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# Examining of athermal effects in microwave-induced glucose/glycine reaction and degradation of polysaccharide from *Porphyra yezoensis*☆

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ABSTRACT

Many reports claim the existence of athermal effects in microwave-induced reactions, and this challenge the assumption that the thermal effect (heating) is the sole factor in microwave heating. Therefore, microwave-induced Maillard reaction of D-glucose/glycine and degradation of polysaccharide from *Porphyra yezoensis* (PSPY) were investigated. Browning reactions were monitored by measuring heating rate, UV-absorbance and brown color, UV–vis and synchronous fluorescence spectra, GC/MS analysis and intrinsic viscosity of degradation. Heating of D-glucose/glycine solution produced brown compounds which were detected at $A_{420}$, and the intermediate products, 2-acetylfuran and 5-methylfurfural, whose fluorescence intensity evidenced their formation. Maximum emission of synchronous fluorescence spectra of samples were at 430–440 nm and 370–390 nm. Both microwave and water bath heating did not cause any compositional changes in the Maillard reaction products. All data failed to show any significant athermal effects of compositional changes in the Maillard reaction products. It can be inferred that some of the reports suggesting the existence of athermal effects, which could ascribe to the different set-up obtained in not well temperature controlled microwave heating systems.

## 1. Introduction

Domestic microwave ovens have been widely in use from the beginning of the 1950s. Since the first study on the use of a microwave system for pasteurization of milk (Hamid, Boulanger, Tong, Gallup, & Pereira, 1969), several works on microwave milk treatment, mainly on microbiological aspects, have been reported (Villamiel, Corzo, Martínez-Castrob, & Olano, 1996). A report in 1986 demonstrated that microwave energy is also suitable for accelerating organic reactions (Giguere, Bray, Duncan, & Majetich, 1986). This has led to a growing interest in the microwave-assisted processes as tools in the synthesis of organic and inorganic substances.

In conventional thermal processing, energy is transferred to the material through convection, conduction, and radiation of heat from the surfaces of the material. In contrast, the microwave energy is delivered directly to materials through molecular interaction with electromagnetic field. In heat transfer, energy is transferred as a result of thermal gradient between two points, but microwave heating involves the transfer of electromagnetic energy to thermal energy, and thus it is a kind of energy conversion rather than heat transfer. This difference in the way energy is delivered can result in many potential advantages to using microwaves for processing of materials (Thostenson & Chou, 1999).

Many researchers have reported on the non-thermal phenomena that have been broadly termed “microwave effects”. Examples of the microwave effect include enhanced reaction rates of thermosetting resins during microwave curing (Marand, Baker, & Graybeal, 1992) and faster densification rates in ceramics sintering (Janney & Kimrey, 1991). Although there is considerable debate over the existence of microwave effects, many papers present unexpected results that do not seem to be a consequence of reduced thermal gradients which is within microwave processed materials. Critics of the microwave effect often claim that differences can be attributed to poor temperature measurement and control of

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experimental conditions that result in systematic error. The existence (or non-existence) of microwave effects continues to be an area of considerable debate and research.

Large numbers of experiments, both academic and industrial, have been conducted over the past few decades to study the enhancement of reaction rate under microwave heating not only for endothermic reactions but also for various classes of reactions. Numerous studies indicated that the overall progress of chemical reactions can be enhanced by using microwave heating (Cherbanski & Mogla, 2009; de la Hoz, Diaz-Ortiz, & Moreno, 2005; Kappe, 2008; Leonelli & Mason, 2010). However, due to difficulties associated with the measurement of local temperature/concentration within the reacting domain, especially under reaction conditions, previous experimental studies lack quantitative correlation between observed enhancement of reaction rate and microwave power absorption within the reacting media.

Absorption of microwave may be highly non-uniform depending on the dimension and dielectric properties of the reacting medium (Bhattacharya & Basak, 2008), which may cause dramatic improvement in the local reaction rate leading to observed enhancement of the overall reaction rate. In the absence of detailed information regarding the local reaction rate induced by microwave, the observed acceleration of chemical reaction was often speculated to be athermal effect of microwave radiations on the chemical reactions (Herrero, Kremsner, & Kappe, 2008). This necessitates detailed theoretical analysis of the microwave heating pattern and its “per se” effect on the local reaction rate in order to quantify as well as provide better understanding about the observed enhancement of reaction rate under microwave radiations in the absence of experimental data.

