



NMR relaxometry as a tool to understand the effect of microwave heating on starch-water interactions and gelatinization behavior



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ABSTRACT

Starch-water interactions during gelatinization by microwave heating was investigated by Low-field ¹H Nuclear Magnetic Resonance (LF NMR) relaxometry experiments. Effect of microwave heating time on longitudinal (T_1) and transverse relaxation times (T_2), gel matrix structures, changes in proton populations and firmness values were evaluated. One to one corn starch (CS) to water ratio samples had the lowest T_2 change during gelatinization indicating lower granule expansion which was attributed to the high amylose content of CS. Non-Negative-Least-Square (NNLS) analysis of the transverse relaxation curves provided detailed information on starch-water interactions during gelatinization. Rice starch (RS) samples had relatively lower changes in peak areas which was related to the better hydration of RS granules. While microscope images revealed the changes in the granule structures after distinct gelatinization intervals, firmness measurements supported the structural changes. With increasing microwave heating time, both T_1 and T_2 showed a decreasing trend. Two distinct proton populations detected on relaxation spectrum indicated the exchanging protons during gelatinization. A noticeable reduction in firmness at the later moments of microwave heating was detected. The results showed that starch gelatinization by microwave heating had some distinct features in terms of mechanism of the gelatinization and could be investigated by NMR relaxometry.

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1. Introduction

Starch has a broad range of function in the food industry, mostly as a stabilizer, gelling and thickening agent. Amylose and amylopectin, that are α -glucan polymers, are the major constituents of starch. Amylose is the linear portion of starch including α -1,4 glucose units, whereas amylopectin consist linear chains of α -1,4 glucose as well as α -1,6 branch points (Hansen et al., 2009). The structural differences of both polymers cause variation on the properties of different starch varieties. While amylose tends to form tough gels and strong films, amylopectin can be dispersed in water and forms soft gels and weak films (Fredriksson, Silverio, Andersson, Eliasson, & Aman, 1998; Perez & Bertoft, 2010). Moreover, amylose has a function of low degree of polymerization compared to amylopectin (Thygesen, Blennow, & Engelsen, 2003).

The main sources of starch, mostly stored in plant tubers and

seed endosperm, are maize, wheat, potato, tapioca and rice (Karntarat, Shobsngob, Triampo, & Triampo, 2008). Based on the botanical origin, starch can be in shapes of spheres, ellipsoids, polygon, platelets or irregular tubules on a size range of 0.1–200 μ m (Witczak, Ziobro, Juszcak, & Korus, 2015). Wheat starch, different from most other starch types, may contain gluten residue which can provide gel structures to acquire elasticity and cohesiveness (Demirkesen, Mert, Sumnu, & Sahin, 2010; Gerits, Pareyt, & Delcour, 2015).

When starch is dispersed in water and heated, the granules start to swell. Swelling of starch granules occurs due to water uptake and it differs for different starch types. Potato starch has greater swelling ability than corn starch (CS), wheat starch (WS) and rice starch (RS) (Witczak et al., 2015).

Thermal treatments of starch and water systems are carried out through granule swelling. These treatments can improve the volume and mechanical properties of starch slurries. When thermal process is performed at lower water levels, which is also called *heat-moisture treatment*, changes in crystalline structure, morphology, chemical and enzymatic susceptibility are achieved.

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Instead, if process is maintained at higher water contents, which is called *annealing*, changes in functional properties are observed. Crystallization of leached amylose into double helices in the continuous phase leads formation of a gel structure. Thus, this phenomenon is referred to gelation (Houben, Höchstötter, & Becker, 2012). Starch gelatinization consists of (i) granule swelling, (ii) leaching of carbohydrate components, mostly amylose, (iii) formation of a three dimensional starch network of leached components and (iv) interactions between granule residues and the leached components (Gerits et al., 2015). After gelation, amylose and amylopectin bind water molecules through hydrogen bonds that cause to gather large amounts of water during cooling (Pycia, Juszczak, Gałkowska, & Witczak, 2012).

Gelatinization of starch is mainly achieved by conventional heating which provides direct heating of medium by means of conductive heat transfer. Apart from the conventional heating, microwave heating which depends on the alternating electricity and magnetic field of the microwave radiation, results in changes on the orientation of dipoles. Polar molecules have fast rotation that results in friction, collision, vibration and generation of heat. Therefore, a rapid increase in temperature is achieved in the medium (Fan et al., 2013; Sumnu, 2001).

LF NMR is a rapid, nondestructive, and low-cost technique that is commonly used in food science (Kirtil & Oztop, 2015). LF ^1H NMR relaxometry is a practical tool to analyze proton relaxation in starch gels, since signals come from all protons in a sample so that distribution and mobility of protons is well-expressed (Hansen et al., 2009; Li et al., 2015). NMR could be used to measure the spin-spin relaxation and mobility of hydrogen molecules. While a hydrogen nucleus in an immobile structure acquires short spin-spin relaxation times, hydrogen nuclei in mobile structure has long spin-spin relaxation times (Thygesen et al., 2003). NMR experiments are usually based on acquiring signals to measure longitudinal relaxation time (T_1) and transverse relaxation time (T_2). When the T_1 and T_2 relaxation curves are analyzed through Non-Negative-Least-Square (NNLS) analysis, a relaxation spectrum is obtained. This spectrum gives information about proton pools which could be associated with water distribution in food samples (Oztop, Rosenberg, Rosenberg, McCarthy, & McCarthy, 2010).

