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Cooking Effects on Water Distribution in Potatoes Using
Nuclear Magnetic Resonance RelaxationMARGIT MORTENSEN,[†] ANETTE K. THYBO,^{*,†} HANNE C. BERTRAM,[†]
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Continuous low-field (LF) ^1H NMR relaxometry was used to monitor the structural changes during cooking of potatoes with two different dry matter (DM) contents. A principal component analysis of the relaxation decay curves revealed major events related to water mobility during cooking, which occur at 53 and 60 °C for potatoes with medium and low DM contents, respectively. Exponential analysis of the relaxation decays reveals two major water populations in the potato: a slow-relaxing (assigned to water in cytoplasm and extracellular cavities) water component, T_{22} (~350–550 ms), and a fast-relaxing component (primarily assigned to water associated with starch and cell walls), T_{21} (~45–65 ms). Significant DM dependent shifts in both the T_{21} and T_{22} relaxation time constants were observed during cooking, indicating that starch gelatinizes between 53 and 70 °C with water exchanging with the hydroxyls of starch (transition in T_{21}) and cells start to disrupt with an increase in diffusion volumes at ~60 °C (transition in T_{22}). The study reveals that continuous LF NMR measurement is an excellent and highly sensitive method to study changes in water mobility and water populations during the cooking of potatoes.

KEYWORDS: Low-field ^1H NMR; water mobility; potato quality; dry matter; cooking; starch gelatinization

INTRODUCTION

Dry matter (DM) content, starch composition, and cell wall structures significantly affect the final texture of potatoes (1–5). Moreover, the DM content has been found to correlate to various texture attributes in cooked potatoes (6–10). Consequently, dry matter content and starch composition as well as storage conditions and processing methods are considered to be critical factors in relation to physiological, biochemical, and structural processes in potatoes during storage and processing. Water content and DM content are inversely proportional, so the distribution and chemical/physical properties of water in the potato during storage and processing must be considered to be significant factors for potato quality. Previous studies on the influence of processing of potatoes have mainly addressed the kinetics in textural changes (11, 12). Conversely, less attention has been given to the effects of starch composition and functionality together with transitions in water states during processing of potatoes. In general, water characteristics such as water distribution and mobility as well as the anatomical structures of raw materials in processed foods have been identified as the most important physical/chemical properties

in relation to technological and sensory properties of food (13). One of the most successful techniques in the characterization of water distribution and anatomical interior of foods, both of which are known to be critical to the texture, is low-field ^1H NMR relaxation (LF NMR) (14–17). This method is potentially nondestructive and enables characterization of the distribution and mobility of water protons during cooking of potatoes. During processing of potatoes water transitions within the potato take place and contribute significantly to subsequent texture development. The transition of water may depend on raw material characteristics, for example, chemical composition, structural characteristics, and dry matter content. However, this area is almost unexplored, despite the fact that knowledge within this field would contribute to a further understanding of how raw material quality interacts with the cooking process in relation to final eating quality.

An increasing number of NMR relaxation studies focusing on water distribution have been carried out in recent years. However, only in a minority of these studies have NMR data been correlated to texture attributes, successfully demonstrated for rice, bread, meat, fish, and potatoes (8–10, 18–22). LF NMR relaxation measurements have also been demonstrated to reflect changes in water characteristics in foods during cooking, for example, dough (22) and meat (23–25). During bread baking, continuous LF NMR measurements displayed the

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onset and offset of the gelatinization process, hereby contributing to the basic understanding of water associations with proteins, starch, and pentosans and subsequent textural changes, which are essential for the prediction of the quality of the final product. The present study shows for the first time that it is also possible to monitor structural changes in potatoes during processing using continuous LF NMR measurements. Considering the limited knowledge about distribution of water in the raw potato tissue and transition of water compartments during the gelatinization process, we set out to enhance our understanding of water transitions during the processing of potatoes as a bulk study by measuring NMR relaxation during cooking of two potato bins that differed in the quality of raw material, especially the dry matter content.

MATERIALS AND METHODS

Potatoes. Two bins (low DM and medium DM) of potatoes of the Sava variety were used as raw material. The potatoes were grown in light sandy soil and harvested in late summer at full maturity (14 days after wilting of leaves) and cured for 14–21 days at 15 °C to allow skin healing. Samples were kept in a clamp for 6 months. The clamp was based on a hole in the earth (20–50 cm deep) in which the potatoes were placed and covered with straw and sheltered by plastic and earth, as this provided a constant low temperature with a high humidity.

