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Keywords: NMR, Relaxation, Egg, Hen egg, FFC, Fast field cycling, CPMG

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A proton NMR relaxation study of hen egg quality

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Abstract

A quantitative analysis of NMR proton relaxation in hen egg albumen and yolk is undertaken to research the causes of quality loss during the first few days of storage and to access the feasibility of an on-line NMR sensor of internal egg quality. It is shown that the change in the transverse relaxation in thick egg albumen mainly results from an increase in proton exchange rate resulting from a pH increase attributed to loss of carbon dioxide by diffusion through the eggshell. The results suggest that the low-field T_1 is the best relaxation time indicator of albumen quality.

Keywords: Egg's quality; Low-resolution NMR; NMRD; Model-free analysis

1. Introduction

Quality deterioration in hen eggs during the first few days of storage has been the subject of several biochemical investigations and has been reviewed [1]. Being noninvasive, MRI is a potentially valuable technique for monitoring these internal quality changes. Jayasundar et al. [2] showed that all the major structures including the yolk, egg white, the chalaza, latebra and airspace could be clearly identified in MR images of raw hen eggs. Even the white and yellow rings inside the egg yolk could be discerned in T_1-weighted images [2,3]. These MRI studies are not merely of academic interest but have potentially important commercial significance for automated egg grading. Modern egg grading machines sort eggs according to size at rates of up to 120,000 eggs per hour, but internal egg quality is usually assessed by random sampling and human visual inspection by shining light through the egg in a process called "candling." To try to circumvent this labor-intensive operation, there is an intense research effort underway to develop noninvasive on-line sensors of both internal and external egg quality [4]. Being noninvasive and capable of revealing internal structure, an on-line MRI sensor is a potential candidate, provided, of course, that it can be developed as a low-cost sensor and useful correlations between image parameters and egg quality can be found. A hint that such correlations exist can be found in the work by Capozzi et al. [5] who showed that the proton longitudinal relaxation time of the egg white measured at 700 kHz follows a pseudoexponential decay with time after laying. More recently, Schwägele et al. [6] showed that the water proton transverse relaxation time of raw egg white measured at 7.5 MHz decreased with increasing storage time over a 40-day period. However, Jayasundar et al. [2], working at a higher proton frequency of 200 MHz, found no MRI-measurable changes in their relaxation-weighted images of either the egg white or yolk during storage of raw eggs over 5 days. They did however see an almost linear increase in the size of the airspace caused by evaporation of water through the shell.

Potentially useful NMR–egg quality correlations can also be explored by in vitro relaxation time measurements on separated egg albumen and yolk, but there have been surprisingly few such studies to date. Klammer and Kimmich [7] found that there were no apparent changes in the transverse relaxation times of resolved spectral components (water and lipid peaks) in the yolk of fertilized hen egg but did not report on the relaxation behavior of the egg albumen. An earlier paper of Koenig and Brown [8] reported

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the effects of cooking on the T_1 frequency dispersions (NMRD profiles) of the albumen but gave no quantitative analysis of the changes. In this paper, we therefore report our systematic quantitative proton relaxation analysis of the effects of aging on raw hen egg white and yolk and try to assess the viability of an on-line NMR-based egg grader.

"Egg white" is actually a generic name that includes both thick and thin egg albumen that exists as two distinct structures of roughly equal volume in the egg white, and these are illustrated in Fig. 1. The two albumen fractions can be separated by filtration through course meshed cloth gauze. In this study, we focus mainly on the NMR properties of the thick albumen component because its high viscoelasticity is an important internal quality factor that, together with water content, decreases during storage [9]. The decrease in water content is caused partly by loss of water by diffusion of vapor through the eggshell and partly by water transport from the albumen into the yolk. These processes cause a gradual increase in the volume of the airspace in the egg (see Fig. 1), and this is another internal quality factor readily measured by MRI.

Changes in the chemical composition and rheological properties of thick egg albumen have been extensively reported in the early biochemical literature [1,10,11]. Egg white is, in essence, an approximately 12% protein solution whose major protein components include ovalbumin (54%), ovotransferrin (12%), lysozyme (3.4%) and the glycoproteins, ovomucoid (11%) and ovomucin (3.5%) [1]. The viscoelasticity of fresh thick egg albumen, which is so crucial to good egg quality, appears to be related to the existence of a cross-linked gel-like protein network consisting of an ovomucin–lysozyme protein complex. The gradual degradation of this network during storage accounts for the progressive decrease in viscoelasticity, but the precise structure of the gel-like network and the reasons for its degradation remain a mystery. One plausible hypothesis relates the breakdown to the loss of carbon dioxide from the albumen by diffusion through the porous eggshell during the first few days of storage. This causes the albumen pH to increase from about 7.5 in newly laid eggs to roughly 9.6, depending on storage temperature. The fact that coating the eggshell with oil or wax to slow the diffusive loss of carbon dioxide and water appears to slow the degradation process [12] lends credence to this hypothesis. In this paper, we test this hypothesis by comparing the relaxation changes in thick egg albumen for both oiled and un-oiled eggs, and with the effect of deliberately changing the pH of the egg albumen.

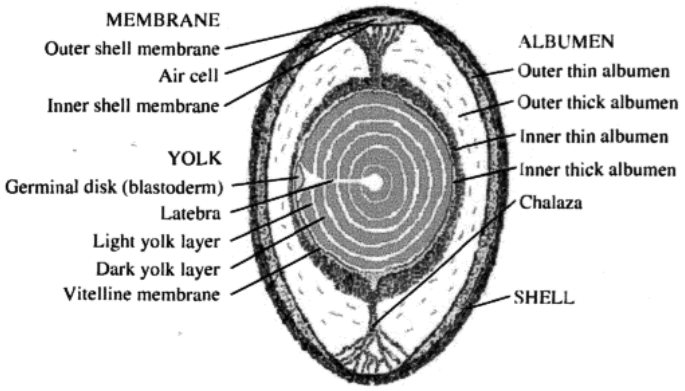

Fig. 1. Schematic of egg morphology.

