey\ protein}\ (FB) \qquad [7]$$

To calculate $SC_{whey\ protein}$ (FB) requires use of the results obtained for the Solid Content of SF emulsions. For Equation 8 to be true, the ingredient proportions—proteins, fat-oil, water—must be the same.

$$SC_{whey\ protein}\ (FB) = SC_{whey\ protein}\ (SF) \qquad [8]$$

In this approach we also suppose that the whey proteins at the droplet interface show the same behavior whatever the level of

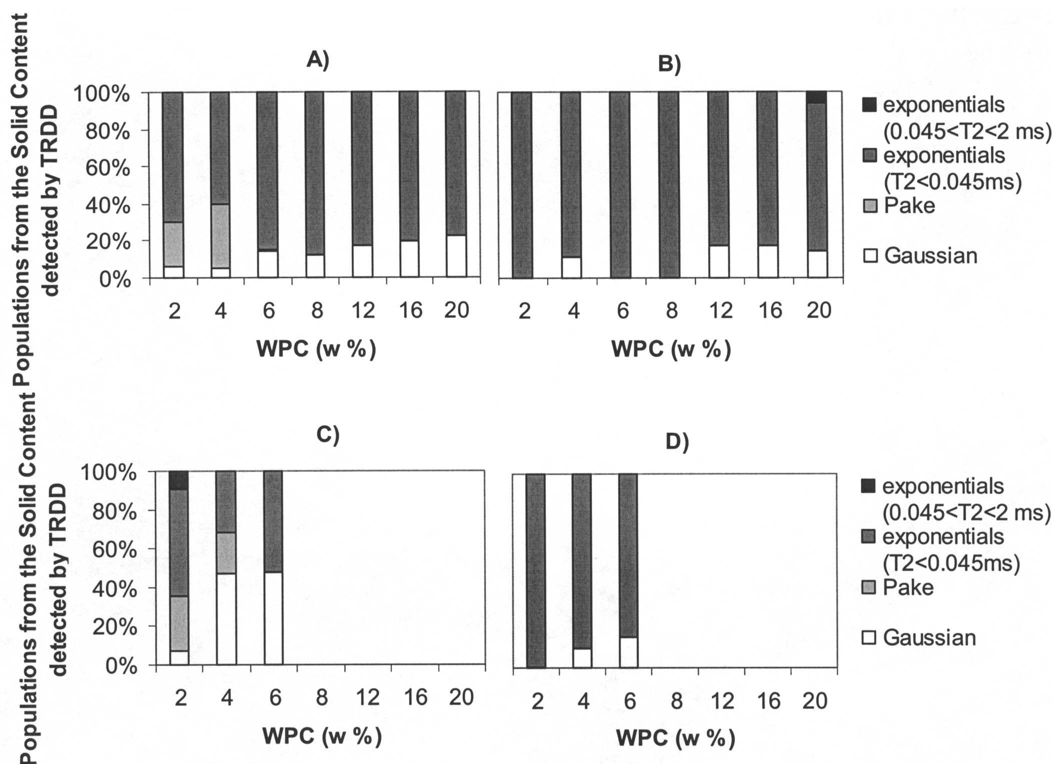

FIG. 6. Solid Content proton populations of sunflower oil emulsions at various WPC content. Neutral emulsions (A) measured at 5°C, (B) measured at 25°C; acidified emulsions (C) measured at 5°C, (D) measured at 25°C. For abbreviations see Figures 1 and 2.

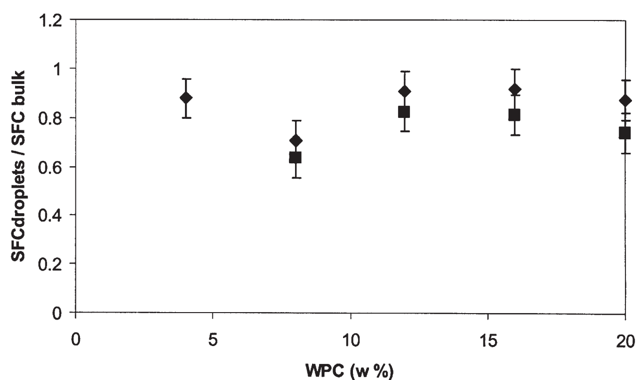

FIG. 7. Relative solid fat content (SFC) for neutral emulsions prepared with a partly crystalline vegetable fat (PC-VF) blend at various WPC concentration measured at 5°C (◆) and 15°C (■). PC-VF is a 1:1 mixture of fully hardened coconut oil and dry fractionated palm oil. For other abbreviation see Figure 2.

crystallized fat in the droplets.

