

# Determination of Damaged Starch and Diastatic Activity in Wheat Flour Using a Flow-Injection Analysis Biosensor Method

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## ABSTRACT

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The determination of damaged starch and diastatic activity in flour was studied using a flow-injection analysis (FIA) biosensor system. The system consisted of an oxygen electrode and an immobilized enzyme column containing purified glucoamylase and glucose oxidase immobilized on activated aminopropyl glass beads. The biosensor system has an optimum pH between 6.5 and 7.5 and an optimum temperature of 35°C for glucose measurement. The response of the FIA biosensor was linear

up to 1.000 g/L of glucose with a lower detection limit of 0.025 g/L. Each assay took about 20 min, and the system showed good reproducibility ( $r = 0.998$ ,  $n = 8$ ). When applied to the measurement of damaged starch and diastatic activity in wheat flour, the results obtained agreed with those obtained using the conventional methods of measurement. This biosensor system is a rapid practical alternative for the measurement of damaged starch and diastatic activity in wheat flour.

It is well known that overgrinding flour damages starch granules and causes problems for preparing breads and other products (Jones 1940, Atkinson and Fuehrer 1960). The consistency of dough in flour depends on the absorption of water by flour, which is affected by the amount of water already in the flour and the amount of damaged starch, gluten, and pentosan. For preparing breads, cakes, and alimentary flour, hard flour, medium hard flour, and soft flour are usually blended depending on the final purpose. Some damage to starch granules occurs during milling, and several methods have been reported for the determination of damaged starch (Sandstedt and Mattern 1960, Sullivan et al 1962, Farrand 1964, Evers and Stevens 1985). Currently, the damaged starch contents of flour are assayed by Approved Method 76-30A (AACC 1995). However, this method is complicated, uses many reagents, and takes a long time.

Recently, new sensing systems using biological molecules as molecular recognition elements have been developed for, among other uses, clinical analysis and environmental monitoring. These methods are advantageous in terms of their sensitivity and selectivity (Karube 1988, Kawashima 1989), and the rapidity and ease with which measurements can be made. Such biosensors can be used in flow-injection analysis (FIA) systems (Wolf and Stewart 1979, Ruzicka and Hansen 1988) to create sensors for the rapid, automated monitoring of various substances.

In this study, a biosensing system was applied to the measurement of damaged starch in flour. An FIA system comprising immobilized glucoamylase and glucose oxidase was used to measure reducing sugars produced by the hydrolysis of starch.

## MATERIALS AND METHODS

### Materials

$\alpha$ -Amylase (EC.3.2.1.1) from *Aspergillus oryzae*, glucoamylase (EC.3.2.1.3), and glucose oxidase (GOD) from *A. niger* were

purchased from Sigma Chemical Co. (St. Louis, MO). Glutaldehyde was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Aminopropylated controlled pore glass beads were purchased from Funakoshi Co. Ltd. (Tokyo, Japan). All other chemicals used were of analytical grade.

### Wheat Flour

Wheat flours were obtained from Nisshin Flour Milling Co., Ltd. (Chiba and Tsurumi, Japan). Damaged starch flour samples were prepared from first- and second-grade soft wheat flours using various milling procedures: pin mill, turbo mill, and variable speed roller mill (Beke 1981).

### Immobilization of Enzymes

Aminopropyl glass beads (200 mg, 200–400 mesh, 50-nm pore size) were washed with distilled water and then activated by treatment with 4 mL of 2.5% glutaraldehyde in 0.1M acetate buffer, pH 4.8, for 1 hr at room temperature. The beads were then washed thoroughly with distilled water to remove excess glutaraldehyde.

Glucoamylase (102 U/mL) and GOD (8305 U/mL) were each suspended in 2 mL of 0.1M acetate buffer, pH 4.8. Enzyme solutions were mixed with activated glass beads and slowly rotated for 20 hr at 4°C. The immobilized enzyme beads were then washed with acetate buffer to remove unbound materials and packed into a column (27 × 3 mm, i.d.) and stored at 4°C in the buffer until use.