The fact that thick albumen is, in essence, just a multicomponent protein gel suggests that it should be possible to analyze the relaxation data quantitatively in terms of biopolymer composition and its changing dynamic state. We will indeed attempt to do this, but the analysis is far from straightforward and the interpretation of water proton relaxation even in single component protein solutions remains a source of controversy. The difficulty revolves around the unknown relative contributions of proton exchange and hydration water to the observed relaxation. The earlier literature on water relaxation in biopolymer systems often neglected the role of proton exchange altogether and interpreted the data in terms of varying amounts of poorly defined states of "bound" water. The current literature generally acknowledges the role of proton exchange in a qualitative way, but there have been few attempts to model exchange contributions quantitatively. For this reason, we begin our relaxation study by reviewing the theoretical interpretation of water proton relaxation in high water content biopolymer systems.

2. The proton exchange cross-relaxation model of water proton relaxation in aqueous biopolymer systems

According to the proton exchange cross-relaxation model [13–15], an aqueous biopolymer system can be considered to comprise three proton pools: namely, the water protons of fraction, P_a; the exchangeable biopolymer protons of fraction, P_b; and the nonexchanging biopolymer protons of fraction, P_m. The water and exchangeable biopolymer protons undergo fast exchange with a proton flux, $P_b k_b$, where $1/k_b$ is the mean lifetime of a proton on the biopolymer. Each proton pool is associated with an intrinsic set of longitudinal and transverse relaxation times, T_{1i} and T_{2i} (i=a, b, m), and resonance frequency offsets, ω_i (i=a, b, m). At high water contents, the hydration water needed to maintain the structural integrity of the biopolymers is a small fraction of the total water and can be included in the biopolymer exchangeable proton pool (P_b). This is considered to be a justifiable approximation because structural hydration water is NMR indistinguishable from the other proton pools in low-resolution relaxation measurements. A fuller discussion of this point can be found in Ref. [14]. The subsequent analysis differs for transverse and longitudinal relaxation so each is considered in turn.

2.1. The proton exchange model for transverse relaxation

In the transverse mode, the nonexchanging biopolymer proton pool, P_m, is uncoupled from the other two pools and

can be neglected. In fact, it sometimes appears as a faster decaying component in the CPMG echo decay envelope. What is loosely described as the "water proton relaxation" observed in the CPMG sequence is actually the combined pools of the water and exchangeable biopolymer protons (P_a+P_b). Dephasing at a rate $1/T_2$ occurs because the P_a and P_b pools differ in resonance frequency by an amount $\delta\omega=(\omega_a-\omega_b)$, which is, of course, proportional to the spectrometer frequency, ω_0. Proton exchange on the NMR acquisition time scale means that the transverse dephasing rate also depends, in general, on the CPMG 90–180° pulse spacing, τ. Higher pulsing rates cause more rapid refocusing of the magnetization, and therefore, in general, a longer measured T_2. The exchange equations describing this process have been presented elsewhere [15] and need not be repeated here. However, considerable care is needed when fitting the CPMG echo decay data with this model. There are five independent variables, namely P_b, T_{2b}, T_{2a}, $\delta\omega$ and k_b, so finding a unique fit to the data would appear to be a near impossible task. Fortunately, the situation is not as hopeless as it might appear. Rough orders of magnitude of each of the parameters can be obtained either by independent measurement or from knowledge of the sample composition. In the case of egg albumen, P_b can be estimated from the known average composition of the mixture. As we shall demonstrate, the transverse relaxation time of the nonexchanging biopolymer proton pool, T_{2m}, can be measured in a separate CPMG experiment where the water magnetization is suppressed. Because they are modulated by the same biopolymer chain dynamics, it would be expected that T_{2b} would not differ greatly from T_{2m} [13]. T_{2a} is the value for pure water (ca. 2 s), and the frequency offset, $\delta\omega$, can be estimated from the known chemical shifts of amino acid residues and the spectrometer frequency. Moreover, if the data are presented in the form of a frequency dispersion by plotting the transverse relaxation rate against pulsing rate, $1/\tau$, then the midpoint of the dispersion is simply the exchange rate, k_b. The effects of independently varying the five parameters on the shape of the dispersion are also found to be quite different, and this greatly assists the fitting process. The number of viable parameter sets can also be reduced by simultaneously fitting the data acquired at several widely differing spectrometer frequencies. In the experiments reported below, we use proton resonance frequencies differing by more than an order of magnitude, namely 23.4 and 300.15 MHz. Despite these fitting aids, it is still necessary to make some assumptions, and in the subsequent analysis, we have chosen to fix the chemical shift difference between the water and exchangeable biopolymer protons at the average value previously found for gelatine solutions [14]. Of course, this is only an approximation but is consistent with the simplification that all the exchangeable biopolymer protons can be grouped into a single pool. An even bigger assumption is that a single "effective" dispersion curve adequately describes what must, in reality, be a superposition of dispersion curves arising from each type of biopolymer in the complex albumen mixture.

2.2. The proton exchange model for longitudinal relaxation

Fitting the $T_1(\omega_0)$ dispersion data (the NMRD curve) provides an additional, independent check on the parameters, P_b and k_b. Unfortunately, longitudinal relaxation is complicated by the possibility of cross-relaxation between the pools of exchangeable and nonexchanging biopolymer protons, characterized by a cross-relaxation rate, k_m, whose magnitude depends sensitively on the mobility of the biopolymer system and on the spectrometer frequency [13]. For rotationally mobile globular proteins, we expect k_m to be much less than k_b so that the nonexchanging and exchanging proton pools remain uncoupled. Our observations with the T_1-null–CPMG sequence described in the next section suggest that this is also the case for egg albumen. Accordingly, we will neglect cross-relaxation in our analysis. The multicomponent nature of egg albumen is the next complicating feature that needs to be taken into account. Consider first the simplest case of a single component biopolymer whose dynamic state can be characterized by a single correlation time, τ_c. For this example, the proton exchange model predicts a dispersion behavior such that [13]

$$T_1^{-1} = \alpha + \beta[0.2J(\omega_0,\tau_c) + 0.8J(2\omega_0,\tau_c)] \quad (1)$$

where $J(n\omega,\tau_c)$ is the usual Lorentzian spectral density function, $\tau_c/(1+n^2\omega^2\tau_c^2)$, and the constants α and β are given, approximately, as