The physical state of the water and its dynamics are crucial for food systems due to impacts on texture, functions and stability (Hansen et al., 2009). Starch-water interactions and the detection of these interactions have been investigated by several methods for microwave and conventional methods in the literature. Differential scanning calorimetry was one of the most favored methods to observe gelling behavior patterns in conventional and microwave heating (Ndife, Şumnu, & Bayındırlı, 1998; Palav & Seetharaman, 2007). In addition, there were some NMR studies as a new approach to discuss starch gelatinization (Bosmans et al., 2012). Starch-water interactions during microwave heating have also been investigated by NMR and T_1 was mainly utilized for interpreting the gelatinization (Fan et al., 2013). Scanning electron microscope, transmission electron microscope and light microscope techniques have been applied to observe morphological changes of starch gel matrices after microwave and conventional heating methods (Xue, Sakai, & Fukuoka, 2008).

NMR Relaxometry approach which is based on analyzing the relaxation spectrum obtained by NNLS analysis could be a useful alternative tool for understanding of the gelatinization properties of a given starch type during microwave heating or detecting the different swelling mechanisms of starch varieties, since the mechanisms of granule swelling can be studied by examining relative peak areas on a spectrum each representing a distinct proton population. Thus, NNLS analysis could be integrated to NMR

studies to analyze starch gelatinization and differentiate the different starch types.

In this study, the objective is to understand the gelling behavior of different starch samples exposed to microwave heating and to propose an explanation for the altered gelling behavior of different starch samples. For this purpose, dynamics of water starch interactions, texture and microstructure of the gel matrices were determined for WS, CS and RS. The present study is expected to enhance the knowledge of microwave treatment of starch slurries with the help of LF NMR. The changes in the T_1 & T_2 as well as variations in the relative peak areas on the relaxation spectra, enabled us to investigate the alterations in gel matrix of starch varieties as the microwave treatment time elapses. Optical microscope images were also analyzed to confirm the results.

2. Materials and methods

2.1. Sample preparation

CS, WS and RS were obtained from Pakmaya Turkey. 1.5 g of starch samples were weighed and mixed with the distilled water at room temperature at 1:1, 1:1.5 and 1:2 proportions (starch: water). Commercial CS and RS did not contain gluten but WS packages were labeled as containing gluten. In order to prove gluten residue in WS, wet gluten analysis was performed. Analysis showed that, only WS sample showed trace amount of gluten presence after washing with salt solution prepared by dissolving 2 g NaCl in 100 ml distilled water. The mixtures were prepared in 16 mm diameter test tubes for all experiments. A homogenizer (Wisemix VW-10, USA) was used to let starch molecules dissolve in distilled water in order to achieve a homogeneous heating in microwave oven.

2.2. Gelatinization of different starch types

All samples were heated in a domestic Arcelik MD 8901 microwave oven operating at a power of 800 W (Arcelik, Turkey). For all starch types, samples were heated for 5, 10, 15, 20, 25 and 30 s in the microwave. Temperature of the samples during microwave treatment was not measured. The gel formation was visually observed between the 5th and the 10th s of microwave heating for all samples. Samples were allowed to reach room temperature before NMR measurements. For the conventional heating experiments to be used as the control samples, 1:1 proportions of all starch types was gelatinized at 60 °C for 30 min in water bath (Ratnayake & Jackson, 2006). All measurements were done for three replicates.

2.3. Moisture content determination

Moisture content of the starch types were measured using an infrared analyzer (Radweg Mac 50, Poland).

2.4. Amylose content determination (iodine binding technique)

Amylose content of the starch types were determined for interpretation of the results. For amylose content determination, method by Zhu, Jackson, Wehling, and Geera (2008) was followed. Absorbance values were recorded at wavelengths of (Mecasys Optizen POP, Korea) 510 nm and 620 nm.

Absorbance values of solutions prepared with iodine binding technique at 510 nm and 620 nm gave amylose contents according to the following equation (R^2 values of 0.9995, 0.9996 and 0.9995 for CS, WS and RS respectively) (Zhu et al., 2008); g amylose/100 g starch = (Absorbance₆₂₀ - Absorbance₅₁₀ + 0.0542)/0.3995.

2.5. Firmness measurement

A penetrometer (PCE-PTR 200, Germany) was used to measure the firmness of the gelatinized samples. The suitable sensor, diameter of 8 mm, was chosen and positioned perpendicular to the sample. The peak force values were recorded as the firmness values. For each starch type, 3 replicates were used.

2.6. Light microscope analysis

The microstructure of starch-water mixture during gelation was observed by optical light microscope (Zeiss Primo Vert, Germany) under 40× magnification. Samples of each starch type (1:1) for each processing time were placed on a microscope slide and images were obtained via Toupview software.