The SFC of the droplets is obtain by the following formula:

$$SFC_{droplets}\,(FB) = SC_{droplets}(FB)/P_{fat\text{-}oil} \quad [9]$$

The calculation of the proton fat-oil fraction, $P_{fat\text{-}oil}$, is done using Equation 4. As a first approximation, one can use the SF proton density instead of the crystallized FB proton density.

The previous section describes the correction needed to extract the SFC of the droplets from the overall Solid Content of the emulsions. By looking at Equation 9 one can see two sources of error: (i) an error in the protein contribution to the Solid Content detected by TRDD, and (ii) an error in the fat-oil calculation.

To illustrate, in the following paragraph the errors on the droplet SFC will be estimated in the case of a 4% WPC concentration and a 30% FB concentration. Low (10%) and high (80%) $SFC_{droplets}$ values will also be taken into account.

Let us first estimate the first source of error. We have seen that whey protein contribution to the Solid Content is function of the denaturation degree of the proteins, of the pH of the product, and to a lesser extent of the concentration of WPC. Based on the measurement of solutions of WPC at various pH values, one can see that 4% of WPC could correspond to a detected Solid Content from the whey protein ranging from 0.8 to 2.8%. If one uses this range in Equation 9, it will correspond to a droplet SFC error of ±3%. With a low SFC value (10 ± 3%), the relative error will be high (±30%), whereas for a high SFC value (80 ± 3%) the relative error is much lower (±4%).

Using *a priori* proton densities could introduce another source of error on the fat-oil phase composition. We have checked two cases. In the first one, the molecular compositions of some ingredients were varied (SF TAG composition, whey protein composition). The effect is extremely limited. In the second case, two extreme values for the FB proton densities, 0.010 and 0.012 mol/g, were taken. As a result, the fat-oil proton fraction ranged from 30 to 34%. With a low SFC value, the SFC ranged from 8.9 to 10%, whereas for a high SFC value the SFC ranged from 70 to 80%. Hence, using *a priori* values can introduce errors in the $SFC_{droplets}$ value (up to SFC ± 5%). At low SFC the whey protein contribution to the Solid Content is the most problematic source of error, whereas at high SFC it is the calculation of the fat-oil phase.

An example of application: SFC in bulk vs. droplet SFC. To study supercooling in O/W emulsions, one should know the exact SFC of the droplet so that it can be compared with the SFC of the FB in bulk. The overall emulsion Solid Content is not enough.

The SFC of O/W emulsions prepared with PC-VF, $SFC_{droplets}$, and the SFC of this FB in bulk, $SFC_{PC\text{-}VF}$, were measured. At 5, 15, and 25ºC the SFC of the vegetable fat in bulk were, respectively, 62.5, 37.5, and 2.9%. The relative SFC ($SFC_{droplets}/SFC_{PC\text{-}VF}$) at five WPC concentrations is shown Fig. 7 for nonlabile protein protons. Owing to the low values at 25°C, it was not relevant to present the relative SFC at this temperature.

The relative SFC values are below a value of one, which seems to indicate that there is more solid in bulk than in the droplets. At low temperatures the effect is small, and more experiments should be done to confirm it. The cooling rate for bulk fat and emulsions was not exactly the same, and cooling rate is known to have an impact on the amount of crystals produced. But at higher temperature the supercooling effect is too important to be an artifact.

Relaxation decays of whey proteins in solutions are determined by the behavior of nonmobile, semimobile, and mobile protons. The detected Solid Content by TRDD can vary as a function of pH, protein content, and protein denaturation. For neutral heated whey proteins solutions, the detected Solid Content corresponds to the amounts of nonlabile whey proteins in solution. The adjusting parameters, the labile and nonlabile proton densities, were found to be of the same magnitude as the calculated one. At low or high pH, the detected Solid Content is not proportional to the amount of whey proteins protons because in some cases water protons have a semimobile behavior (low pH), or in other cases whey proteins protons have a liquid-like behavior (high pH).

The experiments on neutral SF emulsions showed that the Solid Content detected by TRDD corresponds to the amount of whey protein protons in the emulsions. For the acidified emulsions, the Solid Content exceeds the amount of whey proteins in the emulsions. But in both cases, the experiments allowed us to estimate the effective contribution of the whey proteins to the Solid Content of an emulsion.

In O/W emulsions, once the protein Solid Content is estimated, one can then calculate the Solid Content of the fat-oil phase. Knowledge of proton density of each ingredient is essential for quantification of the fat-oil proton percentage. With this value one can then deduce the SFC of the droplets.