### FIA Biosensor System

Figure 1 shows the FIA biosensor system used for the measurement of maltose concentrations. The system consists of a peristaltic pump (type-XV, Alitea, Sweden), precoil (200 × 0.05 cm, i.d.), injector (model 7725, loop volume 20  $\mu$ L, Rheodyne Inc., Cotati, CA), enzyme immobilized column, flow cell (type FLC-42, DKK Co., Ltd., Tokyo, Japan), dissolved oxygen electrode (type 7621L, DKK Co., Tokyo, Japan), potentiostat (type HA-150, Hokuto Denki Co., Tokyo, Japan), recorder, and back pressure control coil (200 × 0.05 cm, i.d.). Polytetrafluoroethylene tubing (0.05 cm, i.d.) was used throughout. The potential between the cathode and anode was set at 700 mV. The carriers were 0.1M phosphate buffer (pH 6–8) for glucose assays and 0.1M acetate buffer (pH 4–6) for maltose assays at a flow rate of 1 mL/min at 30°C. Samples were introduced into the sample injector using a microsyringe. The injected samples reacted with the immobilized enzyme in the flow column, and the oxygen concentration was measured using a dissolved oxygen electrode. The output of the oxygen electrode was displayed on a chart recorder.

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### Measurement of Damaged Starch Content

The determination of damaged starch content and diastatic activity in flour were assayed by Approved Methods 76-30A and 22-15 (AACC 1995). For the determination of damaged starch content using the FIA biosensor system, 100 mg of flour was hydrolyzed in 40 mL of a solution containing 0.1M acetate buffer (pH 4.8) and  $\alpha$ -amylase (50 mg) for 15 min at 30°C. The enzyme reaction was terminated by filtration using a Chromato-disk filter (0.45- $\mu$ m pore size). The clear supernatant was analyzed for maltose using the FIA biosensor system. The damaged starch content was defined as the amount of maltose hydrolyzed by the glucoamylase in the column of the FIA biosensor system. The diastatic activity of flour was assayed in the same way using 500 mg of flour in the same reaction mixture but without  $\alpha$ -amylase.

## RESULTS AND DISCUSSION

The determinations of maltose and glucose are based on the reactions shown in Fig. 2. Dextrin and maltose are enzymatically converted to glucose, and oxygen is consumed when this glucose is oxidized by GOD in the reactor column. Characterization of the FIA biosensor system was conducted for both maltose and glucose.

### Effect of pH

The buffer pH of the reaction mixture drastically affects enzyme activity. The optimum pH levels for native glucoamylase and GOD are 4.5–5.0 and 5.1–5.6, respectively. The pH response of the FIA biosensor system for glucose was studied within the pH range of 6.0–8.0 in 0.1M phosphate buffer. These results (Fig. 3A) show that the optimum pH of this system is between pH 6.5 and 7.5 for glucose measurement.

The pH response of the FIA biosensor system for maltose was studied within the pH range of 4.0–6.0 in 0.1M acetate buffer. As shown in Fig. 3B, the response was basically linear for all maltose concentrations, but showed greater variation at higher maltose concentrations.

### Effect of Temperature

It is well known that enzyme activity increases with temperature in both native and immobilized enzymes (Peterson et al 1989, Arica et al 1995). Figure 4 shows the effect of temperature on the response from immobilized enzymes. The temperature was varied

for the determination of both glucose and maltose. The responses increased with the increase of the temperature from 30 to 60°C for all concentrations of both glucose and maltose. These results suggest that the enzymes are stable under these conditions. Immobilized glucoamylase and GOD from *A. niger* showed good tolerance to temperature; the optimum temperature for glucoamylase from *A. niger* was 60°C (Vandersall et al 1995). However, the responses decreased at 50°C for glucose and at 45°C for maltose (Fig. 4A and B), and the baseline became unstable over 40°C. These results led to the choice of a temperature of 35°C for the determination of glucose and maltose concentration using this system.

### Response of FIA Biosensor System

The calibration curves for glucose and maltose using this system (Fig. 5) were linear between 0.025 and 1.000 mg/L for both sugars with correlation coefficients of  $r = 0.998$  (Fig. 5A) and 0.999 (Fig. 5B) for glucose and maltose, respectively. The response of this sensor plateaus at sugar concentrations  $>1$  g/L.