Although the enhancement of chemical reactions by microwave irradiation is known, few studies have been carried out on chemical changes produced as a result of Maillard reaction caused by microwave heating in a simplified model system. Non-thermal effects are claimed to change the chemical, biochemical, or the physical behavior of some systems (Shazman, Mizrahi, Cogan, & Shimoni, 2007) while the temperature controlled and all other parameters remain unaltered. The possibility of an athermal effect was tested with small molecular (simplified) reaction and macromolecule degradation in a very well controlled, high radiation intensity system in the paper. The purpose of this work was to test the effect of microwave heating on Maillard reaction in a D-glucose/glycine model system and the degradation of polysaccharides.

## 2. Material and methods

### 2.1. Experimental set-up

The experiments were carried out in an 800 W domestic modified microwave system (WP800SL23-2, Galanz, China) with a frequency of 2450 ± 50 MHz. The schematic diagram of experimental set-up for the modified microwave system (MG08S-2B, Huiyan, China) is shown in Fig. 1A. The experimental set-up consisted of a three-necked glass flask, containing the reaction solution, placed in the microwave cavity (350 mm × 215 mm × 330 mm) or in a water bath in case of the control test. An overhead stirrer was placed inside the reaction solution through a central hole at the top of the three-necked glass flask. The microwave power was selected at a linear adjustable level (0–800 W) and for various exposure times (0–99 h).

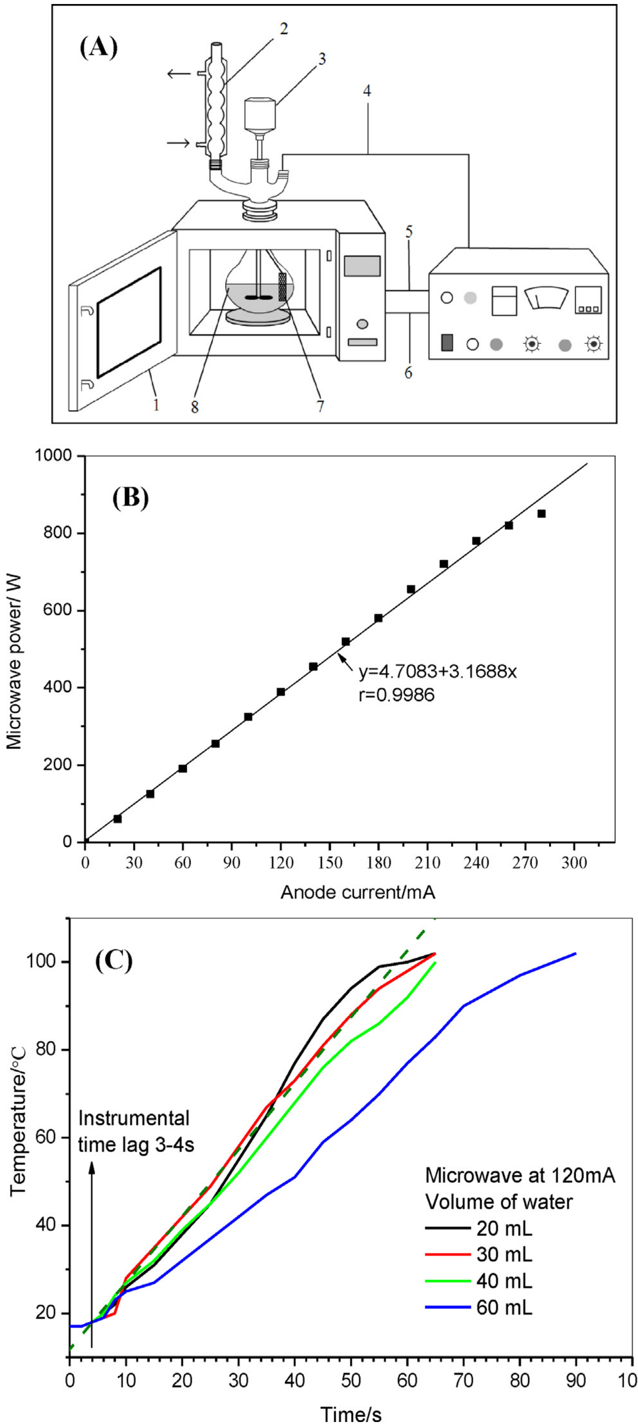


**Fig. 1.** (A) Schematic diagram of the experimental system: (1) oven window, (2) condenser, (3) stirrer, (4) infrared temperature detector, (5) microwave generator, (6) microwave power controller, (7) platinum temperature sensor, and (8) three-necked glass flask; (B) the relationship between anode current and microwaves power in the microwave system; (C) the temperature increase of water in different volumes by microwave irradiation.