2.7. NMR relaxometry experiments

NMR Relaxometry experiments were carried out in a 0.32 T system (Spin Track SB4, Russia). For T_1 measurements, Saturation Recovery sequence having 1s recycle delay was used with delay times changing between 10 ms and 2 s with 16 delay times and 32 scans.

For T_2 measurements, Carr-Purcell- Meiboom-Gill sequence was used with parameters of 1 ms echo time, 400 echoes and 32 scans. 3 replicates of all samples were used for the measurements.

T_1 and T_2 were evaluated by 2 different approaches. First of all, monoexponential fitting was conducted on the relaxation curves using MATLAB. Following the monoexponential fitting, NNLS analysis was conducted on T_2 curves to obtain a relaxation spectrum. Since the number of points on T_1 saturation recovery curve was 16, NNLS analysis was not conducted on T_1 data.

NNLS method is based on Lawson and Hanson algorithm that depends on regularization function which seeks to find a smooth spectrum of exponentials that satisfies the data in a chi-squared sense (Oztop et al., 2010). Prospa 3.1 (Magritek, Wellington, New Zealand) software was used to perform NNLS. Number, amplitude and relative areas of peaks of the samples were analyzed by this method.

2.8. Statistical analysis

In order to evaluate firmness and NMR results, ANOVA was performed with MINITAB (Minitab Ltd, UK). To check the applicability of ANOVA to the data set, assumptions of normal distribution and equality of constant variances were checked using Anderson Darling and Bartlett's test. Tukey test was used as the multiple comparison test with 95% confidence level ($p < 0.05$). For each result, differences were indicated with different letters in graphs or tables.

3. Results

3.1. Firmness measurements & microscope images

Moisture contents of CS, WS and RS were 10.90 ± 0.33 , 12.67 ± 0.15 and 12.32 ± 0.51 g moisture/100 g starch, respectively. In addition, amylose contents were found to be 38.35 ± 0.01 , 36.16 ± 0.01 and 31.62 ± 0.01 g amylose/100 g starch for CS, WS and RS.

In Fig. 1, firmness results of starch gels exposed to different microwave heating times are given for CS, WS and RS. Microwave heating time at 25 s was significantly different on firmness and RS was found to be significantly different ($p < 0.05$). For RS and CS, reduction on firmness at 25 s was observed. ($p < 0.05$).

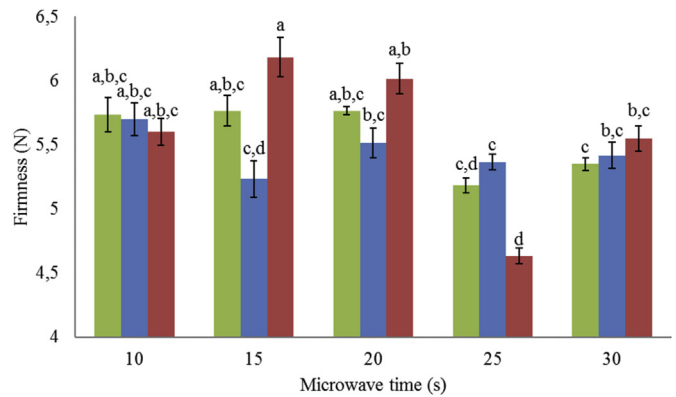


Fig. 1. Firmness results for 1:1 of starch types: ■ corn starch ■ wheat starch ■ rice starch.

Microscope images in Fig. 2 showed that formation and disruption of WS granules occurred with increasing microwave heating time. As observed in the images, granules swelled at 5, 10 and 15 s and started to disrupt at 20 s. Afterwards, disrupted granules came together and formed greater granules at 25 and 30 s. Similar results were also observed by Ratnayake and Jackson (2006) where gelatinization of starch in the presence of excess water was investigated (Ratnayake & Jackson, 2006).

3.2. Spin-lattice (T_1) & spin-spin (T_2) relaxation time measurements

In Figs. 3–5, microwave heating T_1 and T_2 results were shown. Besides, conventional heating T_1 values of 1:1 proportions for CS, WS and RS were found 180.64 ± 1.49 ms, 191.64 ± 5.66 ms and 190.07 ± 3.53 ms, respectively. Furthermore, T_2 values of CS, WS and RS for conventional heating were 24.87 ± 0.78 ms, 35.34 ± 0.43 ms and 25.19 ± 0.36 ms, respectively.

Monoexponential fitting results of relaxation curves gave T_1 and T_2 values for each sample. These monoexponential relaxation times could be considered as a weighted average of the different proton populations on the samples. T_1 and T_2 values of different starch types at different starch water ratios are presented in Figs 3–5. As expected, with increasing water proportion T_1 and T_2 increased. As microwave heating time increased T_1 & T_2 of starch samples decreased for some samples.

3.3. NNLS spectrum analysis

As shown in Table 1, different proportions of different starch types mostly led to two peaks in NNLS results. An additional third peak was observed for only CS having 1:1 starch to water ratio. When holding time in microwave increased, percent contribution of the second population increased while the areas of first peak decreased. Statistical analyses were performed for each set, separately.

