Influence of microwave parameters and water activity on radical generation in rice starch

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ABSTRACT

Radical generation in rice starch under microwave treatment as well as the related chemical bond changes were investigated by electron paramagnetic resonance (EPR) and Raman spectroscopy. Samples with water activity of 0.4 and 0.7 have been treated and analyzed. It was found that microwave power level and water content could influence the amount of radicals along with the radical components and their contribution. Raman spectra showed corresponding changes in vibrational features of chemical bonds. During storage the signal intensity started to drop after a short period of increase. Rice starch radicals were relatively stable and could exist a long time in room temperature. Through signal simulation, 3 main components were separated from the original spectra and the evolving process was investigated. The main component was the radical located on C1 position in the glucose ring.

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1. Introduction

Microwave is used as heating agent for decades in both household and scientific purposes. It has the merit of fast heating and easy to operate, thus becoming more and more popular in food process and widely applied in food thawing, drying, baking, enzyme deactivation and sterilization, etc. Lots of researchers had described in their studies that certain degree of energy would start the accumulation of radicals in starch. Furthermore, both the intensity of energy and duration of treatment can affect the quantity of radicals. Thermal treatment (Ciesielski, Achremowicz, Tomasik, Baczkowicz, & Korus, 1997; Ciesielski & Tomasik, 1996, 1998; Ciesielski, Tomasik, & Baczkowicz, 1998; Dyrek et al., 2007; Labanowska, Weselucha-Birczynska, Kurdziel, & Puch, 2013; Labanowska, Weselucha-Birczynska, Kurdziel, & Sepiolo, 2013), X-ray (Tomasik et al., 2008), γ-ray (Adamić, 1968; Bertolini, Mestres, Colonna, & Raffi, 2001), UV irradiation (Kameya, Nakamura, Ukai, & Shimoyama, 2011) as well as microwave irradiation (Dyrek et al., 2007) could induce radicals in starch from different origins.

Radical is a product of chemical reactions and a middle agent in reactions. For example, thermal degradation in starch leads to the production of radicals and intramolecular or intermolecular dehydration, hence producing furan, aldehyde, ketones and CO_2, CO, H_2O, etc. (Liu, 2011). It also has been reported that long-term radicals in polysaccharides can be considered as traps for free electrons, neutralizing in this way the dangerous reactive species (Labanowska, Weselucha-Birczynska, Kurdziel, & Puch, 2013). Therefore, deciphering starch radical generation process and the influence of different factors during process may be essential for food safety and quality control.

So far there have been several researches on the aspect of microwave impact on starch, e.g., granule properties, gelatinization and swelling properties, thermal dynamics and dielectric properties of the starch, etc. Evidence showed microwave irradiation brought inevitable changes to starch granules and even to their molecule structures. However, the field of microwave induced starch radicals has merely been explored. The present discussions were about the identification of radicals and the determination of the attenuation characteristics during storage. The radical generation progress was yet to be investigated, with regard to the changes of microwave power, duration of treatment and properties of starch.

This study was focused on the forming of radicals in rice starch with low water activity, as well as the corresponding changes in

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chemical bonds during microwave treatment. The microwave frequency used in this study was 2450 MHz which is commonly applied in domestic and industrial ovens. In our present work, the electron paramagnetic resonance (EPR) spectroscopy was used to determine the nature of radicals, their content and relative quantities. Information collected by Raman spectroscopy was used to analyze the bond changes occurring in carbohydrate polymers and confirm the corresponding radical evolution process. The influence of microwave parameters and water activities of starch on radicals and chemical bond will be discussed.

2. Materials and methods

2.1. Materials

Native rice starch purchased from Golden Agriculture biotech Co., Ltd., was used for the investigation. The purity of the starch was up to 95%. The amylose content of the starch was equal to 30.8% (dry mass) determined according to GB/T 15683-2008 iodine colorimetry. AACC Method 46-10 (Improved Kjeldahl method) was applied to determine the protein content of the starch, which was equal to 0.37% (dry mass).

To adjust the water content in the starch sample, two kinds of saturated solution (K_2CO_3 and K_2SO_4) were prepared and kept in two separate air-tight containers each with a porous china plate in the middle of the container. The saturated solution was kept underneath the plate. Beakers containing starch were kept on the plate for 2 weeks to balance the water content. The water activity of the starch detected by a FA-st lab water activity meter (GBX, Romans sur Isere, France) was about 0.4 (K_2CO_3 solution) and 0.7 (K_2SO_4 solution).

For EPR measurements, 60 mg of each starch samples were put in a special glass tube (inner diameter = 3 mm) with a plastic lid, which showed no signal during EPR measurements.