Different microwave powers were produced by altering the anode current supply of the microwave generator transformer (Fig. 1B). Thus, in order to generate a microwave with a desired microwave power density, the anode current of the microwave generator is adjusted to an appropriate current. Measurement of process temperature was done by platinum temperature sensor connected to data acquisition system (temperature accuracy of ±0.5 °C), and this was linked to proportional-integral-derivative (PID) controller (TCG-6061, HUAYOU) for continuous recording of data. The platinum temperature sensor was inserted into the reaction solution and an infrared temperature detector was held just above the reaction solution. However, the infrared temperature detector (Cole-Parmer Inc. 08406 series, IL, U.S.A) with an accuracy of ±1 °C was used for further reassurance. During experiments, the readings of platinum temperature sensor and the infrared temperature detector almost agreed with a difference of 1 or 2 °C.

### 2.2. Maillard reaction

In order to test the formation of Maillard reaction products, equimolar solutions (0.5 mol/L) of D-glucose and glycine were prepared separately in a phosphate buffer (0.1 mol/L, pH 5.0), filtered (0.2 μm, Schleicher & Schuell) and then mixed together for the experiment as described by Shazman et al. (2007). The test solution was heated by microwave or by the water bath (control). At predetermined heating time, the sample was withdrawn and cooled immediately in ice water prior to analyses. The drop in temperature upon cooling resulted in the virtual termination of the reaction. Each reaction mixture was prepared, heated and analyzed at least in triplicate. The collected samples were lyophilized in a freeze dryer (ALPHA 1-2, Martin Christ Inc., Osterode, Germany). The sample was dissolved in acetone to prepare a solution with concentration of 1 mg/mL (w/v). Then 1 μL of this solution was injected into GC/MS for analysis.

### 2.3. The degradation of polysaccharide from *Porphyra yezoensis*

*Porphyra yezoensis* was purchased from Nantong Lanbo Industry Co. Ltd. (Jiangsu, China). Polysaccharide from *P. yezoensis* (PSPY) was prepared as described in our previous study (Zhou & Ma, 2006). The effect of microwave intensity (60 and 240 mA), with or without $H_2O_2$ solution, on the degradation of PSPY (0.5%, w/w) at 85 °C was examined.

### 2.4. Measurement of browning

The absorbance of Maillard reaction products of the samples was measured according to the method of Ajandouz, Tchiakpe, Ore, Benajibas, and Puigserver (2001). A 10-fold diluted aqueous sample solution was prepared and the browning intensity measured at 420 nm, using a Cary 100 UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan).

### 2.5. Ultraviolet visible spectroscopy analysis

Light absorption measurements were made on a Shimadzu double-beam spectrophotometer; model UV-2450 at 25 °C using quartz cuvettes of 1 cm path length. The absorption spectra of sample solutions were recorded in the wavelength range of 190–700 nm against deionized water as a blank.

### 2.6. Synchronous fluorescence spectra

All synchronous fluorescence (SyF) measurements were made with a Hitachi F-4500 fluorescence spectrophotometer (Tokyo, Japan) equipped with a plotter unit and a 1 cm quartz cell. The SyF spectra were recorded with excitation between 200 and 800 nm and with the following settings: 5 nm excitation and emission slits width; wavelength difference between the excitation and emission monocromators of 50 nm; scan rate of Super. The spectra were digitized at every 1 nm throughout the spectral range (Esteves da Silva, Machado, & Oliveira, 1997).

### 2.7. Gas chromatography–mass spectrometric analysis

GC/MS analysis was performed with an Agilent 6890 N gas chromatography instrument (SUPELCOWAX® 10 Capillary GC Column, $L \times$ I.D. 30 m × 0.25 mm, $d_f$ 0.25 μm), combined with an Agilent-5973 mass spectrometer equipped with an electron ionization (EI) and quadruple analyzer. The temperatures of the ion source and interface were set at 200 and 300 °C, respectively. A split injection (injection 1 μL, split ratio 40:1, v/v) and injector temperature of 220 °C were employed and a mass scan range was from 40 to 300 amu. The electron energy was set at 70 eV. The oven temperature was programmed from 40 °C to 250 °C at a rate of 10 °C/min. Total runtime was 30 min. Helium was used as a carrier gas with initial pressure of 5 psi (Lee et al., 2012).