### Reproducibility

The reproducibility of the system was examined by analyzing 0.5 and 0.25 g/L of glucose and maltose standard solutions. Figure 6 shows the responses to both sugars for five consecutive samples. These results indicate that the responses are reproducible over 25 min even for different concentration of sugars. The coefficients of variation (CV) are 0.8 and 1.2% (Fig. 6A) at glucose concentrations 0.5 and 0.25 g/L, and 0.5 and 1.4% (Fig. 6B) at maltose concentrations 0.5 and 0.25 g/L, respectively.

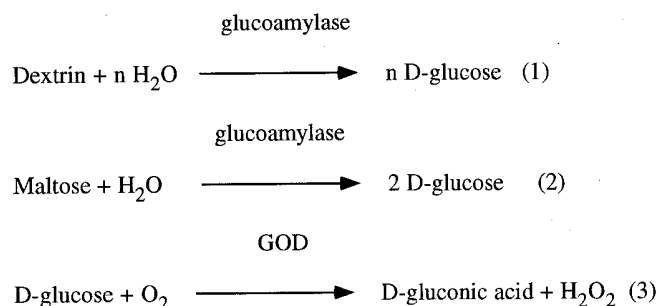


Fig. 2. Reactions for determination of maltose and glucose.

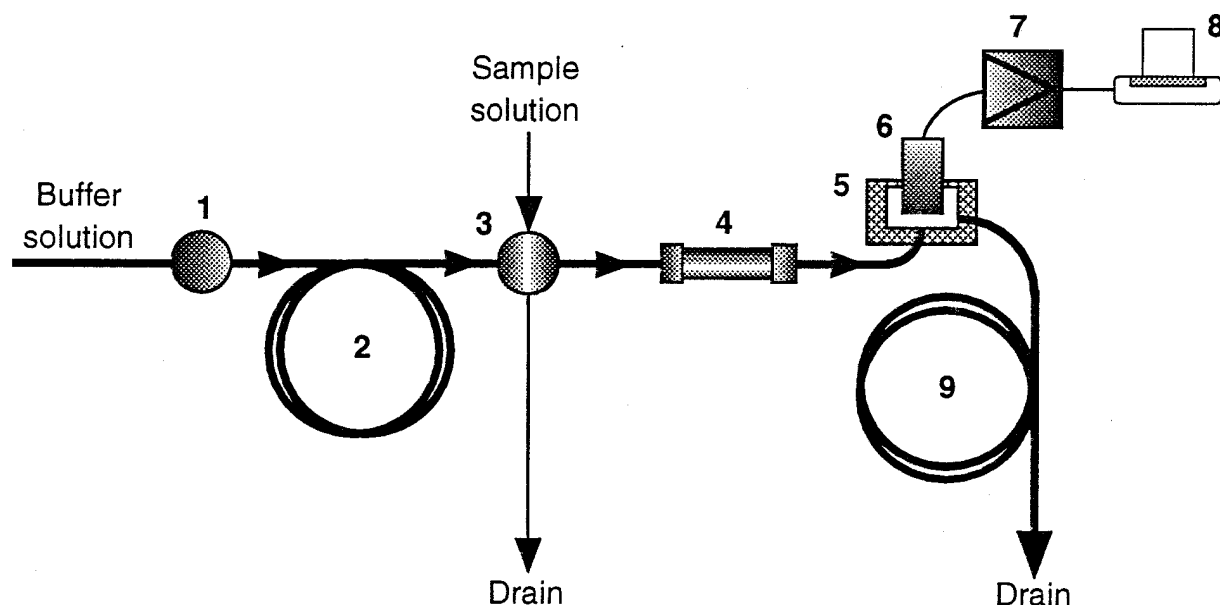
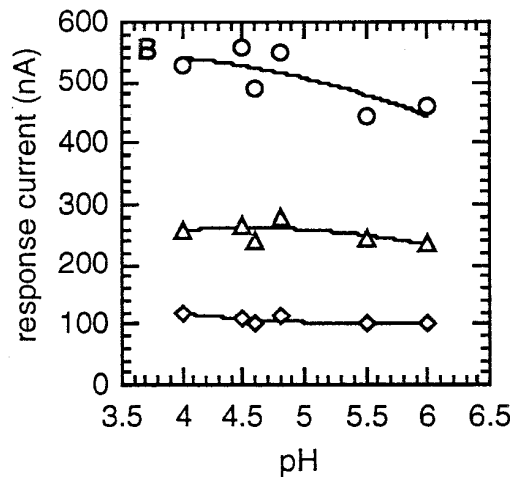
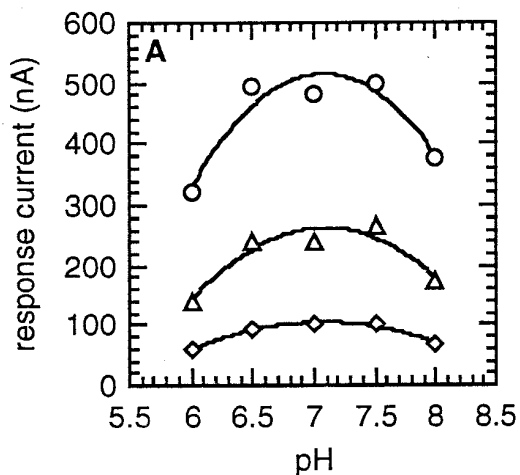
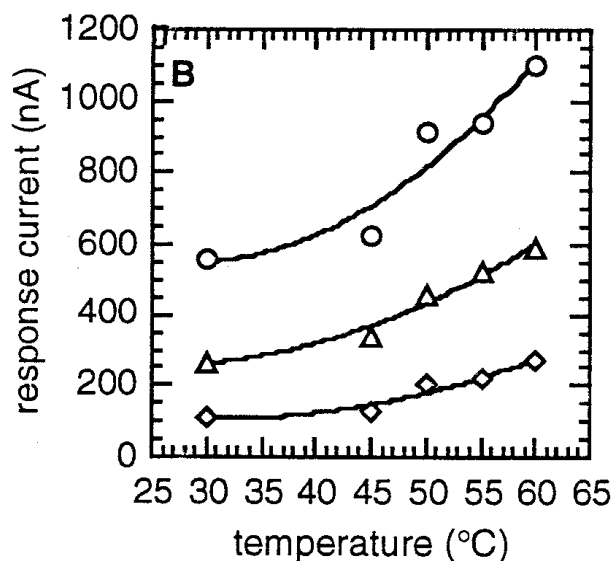
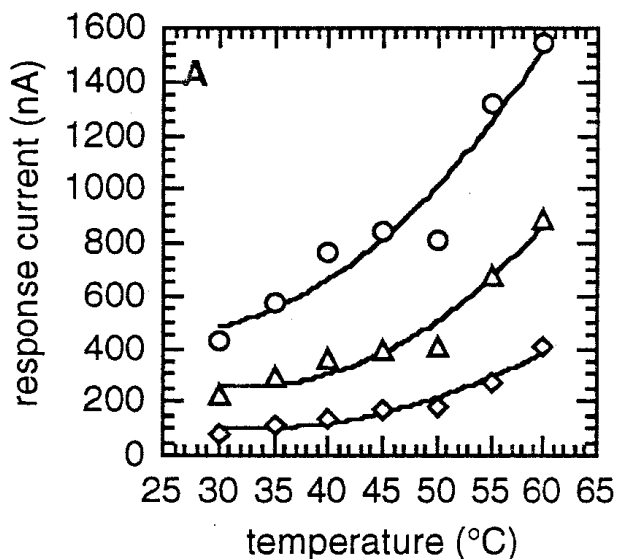