For determination of LF-NMR, 0.8 g of each starch samples were put in a glass tube (inner diameter = 15 mm). The temperature of the samples was adjusted to 35 °C before LF-NMR measurements.

2.2. Methods

2.2.1. Irradiation with microwaves

2.0 g of starch sample was uniformly distributed in a glass Petri dish (inner diameter = 6 mm) without its lid. The average thickness of the sample in each dish was 1.5 ± 0.1 mm. 5 Petri dishes containing starch sample were placed in the center of the microwave chamber to form a circle. The samples were irradiated with microwave at 800 W (80 W/g) or 1600 W (160 W/g) for 1–5 min using a custom made microwave station (XO-SM400 Xian Ou, China) at 2450 MHz frequency. After microwave irradiation, samples in 5 Petri dishes were mixed for later analysis. A fiber optic temperature sensor (Fiso, Canada) was used to detect the temperature of the sample. The temperature sensor was connected to a computer station which could display the temperature in real time. Each experiment was conducted three times.

2.2.2. EPR measurements

The EPR measurements of the starch sample before and after microwave treatment were performed with a Bruker EMX-8/2.7 B spectrometer (Karlsruhe, Germany) operating in X band (9.85 GHz) with the modulation frequency of 100 kHz. The EPR spectra were recorded at room temperature with modulation amplitude = 0.6 mT, microwave power = 20 mW and receiver gain = 3.17 * 10⁵.

g value was measured using a standard mark (Bruker, $g_s = 1.9800$). $H_{s(x)}$ is a value of magnetic resonance field for the standard and the sample, respectively. g_x could be calculated using the formula below:

$$hv = g_s\beta H_s = g_x\beta H_x$$

$$g_x = g_s H_s/H_x$$

In the equations, h stands for Planck constant ($h = 6.626 \times 10^{-34}$ J s); β stands for Bohr magneton; v stands for the frequency of the microwave added perpendicular to the magnetic field.

Peak to peak height of each spectra was calculated as the signal intensity. The EPR acquisition and processing was conducted by WinEPR (Bruker, Germany). The signal simulations were conducted using the program EasySpin 4.5.3 (Stoll, 2013; Stoll & Britt, 2009; Stoll & Schweiger, 2006, 2007) running on Matlab R2013a (The MathWorks, Inc., US). The parameters determined by EasySpin had an accuracy of ±0.0005 for g value and ±0.1 mT for hyperfine splitting constant A.

2.2.3. Raman spectra

All the Raman spectra were recorded with a Multi-purpose Laser Raman System Raman Tracer-200-WF-B (Opto Trace Technologies, Inc., US). Samples were excited with 785 nm laser line. Registrations were repeated three times for each sample. Layer thickness and spot laser size were the same for all samples.

2.2.4. LF-NMR relaxometry

The low-field nuclear magnetic resonance (LF-NMR) relaxation measurements were performed on a NM20 NMR analyzer (Niumag, China) with a magnetic field strength of 0.28 T corresponding to a resonance frequency for protons of 21 MHz.

The temperature of the NMR instrument was set to 35 °C. The transverse relaxation time constant, T_2, was measured using the sequence based on Carr–Purcell–Meiboom–Gill (CPMG) (Carr & Purcell, 1954; Meiboom & Gill, 2004). The T_2 measurements were performed with a τ value of 50 μs (time between 90° and 180° pulses). The 90° and 180° pulses were 10 μs and 20 μs, respectively. Data from 500 echoes were acquired; they were obtained as a 32 scan repetition, with 1 dummy scan in front to ensure that a spin system is in a steady state before data are collected.

2.2.5. Infrared thermal imaging

After each period of microwave irradiation, the samples were immediately removed from the cavity and photographed using an infrared thermal camera (IRI4010, Northants NN4 9BG, UK). The photographs were then processed using the software (IRISYS 4000 Series Imager).