4. Discussion

4.1. Firmness measurements & microscope images

Although firmness measurements for all types of starches and starch-water fractions did not show any significant difference, a sudden decrease in firmness of all samples at the 25th s of the heating process was observed in Fig. 1. Since microwave heating provides a very quick gelatinization between 5 and 10 s of heating

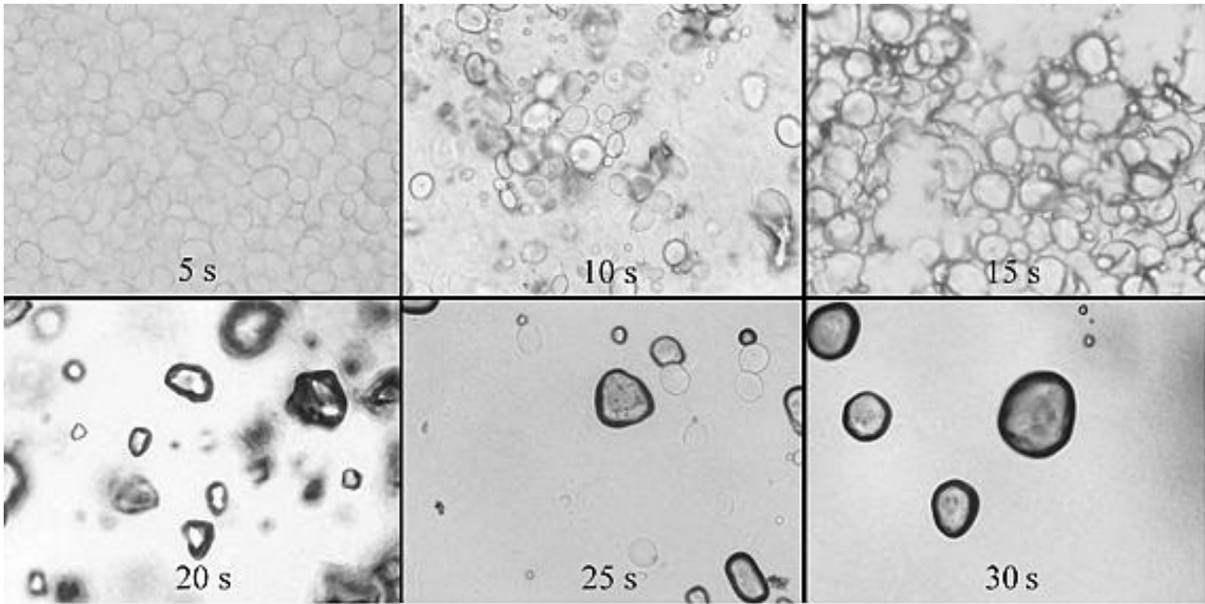


Fig. 2. Microscope images of 1:1 wheat starch for 5, 10, 15, 20, 25 and 30 s under 40× magnification.

of the samples, the gelatinized starch network could be damaged at 25th s of microwave heating (Bilbao-Sáinz, Butler, Weaver, & Bent, 2007; Lewandowicz, Jankowski, & Fornal, 2000). Although onset temperature of gelatinization depends on the botanical origin of the starch and the amount of water present, starch gelatinization usually starts to occur at around 60 °C in conventional heating methods and this takes a much longer time when compared to microwave heating (Hansen et al., 2009). Therefore, the sudden decrease in firmness of the samples at 25th s is claimed to be due to the disruption of the swollen granules and the damages in the gel network after prolonged heating times in microwave (Patel & Seetharaman, 2006). This claim was justified by the microscope images of gelatinized starch samples. Fig. 2 shows the microscope images of WS under 40× magnification. It was observed that after 20 s of microwave treatment, granules started to disrupt and gel matrix was severely damaged. Another indication of starch granule disruption is the stabilization of the T_2 values almost to a specific value starting at 25th s. The reason for this stabilization was that

starch granules ruptured due to gelatinization caused a stabilized interaction between water and starch (Fan et al., 2013; Tester & Morrison, 1990). Kovrlija and Rondeau-Mouro also discussed that during heating of starch suspensions between 50 and 90 °C, swollen granules are heated further and some hydrogen bonds between adjacent glucose units are disrupted then, the crystalline arrangement is partially destroyed. Authors also claimed that the gelatinization of starch granules are achieved due to temperature-associated changes and this is accompanied by damage to their granular structure and high water-starch interactions (Kovrlija & Rondeau-Mouro, 2016). Thus, after 20 s of microwave heating, the temperatures of samples were clearly above 50 °C. In addition to this, rice 1:1 sample indicated the lowest firmness at 25 s of treatment. Amylose content of RS (31.62 ± 0.01 g amylose/100 g starch) was the lowest among other starch types verifying the relatively higher reduction of RS firmness. During gelatinization in microwave heating, high amount of amylose was leached out from the starch molecules and in the RS case the remaining amylose