$$\alpha = (1-P_b)T_{1a}^{-1} \quad (2)$$

which is just the bulk water contribution, and

$$\beta = (3/2)C_p^2P_b. \quad (3)$$

Here, C_p is the effective dipolar coupling constant for the biopolymer protons. In writing Eq. (1), we have assumed that $T_{1b}k_b \gg 1$, which implies that proton exchange is fast compared to the intrinsic longitudinal relaxation of the biopolymer protons. If this is not the case and proton exchange is rate limiting, then the relaxation rate becomes independent of frequency and proportional to the exchange rate:

$$T_1^{-1} = \alpha + P_bk_b \quad (4)$$

In a complex, multicomponent biopolymer mixture, each biopolymer component, i, would be expected to give rise to its own independent dispersion curve, which may not necessarily follow the theoretical curve described by Eq. (1). Many cases have been reported in which, even in single component protein solutions, the curves appear "stretched" on the logarithmic ω_0 scale (see, e.g., Refs. [9–29] in Ref. [16]), thus calling for a more complex analysis than simple fitting of the NMRD data to Eq. (1). The twofold level of complexity (i.e., the possibility of stretched NMRD

curves in the presence of a mixture of components) has been faced in this work by calculating average NMRD parameters through the "model free" approach of Halle et al. [16]. In this method, each NMRD curve is fitted to a modified Eq. (1) where $J(n\omega, \tau_c)$ has been redefined as

$$J(n\omega, \tau_c) = \frac{\sum_{n-1}^{N} C_n \frac{\tau_n}{1+(n\omega\tau_n)^2}}{\sum_{n=1}^{N} C_n} \quad (5)$$

Although the τ_n's obtained by Eq. (5) lack any physical significance, it has been pointed out [16] and more recently shown experimentally [17] that their weighted average over the c_n's, $\langle\tau_c\rangle$ is phenomenologically meaningful in that it correlates linearly with the rotational correlation time calculated from the Stokes–Einstein relationship for many protein systems. As we shall show, the albumen dispersion data require at least a double dispersion ($N \geq 2$) for proper characterization.

3. Methods

Resonance Instruments DRX spectrometers operating at proton frequencies of 23.4 and 300.15 MHz were used in all transverse relaxation measurements. Samples were thermostated in the probe at 23°C, and the transverse relaxation time of the exchangeable proton pool was determined with the standard CPMG sequence with full-phase cycling and a recycle delay of 10 s, and sufficient echoes to reach the baseline. The echo decay envelopes were analysed as a continuous distribution of exponential by inverse Laplace transformation with WINDXP software (Resonance Instruments, Whitney, Oxford, UK). To observe the transverse relaxation times of the nonexchanging biopolymer protons in albumen, the exchangeable proton signal was suppressed with a T_1-null inversion recovery preparation prior to the CPMG sequence. The CPMG data were then deconvoluted in the usual way with WINDXP to reveal the nonexchanging biopolymer proton transverse relaxation peak.

T_1 dispersions (NMRD curves) were measured at frequencies between 10 kHz and 15 MHz (5 kHz to 30 MHz for yolk) on a Stelar Fast Field Cycling spectrometer with the standard prepolarizing pulse sequence. The NMRD data were analysed according to the model free approach by Halle et al. [16]. The choice of N in Eq. (5) was based on the F statistics.

Newly laid hens eggs were obtained from a local farm and either used within the first hour or stored, intact, for 7 days in a thermostated room at 23°C. Because the extraction of the thick egg albumen or yolk is destructive, it was necessary to test the variation in relaxation data arising from biological diversity. This was done by repeating all NMR measurements on five different eggs both at days 0 and 7.

The eggs were analyzed for albumen pH and Haugh Index (a phenomenological measure of the albumen firmness that depends on the albumen height and the egg weight) [18,19]. Each egg was carefully opened and the content laid on a flat glass surface placed on a metal stand having adjustable legs for leveling. Albumen height was measured with a digital micrometer following the U.S.A. Department of Agriculture guidelines [18] and converted to Haugh units [19]. The value of the pH was measured with a Crison 507 pH meter equipped with a Double Bore electrode (Hamilton) that was inserted directly into the thick albumen.

Comparison of the in vitro relaxation data with in vivo MR images of each egg would have been beneficial, but

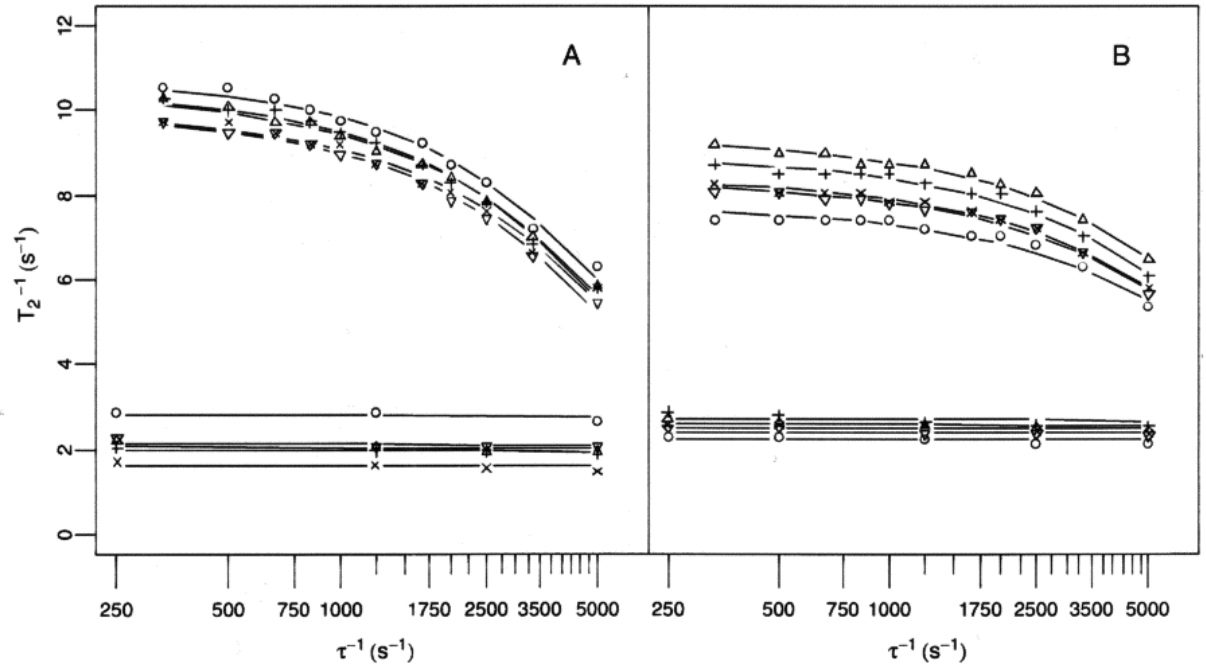

Fig. 2. Transverse relaxation rate calculated for several 90–180 pulse spacings on fresh (A) and 7-day-old (B) non-oiled eggs.