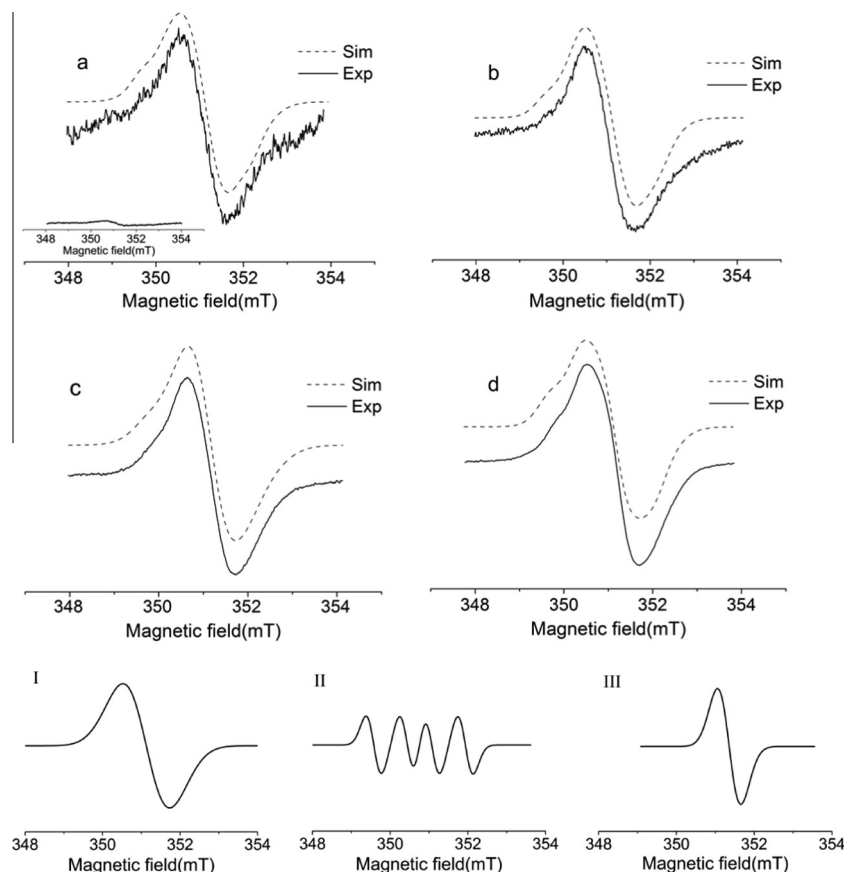#### 2.2.6. Statistical analysis

Spectra analysis and statistic calculations were performed using OriginPro 8 SR1 (OriginLab Corporation, USA) and Microsoft® Office Excel 2007 software. Raman spectra were analyzed using OMNIC v6.2 software (Thermo Nicolet, US).

3. Results and discussion

3.1. Radical generation during microwave irradiation

3.1.1. Identification

The native rice starch did not show an obvious EPR signal (Fig. 1a – insert). EPR signals of starch after microwave treatment were presented in Fig. 1. Different settings of parameters resulted in similar g factors of EPR signals. The signal shapes detected in our

Fig. 1. Simulated (Sim) and experimental (Exp) EPR spectra of rice starch after 5 min of microwave irradiation: (a) starch (Aw = 0.7) irradiated under 800 W, insert – native starch, (b) starch (Aw = 0.4) irradiated under 800 W, (c) starch (Aw = 0.7) irradiated under 1600 W, (d) starch (Aw = 0.4) irradiated under 1600 W, I–III – components of the signals.

experiments were almost identical to the thermally generated radicals in potato and corn starches (Labanowska, Weselucha-Birczynska, Kurdziel, & Puch, 2013; Labanowska, Weselucha-Birczynska, Kurdziel, & Sepiolo, 2013). Thus the radicals in rice starch treated with microwave were identified as carbon-centered radicals which coincided with previous research done by Dyrek et al. (2007).