Protein contribution to the Solid Content may be obtained by doing a measurement on the emulsion at a temperature above the m.p. of the fat. The present results have shown that the protein contribution is similar in the 5–25ºC range. However, in another temperature range the protein aggregation may

be different, leading to a variation in the protein contribution. Further investigation may be needed then.

ACKNOWLEDGMENTS

Dr. Elena Trezza is acknowledged for stimulating discussions. The European Union "Marie Curie" fellowship program supported this work under contract number HPMI-CT-2001-00154.

REFERENCES

1. Gidley, M.J., S.A. Ablett, and D.R. Martin, The Food Supply Chain: The Present Role and Future Potential of NMR, in *Magnetic Resonance in Food Science—Latest Developments*, edited by P.S. Belton, A.M. Gil, G.A. Webb, and D. Ruthledge, The Royal Society of Chemistry, Cambridge, 2003, pp. 3–16.
2. Bot, A., E. Flöter, J.G. Lammers, and E.G. Pelan, Controlling the Texture of Spreads, in *Texture in Food: Semi-solid Foods,* edited by B.M. McKenna, Woodhead, Cambridge, United Kingdom, 2003, pp. 350–372.
3. Champion, D., M. Le Meste, and D. Simatos, Towards an Improved Understanding of Glass Transition and Relaxations in Foods: Molecular Mobility in the Glass Transition Range, *Trends Food Sci. Technol. 11*:41–55. (2000).
4. Ruan, R.R., and P.L. Chen, *Water in Foods and Biological Materials: A Nuclear Magnetic Resonance Approach*, CRC Press, Boca Raton, 1998.
5. Widlak, N. (ed.), *Physical Properties of Fats, Oils and Emulsifiers*, AOCS Press, Champaign, 2000.
6. van Putte, K., and J. van den Enden, Fully Automated Determination of Solid Fat Content by Pulsed NMR, *J. Am. Oil Chem.Soc. 51*:316–320 (1973).
7. Clark, A.H., and P.J. Lillford, Evaluation of a Deconvolution Approach to the Analysis of NMR Relaxation Decay Functions, *J. Magn. Reson. 41*:42–60 (1980).
8. Trezza, E., A.M. Haiduc, and J.P.M. van Duynhoven, Comprehensive Phase-Compositional Analysis of Lipid Based Food Products, in *Magnetic Resonance in Food Science,* edited by P.S. Belton, A.M. Gil, G.A. Webb, and D. Rutledge, RSC Books, London, 2005, pp. 217–224.
9. Trezza, E., A.M. Haiduc, G.J.W. Goudappel, and J.P.M. van Duynhoven, Rapid Phase Compositional Assessment of Lipid Based Food Products by Time Domain NMR, *Magn. Reson. Chem*. (in press):doi number 10.1002/mrc.1893.
10. van den Dries, I.J., N.A.M. Besseling, D. van Dusschoten, M.A. Hemminga, and E. van der Linden, Relation Between a Transition in Molecular Mobility and Collapse Phenomena in Glucose-Water Systems, *J. Phys. Chem. B 104*:9260–9266 (2000).
11. Derbyshire, W., M. van den Bosch, D. van Dusschoten, W. MacNaughtan, I.A. Farhat, M.A. Hemminga, and J.R. Mitchell, Fitting of the Beat Pattern Observed in NMR Free-Induction Decay Signals of Concentrated Carbohydrate-Water Solutions, *J. Magn. Reson. 168*:278–283 (2004).
12. LeBotlan, D., and I. Helie-Fourel, Assessment of the Intermediate Phase in Milk-Fat by Low-Resolution Nuclear-Magnetic-Resonance, *Anal. Chim. Acta 311*:217–223 (1995).
13. Goetz, J., and P. Koehler, Study of the Thermal Denaturation of Selected Proteins of Whey and Egg by Low Resolution NMR, *Food Sci. Technol./Lebensm. Wissen. Technol. 38*:501–512 (2005).
14. LeBotlan, D.J., and L. Ouguerram, Spin-Spin Relaxation Time Determination of Intermediate States in Heterogeneous Products from Free Induction Decay NMR Signals, *Anal. Chim. Acta 349*:339–347 (1997).