Table 1
Proton exchange parameters derived by fitting the transverse relaxation data of albumen samples taken from fresh and 7-day-old non-oiled eggs in Fig. 2

	Sample	T_{2b} (ms)	k_b ($s^{-1}\times10^3$)	P_b ($\times10^{-3}$)	pH
Day 0,	Egg 1	3.07	6.23	7.29	7.90
non-oiled	Egg 2	4.95	6.94	7.70	8.20
eggs	Egg 3	5.17	6.65	7.63	7.95
	Egg 4	7.35	7.71	8.06	8.10
	Egg 5	4.22	6.27	6.90	7.90
	Average	4.95•	6.76•	7.52•	8.01•
Day 7,	Egg 1	4.20	11.34	7.31	9.56
non-oiled	Egg 2	4.14	10.85	8.66	9.65
eggs	Egg 3	3.40	9.93	7.58	9.65
	Egg 4	3.60	10.23	7.29	9.50
	Egg 5	3.81	10.45	7.39	9.50
	Average	3.83•	10.56▲	7.64•	9.57▲

T_{2a} and $\delta\omega$ were fixed at 2 s and 1.674 ppm, respectively. Different symbols indicate statistically significant differences between average values based on the Student's t test ($P<.05$).

unfortunately, the bore of our NMR microimager is too small to accommodate whole eggs.

4. Results

4.1. Transverse relaxation of thick egg albumen

Because of its relatively high water content, the nonexchanging protein proton pool in thick egg albumen makes a negligible contribution to the CPMG echo decay envelope which can, in all cases, be adequately fitted as a single exponential decay. Fig. 2A shows the transverse relaxation dispersions for five fresh, non-oiled eggs measured at both 23.4 and 300.15 MHz. The lines are fits of the proton exchange model with the parameters listed in Table 1. Note that when fitting these data, the bulk water relaxation time was fixed at 2 s and the frequency offset between bulk water and the exchangeable biopolymer protons was set to 1.674 ppm. This is the average value derived by fitting the experimental dispersion for gelatine sols [14]. The fitted values of P_b can be compared with the values calculated at different pH values from the known average (glyco)protein composition of thick egg albumen and their amino acid compositions [20] as listed in Table 2. Given the complexity of the biopolymer composition, the agreement between the predicted and experimental values of P_b is remarkably good. The fact that the same set of exchange parameters fits the data within the experimental error at both frequencies lends strong support to the assumptions underlying the proton exchange model for transverse relaxation in egg albumen. A comparison with the corresponding data for thick egg albumen from 7-day-old non-oiled eggs (Fig. 2B and Table 1) shows that the only statistically significant effect of aging is to increase the average proton exchange rate, k_b, from about 6800 s^{-1} in fresh eggs to 10,600 s^{-1} in 7-day-old eggs. No significant changes in the exchange parameters were found after 7 days for eggs that had been covered in oil to prevent CO_2 loss (data not shown). These observations are consistent with the hypothesis that loss of carbon dioxide and the resulting increase in pH are the major cause of the relaxation changes in the thick egg albumen during the first few days of storage. As an additional check on this conclusion, the proton exchange analysis was repeated with thick egg albumen whose pH had been deliberately varied by addition of small amounts of either sodium hydroxide or hydrochloric acid. The results in Table 3 show that increasing the pH from 6.75 to 9.14 leads to a significant increase in k_b consistent with the changes seen in the non-oiled stored eggs.

Table 2
Values of P_b and P_m calculated from the known composition of thick egg albumen

pH	P_b ($\times10^{-3}$)	P_m ($\times10^{-3}$)
5.2	2.37	70.5
8.0	4.92	67.9

Table 3
The effect of deliberately changing the pH of thick egg albumen

pH	T_{2b} (ms)	k_b ($s^{-1}\times10^3$)	P_b ($\times10^{-3}$)
9.14	3.44	9.61	6.90
6.75	3.80	4.82	5.10
5.70	3.80	10.80	3.33
4.12	3.76	8.29	2.89
1.00	1.66	48.70	6.30

T_{2a} and $\delta\omega$ were fixed at 2 s and 1.674 ppm, respectively.

4.2. Water-suppressed transverse relaxation of thick egg albumen

The transverse relaxation time behavior of the nonexchanging biopolymer proton pool in egg albumen can be observed directly if the magnetization of the exchangeable proton pool is suppressed with the T_1-null–CPMG sequence (see Methods). The resulting CPMG echo decay envelopes showed double exponential relaxation arising from the nonexchanging and exchanging proton pools. The fact that separate inversion recovery curves are observed for the exchangeable and nonexchangeable proton pools shows that cross-relaxation between the two pools cannot be in the fast exchange regime and therefore supports our earlier assumption that $k_m < k_b$. Table 4 shows the dependence of the intrinsic transverse relaxation rate of the nonexchanging biopolymer proton pool, T_{2m}, on both pH and spectrometer frequency. As expected, T_{2m} has the same order of magnitude as the values of T_{2b} derived from the exchange analysis. Nevertheless, the T_{2m} are all significantly longer than T_{2b} for reasons that are not yet clear. The relaxation

Table 4
The pH dependence of the measured nonexchanging biopolymer proton transverse relaxation time, T_{2m} (ms)

pH	23.4 MHz	300.15 MHz
9.2	6.6	6.8
6.3	6.4	6.6
5.2	5.8	7.0

Table 5
The dependence of the average measured nonexchanging biopolymer proton transverse relaxation time, T_{2m} (ms), on aging.