Samples. The potatoes in the two bins were of the same size (70–106 mm in length and 52–66 mm in width). The following procedures were carried out for 10 potato tubers (replicates) from each dry matter bin: (i) determination of specific gravity (sg) and (ii) DM content as well as measurement of (iii) LF NMR relaxation and (iv) thermal characteristics using differential scanning calorimetry (DSC). After removal from the clamp, the potatoes were kept at 4 °C, and 1 day before analysis they were placed at room temperature.

Determination of Specific Gravity (sg). The sg of each tuber was nondestructively determined according to

$$sg = \frac{w}{w - w_w} \quad (1)$$

where $w \equiv$ weight in air and $w_w \equiv$ weight in water.

LF NMR Measurement. Each potato tuber was divided transversely into two halves, ensuring that the bud end was more than 45 mm long. The bud end was used for the LF NMR, DM content, and DSC measurements. Two cylindrical samples with a diameter of 11.5 mm were cut with a metal cork bore. Both were taken out of the storage parenchyma, but with caution to minimize material from the core, cortex, and stem end of the potato. One cylindrical sample was used for LF NMR measurements, whereas the other was used for DM content and DSC analysis. The sample for LF NMR was cut to a length of ~40 mm. The sample was placed in a glass tube with a lid, which in turn was placed in a measuring glass tube before thermostating the sample in a 25 °C water bath for 5–10 min followed by LF NMR measurement.

The LF NMR relaxation measurements were performed on a Maran benchtop pulsed NMR analyzer (Resonance Instruments, Witney, U.K.) with a resonance frequency for protons of 23.2 MHz. The NMR instrument was equipped with an 18 mm variable-temperature probe. Transverse relaxation, T_2 , was measured using the Carr–Purcell–Meiboom–Gill sequence (CPMG) (26, 27). The T_2 measurements were performed with a τ value (time between 90° pulse and 180° pulse) of 150 μ s. The 90° and 180° pulses were 8.2 and 16.4 μ s, respectively. The repetition time between consecutive scans was 6 s. The quadrature data were phase corrected using principal phase correction (28). Data from 4096 echoes were acquired; they were obtained as a four-scan repetition with one preceding dummy scan. Only even-numbered echoes acquired in the CPMG data were used in the data analysis. The temperature of the NMR probe was set at 110 °C. The measurements on each potato cylinder were performed as continuous measurements during heating of the sample, and measurements were performed every 55 s after placing the potato in the NMR probe. An initial investigation

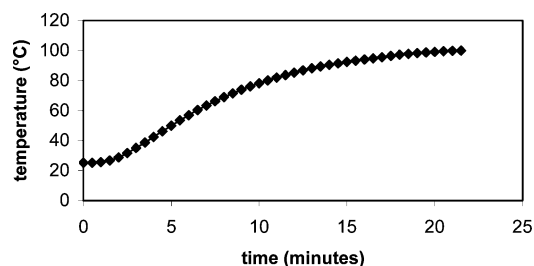


Figure 1. Temperature profile for heating of potatoes in the LF NMR probe.

with a thermocouple inserted in the center of the potato cylinder showed that when using the above-described LF NMR setup, it was possible to make 22 measurements, which correspond to measurements at 25.2, 27.3, 32.5, 39.2, 46.3, 53.2, 59.7, 65.4, 70.4, 74.9, 78.8, 82.4, 85.4, 88.0, 90.1, 91.8, 93.4, 95.0, 96.4, 97.4, 98.3, and 98.8 °C. The LF NMR measurements were performed on 10 replicates from each bin. The temperature profile for cooking potato in the NMR probe is shown in Figure 1.

Handling of NMR Data. The NMR data were analyzed as (1) CPMG relaxation curves, (2) distributed exponential fitting, and (3) discrete exponential fitting. The CPMG relaxation curves were maximum-normalized (by first echo amplitude).

Distributed exponential fitting analysis was performed according to the regularization algorithm of Butler et al. (29) and carried out in MatLab version 6.5 (The MathWorks Inc., Natick, MA) using in-house scripts. This analysis resulted in a plot of relaxation amplitude for individual relaxation processes versus relaxation time.