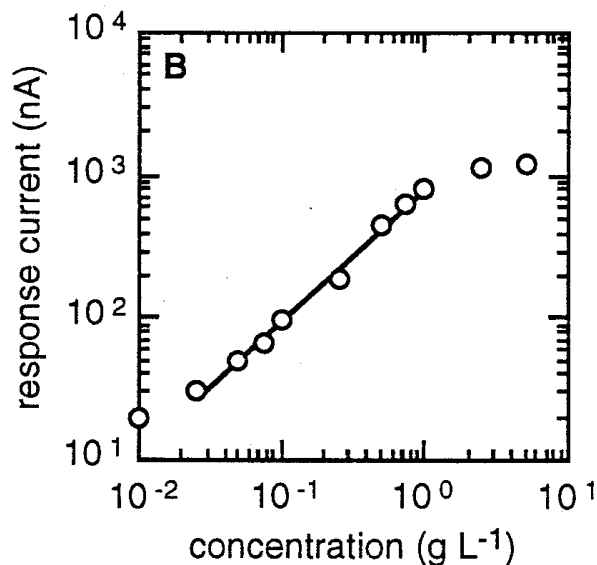
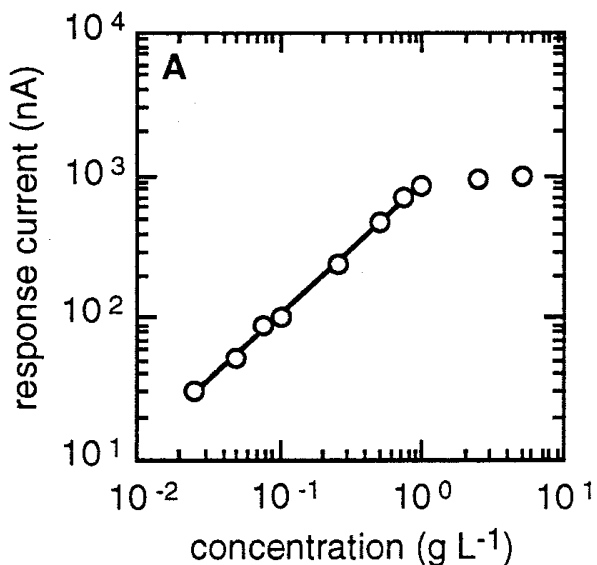
Fig. 1. Flow-injection analysis maltose sensor system: 1 = peristaltic pump, 2 = precoil, 3 = injector, 4 = enzyme column, 5 = flow cell, 6 = dissolved oxygen electrode, 7 = potentiostat, 8 = recorder, 9 = back pressure coil. Temperature maintained using a waterbath. Flow rate, 1 mL/min.



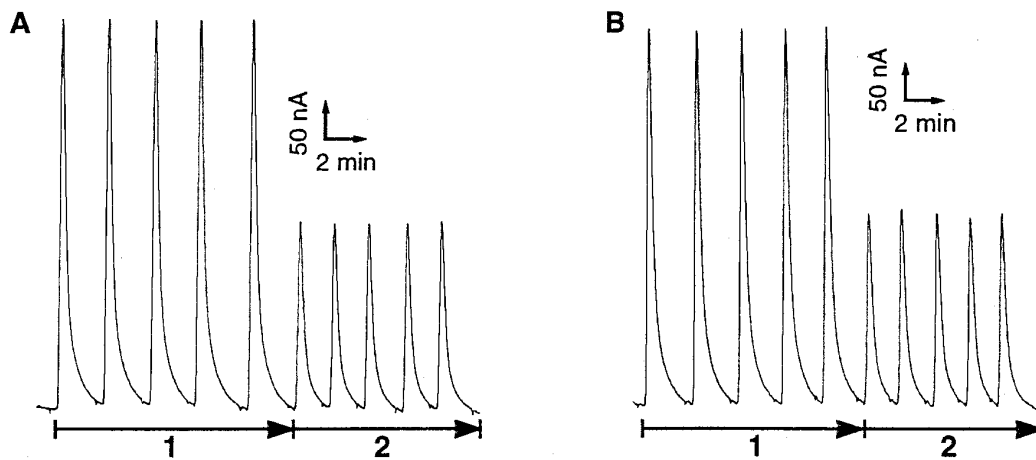
**Fig. 3.** pH Profiles of the flow-injection analysis biosensor system. **A**, Glucose solutions 0.50 g/L ( $\circ$ ), 0.25 g/L ( $\Delta$ ), 0.10 g/L ( $\diamond$ ). Carrier, phosphate buffer (0.1M, 30°C). Flow rate, 1 mL/min. **B**, Maltose solutions 0.50 g/L ( $\circ$ ), 0.25 g/L ( $\Delta$ ), 0.10 g/L ( $\diamond$ ). Carrier, acetate buffer (0.1M, 30°C). Flow rate, 1 mL/min. Each point represents the average of at least two measurements.