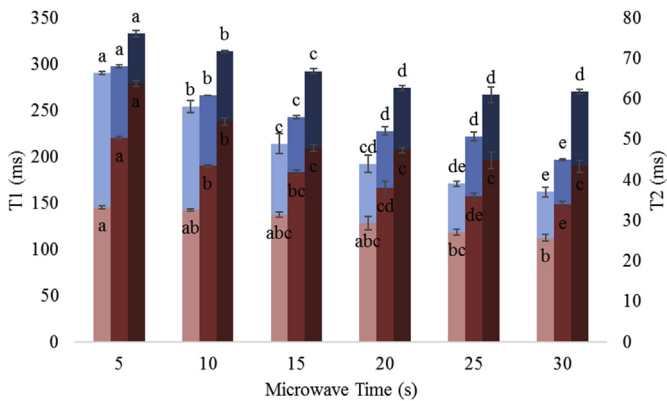


Fig. 3. T_1 and T_2 values of corn starch at different starch: water ratios: T_1 values for 1:1 corn starch; T_1 values for 1:1.5 corn starch; T_1 values for 1:2 corn starch;; T_2 values for 1:1 corn starch; T_2 values for 1:1.5 corn starch; T_2 values for 1:2 corn starch. Different small letters mean that T_1 or T_2 belonging to each corn starch: water ratio, significantly differs in each microwave treatment time ($p < 0.05$).

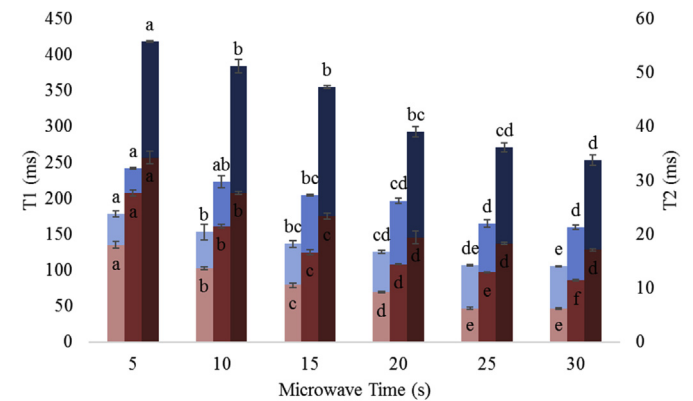


Fig. 4. T_1 and T_2 values of wheat starch at different starch: water ratios: T_1 values for 1:1 wheat starch; T_1 values for 1:1.5 wheat starch; T_1 values for 1:2 wheat starch;; T_2 values for 1:1 wheat starch; T_2 values for 1:1.5 wheat starch; T_2 values for 1:2 wheat starch. Different small letters mean that T_1 or T_2 belonging to each wheat starch: water ratio, significantly differs in each microwave treatment time ($p < 0.05$).

Table 1
NNLS results of T₂ values of corn, wheat and rice starches for 1:1, 1:1.5 and 1:2 starch to water proportions.