### 2.8. Determination of intrinsic viscosity

Intrinsic viscosity is an important index for studying degradation of large biological molecules, because it is relative to the change of molecular weight and molecular configuration (Cyrille & Marc, 1989). The intrinsic viscosity [$\eta$] of PSPY was determined by an Ubbelohde capillary (type Φ0.5–0.6 mm, 0.01187 $mm^2/s^2$) at 25 ± 0.1 °C as in the method of our previous publication (Zhou, Yu, Zhang, He, & Ma, 2012). The [$\eta$] value was determined by the mean intercept of Huggins and Kraemer plots (Young & Lovell, 1991).

### 2.9. Statistical analysis

All analyses were run in triplicate. Analysis of variance (ANOVA) was performed and means comparison were done by Duncan's multiple range test at $P < 0.05$. Analysis was performed using a SPSS package (SPSS 8.0 for windows, SPSS Inc., Chicago, IL).

## 3. Results and discussion

### 3.1. Temperature–time profiles of the microwave system

In order to inspect the heating characteristics of microwave system, different volumes (20, 30, 40 and 60 mL) of distilled water (the initial temperature was 17 °C) were prepared in the three-necked glass flask, and the microwave anode current was set at 120 mA. The temperature–time profile of the heating characteristics of the microwave system is shown in Fig. 1C. As can be seen from Fig. 1C, the time lag of the microwave system was about 3–4 s. *t*-Test analysis revealed that there was no significant difference in terms of the rate of increase in temperature among water of 20, 30 and 40 mL (linear regression analysis: 20 mL, slope of 1.56 ± 0.13, $r = 0.9902$; 30 mL, slope 1.46 ± 0.15, $r = 0.9959$; 40 mL, slope of 1.35 ± 0.12, $r = 0.9989$). However, the *t*-test indicated that the rate of increase in temperature for the water volume 60 mL was significantly lower than those for 20, 30 and 30 mL (linear regression analysis: 60 mL, slope of 1.05 ± 0.14, $r = 0.9929$). In addition, the results showed that the difference between 20 and 60 mL had $P = 0.0099$, 30–60 mL ($P = 0.0258$), and 40–60 mL ($P = 0.0479$), at least 0.05 levels above the difference. Accordingly, linear relationship between temperature and microwave irradiation-time was established.

### 3.2. The effects of microwave radiation on Maillard reaction

The chemistry involved in Maillard reaction is complex. Therefore, we suggest that, if any of the many stages of this process are athermally affected by microwave radiation, we may expect a detectable effect on the formation of the final color pigments. Thus, using D-glucose/glycine solutions, we explored this possibility by heating these solutions in a similar manner, using both microwave and conventional water bath. Fig. 2A and B shows the results of a triplicate measurement made at absorbance of 420 nm. A significant difference in the absorbance readings of solutions heated by the microwave and the water bath was observed (Fig. 2A). Possible

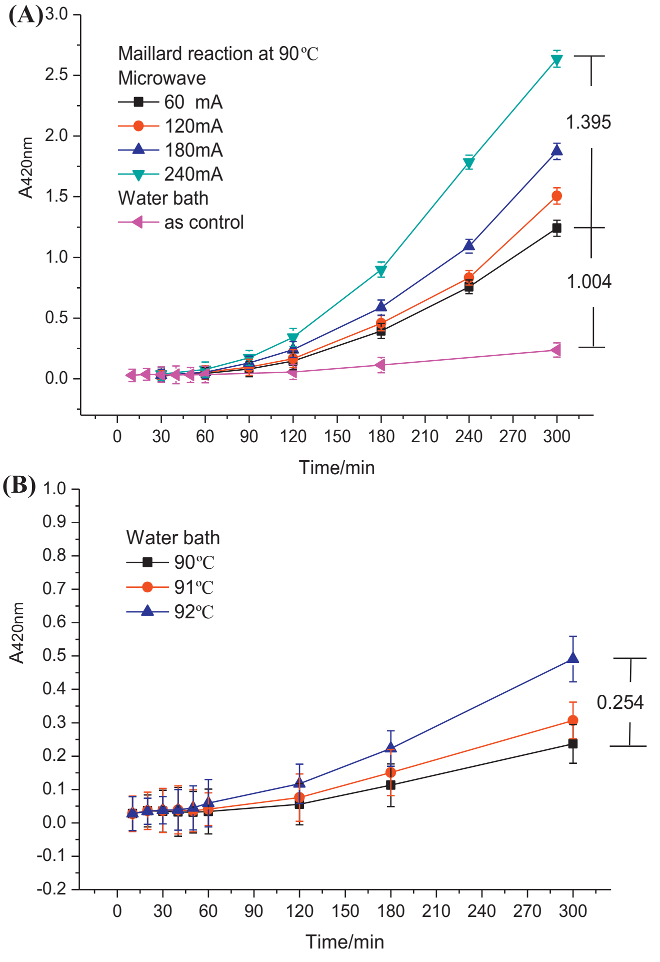


**Fig. 2.** The effect of different microwave anode currents (A) and water bath temperatures (B) on the development of brown colors due to Maillard reactions.