As can be seen in the EPR spectra of starch after microwave treatment, the signals consisted of several different components (Fig. 1). Through simulation process, 3 main ingredients were separated from the signals. According to the researches by Labanowska, Weselucha-Birczynska, Kurdziel, and Puch (2013) and Labanowska, Weselucha-Birczynska, Kurdziel, and Sepiolo (2013), signal I was the radical formed by abstraction of α hydrogen atom on C_1 position. The β hydrogen atom situated at C_2 interacted with the unpaired electron gave the signal a two-line structure. Signal II had a four-line hyperfine structure (HFS). It was the result of interactions between magnetic moments of the unpaired electron located on C_6 and two non-equivalent nucleic atoms, α hydrogen atom on C_6 and β hydrogen atom on C_5. Signal III, which had a lower g value and more narrow line width than signal I, was also the radical of C_1. The appearance of signal III was accompanied by the decrease of signal I. The dehydration progress introduced a double bond into the glucose ring between C_2 and C_3 atoms. The abstraction of β-hydrogen atom from C_2 depleted the HFS structure in signal I. Furthermore, the removal of OH group from C_3 weakened the influence of oxygen on the unpaired electron. The double bond formation also explained why the g value of signal III was lower than that of signal I.
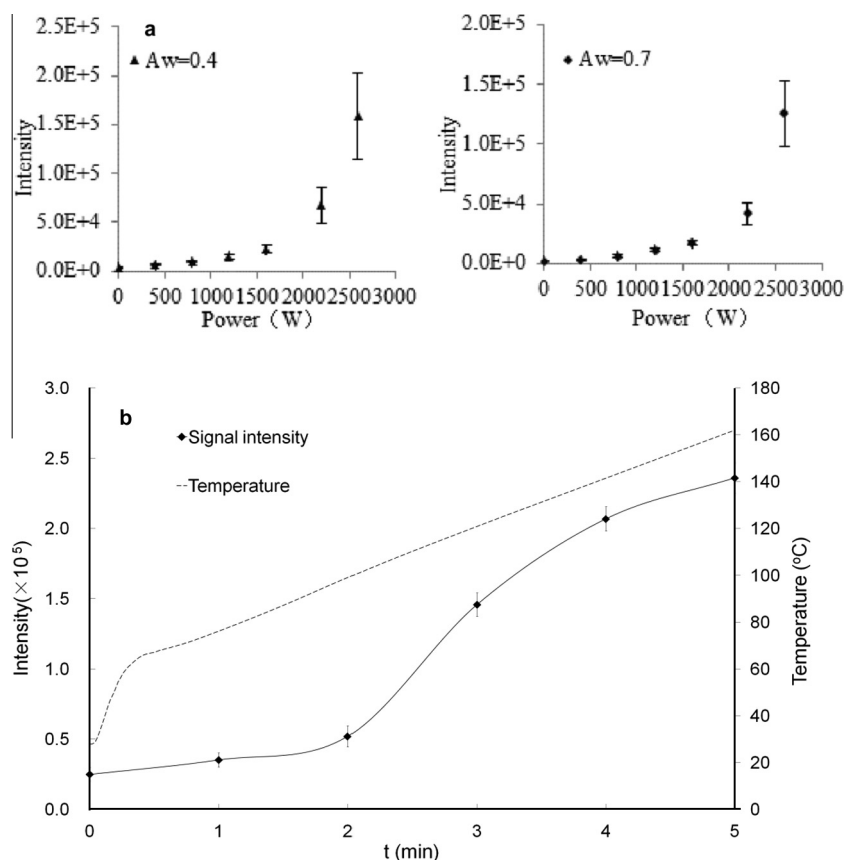
3.1.2. Effect of microwave power on starch radicals

It was shown in previous work (Andersen, Erichsen, Skibsted, Graversen, & Rodrigues-Filho, 2011; Ciesielski et al., 1997, 1998; Ciesielski & Tomasik, 1996, 1998) that heat can induce radicals in starch from different origins. However, in our study, microwave was considered as a source of energy contributing to heat generation and radical reactions in starch molecules. Therefore, it was reasonable to assume that the electromagnetic field will affect the progress of radical generation (Dyrek et al., 2007). All samples for EPR spectra were the same weight and measured in the same condition to ensure the signals were comparable in shapes and intensities.

The peak height of radical signals under different microwave power were calculated to get the relationship between the free radicals and power (Fig. 2a). The results showed that the greater the power of microwave processing, the larger the intensity of free radicals. However, when the microwave power was more than 1600 W, the color of rice starch was changed obviously. Part of the sample color turned to brown, starch may have been degraded, and with the increase of power the absolute error of the signal intensity was also increased. When the microwave power was less than 800 W, the signal intensity of starch radicals was too small to detect. In order to get more stable experimental result, 800 W and 1600 W were chosen to analyze the effects of microwave treatment on rice starch.

3.1.3. Time related evolution of starch radicals

The changes in the EPR signals of rice starch (Aw = 0.7) irradiated under 1600 W for 1–5 min depicted the trend of how radicals evolved in the electromagnetic field (Fig. 2b). Supposedly,

Fig. 2. (a) Signal intensity of starch rice radical after treated for 3 min with microwave operating in different power levels; (b) time related evolution of radicals generated in starch (Aw = 0.7) and temperature curve under 1600 W microwave irradiation.

the overall concept is that the more energy the starch absorbs, the more radical reactions occur. However, results showed that barely any signals were detected to prove the existence of stable radicals after the first minute of irradiation. In the early period of irradiation, the sample had a relatively high dielectric constant considering the water within. The water helped starch to absorb microwave energy and to convert the energy into heat allowing a large temperature increment to occur. Starch irradiated for 2 min showed no significant increase in radical numbers either, which was the same when the sample was irradiated for 1 min. From the information collected by temperature sensor in Fig. 2b and by infrared camera in Fig. 3, it was clear that the temperature of the starch in the first 2 min of treatment stayed below 100 °C. So the insufficient of heat energy may be a reason for small signals. Also extra water in the system can annihilate radical molecules once they were produced. As was revealed in Fig. 4a, the LF-NMR inversion spectra only gave one signal for each starch sample. The transversal relaxation time (T_2) in low water activity natural starch was 0.5–2 ms. During microwave treatment, water loss in starch was intense in the first third minute (Fig. 4a and b), demonstrating a shorter T_2 (0.02–0.2 ms) and much smaller area under the curve. The shortening of T_2 indicated tighter bonds formed between water and starch molecule after microwave irradiation. The signal at the third minute only has 2 components with the contribution of 87% for signal I and 13% for signal II. It proved that the dominant part of the signal was attributed by the unpaired electron located on C_1. Another important part was donated by the formation of C_6 radical, because of the abstraction of one α hydrogen atom.