Discrete exponential fitting of relaxation data was performed in MatLab version 6.5 (The MathWorks Inc.) using in-house code (available at www.models.kvl.dk). To determine the number of components, the NMR relaxation curves were fit to a sum of a few significant decaying exponentials by discrete exponential fitting (28), each with different characteristic time constants and corresponding amplitudes. In the time domain, transverse LF NMR relaxation data are assumed to be a sum of exponentials

$$R_{\text{mag}}(t) = \sum_{n=1}^N M_n \times \exp\left(\frac{-t}{T_{2n}}\right) + e \quad (1)$$

where R_{mag} is the residual magnetization as a function of acquisition time t , N is the number of exponential functions or components in the sample, M_n is the concentrations or amplitudes of the n th exponentials, T_{2n} is the corresponding relaxation time constants, and e is the residual error. Whereas the T_{2n} values provide a qualitative description of the n th transverse relaxation time constant, M_n is the amplitude of the n th exponential constant, which holds the quantitative description.

Determination of Dry Matter. Sampling from the individual tubers was carried out as mentioned under LF NMR measurements. The cylinder was cut into smaller bits (approximately 16 half-cylinders) and placed in a ceramic crucible, which had been weighed beforehand. The crucible and potato were weighed together and placed in an oven at 100 °C for 24 h, cooled in an excicator, and weighed again. The DM content was calculated as the percentage of dry matter to fresh weight:

$$\% \text{ dry matter} = \left(\frac{\text{weight_after_crucible+potato} - \text{weight_crucible}}{\text{weight_before_crucible+potato} - \text{weight_crucible}} \right) \times 100 \quad (2)$$

Determination of Thermal Characteristics using Differential Scanning Calorimetry. Sampling from the individual tubers was carried out as described under LF NMR measurements. Small samples were taken (1 × 1 × 0.5 mm) from the cylinders used in the determination of the DM content, weighed, and placed in the DSC aluminum crucibles, which were subsequently closed hermetically with a matching lid. Samples were measured using a scanning rate of 4 °C/min over the range from 4 to 120 °C using an empty crucible as reference. DSC measurements were performed using a Seiko DSC220C

Table 1. Data for the Potatoes (Sava Variety in Two Dry Matter Bins), with Minimum and Maximum Observations (Interval), Least-Squares (LS) Mean, Standard Error (in Parentheses), and Statistical *P* Value

	bin I (medium dry matter) (<i>n</i> = 20)		bin II (low dry matter) (<i>n</i> = 20)		<i>P</i> value
	interval	LS mean	interval	LS mean	
specific gravity	1.071–1.079	1.076 (0.002)	1.058–1.077	1.068 (0.002)	0.0044
dry matter	15.01–19.29	17.25 (0.50)	12.20–18.00	15.54 (0.50)	0.0267
DSC onset (°C)	61.28–65.40	63.45 (0.40)	63.92–68.55	65.99 (0.40)	0.0003
DCS peak (°C)	64.58–67.25	66.02 (0.32)	67.30–69.89	68.42 (0.32)	<0.0001
DSC area (mJ/mg)	1491–2432	1978 (170)	422–2745	1382 (170)	0.0234

(Seiko Instruments Inc., Torrance, CA), which was provided with a liquid nitrogen subambient assembly, at a temperature range of -150 to 725 °C and a detection range of ± 100 μ W/fs to ± 100 mW/fs.

Only one thermal transition was found in all samples. The temperature at the onset of the peak (DSC onset), the temperature at the peak (DSC peak), and the estimated area below the transition curve (DSC area) were measured according to the method of Parsons and Patterson (30).

Chemometric and Statistical Analysis. Principal component analysis (PCA) is a descriptive bilinear method that allows the main variability aspects of a multivariate data set on many samples to be visualized without the need of an initial hypothesis concerning the relationships between samples or between samples and variables. The analysis aims at finding relationships between the many different parameters (samples and variables) and to display possible clusters within samples and/or variables. PCA gives an overview of the data set, as the objects are projected onto the principal components (PCs), and an estimate of the number of components needed to explain the variation in the data set (31). The main advantage is the ability to treat many different data types simultaneously and the projection into fewer components/variables.