	23.4 MHz	300.15 MHz
Fresh	6.6	7.6
Day 7, non-oiled	7.1	9.5
Day 7, oiled	6.6	8.2

times at 300.15 MHz are also systematically greater than those at 23.4 MHz, but this is an expected consequence of the spectral densities contributing to T_{2m}, and the average experimental ratio $T_{2m}(300.15)/T_{2m}(23.4)$ of 1.15 agrees reasonably well with the average theoretical ratio of 1.05 calculated using

$$T_{2m}^{-1} = 0.1C_p^2[6J(0,\tau_R) + 10J(\omega_0,\tau_R) + 4J(4\omega_0,\tau_R)] \quad (6)$$

It is especially interesting to note that T_{2m}, which reflects the biopolymer dynamics, shows a significant increase after 7 days in the non-oiled egg (see Table 5). This is to be expected if the thinning of the albumen during storage results in increased chain mobility.

4.3. Longitudinal relaxation dispersions of thick egg albumen

Fig. 3 shows a representative NMRD curve for fresh thick egg albumen together with the theoretical single correlation time dispersion predicted using the average P_b and k_b derived from the above transverse relaxation analysis. Although there is an order-of-magnitude agreement, it is obvious that the experimental curve is stretched, and therefore, a single correlation time model fails to accurately describe the data. Fig. 4A and B shows the much improved fit obtained by model-free analysis (see Methods) and Table 6 summarizes the fits for all the eggs.

It is apparent in Fig. 4 that the relaxation profiles of fresh albumen samples display lower R_1 values than aged ones. Moreover, the relaxation rates of albumen from non-oiled eggs are, on the average, higher than the ones of the samples taken from oiled eggs. These differences are reflected in the parameters of the model-free analysis listed in Table 6. Consider first the average correlation time, $\langle\tau_c\rangle$. Although there is no significant effect of aging (i.e., day 0 vs. day 7) on $\langle\tau_c\rangle$, there is an obvious difference between oiled and non-oiled eggs. The day 0 samples were analysed about 6 h after laying. In this short time, the pH of the non-oiled eggs albumen increased from ca. 7.8 to 8.5, and the Haugh Index decreased from 77.7 to 68.5 units ($P<0.05$), thus indicating that albumen from non-oiled eggs was more liquid than albumen from oiled eggs as early as 6 h after laying. This macroscopic behavior is nicely recognized by NMRD, as the average albumen $\langle\tau_c\rangle$ decreased significantly on passing from oiled to non-oiled fresh eggs. The fact that $\langle\tau_c\rangle$ of albumen shows no further change upon aging in non-oiled eggs (i.e., where pH keeps increasing) means that whatever process is responsible for inducing albumen liquefaction acts in the very first hours after laying and only as a consequence of a rapid pH increase. The observation that neither the Haugh Index nor $\langle\tau_c\rangle$ change even after a week in albumen from oiled eggs, where pH remains unchanged, supports this conclusion. Table 6 also shows that there is a significant increase in both the "baseline" term α and in the amplitude of the dispersion, represented by β in Eq. (1). The increase in β is to be expected because Eq. (3) shows that it is proportional to P_b, the fraction of exchangeable biopolymer protons, and this would increase if the lysozyme–ovomucin complexes disassemble during storage. The observation that this is accompanied by an increase in α, which, according to Eq. (2) is proportional to the free water term, suggests that hydration water is liberated from the lysozyme–ovalbumin complex during this depolymerization process.

4.4. Transverse relaxation in egg yolk

Egg yolk is an exceedingly complex, microheterogeneous substance. Its total solid content is about 50% by weight, of which ca. 16% is protein and 34% lipid with minor amounts of carbohydrate and minerals [21]. Optical microscopy reveals a large number of granular structures, including yolk spheres, granules and lipoprotein complexes suspended in a clear aqueous solution, called yolk plasma. Ultracentrifugation leaves the plasma as a supernatant and this comprises about 78% by volume of the yolk and contains about 18% protein and 77–81% lipid. The yolk is surrounded by the vitelline membrane whose permeability increases with time permitting water to transport from the albumen into the yolk during the first few days of storage.

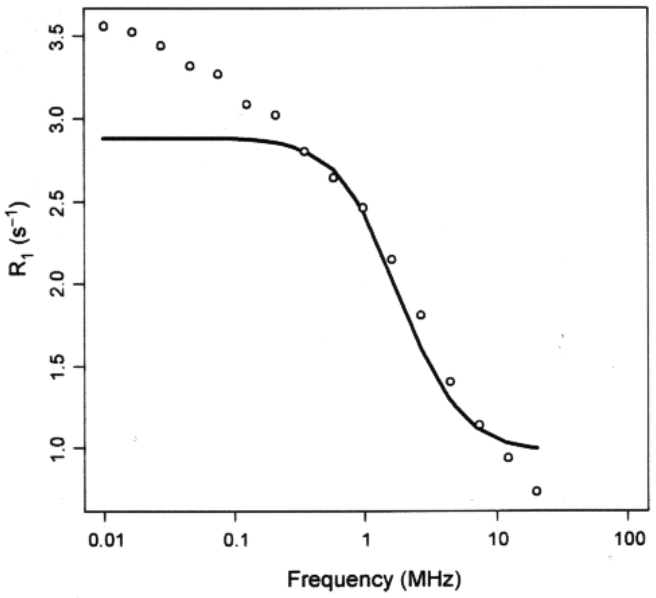

Fig. 3. Representative NMRD profile obtained from a fresh albumen sample (points) and theoretical dispersion (solid) predicted using average P_b and k_b derived from T_2 analysis.