The total number of radicals came to a rapid increase in the 2–4 min period of irradiation, which appeared to be the result from

series of factors. The temperature curve and thermal images (Figs. 2b and 3) indicated some part of the sample reached a certain point, probably around 100 °C, at which breaking of glycosidic bonds may occur when the activation energy achieves the defined value. At the same time, the water activity kept dropping. It can be seen in Fig. 4a, that the area under the curve became smaller and T_2 was shorter indicating the strengthen of water–starch bond. There was insufficient water molecules left in the system to counteract the radical generation process but was enough to stimulate a chain reaction, which induces a considerable amount of radicals. The lack of water molecules in the system also led to the appearance of a third content in the sample at the fourth minute of the treatment. The hydrogen atom on C_2 and OH group situated on C_3 started to react and left as water molecules. This content was described as signal III, which was also C_1 radical but with double bond between C_2 and C_3 atoms in the glucose ring.

In the last period of the 5 min microwave treatment, the water activity had dropped to 0.05. The drop of the water activity decreased the dielectric constant even more, meaning the starch's ability to convert electromagnetic energy into heat or energy for chemical reactions had been weakened. It was also seen from temperature information in Fig. 3 that heat was not uniformly distributed in the samples. However, each part of the samples temperature rises simultaneously and most part of the sample exceeded 100 °C after 4 min. Meanwhile, the contribution of signal III increased from 2% to 4%. The lwpp (line width peak to peak) increased for each component of the signal, which may be induced by the increase of dipole–dipole interactions among the existing high amount of radicals. That may be the reason why the radical generation rate appeared to slow down. During the last 3 min of the treatment, signal III started to form and grow while the
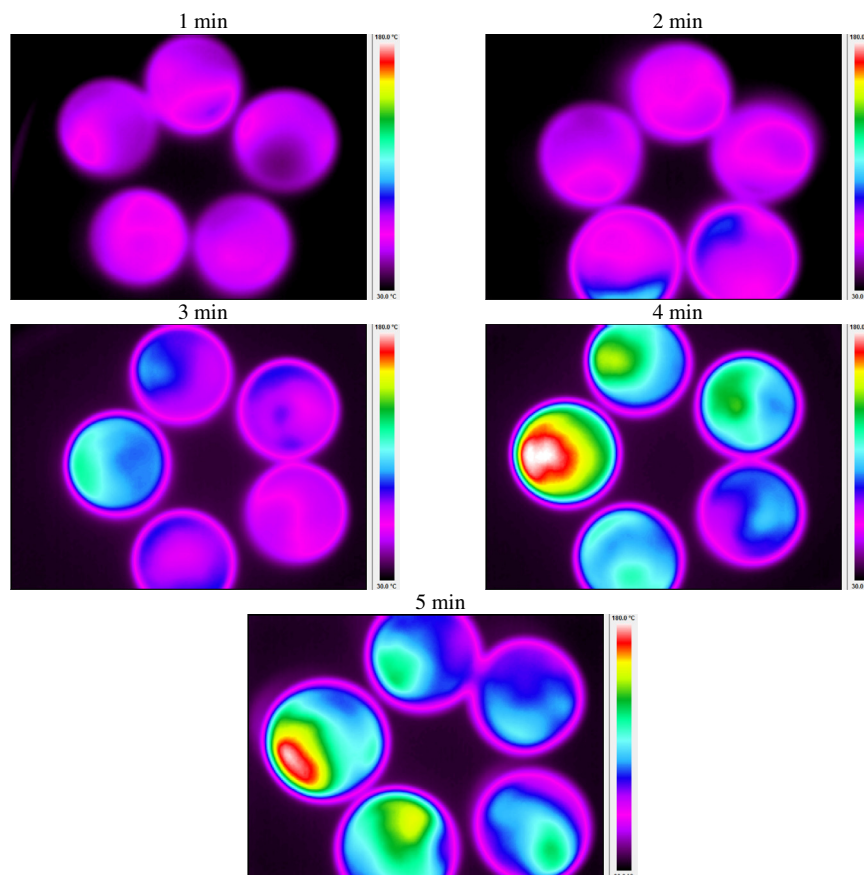
Fig. 3. Infrared image of starch sample after treated with 1600 W microwave for 1–5 min.

contribution of signal I decreased. Although the total number of radicals appeared to be accumulating, the content of C_6 radicals slightly decreased. It was assumed that the radicals formed faster on C_1 than on C_6.