PCA was performed using Unscrambler version 8.0 (Camo, Oslo, Norway; www.camo.com).

Classical statistical analysis was performed using the Statistical Analysis System package (version 6.12, SAS Institute, www.sas.com). To test the presence of statistical differences in score values between the different temperatures, a statistical analysis of the score values from each of the continuous measurements (22 temperatures) was carried out using PROC GLM of SAS for each DM bin with temperature as a fixed effect.

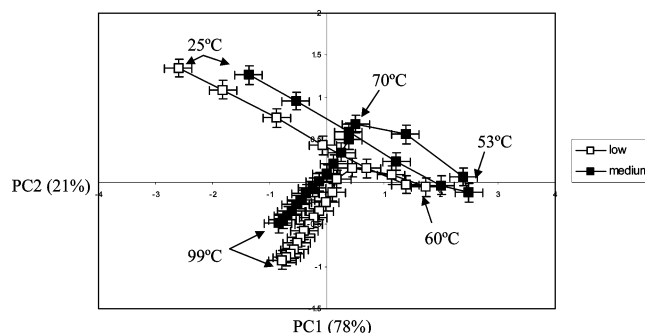
The statistical effects of DM bin on specific gravity, DM content, DSC measurements, and the relaxation times (T_{2n}) and the amplitudes (M_{2n}) from the discrete exponential fitting of the relaxation curves were investigated using the PROC MIXED of SAS.

RESULTS AND DISCUSSION

Differential Scanning Calorimetry. Mean values for specific gravity, DM content, and DCS characteristics are shown in **Table 1**. A significant difference in all variables is found between the two potato bins. The statistical difference in specific gravity is more pronounced than the difference in DM content. This might be explained by the fact that specific gravity was determined on the entire potato, whereas DM content determination was carried out on a cylindrical sample from the inner medulla.

The thermal characteristics of the potatoes using the onset and peak temperature show that the bin with the medium DM content has lower onset and peak temperatures. Accordingly, the present data show that a higher DM content results in a lower gelatinization temperature. These data thereby support data by Burton (2), who suggested that this could be explained by the simultaneous increase in the ratio between amylose and amylopectin with increasing dry matter content.

Unsupervised Analysis of the Cooking Experiments. The entire data set, including all LF NMR measurements obtained

**Figure 2.** PC1 versus PC2 for score values from continuous measurements on cooking of potatoes (CPMG curves). The curves represent least-squares mean values of the two bins. Bars show standard errors.

during heating of samples, was first studied using PCA. The resulting average score plots are shown in **Figure 2**.

A characteristic loop course is observed for the mean score values of the two bins. There is also a significant difference with respect to water characteristics and water transition between the two bins, as the course of the loop differs and occurs at different temperatures. The loop for the medium DM bin is broader and longer, as it goes halfway back before crossing “down”, whereas the low DM loop only goes one-fourth of the way back before crossing down. Another difference between medium and low DM bins is the temperature of the first turn in the loop. For the low DM bin the turn is sharp and occurs at 60 °C, whereas the turn for the medium DM bin is less sharp and occurs at 53 °C. The second turn does not differ between the two bins (70 °C). The results in **Figure 2** clearly show that NMR is a sensitive method for monitoring the cooking events in potatoes and that two significant PCs were sufficient to describe the structural changes with respect to water associations in potatoes during cooking.

From the PCA we can deduce that the major events related to water mobility in the cooking of a potato occur at 53 and 60 °C for potatoes with medium and low DM contents, respectively. This transition temperature determined with great precision by LF NMR must be attributed to a softening of cell walls and an initiation of the gelatinization process; that is, the starch grains swell as a consequence of the water uptake, and subsequent starch gelatinization. In contrast, the DSC measurements showed slightly higher gelatinization temperatures, that is, 63 and 66 °C, respectively (**Table 1**), which correspond to previously reported gelatinization temperatures for potato starch (2). However, it must be noted that the relatively poor temperature resolution (steps of ~ 5 – 7 °C for LF NMR measurements) do not fully justify the quantitative differences between LF NMR and DSC measurements in the critical range where gelatinization occurs. In contrast to the registered difference in initiation temperature for the gelatinization process as a consequence of difference in dry matter content of the two potato bins, the termination of the gelatinization appeared at 70 °C, independent