		Corn						Wheat						Rice					
		1:1		1:1.5		1:2		1:1		1:1.5		1:2		1:1		1:1.5		1:2	
		Time (ms)	Area (%)	Time (ms)	Area (%)	Time (ms)	Area (%)	Time (ms)	Area (%)	Time (ms)	Area (%)	Time (ms)	Area (%)	Time (ms)	Area (%)	Time (ms)	Area (%)	Time (ms)	Area (%)
First Peak	5	4.5 ± 0.2 ^c	19.0 ± 0.8 ^a	4.9±0 ^a	12.7 ± 0.6 ^a	3.4 ± 0.1 ^b	9.2 ± 0.6 ^a	4.1±0 ^d	35.0 ± 3.8 ^b	3.3±0 ^b	18.2 ± 0.7 ^a	5.2±0 ^a	13.6 ± 0.1 ^a	4.5±0 ^a	7.8 ± 0.3 ^a	6.9 ± 0.3 ^a	7.2 ± 0.2 ^a	4.1±0 ^a	2.0±0 ^b
	10	7.2±0 ^a	17.1 ± 1.1 ^a	4.5 ± 0.4 ^{ab}	7.3 ± 0.7 ^b	4.8 ± 0.3 ^a	6.4 ± 0.7 ^{ab}	4.1±0 ^d	24.7 ± 0.1 ^c	3.2±0 ^b	3.3 ± 0.2 ^b	3.4±0 ^c	2.4 ± 0.3 ^{bc}	3.3±0 ^b	4.8 ± 0.1 ^b	3.2±0 ^c	3.6 ± 0.2 ^d	4.1±0 ^a	3.4 ± 0.2 ^{ab}
	15	7.2±0 ^a	19.4 ± 0.8 ^a	4.6 ± 0.3 ^a	7.3 ± 0.8 ^b	4.7 ± 0.2 ^a	6.6 ± 0.3 ^{ab}	11.0±0 ^a	100.0 ± 0.1 ^a	3.2±0 ^b	1.3 ± 0.3 ^c	3.2±0 ^c	2.0 ± 0.1 ^c	3.2±0 ^b	3.5 ± 0.2 ^b	4.1±0 ^b	5.0 ± 0.1 ^c	3.2±0 ^b	2.3 ± 0.5 ^b
	20	6.0±0 ^b	13.3 ± 1.8 ^b	4.7 ± 0.2 ^a	7.0 ± 0.6 ^b	4.5±0 ^a	4.5 ± 1.1 ^b	8.3±0 ^b	100.0 ± 0.1 ^a	3.2±0 ^b	1.7 ± 0.3 ^c	3.2±0 ^c	1.1±0 ^d	2.9 ± 0.2 ^b	3.4 ± 0.2 ^b	3.3±0 ^c	4.2 ± 0.3 ^{cd}	4.1 ± 0 ^a	4.3 ± 0.5 ^{ab}
	25	6.0±0 ^b	10.8 ± 1.6 ^b	4.7 ± 0.2 ^a	6.9 ± 1.4 ^b	4.5±0 ^a	6.3 ± 0.5 ^{ab}	5.7 ± 0.2 ^c	100.0 ± 0.2 ^a	3.8 ± 0.2 ^a	2.7 ± 0.2 ^{bc}	3.9 ± 0.1 ^b	1.0±0 ^d	4.1±0 ^a	4.2 ± 0.3 ^b	3.3±0 ^c	6.4 ± 0.3 ^{ab}	4.1±0 ^a	4.4 ± 0.3 ^{ab}
	30	4.9±0 ^c	8.4 ± 0.9 ^b	3.3±0 ^b	6.8 ± 1.1 ^b	3.3±0 ^b	4.9 ± 1.0 ^b	5.7 ± 0.2 ^c	100.0 ± 0.1 ^a	3.3±0 ^b	3.9 ± 0.1 ^b	4.1±0 ^b	2.6±0 ^b	4.3 ± 0.2 ^a	6.3 ± 0.5 ^a	4.3 ± 0.2 ^b	6.2 ± 0.1 ^b	4.3 ± 0.2 ^a	7.3 ± 2.1 ^a
Second Peak	5	27.0±0 ^c	68.2 ± 2.6 ^d	52.7 ± 2.9 ^a	87.3 ± 0.6 ^b	71.0 ± 1.0 ^a	90.8 ± 0.5 ^a	22.5 ± 0.5 ^a	65.0 ± 4.1 ^b	29±0 ^a	81.8 ± 0.6 ^b	36.0±0 ^a	86.4 ± 0.9 ^b	21.3 ± 0.3 ^a	92.2 ± 1.8 ^a	29.0±0 ^a	92.8 ± 0.8 ^a	36.0±0 ^b	98.0 ± 0.7 ^a
	10	40.0±0 ^a	64.0 ± 0.1 ^c	48.0±0 ^{ab}	92.7 ± 0.7 ^a	60.0 ± 2.0 ^b	93.6 ± 1.2 ^a	16.7 ± 0.8 ^b	75.3 ± 0.2 ^a	22±0 ^b	96.7 ± 0.5 ^a	29.0±0 ^b	97.6 ± 0.6 ^a	19.0 ± 1.0 ^b	95.2 ± 0.6 ^a	29.0±0 ^a	96.4 ± 0.6 ^a	46.0±0 ^a	96.6 ± 0.6 ^{ab}
	15	33.0±0 ^b	80.6 ± 0.7 ^{bc}	42.7 ± 2.7 ^{bc}	92.7 ± 1.0 ^a	55.3 ± 1.4 ^{bc}	93.4 ± 1.3 ^a *	*	18±0 ^c	98.7 ± 0.5 ^a	22.0±0 ^c	98.0 ± 0.9 ^a	14.3 ± 0.3 ^c	96.5 ± 0.7 ^a	22.0±0 ^b	95.0 ± 0.4 ^a	46.0±0 ^a	97.2 ± 1.1 ^{ab}	
	20	33.0±0 ^b	86.7 ± 1.8 ^{abc}	39.3 ± 0.3 ^c	93.0 ± 0.7 ^a	49.6 ± 1.7 ^c	95.5 ± 0.9 ^a *	*	14±0 ^d	98.3 ± 0.2 ^a	19.3 ± 1.3 ^{cd}	98.9 ± 0.8 ^a	14.0±0 ^c	96.6 ± 1.5 ^a	21.3 ± 0.3 ^b	95.8 ± 0.9 ^a	36.0±0 ^b	95.7 ± 0.9 ^{ab}	
	25	29.0 ± 2.0 ^{bc}	89.2 ± 0.8 ^{ab}	39.3 ± 0.3 ^c	93.1 ± 1.3 ^a	50.6 ± 1.4 ^c	93.7 ± 0.9 ^a *	*	11±0 ^e	97.3 ± 0.4 ^a	18.0±0 ^d	99.0 ± 0.7 ^a	11.0±0 ^d	95.8 ± 0.9 ^a	21.3 ± 0.3 ^b	93.6 ± 1.3 ^a	29.0±0 ^c	95.6 ± 0.9 ^{ab}	
	30	27.0±0 ^c	91.6 ± 0.8 ^a	31.0 ± 1.2 ^d	93.2 ± 1.2 ^a	51.3 ± 1.7 ^c	95.1 ± 1.3 ^a *	*	11±0 ^e	96.1 ± 0.8 ^a	18.0±0 ^d	97.4 ± 0.2 ^a	11.0±0 ^d	93.7 ± 1.7 ^a	19.5 ± 1.5 ^b	93.8 ± 0.4 ^a	29.0±0 ^c	92.7 ± 1.5 ^b	
Third Peak	5	180±0 ^a	12.8 ± 1.9 ^b	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
	10	70±0 ^b	18.8±0 ^a	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
	15	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
	30	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	