15. Mariette, F., and T. Lucas, NMR Signal Analysis to Attribute the Components to the Solid/Liquid Phases Present in Mixes and Ice Creams, *J. Agric. Food Chem. 53*:1317–1327 (2005).
16. Bot, A., F.A.M. Kleinherenbrink, M. Mellema, and C.K. Reiffers-Magnani, Cream Cheese as an Acidified Protein-Stabilized Emulsion Gel, in *Handbook of Food Products Manufacturing,* edited by Y.H. Hui *et al*., Wiley, New York, 2007, Chapter 28, in press.
17. Kiokias, S., C.K. Reiffers-Magnani, and A. Bot, Stability of Whey-Protein-Stabilized Oil-in-Water Emulsions During Chilled Storage and Temperature Cycling, *J. Agric. Food Chem. 52*:3823–3830 (2004).
18. Kiokias, S., and A. Bot, Temperature Cycling Stability of Preheated Acidified Whey Protein-Stabilised O/W Emulsion Gels in Relation to the Internal Surface Area of the Emulsion, *Food Hydrocolloids 20*:245–252 (2006).
19. Meiboom, S., and D. Gill, Modified Spin-Echo Method for Measuring Nuclear Relaxation Times, *Phys. Rev*. *29*:688–691 (1958).
20. Goudappel, G.J.W., M.C.M. Gribnau, V.K.S. Shukla, and J.P.M. van Duynhoven, Solid Fat Content Determination by NMR, *INFORM 10*:479–484 (1999).
21. Mills, B.L., and F.R. Vandevoort, Comparison of the Direct and Indirect Wide-Line Nuclear Magnetic-Resonance Methods for Determining Solid Fat Content, *J. Am. Oil Chem. Soc. 58*:776–778 (1981).
22. Gribnau, M.C.M., Determination of Solid/Liquid Ratios of Fats and Oils by Low-Resolution Pulsed NMR, *Trends Food Sci. Technol. 3*:186–190 (1992).
23. van Duynhoven, J.P.M., I. Dubourg, G.J. Goudappel, and E. Roijers, Determination of MG and TG Phase Composition by Time-Domain NMR, *J. Am. Oil Chem. Soc. 79*:383–388 (2002).
24. Hills, B.P., S.F. Takacs, and P.S. Belton, The Effects of Proteins on the Proton N.M.R. Transverse Relaxation Times of Water. 1. Native Bovine Serum Albumin, *Mol. Phys. 67*:903–918 (1989).
25. Hills, B.P., S.F. Takacs, and P.S. Belton, The Effects of Proteins on the Proton NMR Transverse Relaxation Time of Water. 2. Protein Aggregation, *Ibid. 67*:919–937 (1989).
26. Denisov, V.P., and B. Halle, Hydrogen Exchange Rates in Proteins from Water ^{1}H Transverse Magnetic Relaxation, *J. Am. Oil Chem. Soc. 124*:10264–10265 (2002).
27. Belton, P.S., Can Nuclear Magnetic Resonance Give Useful Information About the State of Water in Foodstuffs, in *Comments Agric. Food Chem. 2*:179–209 (1990).
28. Le Dean, A., F. Mariette, and M. Marin, ^{1}H Nuclear Magnetic Resonance Relaxometry Study of Water State in Milk Protein Mixtures, *J. Agric. Food Chem. 52*:5449–5455 (2004).
29. Belton, P.S., NMR and the Mobility of Water in Polysaccharide Gels, *Int. J. Biol. Macromol. 21*:81–88 (1997).
30. van den Dries, I.J., D. van Dusschoten, and M.A. Hemminga, Mobility in Maltose-Water Glasses Studied with ^{1}H NMR, *J. Phys. Chem. B 102*:10483–10489 (1998) .
31. Alting, A.C., R.J. Hamer, C.G. de Kruif, and R.W. Visschers, Formation of Disulfide Bonds in Acid-Induced Gels of Preheated Whey Protein Isolate, *J. Agric. Food Chem. 48*:5001–5007 (2000).
32. de Kruif, C.G., M.A.M. Hoffmann, M.E. van Marle, P.J.J.M. Van Mil, S.P.F.M. Roefs, M. Verheul, and N. Zoon, Gelation of Proteins from Milk, *Faraday Discuss. 101:185*–200 (1995).
33. Verheul, M., and S.P.F.M. Roefs, Structure of Particulate Whey Protein Gels: Effect of NaCl Concentration, pH, Heating Temperature, and Protein Composition, *J. Agric. Food Chem. 46*:4909–4916 (1998).
34. Vasbinder, A.J., A.C. Alting, R.W. Visschers, and C.G. de Kruif, Texture of Acid Milk Gels: Formation of Disulfide Cross-Links During Acidification, *Int. Dairy J. 13*:29–38 (2003).

[Received May 3, 2006; accepted July 25, 2006]