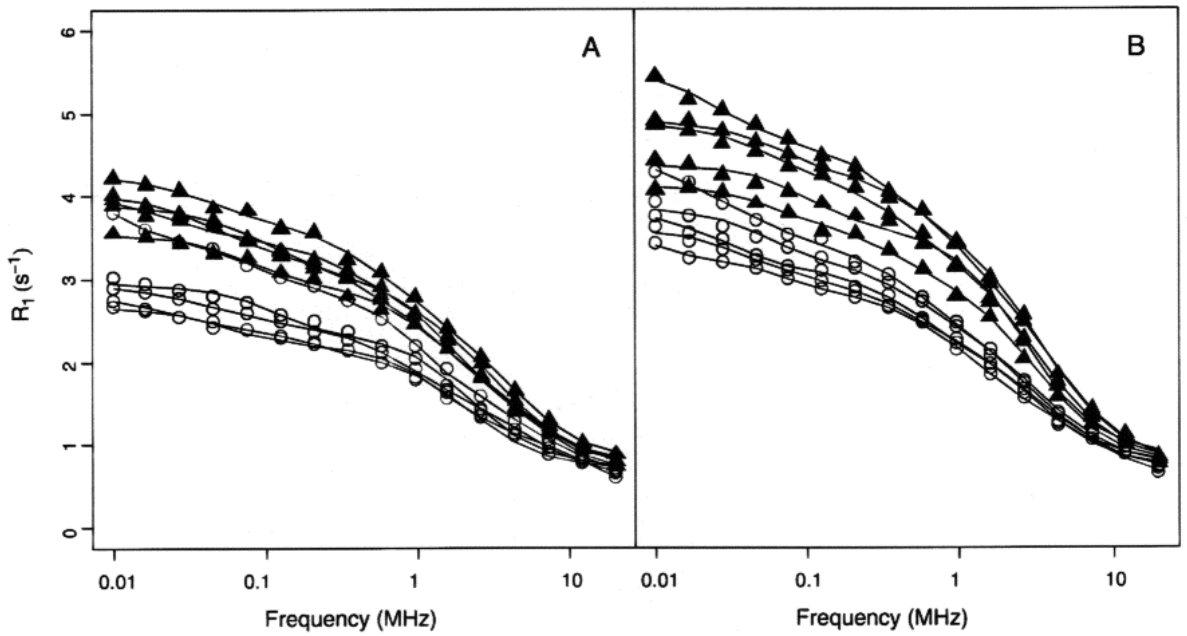

Fig. 4. NMRD profiles of albumen samples from (A) oiled and (B) non-oiled eggs. Circles and triangles represent fresh and 7-day-old samples, respectively.

Given this compositional complexity, it is not surprising that the distribution of proton transverse relaxation times for fresh egg yolk obtained by inverse Laplace transformation of the CPMG echo decay envelope acquired with a short 90–180° pulse spacing of 200 ms displays five relaxation peaks (see Fig. 5). No attempt has been made to assign these peaks to the various lipoprotein fractions, though the dominant peak at ca. 12 ms probably arises from water protons. Fig. 6 shows the relaxation rate of this dominant peak plotted as a function of pulsing rate for the two spectrometer frequencies (23.4 and 300.15 MHz). No significant pulse spacing dependence is observed. The apparent decrease in relaxation rate at very long-pulse spacings is an artifact caused by failure to properly deconvolute the shorter relaxation time components when the pulse spacing becomes comparable to the relaxation

Table 6
Best-fit parameters obtained through model-free analysis [16] for the NMRD profiles and pH values registered on hen egg thick albumen

		N^a	β ($s^{-2}\times10^7$)	α ($s^{-1}\times10^{-1}$)	$\langle\tau_c\rangle$ ($s\times10^{-8}$)	pH
Day 0, oiled eggs	Egg 1	3	3.58	7.16	5.84	7.60
	Egg 2	2	4.17	7.34	5.30	8.15
	Egg 3	4	6.47	6.12	3.58	7.74
	Egg 4	3	4.57	6.64	4.89	8.16
	Egg 5	4	8.86	5.90	5.53	7.20
	Average		5.53•	6.63•	5.03•	7.77•
Day 7, oiled eggs	Egg 1	3	6.79	6.96	4.58	7.80
	Egg 2	4	9.17	5.51	5.34	7.90
	Egg 3	3	5.65	7.34	5.08	8.05
	Egg 4	4	7.21	7.30	5.18	7.58
	Egg 5	3	6.12	7.83	5.03	7.60
	Average		6.99•▲	6.99•▲	5.04•	7.79•
Day 0, non-oiled eggs	Egg 1	3	8.23	6.78	3.48	8.60
	Egg 2	4	7.72	7.42	4.26	8.26
	Egg 3	3	8.05	8.51	4.65	8.42
	Egg 4	3	9.37	6.86	3.41	8.22
	Egg 5	3	7.39	8.04	4.32	9.00
	Average		8.15▲■	7.52▲■	4.02▲	8.50▲
Day 7, non-oiled eggs	Egg 1	3	8.14	7.79	4.11	9.25
	Egg 2	2	8.01	8.75	4.36	9.30
	Egg 3	4	11.85	7.72	3.48	9.18
	Egg 4	3	9.47	8.10	4.27	8.80
	Egg 5	3	11.03	8.09	4.26	8.95
	Average		9.70■	8.09■	4.09▲	9.10■

Different symbols indicate statistically significant differences between average values ($P<.05$).

[a] Number of Lorentzian functions used in Eq. (5).

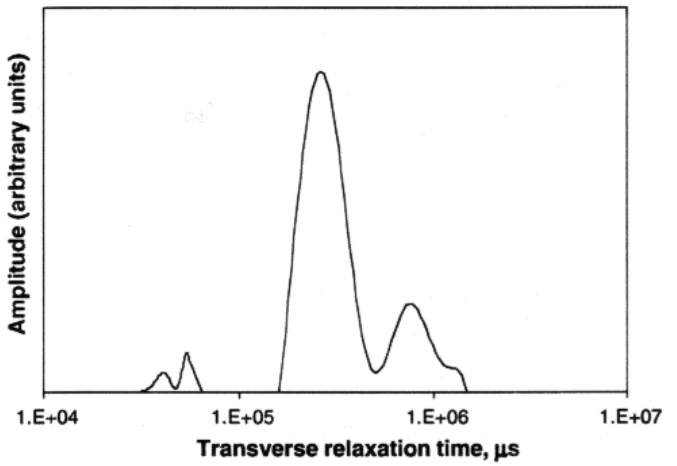

Fig. 5. Inverse Laplace transformation of the CPMG signal envelope of fresh egg yolk.

time. The increase in relaxation rate with increasing spectrometer frequency suggests that proton exchange makes a significant contribution to plasma transverse relaxation. However, given the compositional and structural complexity of the system, no attempt was made to quantitatively analyze the data with the exchange model.