* No relaxation peak was observed. Different small letters denote that T₂ values and the relative areas (%) of each peak for each starch type and the corresponding starch: water ratio, significantly differs for each microwave treatment time (p<0.05).

content after microwave heat treatment possibly were lower than in the wheat or corn starch granules. Microscope images also supported the attribution of fast gelatinization process in microwave heating.

4.2. Spin-lattice (T_1) & spin-spin (T_2) relaxation time measurements

NMR experiments revealed that there was a decreasing trend in both T_1 and T_2 values with increasing microwave heating time. T_1 is called the spin-lattice relaxation time, because it refers to the time it takes for the spins to give the energy they obtained from the radio frequency pulse back to the surrounding lattice, in order to go back to their equilibrium state (Hashemi, Bradley, & Lisanti, 2010). As shown in Figs. 3–5, T_1 values of three starch types (CS, WS and RS) showed a decreasing trend when microwave treatment of the mixtures increased from 5 to 30 s. This phenomenon was mainly due to the gelatinization of starch with increasing microwave processing time. During gelatinization, starch granules start to absorb water and upon cooling this process leads to formation of a gel network. T_1 is highly dependent on the mobility of water protons (Zhang, 2007). The formed gel network reduced the mobility of water protons since water molecules formed hydrogen bonds with the surrounding starch molecules as gelatinization took place. Lower movements of water molecules reduced the T_1 value thus induced faster longitudinal relaxation. Therefore, the decreasing trend of T_1 's in all three types of starch slurries was expected and validated the gelatinization process under short time microwave treatment. In each starch type as the water ratio in the starch solution increased, T_1 values also increased since higher amount of water contributed to longer longitudinal relaxation time of the protons.

Another important parameter to be investigated in this study was the T_2 value. T_2 is also called as transverse relaxation time or spin-spin relaxation time, since it refers to time required for an excited spin-spin proton to reach dynamic equilibrium after energy exchange with adjacent protons (Hashemi et al., 2010; Pitombo & Lima, 2003). Similar to T_1 data, all starch types showed a decreasing trend of T_2 as the microwave heating time increased as seen in Figs. 3–5. Since gelatinization took place during microwave heating of starch solutions and granules absorbed water, thus reducing the mobility of protons inside a starch granule compared to water protons around the starch molecules (Fan et al., 2013). When the T_2 data of all 1:1 starch types were examined with

respect to increase in microwave heating time, it was seen that the percent change of the T_2 of corn starch solution was $22.57 \pm 3.02\%$ which was much lower than the WS and RS solutions, $65.31 \pm 3.57\%$ and $44.23 \pm 2.76\%$, respectively. The reason could be the higher amylose content of the corn starch (38.35 ± 0.01 g amylose/100 g starch) compared to other starch types used in the experiment. The different amylose/amylopectin ratio was reported to affect the gelatinization process. Amylose is known to be a structure builder and a mobility reducing entity. Therefore, lower amylose containing starches are attributed to have higher swelling power (Liu, Yu, Xie, & Chen, 2006; Xie et al., 2009). In 1:1 corn starch, the percent change of T_2 was quite little since it is possible that high amylose containing corn starch granules were not able to absorb as much water as WS or RS granules resulting in lower expansion of the corn starch granule. Lower granule expansion also reduced the water-starch molecule interactions and most of the water protons in the system remained free outside the granules causing a slight decrease in T_2 at the end of the heating process. For corn starch, as the water fraction increased, the T_2 values decreased more. The same pattern was observed in the T_2 changes of RS set. However, wheat starch showed a slight difference of T_2 between 1:1.5 and 1:2 starch to water ratios after 30 s microwave treatment which indicated that there was not a considerable expansion of the WS granules after 1:1.5 starch to water ratio during microwave heating. Here, it is hypothesized that the gluten residue in wheat starch samples may be in competition with starch granules for water and this could have resulted in less water absorption by the starch granules hence less swelling of these starch granules during heat treatment (Bosmans et al., 2012; Wang, Choi, & Kerr, 2004; Wynne-Jones & Blanshard, 1986).