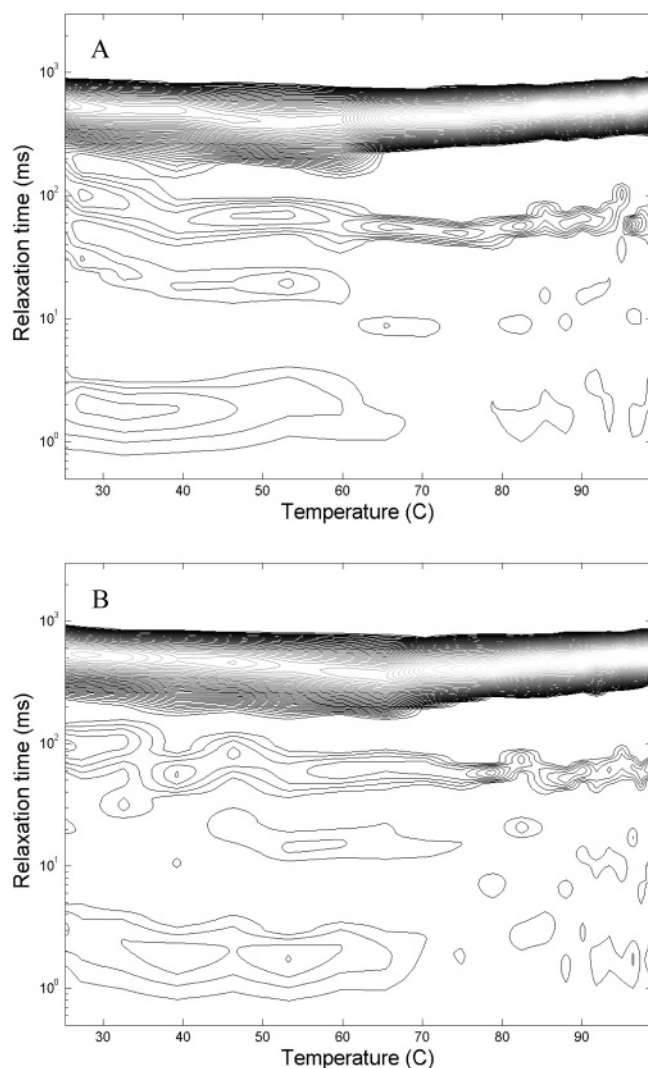


Figure 3. Contour plot of the development in water populations during cooking of potatoes with medium DM content (A) and low DM content (B) performed on data from the distributed exponential fitting of the CPMG curve from one potato.

of the quality of the potatoes. This is explained by the fact that thermal motions dominate at this point and the Boltzmann distribution thus makes contrasting less favorable and that the DM content does not significantly influence the end-point gelatinization temperature.

Distributed Exponential Curve Fitting. To further investigate the relaxation data in relation to water compartmentalization, distributed exponential curve fitting was performed on obtained CPMG curves to obtain a qualitative interpretation. **Figure 3** displays a contour plot of the development in water populations during continuous cooking of a potato.

Figure 3 reveals two major water populations in potatoes: a population with a relaxation time at ~ 50 ms (T_{21}) and a population with a relaxation time at ~ 500 ms (T_{22}), which is in accordance with previous findings (8–10).

Only minor changes in the characteristic relaxation times are observed as a function of temperature (T_{21} , 65–85 ms; T_{22} , 350–550 ms). However, the populations change characteristics at ~ 62 – 70 °C and become more well-defined with an increase in the T_{22} population during cooking. The T_2 relaxation populations display slower mean relaxation times during cooking. Whereas the PCA (**Figure 2**, see above) suggested the presence of two independent components, the T_2 distribution

analysis indicates the presence of a small population T_{20} of very fast-relaxing waters (~ 3 – 4 ms), which is present up to 60 °C. Hills and Le Floch (32) reported the presence of four water populations in raw potato tissue. These authors tentatively assigned the components to water in starch granules (~ 2 – 4 ms), water associated with the cell walls (10 ms), and two fast-relaxing dominating components at ~ 100 – 500 ms to water in cytoplasm and extracellular regions. The fact that the shortest relaxing component T_{20} in the present study appears to vanish at temperatures > 60 °C when gelatinization and cell disruption occur supports the assignment of this component.