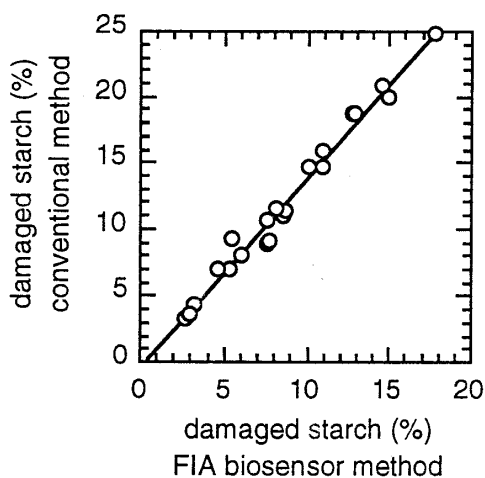
**Fig. 4.** Temperature profiles of the flow-injection analysis biosensor system. **A**, Glucose solutions 0.50 g/L ( $\circ$ ), 0.25 g/L ( $\Delta$ ), 0.10 g/L ( $\diamond$ ). Carrier, phosphate buffer (0.1M, pH 7.0). Flow rate, 1 mL/min. **B**, Maltose solutions 0.50 g/L ( $\circ$ ), 0.25 g/L ( $\Delta$ ), 0.10 g/L ( $\diamond$ ). Carrier, acetate buffer (0.1M, pH 4.8). Flow rate, 1 mL/min. Each point represents the average of at least two measurements.



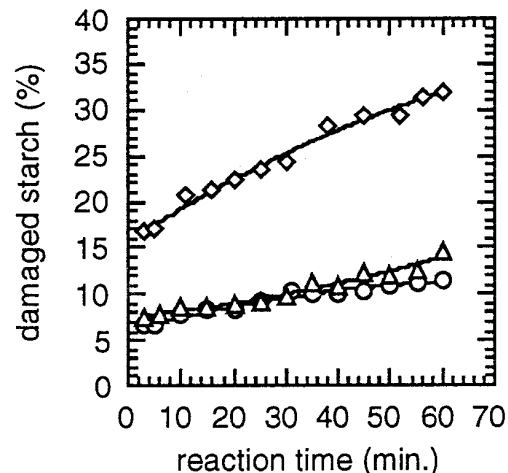
**Fig. 5.** Calibration curves for the flow-injection analysis biosensor system. **A**, Glucose solutions,  $r = 0.998$ . Carrier, phosphate buffer (0.1M, pH 7.0, 30°C). Flow rate, 1 mL/min. **B**, Maltose solutions,  $r = 0.999$ . Carrier, acetate buffer (0.1M, pH 4.8, 30°C). Flow rate, 1 mL/min.



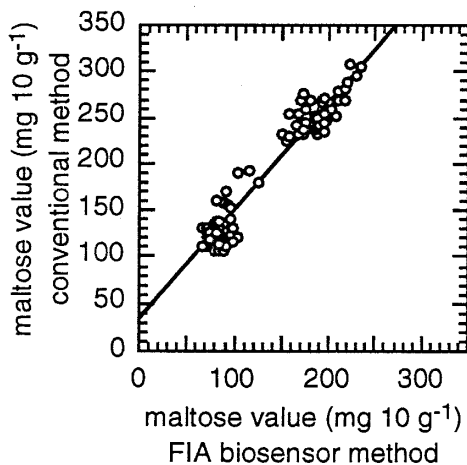
**Fig. 6.** Reproducibility of the flow-injection analysis biosensor system. **A**, Glucose solution 1: 0.5 g/L of glucose concentration, 494 nA average response current, 0.8% CV. Glucose solution 2: 0.25 g/L of glucose concentration, 237 nA average response current, 1.2% CV. Carrier: phosphate buffer (0.1M, pH 7.0, 30°C). Flow rate: 1 mL/min. **B**, Maltose solution 1: 0.5 g/L of maltose concentration, 487 nA average response current, 0.5% CV. Maltose solution 2: 0.25 g/L of maltose concentration, 247 nA average response current, 1.4% CV. Carrier, acetate buffer (0.1M, pH 4.8, 30°C). Flow rate, 1 mL/min.