4.3. Relaxation spectrum analysis

In addition to T_1 and T_2 measurements, relaxometry analyses were also performed to inspect gelatinization in detail. Relaxometry results were summarized in Table 1. The results showed that there were mainly two proton populations (two peaks) in all starch solutions and fractions except for 1:1 of CS which had three distinct proton populations. The first peak represented the rigid proton part interactions which were not exposed to water in the first place. So, this proton population was representing starch-starch interactions. The second peak, on the other hand, represented the protons coming from a more mobile environment which was the water interacting with hydroxyl protons of starch (Tang, Brun, & Hills, 2001). As the microwave heating time increased from 5 to 30 s, the area of the first peaks of almost all starch types decreased while the area of the second peaks increased. This was coherent with our decreasing T_1 and T_2 results. As the microwave heating time increased, protons of water molecules increased their exchange with protons of starch. The starch-starch interactions decreased since the starch-water interactions took place of starch-starch interactions. The presence of a third peak in 1:1 of CS was associated with the presence of an additional proton population in the system. This less abundant population could be attributed to the water entrapped and immobilized in the various parts of the corn starch gel matrix, such as confined water protons that exchanged with starch hydroxyl protons in the intergranular space (Ritota, Gianferri, Bucci, & Brosio, 2008). This third peak encountered, disappeared in the higher water fractions 1:1.5 and 1:2 of CS solutions due to the merging of the second and the third peaks. The reason was that the higher amount of water provided a better starch-water interaction during microwave heating, thus a better gelatinized network and the further expansion of the corn starch granules after water uptake led to a faster exchange of water molecules between two different regions (Tananuwong & Reid,

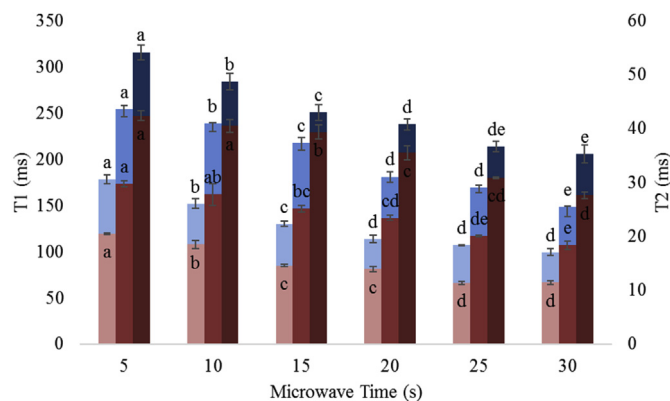


Fig. 5. T_1 and T_2 values of rice starch at different starch: water ratios: T_1 values for 1:1 rice starch; T_1 values for 1:1.5 rice starch; T_1 values for 1:2 rice starch; T_2 values for 1:1 rice starch; T_2 values for 1:1.5 rice starch; T_2 values for 1:2 rice starch. Different small letters mean that T_1 or T_2 belonging to each rice starch: water ratio, significantly differs in each microwave treatment time ($p < 0.05$).

2004). When 1:1 WS spectrum was analyzed it was observed that there was an increase in the first peak area on the contrary to other samples and a quick merge of the first and the second peaks. This unexpected event might have stemmed from the presence of gluten residue in wheat starch samples which was also considered in T₂ measurement analysis. Residual gluten, despite the trace amount, which was competing with starch molecules for water might have increased the solid-solid interactions in the absence of water (Bosmans et al., 2012). Insufficient water content may have led to a merge in the two peaks. In 1:1.5 of WS samples, this incident was not observed since at that fraction there was enough water to fully hydrate the solid fractions. The relative change in the peak areas in RS sample was less with respect to other starch samples. Furthermore, the first peak areas that were responsible for solid-solid interactions after microwave heating of RS solutions were lower than the others whereas the second peak areas of rice solutions were larger again than the others. The reason could be that the RS granules were better hydrated than other starch granules because of their small size granules and this led to smaller changes in the peak areas. The sudden rise in the second peak areas of RS even in low water fractions, suggests a quick gelatinization. Therefore, it can be claimed that the gelatinization of RS possesses a more homogeneous gel matrix structure. When the dielectric properties of each starch types were examined, Ndife et al. showed that at the beginning of the gelatinization (~ 25 °C), the loss factors of 1:2 starch to water ratio CS, WS and RS were close to each other. When solution temperature increased during microwave gelatinization, the loss factors of CS and WS samples experienced a dramatic reduction, whereas the loss factor of RS samples were almost stable and did not experience such a dramatic reduction even at high temperatures above 50 °C (Ndife et al., 1998). Consequently, the hypothesized quick and homogenous gelatinization of RS might also be attributed to the dielectric properties of RS samples.

5. Conclusion

LF NMR Relaxometry technique has been widely used to investigate the interactions between starch and water. This study provides information about the gelatinization of three different starch types namely CS, WS and RS by microwave heating. T₁ and T₂ for each starch type and starch to water ratio were determined and evaluated by LF NMR. Gelatinization was characterized by the decrease in the T₁ and T₂ values. In addition, relaxation spectrum was obtained and analyzed in order to understand the gelling mechanism. Microscope images for each starch solution and time interval were obtained. Firmness values of gels at distinct microwave heating time intervals were also measured. All in all, data analysis based on mentioned methods revealed an alternative way of understanding the microwave gelatinization of starch solutions. The effects of rapid heating mechanism of microwave heating due to fast vibration of polar molecules was clearly observed in gelatinization. Quick gelatinization with abrupt changes in the gel network justified the effects of gelatinization achieved by microwave heating. LF NMR was shown in this study to be an effective and simple method for the study of gelatinization of various types of starch solutions by microwave heating.

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