It was particularly noteworthy that no significant change in relaxation behavior of the yolk was observed during storage over a 7-day period, either with oiled or non-oiled eggs. This agrees with earlier reports that yolk viscosity shows little change during aging [21] and that the water content of the yolk increases by less than 4% over a 16-day storage period.

4.5. Longitudinal relaxation in egg yolk

Fig. 7 shows the NMRD profile of egg yolk that obviously does not conform to any single correlation time model. Indeed, even a combination of BPP-like curves with half a dozen adjustable correlation times does not lead to a satisfactory fit. The solid line in Fig. 7 shows that the data

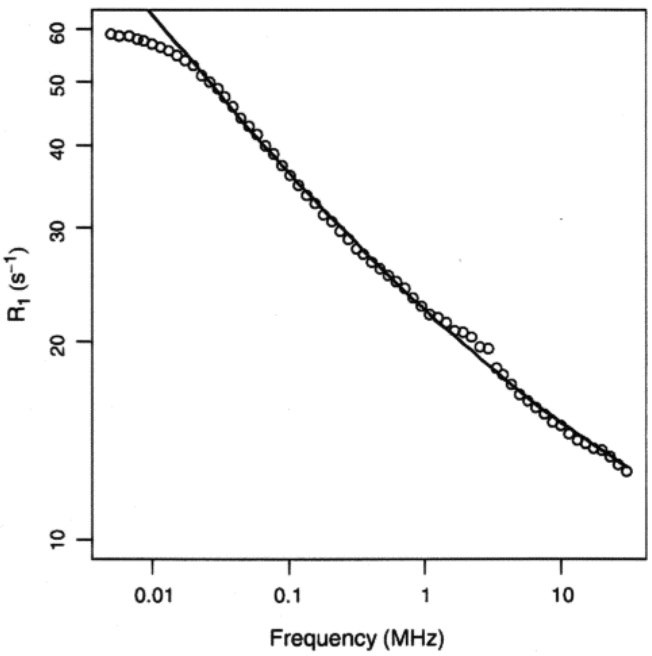

Fig. 7. Experimental NMRD profile (plotted for clarity on double log scale) of egg yolk (circles). The solid line represents the best fit of the 10-kHz to 30-MHz part of the experimental profile to the equation $R_1 = A^*\omega^{-b} + C$ [23]. Here, $A = 15.45$ s$^{-0.72}$, $b = 0.28$ and $C = 7.01$ s^{-1}.

conforms to a power law relationship such that $T_1 \propto \omega^{0.28}$ down to frequencies of about 20 kHz. Such power law relationships have been observed for many polymers and biopolymers [22,23]. The shape of the dispersion curve matches closely those of several bulk polybutadiens and, somewhat less exactly, those of natural rubber and polyisoprene (unpublished bulk polymers survey by one of the authors). In such systems, the dipolar relaxation mechanism is governed by elastic-like internal motions that do not involve a complete reorientation of proton–proton pairs and thus exclude the applicability of formulae of the BPP type. The lack of complete reorientation (within the time scale defined by the T_1 values) is further supported by

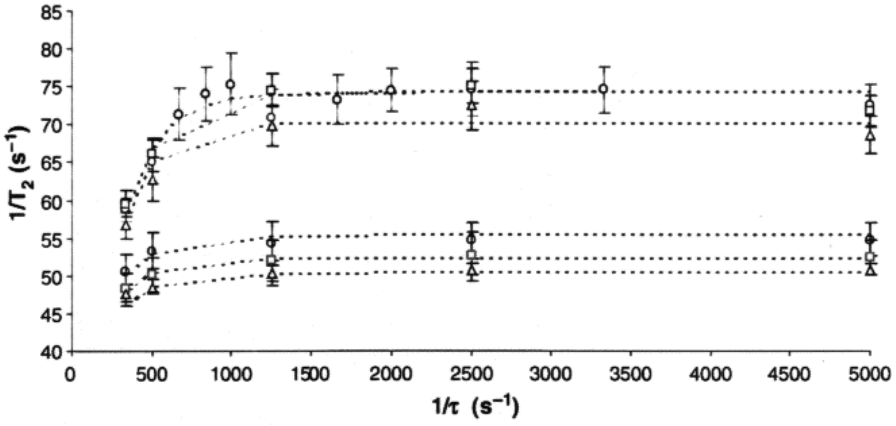

Fig. 6. Relaxation rates registered at different τ values for samples of yolk taken from fresh, non-oiled eggs (circles), 7-day-old non-oiled eggs (squares) and 7-day-old oiled eggs (triangles) at 300.15 MHz (upper curves) and 23.4 MHz (lower curves).

the clear visibility of two nitrogen cross-relaxation glitches in the interval between 2 and 3 MHz, which would be otherwise too broad to be detected.

This points to a model in which the relaxation occurs prevalently within large and nearly static blocks of protein matter where it is stimulated by abundant but highly restricted internal motions. At the same time, however, the exchange of nuclear magnetization between the solidlike matrix and the sample components with low molecular weight is quite efficient. The individual decay curves that gave rise to the individual points in Fig. 7 were in fact all nearly monoexponential (though deviations from monoexponentiality were detectable by analyzing the fitting residues, their overall impact on the fit was in all cases smaller than 2%).

It appears, therefore, that yolk behaves somewhat like a microsuspension of solidlike particles in water rather than as a solution. The transition from a suspension to a solution is of course by itself a complex physical topic. In any case, a detailed elucidation of the dynamic behavior of yolk and of its NMR aspects is beyond the scope of this paper.