3.1.4. Component of overheated starch radicals under microwave heating

The rice starch (Aw = 0.7) was treated with 1600 W for 15 min, the color of rice starch turned to dark brown, and the internal structure became loose and expand. The samples were detected by EPR, as shown in Fig. 5, the results indicated that the g value of the sample was still carbon-centered radicals. The signal intensity was about 2 orders of magnitude greater than non-overheated samples, and the spectral line width was significantly narrower than that of the non-overheated sample. The overheated starch was analyzed by signal simulation, a new component of free radical was found to be about 11%, which was not present in the starch samples, and it was determined to be IV. There is no signal of II in the overheated samples, the reason may be that the signal is too small to be separated from the radicals produced by the side chain. While the content of I which was the main components of radicals was significantly decreased to about 21%, the component of III was in the dominant position after over heating, the content of III is about 68%. This showed that the molecular chain structure of starch and glucose ring structure were destroyed, a large number of double bonds in C_2 and C_3 and carbon compounds were formed. As the appearance of signal IV, carbon-centered radicals had impacted on each other, which led to the hyperfine splitting of the signal, and the g value of the signal was decreased, and the width of the signal was narrow. Signals I–III were Gaussian type paramagnetic resonance spectra, while signal IV was close to
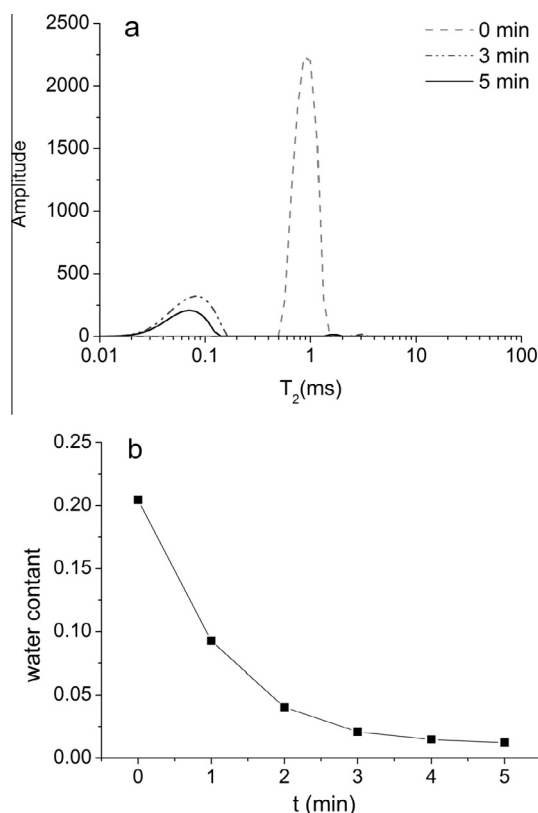
Lorentz type paramagnetic resonance spectra, which was due to the interaction of carbon component to the radical center that caused the inhomogeneity of the magnetic field.

3.2. Raman spectra of bond changes in irradiated starch

The Raman spectra of starch before and after microwave treatment were demonstrated. It was clear that the positions of the bands were almost the same for each spectrum but the intensities were different due to the length of treatment period. After 3 min and 5 min of microwave treatment, the spectra have a significant rising of the background. According to Katumba, Mwakikunga, and Mothibinyane (2008), the length of polysaccharide particles might have been shortened by the microwave process.

The region of 2800–3100 cm^{-1} was O—H and C—H stretching region, which had some differences in the spectra but was rather poorly resolved. The region 400–1600 cm^{-1} could provide valuable information of chemical bond changes in polysaccharide structures. The decipher of the information could help explain how the chemical bond vibration changed after microwave treatment.

Because the polysaccharide chains were composed by glucose rings, the spectra of starch and its fractions were supposed to exhibit similar spectra as the glucose molecule (Cael, Koenig, & Blackwell, 1973). A strong band at 472 cm^{-1} was one of the dominant bands in the spectra. It was the skeletal mode of pyranoid ring involving (C—O—C) ring mode and δ(C—C—O). The glucose ring constituting the starch molecule provides most of the spectra information in the region 800–1500 cm^{-1} and the bands were highly overlapped and very intricate. The characteristic bands for starch were listed as follows. 860 cm^{-1} was originated from v_s(C—O—C) ring mode and C_1—H bending α-configuration. As there

Fig. 4. (a) LF-NMR inversion spectra of transversal relaxation time of starch (Aw = 0.7) irradiated under 1600 W for 0 min (unirradiated), 3 min and 5 min; (b) time–water content curve of starch (Aw = 0.7) treated under 1600 W.

