

## Effect of Microwave Irradiation on Acid-Catalyzed Hydrolysis of Starch

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Recently, there has been growing interest in applying microwave heating to rapid thermal digestion prior to elemental and chemical analysis of inorganic and biological samples.<sup>1</sup> Microwave heating involves direct absorption of energy by functional groups that bear ionic conductivity or a dipole rotational effect, and this energy is then released into the surrounding solution. This absorption of energy causes the functional groups involved to have higher reactivity with surrounding reactants than when they are simply incubated with the reactants at the same temperature. Because much debate still remains with regard to the contribution of the nonthermal effects of microwaves on hydrolysis, the present study examined the effects of isothermal microwave irradiation on the hydrolysis of starch.<sup>2</sup>

Starch (10%) suspended in dilute hydrochloric acid (0.5 M) was hydrolyzed using either microwave irradiation (a household microwave, 2.45-GHz microwaves, 10- $\mu$ s pulses, 20–40% full power, temperature setting at 95 °C)<sup>3</sup> or a traditional heating block (100 °C). The hydrolysis of starch to glucose was monitored using HPLC and gel filtration columns (see figure legends for details). For reactions under heated conditions, the starch suspension

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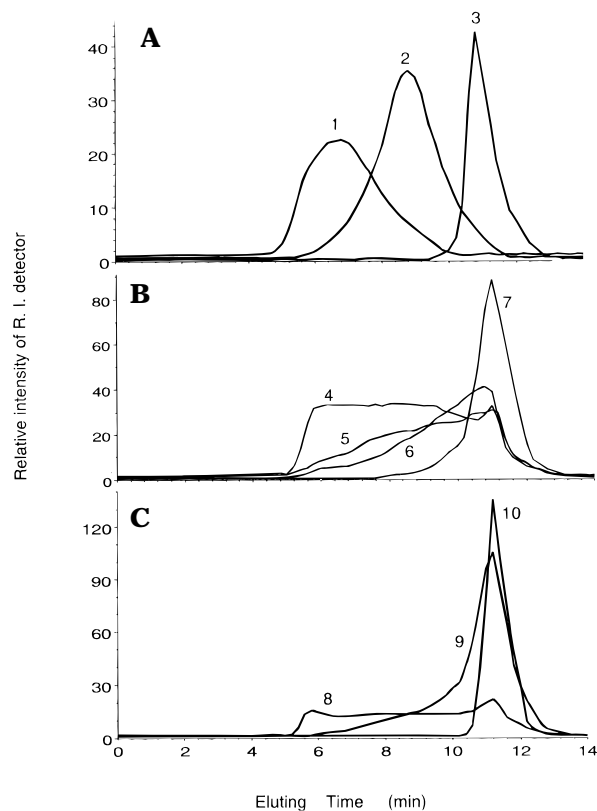
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(3) The microwave oven used was a commercially produced cooking apparatus without any modification (Tatung microwave oven TMO-110, Tatung Co., Taipei, Taiwan). The total power of the microwave was 650 W with nine power settings, the lowest of which was 72 W. In this study, 20–60% full power was used.

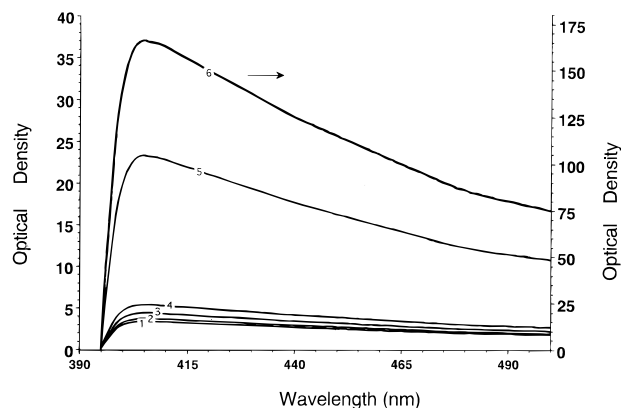


**Figure 1.** Analysis of hydrolysate of starch solution after microwave irradiation or thermohydrolysis in acidic suspension. The HPLC consisted of a Hitachi 6200 pump and a Hitachi Reflex Index Detector, and the data were collected on a Macintosh LCII using Rainin Chrompic software. The conditions for analysis were as follows: column, TSK G2000SW, 7.5 × 300 mm; eluent, phosphate buffer 0.05 M, pH 5.0; flow rate, 1 mL/min; RI, 4 RIU/FS. 1 is the HPLC profile of dextran (MW 40 000); 2 is dextran (MW 8800); 3 is glucose; 4, 5, 6, and 7 represent starch suspensions incubated at 100 °C for 5, 10, 20, or 60 min, respectively; 8, 9, and 10 represent the starch suspensions subjected to microwave irradiation at 40% full power for 3, 4, or 5 min, respectively.