explanation of the phenomenon was ascribed to variations or slow drift found in the readings of the spectrophotometer (Shazman et al., 2007). There was no consistent pattern in these results; e.g. sometimes the microwave heating seems to produce slightly higher readings and sometimes vice versa. Since the drift could not be accounted for quantitatively, another test was conducted where the solutions were heated at temperatures of 91 and 92 °C in a water bath (Fig. 2B). Here too, the results suggest differences between microwave heating at 90 °C and water bath heating at 90, 91 and 92 °C.

D-Glucose/glycine solution is among the most frequently used model systems. The intermediate Maillard reaction products identified in aqueous D-glucose/glycine solutions include quinoxalinone, alkyl-substituted pyrazinones, 5-hydroxy-1,3-dimethyl-2(1H)-quinoxalinone (Keyhani & Yaylayan, 1997; Yaylayan, Matni, Pare & Belanger, 1997), pyrrole-like or furanone-like compounds (Bailey, Ames, & Monti, 1996), and 2-acetyl-6-hydroxy-7-hydroxymethyl-1,5,6,7-tetrahydro-4H-azepinone (Ames, Bailey, & Mann, 1999).

Woodward's rules, named after Robert Burns Woodward and also known as Woodward–Fieser rules are several sets of empirically derived rules which attempt to predict the wavelength of the absorption maximum ($\lambda_{max}$) in an UV–vis spectrum of a given compound. Absorption maximum of furaneol (2,5-dimethy-4-hydroxy-3(2H)-furanone): Maternal conjugated two dilute base 215 + 35(α-OH) + 30(β-OCR) + 12(β-$CH_3$) equal to 292 nm (Figs. 3A and 4).

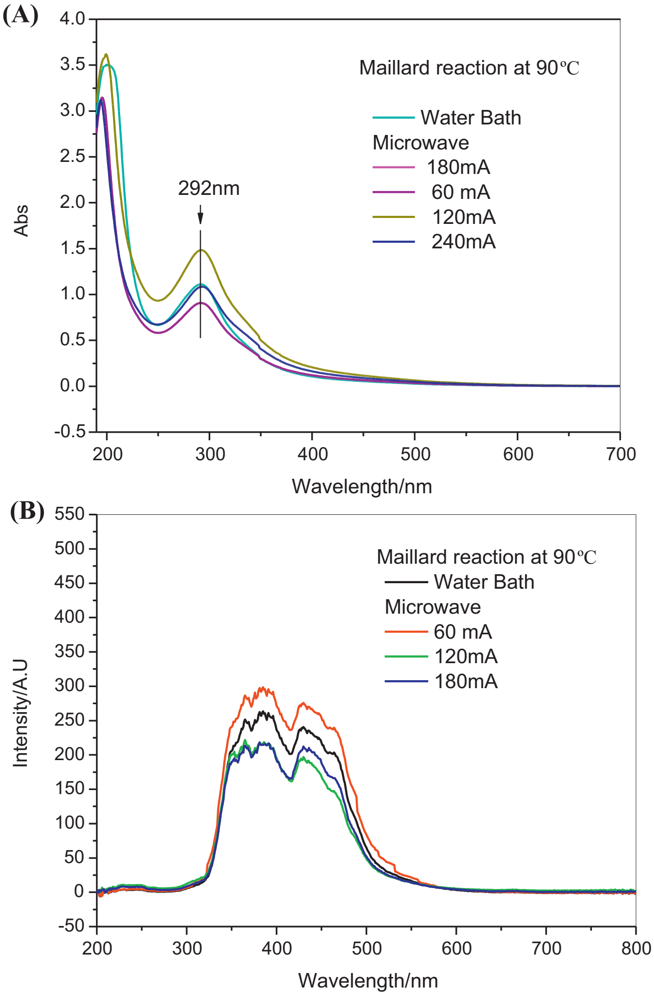
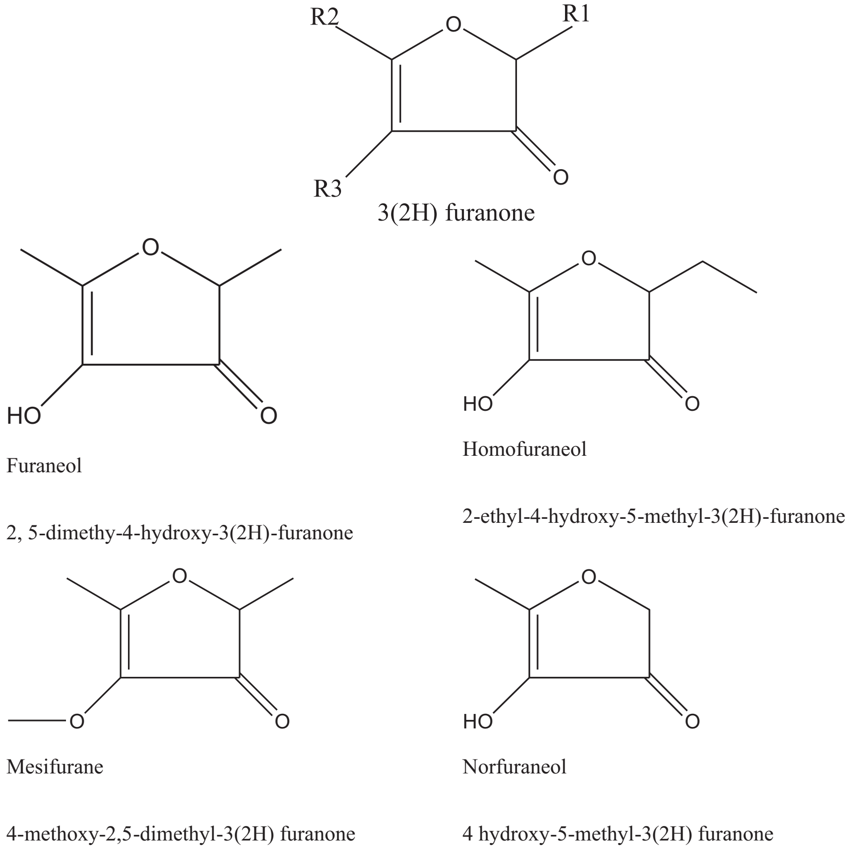