Discrete Exponential Curve Fitting. To obtain more qualitative information about the water populations in relation to cooking and DM content, discrete biexponential fitting of the CPMG curves obtained during cooking of the potatoes was carried out (**Figure 4**). This resulted in relaxation components characterized by time constants of ~ 45 – 65 ms (T_{21}) and ~ 350 – 550 ms (T_{22}).

The characteristic relaxation time of the fast-relaxing component, T_{21} (**Figure 4A**), has a more complex cooking behavior than T_{22} (**Figure 4B**). Like the T_{22} , the T_{21} time constant decreases steadily from 25 to 53 °C because of a decreased correlation time for the chemical exchange, yet it reaches a local minimum at 53 °C. From 53 °C to ~ 65 °C T_{21} increases as gelatinization occurs, which makes the water exchange with the hydroxyls of the starch. From 65/70 °C to the end of cooking, T_{21} is nearly steady, because increased exchange rate and increased mobility cancel out.

The characteristic relaxation time of the slow relaxation component, T_{22} , steadily decreases from 25 to 60 °C, from which it continuously increases to the end of cooking with an indication of a tiny bump around 70 °C (**Figure 4B**). The T_{22} relaxation component is assigned to originate from relatively free bulk water in the extracellular cavities and cytoplasm, where no associations with starch exist, which is in accordance with assignments by Hills and Le Floch (32). As for T_{21} , the decrease in relaxation time up to 60 °C is due to a decreased correlation time for the chemical exchange and cell expansion. The increase in relaxation time above 60 °C is caused by cell disruption and consequently larger diffusion volumes. This is corroborated by the abrupt change in the ratio between the two populations of water (M_{21} and M_{22} in **Figure 4C**).

In a previous more subtle investigation we had tentatively ascribed the T_{22} water population to intracellular water primarily because the T_{22} time constant was reduced by 50% when T_{22} of raw and cooked potatoes was compared (8). However, as mentioned above, the present time course study combined with observations by Hills and Le Floch (32) strongly suggests a model with two components in which the fast relaxation T_{21} time constant is primarily ascribed to water in starch granules and cell walls and T_{22} is not only ascribed to intracellular water in cytoplasm but also to extracellular water. The latter interpretation is primarily based on the fact that the relative amount of T_{21} and T_{22} is approximately 12:88% (**Figure 4C**), which is roughly the ratio of water in starch/cell wall material to extracellular and cytoplasmic water in a potato with $\sim 80\%$ water (33). The fact that the T_{21} water population appears to be only slightly less mobile in the cooked potato compared to the raw stage (12–15% reduction) also supports this hypothesis. However, Hills and Le Floch (32) found two separate fast relaxation components assigning for water in starch granules and cell walls, respectively. A triexponential fitting of the present data revealed a third very fast-relaxing component with a T_2 of ~ 3 – 4 ms and with a relative amount of $\sim 10\%$ up to 60 °C,