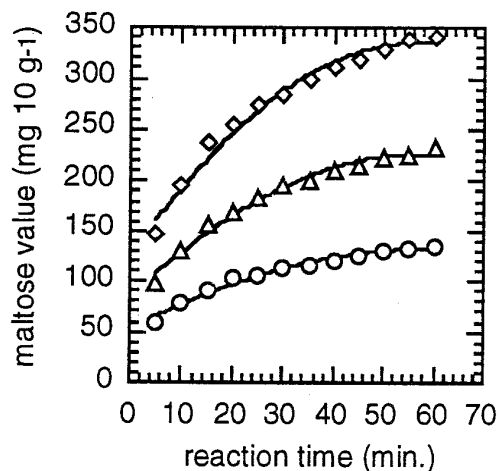
**Fig. 7.** Comparison of the flow-injection analysis biosensor method and the conventional methods for measurement of damaged starch in flour,  $r = 0.99$ . Hydrolysis conditions: acetate buffer (40 mL, 0.1M, pH 4.8, 30°C); sample, 100 mg;  $\alpha$ -amylase, 50 mg; hydrolysis time, 15 min.



**Fig. 8.** Hydrolysis of starch samples from 1st grade soft flour (○ and Δ) measured using the flow-injection analysis biosensor method. ◇ = Starch from lower grade soft flour. Hydrolysis conditions: acetate buffer solution (40 mL, 0.1M, pH 4.8, 30°C), sample 100 mg,  $\alpha$ -amylase 50 mg. Each point represents the average of at least two measurements.



**Fig. 9.** Comparison of the flow-injection analysis biosensor method and the conventional method for measurement of diastatic activity in flour,  $r = 0.97$ . Hydrolysis conditions: acetate buffer solution (40 mL, 0.1M, pH 4.8, 30°C), sample 500 mg, hydrolysis time 15 min.



**Fig. 10.** Diastatic activity of flour samples measured using the flow-injection analysis biosensor method: ○ = soft flour, Δ = hard flour, ◇ = medium hard flour. Hydrolysis conditions: acetate buffer solution (40 mL, 0.1M, pH 4.8, 30°C), sample 500 mg. Each point represents the average of at least two measurements.

### Determination of Damaged Starch

The FIA biosensor system was applied to the determination of damaged starch in flour. The results were compared with those obtained using conventional methods. The Approved Method (AACC 1995) is extremely complicated because samples are titrated in four steps. There have been several studies of the determination of starch damage in flour (Sandstedt and Mattern 1960, Donelson and Yamazaki 1962, Sullivan et al 1962). The methods described in these reports are also troublesome. In contrast, the FIA biosensor system for the assay of damaged starch content and diastatic activity in flour is rapid and simple. And the FIA biosensor system has the advantage of being less costly once the system is established.

The enzymatic hydrolysis of flour samples using  $\alpha$ -amylase was stopped by filtration. Because  $\alpha$ -amylase contains stabilizing sugars, only the value for  $\alpha$ -amylase was subtracted from the values of samples treated with  $\alpha$ -amylase to give values for the glucose and maltose of damaged starch in flour. Figure 7 shows the correlation of the results obtained using conventional methods and those of the FIA system. The levels of damaged starch as measured by the FIA biosensor system agreed excellently with those using the Approved Method ( $r = 0.99$ ) (AACC 1995). These results suggest that the FIA maltose sensor is an excellent system for the monitoring of damaged starch in wheat.

After hydrolyzing soft flour by enzyme pretreatment, the level of damaged starch was monitored using the FIA biosensor system over 60 min. As shown in Fig. 8, the amount of damaged starch in soft flours increased with time. The increase for lower grade soft flour was remarkable. The difference in flour quality was evident from these results.

### Determination of Diastatic Activity

Because diastatic activity is used as an index of damaged starch content, comparative measurements were also made of the diastatic activity of flour. The maltose values obtained using the FIA biosensor system agreed with measurements made using the Approved Method (AACC 1995). The correlation coefficients between the results obtained using the FIA biosensor system and those of the conventional method was  $r = 0.97$  (Fig. 9). Excellent agreement was observed between the results of the two methods over a wide range of maltose concentrations.

After hydrolyzing soft flour by enzyme pretreatment, the maltose concentration was monitored over 60 min using the FIA biosensor system and three kinds of flour. As shown in Fig. 10, the maltose concentration increased with time. The highest value of maltose was observed in medium hard flour, and the value for soft flour was lower than those of the hard flours. These results illustrate the relationship between flour quality and diastatic activity. From our results, the FIA biosensor system could be applied to the measurement of damaged starch and diastatic activity in flour.

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