**Fig. 3.** Spectra of UV absorption (A) and synchronous fluorescent scanning (B) at different microwave anode currents.

It has previously been shown in model systems (Leclère & Birlouez-Aragon, 2001; Masaki, Okano, & Sakurai, 1999) and in biological samples (Murthy & Sun, 2000) that fluorescent products from Maillard reaction have a maximum excitation at wavelengths between 340 and 370 nm, and that the wavelengths of maximum emission are between 420 and 450 nm. The fluorescence from these products is clearly distinguishable from that of tryptophan in proteins (for which the wavelengths for maximum excitation and emission are 290 and 336 nm, respectively). Synchronous fluorescence spectra were thus selected to analyze the samples (Fig. 3B). The wavelengths of maximum emission of fluorescent products were at 370–390 nm and 430–440 nm, which agreed with the above-mentioned range (420–450 nm). However wavelengths between 370 and 390 nm were not detected in the previous papers of the above-mentioned authors. Although the chemical structures of the fluorescent products were only described for a few of those compounds, all the available structures indicated that they always have one or more nitrogen atoms originating from the reactant amino-compounds.

It is assumed that furfural is formed during the catalytic degradation of glucose in the presence of amino compounds. According to the assumption, the enol form of 3-deoxy-D-erythrohexosulose appeared during the degradation that was followed by dehydration, oxidation and disproportionation (Olsson, Pernemalm, & Theander, 1978). There were also other compounds in large amounts



3(2H) furanone

Furaneol

2, 5-dimethy-4-hydroxy-3(2H)-furanone

Homofuraneol

2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone

Mesifurane

4-methoxy-2,5-dimethyl-3(2H) furanone

Norfuraneol

4 hydroxy-5-methyl-3(2H) furanone

2, 5 -dimethy-4-hydroxy-3(2H)-furanone: Maternal conjugated two dilute base 215 + 35(α -OH) +

30(β-OCR) + 12(β-$CH_3$) = 292 nm

**Fig. 4.** Structures of Maillard reaction products at the maximum absorption of 292 nm.

experimentally detected in the systems tested. In our experiments, the 2-acetylfuran and 5-methylfurfural, Maillard reaction products, were detected and that agreed with the reports of other authors about the key-compounds of the Maillard reaction (Shen, Tseng, & Wu, 2007). It should be emphasized that in our study, the GC/MS investigation was not aimed at the identification and quantification of new and/or minor compounds in model systems, but only major constituents were considered. There were no changes in composition of Maillard reaction with microwave and water bath heating (Fig. 5). These observations are identical to those under conventional Maillard reaction, which might suggest that microwaves seem not to have affected the reaction path thermodynamically but have affected the reaction in a kinetic way.