was no information given in 914–998 cm^{-1} for glucose monomers, the band in this particular region was the attribution of the glycosidic bond in starch. Therefore the band at 940 cm^{-1} was assigned to v_s(C—O—C) α-1,4-glycosidic linkage. The band at 1060 cm^{-1} was from δ(C—OH) and γ(C—OH), while 1120 cm^{-1} attributed to v(C—O) and C—O—H deformation. The band at 1145 cm^{-1} was associated with v_a(C—O) α-1,4-glycosidic linkage between glucose units in starch molecule. The band at 1260 cm^{-1} was characterized as a complex mode involving CH$_2$OH side-chain. In the range 1500–1300 cm^{-1} located the band relevant to the bending and deformation concerning C and H atoms. At about 1334 cm^{-1} the band related to CH$_2$–, δ(CH$_2$) and C—OH bending could be observed, whereas the band correlated to δ(C—H), C—H bending and C—H

scissoring vibrations were to be found at 1374 cm^{-1}. And finally, the band at 1455 cm^{-1} could be attributed to δ(CH$_2$) twisting and C—H bending (Cael et al., 1973; Colthup, Daly, & Wiberley, 1990; Galat, 1980; Kizil, Irudayaraj, & Seetharaman, 2002; Labanowska, Weselucha-Birczynska, Kurdziel, & Puch, 2013; Labanowska, Weselucha-Birczynska, Kurdziel, & Sepiolo, 2013; Söderholm, Roos, Meinander, & Hotokka, 1999).
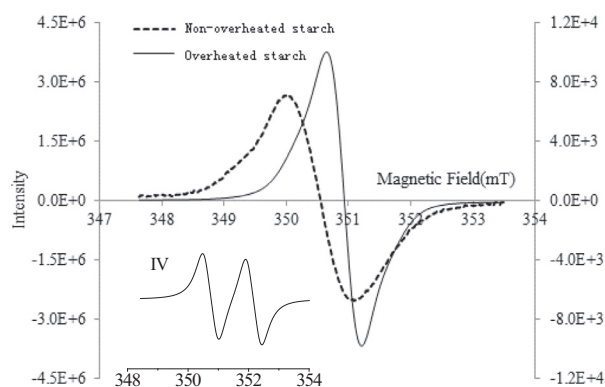
Observed from Raman scans, the C—C and C—O stretching region (1300–800 cm^{-1}) was very sensitive to the process, providing changes of intensity at almost all bands. For example, intensity of the bands at 940 cm^{-1} and 860 cm^{-1} decreased along treatment, suggesting severe inflictions on the molecule structure.

To determine the degree of impact on bands at each position, the relatively stable peaks at 1120 cm^{-1} and 1333 cm^{-1} were set as reference peaks separately, while the same pattern occurred in the change of other peaks. The ratio of other peaks to each reference peak was listed in Table 1. Though all bands were affected after 3 min of microwave irradiation, the band at 472 cm^{-1} diminished distinguishingly. The phenomenon indicated a 3-min period of large power microwave treatment had a serious impact on the (C—O—C) ring mode and δ(C—C—O). It proved disarrangement of the starch structure occurred in the glucose ring. On the other hand, in the last 2 min of the 5-min treatment, the most obvious changes happened to the bands at 860 cm^{-1}, 930 cm^{-1} and 1455 cm^{-1}. The observed significant decrease in their intensities might be caused by the changes in pyranoid ring, v_s(C—O—C) α-1,4-glycosidic linkage, C$_1$—H bending α-configuration and the changes in the hydrogen bond network involving CH$_2$OH groups, e.g., δ(CH$_2$) twisting (Labanowska, Weselucha-Birczynska, Kurdziel, & Puch, 2013; Labanowska, Weselucha-Birczynska, Kurdziel, & Sepiolo, 2013).

It was shown in Raman spectroscopy that most bands in starch were influenced by microwave treatment. The disturbance in the pyranoid ring, glycosidic bonds, CH$_2$OH side chain, as well as C$_1$—H bonds seconded the formation of carbon radicals during microwave treatment, which could be considered as an intermediate step of polysaccharides degradation.