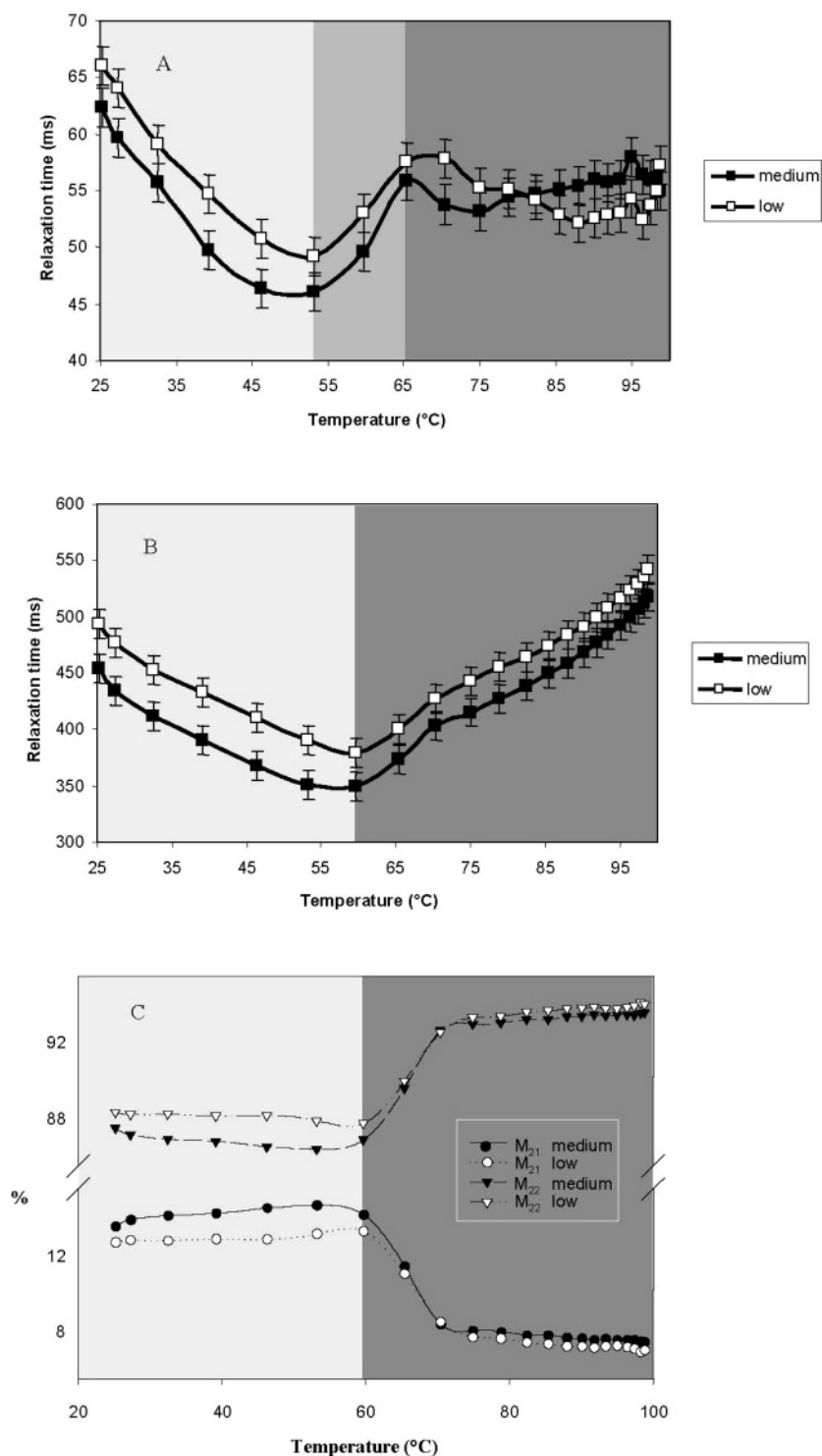


Figure 4. Development in the relaxation time for the fast relaxation component T_{21} (A) and the slow relaxation component T_{22} (B) and development in the relative amount of the population sizes (C) (M_{21} and M_{22}) during cooking of potatoes. Each dry matter bin is an average of 10 samples and based on the discrete exponential fitting of the LF NMR data. Bars show standard errors.

which is in accordance with findings by Hills and Le Floc'h (32), who attributed this component to water in starch granules. However, due to only a few data points in the beginning of the relaxation measurements, the component is not determined precisely.

Besides the qualitative information about the gelatinization process of potatoes during cooking, **Figure 4A,B** also shows the influence of dry matter content on the mobility of the water populations, which is primarily an offset in relaxation time (the more dry matter, the faster relaxation). The gelatinization process

in the medium DM bin appears at a lower temperature, which is in accordance with the DSC data (**Table 1**). In accordance with theory, a lower DM content results in a less restricted mobility of water and thus longer relaxation times of both water population times. As mentioned in the Introduction, DM content is highly relevant for the texture of many food products, for example, potatoes (8). Therefore, the relaxation data of cooked potatoes reflect an entirely amorphous average system in contrast to the relaxation data from the well-structured raw potatoes. Such knowledge is therefore highly relevant for the use of LF

NMR relaxation as a noninvasive method for the determination of structural changes during processing and final texture. In conclusion, continuous LF NMR measurements obtained during processing determined the changes in water mobility and water populations. This study demonstrates for the first time the potential of LF NMR to obtain information of considerable importance for a basic understanding of the influence of processing on gelatinization of cooked potatoes in relation to variability in raw material characteristics.

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