Maillard reaction is a form of non-enzymatic browning, resulted from a chemical reaction between an amino acid and a reducing sugar, which is very common in agriculture and food processing. Microwave heating has vast applications in the field of food processing (including drying, pasteurization, sterilization, thawing, tempering, and baking of food materials) over a period of several decades (Chandrasekaran, Ramanathan, & Basak, 2013). In conventional thermal processing, energy is transferred to the material through convection, conduction, and radiation of heat from the surfaces of the material. In contrast, microwave energy is delivered directly to materials through molecular interaction with the electromagnetic field. The difference in the way energy is delivered can result in many potential advantages to using microwaves for processing of materials. Microwaves may be able to initiate chemical reactions not possible in conventional processing (Venkatesh & Raghavan, 2004). So many reports claim the existence of athermal effects in microwave-induced reactions (Herrero et al., 2008). But, all tests here failed to produce a significant difference between the microwave and conventional bath heating methods. As the controversy, the study of Gedye (1997) is of great interest. In his experiments, which had a very careful and well temperature control, Gedye did not detect any microwave athermal effects. The security in terms of applications of microwave in food industry is positive.

### 3.3. *The effects of microwaves on degradation of polysaccharide*

Fig. 6A illustrates that under the experimental conditions, even in the larger anode current (240 mA), microwave itself did not cause the degradation of PSPY. However, even in microwave with small anode current (60 mA), microwave can assist $H_2O_2$ in the degradation of PSPY (Fig. 6B). Meanwhile, the effects of microwave on the degradation of PSPY were verified with the Maillard reaction results. Linear regression analysis of Fig. 6B shows that, under these conditions there was a good linear dependence ($r > 0.98$) between intrinsic viscosity and degradation time for both water bath and microwave heating. The degradation process rule of PSPY is similar to microwave degradation of λ-carrageenan, which contains a sulfate and 3, 6-anhydrogalactose unit in its structure similar to

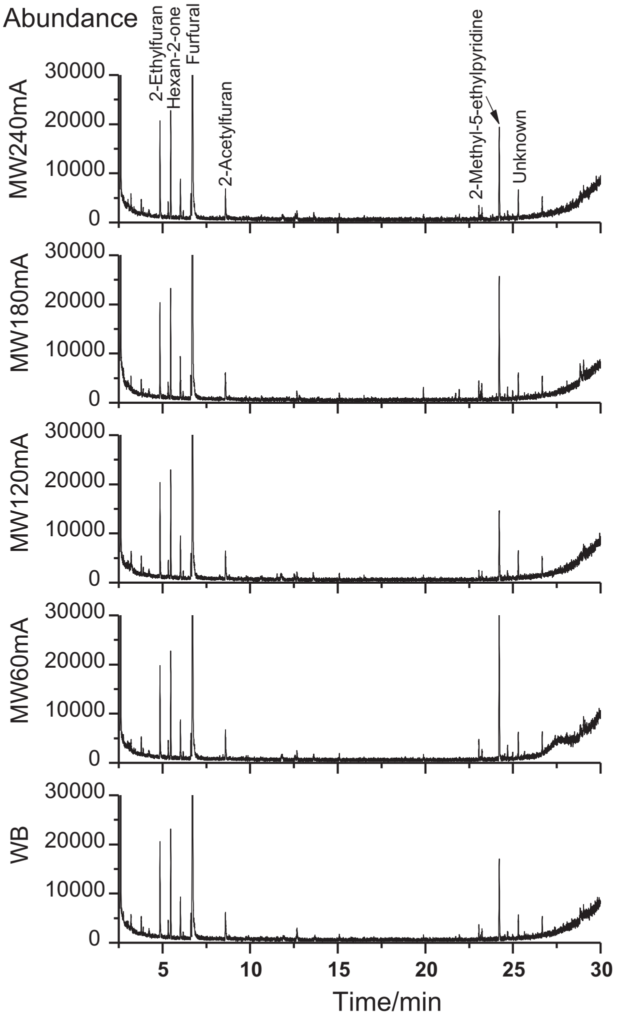


**Fig. 5.** GC/MS chromatogram of Maillard reactions (acetone solubility components) at different microwave anode currents and water bath heating.