3.3. Attenuation of starch free radicals during storage

After analyzing the results of non overheated starch samples, we knew that with the extension of storage time, the interaction of dipole–dipole from a large number of radicals gradually reduced, the free radical signals that were previously not detected, the signal of radicals which was not detected previously the signal of free radicals appeared in the first 1 day, and the intensity of the free radicals increased gradually. In the first 1–5 days, the growth trend of free radicals had disappeared and appeared the trend of the growth on the contrary. The number of free radicals after microwave heating for 5 min was the same as the end of treatment in 3 weeks, while the same state was in 9 days for samples after treated 3 min. Further analyzing the component of free radicals during decay processing, the signal III was gradually reduced to disappear in 5 days after irradiated for 5 min. This was contrary to the composition of signal III during microwave processing. Suggested the reason was that the C$_2$=C$_3$ which formed by the internal dehydration of glucose molecules absorbed moisture in the environment during storage, then the double bond is reduced that promoted component III of free radicals gradually transformed to component I. It is showed that the free radicals containing double bonds were not stable in storage, while the C$_1$ and C$_6$ radicals of glucose cyclical structure were relatively stable. The components showed little change in the growth and decay stage of starch radicals after treated for 3 min, the component of radicals was still based on signal I which accounting for more than 90% of the total radicals, and the rest were most component II of side chain radicals.

Fig. 5. EPR spectra of overheated and non-overheated starch treated with microwave and simulated spectrum of component IV.

Table 1
Peak height ratio change in different stage of microwave irradiation using peak at 1333 cm^{-1} and 1120 cm^{-1} as references.

Position (cm^{-1})	Height ratio of natural starch	Height ratio of starch irradiated for 3 min	Intensity change in 3 min	Height ratio of starch irradiated for 5 min	Intensity change between 3 min and 5 min	Height ratio of natural starch	Height ratio of starch irradiated for 3 min	Intensity change in 3 min	Height ratio of starch irradiated for 5 min	Intensity change between 3 min and 5 min
471	0.70	0.55	**−21.1%**	0.56	+0.9%	0.68	0.53	**−22.3%**	0.53	−1.2%
860	0.41	0.40	−3.1%	0.21	**−48.1%**	0.40	0.38	−4.6%	0.19	**−49.2%**
933	0.56	0.55	−1.4%	0.40	**−28.5%**	0.55	0.53	−2.9%	0.37	**−30.0%**
1077	0.84	0.85	+1.0%	0.85	+0.7%	0.82	0.81	−0.5%	0.80	−1.3%
1120	**1.00**	–	–	–	–	0.97	0.96	−1.5%	0.94	−2.1%
1256	0.68	0.80	+17.3%	0.93	+16.8%	0.66	0.76	+15.5%	0.87	+14.4%
1333	1.03	1.04	+1.5%	1.06	+2.1%	**1.00**	–	–	–	–
1376	0.91	0.98	+7.4%	0.96	−1.8%	0.89	0.94	+5.7%	0.90	−3.9%
1453	0.72	0.79	+8.6%	0.65	**−17.6%**	0.71	0.75	+7.0%	0.61	**−19.3%**

Overheated starch radicals decayed rapidly in the first 2 h, causing the signal strength dropped to about 80% of just after the treatment, and the signal intensity began to increased until 1 day, then the signal strength began to maintain the trend of decline. Free radical signals within 2 h decreased rapidly was probably due to the radicals produced by overheated starch, some of which had extremely strong ability of reaction for its fast spin. So that the radicals could be quenched in an instant. The slow rise of radicals after 2 h and until 1 day the radicals continued decline may be caused by the interaction of some more stable free radicals in the material. With similar to the decay trend of non-overheated starch radicals, the slowly and steadily increase of free radicals was mainly due to the dipole–dipole interaction between the large number of free radicals which led to the signal broadening and even disappeared. From the composition of free radicals perspective, the content of signal I had been increasing, the composition of signal III and IV has been relatively reduced. The reason was that the structure of signal I is the most stable, and the signal I can be long time in the same time. The content of signal IV which was the most unstable decreased by 9% in 10 days and completely disappeared in 51 days, then the composition of the overheated starch was no longer changed.

4. Conclusions

The generation rate of radicals in starch under microwave irradiation was neither in proportion to the microwave power nor to the length of time. The EPR results of starch radicals indicated radicals formation had a prominent phase of fast-growing around 100 °C. The heat generated by microwave treatment was an important factor to the final quantity of long-term radicals. The EPR simulation showed that the signals had three different components indicating three kinds of radicals were formed during the process. Starch with lower water activity (Aw = 0.4) was more seriously affected by the increase of microwave power. It was detected with stronger radical signals and more double bonds formation between C_2 and C_3 atoms after microwave irradiation.

Vibrational information provided by Raman spectra also confirmed chemical bond changes in carbohydrate polymers evolving through the process of microwave treatment. The results supported the theory of chemical structure changes during radical formation under irradiation. The signal intensity of overheated starch was two orders of magnitude larger than that of non-overheated starch. Using simulation software, a new component was found in the radical signals. During storage, the annihilation progress went through a very short period fast decreasing, then a period of steady growth and finally the stage of slow decrease.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.09.012.

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