(4.0 mL) was placed in a sealed test tube, purged with nitrogen, and heated at 100 °C. After 5, 10, 20, or 60 min, an aliquot (10  $\mu$ L) of reaction suspension was collected, and the extent of hydrolysis was measured using HPLC. For reactions with microwave irradiation, the starch suspension (1.0 mL) was placed in reaction vessels and irradiated for 3.0, 4.0, 4.5, 5.0, 5.5, or 6.0 min, and each timed suspension was analyzed using HPLC.

Figure 1 shows the results of the reaction. Three samples, dextran (MW 40 000), dextran (MW 8800), and glucose, were used as references (Figure 1A). Figure 1B shows the results of HPLC analysis of the starch suspensions incubated at 100 °C for 5, 10, 20, or 60 min. Following 60 min, the soluble starch was completely converted to glucose and the retrograded starch remained suspended in the solution. This suspension was centrifuged at 7000 rpm for 10 min at 25 °C and decanted to isolate the retrograded starch.<sup>4</sup> The four types of starch used (rice starch, corn starch, and wheat starch from Sigma, St. Louis, MO, and tapioca starch from Thailand)

(4) The resulting suspension containing retrograded starch was centrifuged (7000 rpm) for 10 min at 25 °C, and the supernatant was decanted off. The precipitate was resuspended in water and centrifuged. This procedure was repeated three times, and the final precipitate was lyophilized to yield the retrograded starch.



**Figure 2.** Measurement of the absorbance (395–500 nm) of the starch hydrolysate in 0.5 M hydrochloric acid after microwave irradiation for 5 min (curve 2) or after incubation at 100 °C for 5 min (curve 1), 30 min (curve 3), 1 h (curve 4), 8 h (curve 5), and 24 h (curve 6). The scale of the vertical column for curve 6 is plotted on the right and all others are on the left. The spectra were measured by diluting the hydrolysates with water (0.1–3.0 mL), centrifuging to remove the precipitates, and directly measuring the absorbance of the supernatants at wavelengths ranging from 395 to 500 nm.

contained 5.1%, 6.6%, 9.8%, and 2.5% retrograded starch, respectively. To confirm the results, the completeness of the starch hydrolysis was also measured with HPLC using an amine-bonded column (data not shown).

Figure 1C shows the gel filtration HPLC analysis of the starch suspensions subjected to microwave irradiation for 3, 4, or 5 min at 30% full power. After irradiation for 5 min, the solution was clear and no retrograded starch remained in suspension. This result led to the hypothesis that the  $\beta$ -structure of the retrograded starch was deformed by microwave irradiation, making it soluble in the solution and, therefore, subject to hydrolysis. To test this hypothesis, the isolated retrograded starch from

the thermohydrolysis experiments (25 mg/mL) was re-suspended either in water or in dilute hydrochloric acid (0.5 M) and irradiated in the same manner as above for 5 min. The retrograded starch became soluble in water and was completely hydrolyzed in hydrochloric acid, resulting in a clear solution as measured by HPLC in the same manner as above.

When the thermohydrolysis was prolonged at high temperature, the reaction solution became colored, and the absorbance of the solution increased significantly at wavelengths between 400 and 500 nm. Figure 2 shows the absorbance of the starch solution at wavelengths between 395 and approximately 500 nm. When the solution was digested for 5 min via either microwave irradiation or incubation, the absorbance of the starch solution was near zero. In the samples heated at 100 °C, the absorbance increased as the incubation time increased. After incubation for 7 days, the solution was dark-brown, and the retrograded starch remained suspended in the solution. Using standard methods, the retrograded starch in soluble starch does not hydrolyze and can be problematic by sticking onto the filter in large-scale separation experiments.

The results show that, with microwave irradiation, the starch in a suspension of starch (10%) in dilute hydrochloric acid (0.5 M) is completely hydrolyzed within 5 min without the formation of colored byproducts. In contrast, when standard methods using heat are used, the retrograded starch in a similar solution does not hydrolyze. In conclusion, microwave irradiation may not only increase the rate of energy transfer to accelerate reaction time but also change the structure (or conformation) of the reactant to facilitate the reaction.

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