5. Discussion

The detailed analysis of the relaxation behavior of albumin and yolk presented above provides the necessary information for optimized on-line detection of egg quality in the low-field on-line MRI sensors under development. A comparison of Fig. 2A and B shows that the effect of aging on the transverse relaxation time of the thick egg white is greatest at long-pulse spacings and at high-field strengths (300.15 MHz). Unfortunately, such high fields are conventionally produced with expensive superconducting magnets that are unlikely to be used in any commercially viable on-line NMR sensor. At the lower field of 23.4 MHz, there is a tendency for the thick egg albumen T_2 to decrease from an average of 4.5 ms for fresh eggs to 3.8 ms over a 7-day period, but this is a marginal trend and unlikely to be a useful basis for a commercial quality sensor.

The longitudinal relaxation data in Fig. 4A and B are more promising. Although there is no obvious difference in the high-field T_1 for thick egg albumen, the low field longitudinal relaxation time is significantly shorter in the 7-day-old eggs. The difference is actually greatest at a spectrometer frequency of 2–3 MHz, which is suitably low for use in an on-line NMR sensor. In this respect, it is interesting to note that there are already some preliminary studies on hens eggs at frequencies between 100 kHz and 1 MHz using low-cost hardware [24].

There are no significant changes in the relaxation characteristics of the egg yolk during storage, but the fact that the transverse relaxation times are shorter for yolk than for albumen confirms the ease with which these two structures can be contrasted in the MRI images reported by Jayasundar et al. [2]. Yolk quality factors such as the existence of double yolks and yolk volume are therefore amenable to MRI sensing. The position of the yolk in the egg could, perhaps, be used to estimate egg white thickness by observing the effect of end-over-end rotation of the egg on the yolk position. Another significant internal quality factor amenable to MRI measurement is the volume of the airspace, which increases almost linearly with storage time as water is lost from the egg.

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References

[1] Robinson DS. The chemical basis of albumen quality. In: Wells RG, Belyavin CG, editors. Egg quality — current problems and recent advances. London: Butterworths; 1987. p. 179–89.

[2] Jayasundar R, Ayyar S, Raghunathan P. Proton resonance imaging and relaxation in raw and cooked hen eggs. Magn Reson Imaging 1997; 15:709–17.

[3] Hutchison MJ, Lirette A, Etches RJ, Towner RA, Janzen EG. An assessment of egg-yolk structure using magnetic resonance imaging. Poult Sci 1992;71:2117–21.

[4] De Baerdemaeker J, Bamelis F, Kemps B, Govaerts T, Decuypere E, De Ketelaere B. Non-destructive measurements of egg quality. Proceedings of the Tenth European Symposium on the Quality of Eggs and Egg Products. St. Brieuc (France): Yves Nys (INRA); 2003. p. 77–90.

[5] Capozzi F, Cremonini MA, Franchini A, Luchinat C, Placucci G, Vignali C. Thinning of hen egg albumen as followed by low field 1H-NMR. In: Webb GA, editor. Book of abstracts: Fourth International Conference on Applications of Magnetic Resonance to Food Science. Norwich (UK): Royal Society of Chemistry; 1998. p. 72.

[6] Schwägele F, Poser R, Krockel L. Application of low resolution ^{1}H NMR spectroscopy for determining internal egg quality — correlations between relaxation times and internal egg quality parameters. In: Nys Y, editor. Proceedings of the Tenth European Symposium on the Quality of Eggs and Egg Products. St. Brieuc (France): Yves Nys (INRA); 2003. p. 112–8.

[7] Klammer F, Kimmich R. Volume-selective and spectroscopically resolved NMR investigation of diffusion and relaxation in fertilised hen eggs. Phys Med Biol 1990;35:67–79.

[8] Koenig SH, Brown RD. The raw and the cooked, or the importance of the motion of water for MRI revisited. Invest Radiol 1988;23:495–7.

[9] Overfield ND. Evaluation of egg quality in commercial practice. In: Wells RG, Belyavin CG, editors. Egg quality — current problems and recent advances. London: Butterworths; 1987. p. 71–86.

[10] Robinson DS, Monsey JB. Changes in the composition of ovomucin during liquefaction of thick egg white. J Sci Food Agric 1972;23: 29–38.

[11] Robinson DS, Monsey JB. Changes in the composition of ovomucin during liquefaction of thick egg white: the effect of ionic strength and magnesium salts. J Sci Food Agric 1972;23:893–904.

[12] Romanoff AL, Romanoff AJ. The avian egg. New York: Wiley; 1949.

[13] Hills BP. The proton exchange cross-relaxation model of water relaxation in biopolymer systems. Mol Phys 1992;76:489–508.

[14] Hills BP. The proton exchange–cross relaxation model of water relaxation in biopolymer systems — the sol and gel states of gelatine. Mol Phys 1992;76:509–23.

[15] Hills BP. Magnetic resonance imaging in food science. New York: Wiley; 1998.
[16] Halle B, Jóhannesson H, Venu K. Model-free analysis of stretched relaxation dispersions. J Magn Reson 1998;135:1–13.
[17] Bertini I, Fragai M, Luchinat C, Parigi G. Proton NMRD profiles of diamagnetic proteins: a model-free analysis. Magn Reson Chem 2000; 38:543–50.
[18] Anonymous. Egg grading manual. USDA. Washington, DC: USDA; 2000.
[19] Haugh RR. The Haugh unit for measuring egg quality. US Egg Poultry Magazine 1937;43:522–55, 572–3.
[20] Li-Chan E, Nakai S. Biochemical basis for the properties of egg white. Crit Rev Poul Biol 1989;2:21–59.
[21] Powrie WD, Nakai S. The chemistry of eggs and egg products. In: Stadelman WJ, Cotteril OJ, editors. Egg science and technology. 3rd ed. Westport (Conn): AVI Publishing; 1986. p. 97–139.
[22] Kimmich R, Gille K, Fatkullin N, Seitter R, Hafner S, Müller M. Field-cycling nuclear magnetic resonance relaxometry of thermoreversible polybutadiene networks. J Chem Phys 1997;107: 5973–8.
[23] Korb J-P, Bryant RG. Magnetic field dependence of proton spin-lattice relaxation times. Magn Reson Med 2002;48:21–6.
[24] Capozzi F, Cremonini MA, Luchinat C, Placucci G, Vignali C. A low frequency H-1-NMR external unit for the analysis of large foodstuff samples. J Magn Reson 1999;138:277–80.