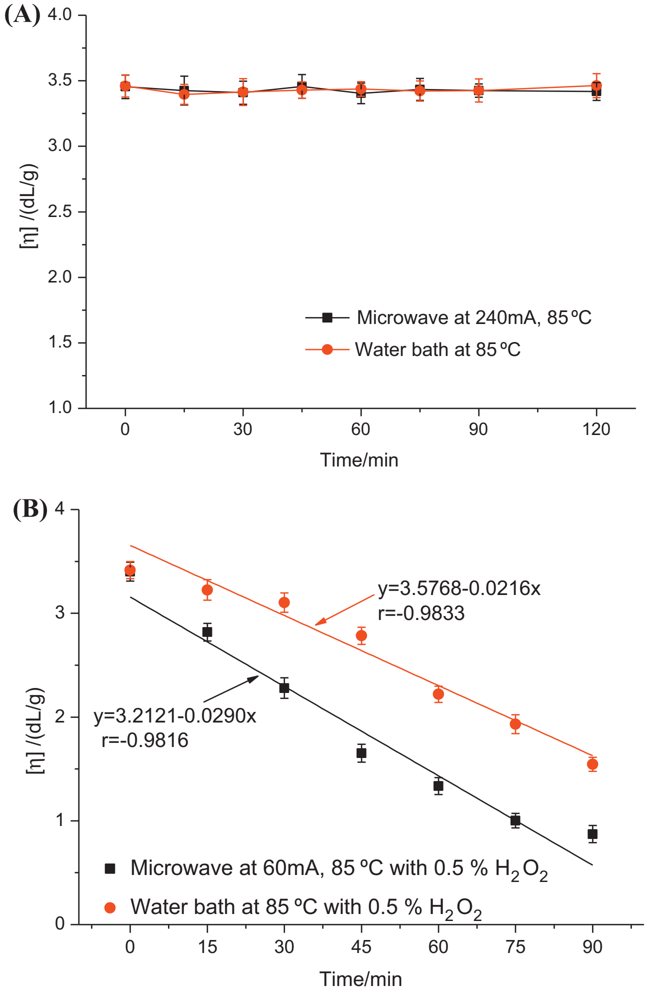


**Fig. 6.** The effects of microwave and water bath heating without $H_2O_2$ (A) and with $H_2O_2$ (B) on the intrinsic viscosity of polysaccharide from *Porphyra yezoensis*.

that of PSPY. Therefore, a degradation consistency between the two polysaccharides was observed. Interestingly polysaccharide sulfate for either microwave degradation or acid–base hydrolysis have similar degradation regularity (Hjerde, Smidsrød, Stokke, & Christensen, 1998). As effects of microwaves on degradation of polysaccharide, the resulting degradation curves were practically identical in Fig. 6A. Thus, we suggest that the differences in Fig. 6B, as compared to bath heating, may due to the two heating systems with different heating procedures, and cannot be attributed to athermal effects. An extensive review reflecting this debate about athermal effects was published by the Royal Society of Canada (Krewski et al., 2001). The comparison of these studies in Krewski's review is difficult, if not impossible, due to the lack of common experimental conditions. Extensive researches have been conducted and found athermal effects are always associated with microwave radiation (Bohr & Bohr, 2000; de Pomerai et al., 2003; Pagnotta, Pooley, Gurland, & Choi, 1993). However, many other studies could not detect any athermal effect (Meissner & Erbersdobler, 1996; Welt, Steet, Tong, Rossen, & Lund, 1993). Similarly, under the well temperature controlled conditions, the results of the present study cannot support the hypothesis of athermal effects induced by microwave radiation. Some of the reports suggesting the existence of athermal effects could ascribe to not using well temperature controlled microwave heating systems.

## 4. Conclusions

The results demonstrated the penetration effect of microwave heating, and this occurred in a reaction solution of volume 60 mL from temperature–time profiles in the microwave system. The PSPY degradation produced two major intermediate products, 2-acetylfuran and 5-methylfurfural, whose formation was detected from their fluorescence intensity. Maillard reaction intermediate products were observed, evidenced by $A_{292}$ and fluorescence intensity. Further development of browning was noticeable at $A_{420}$. Maximum emission of synchronous fluorescence spectra at 370–390 nm was not detected in previous works. All data failed to show any significant athermal effects of compositional changes in the Maillard reaction products.

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