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Identification of botanical origins of starches using a glucose biosensor and amyloglucosidase

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Abstract

New sensory techniques for the identification of botanical origins of starches are developed with two point data of enzymatic glucose production from a starch. The used starches are from rice, corn, potato, sweet potato, wheat, banana, chestnut, and tapioca. The amylose contents of all the starches are in the range of 20–30%. The two point glucose production are measured with free amyloglucosidase and a glucose biosensor in 0.6 M phosphate buffer of pH 6.5 at 37 °C after heat treatment of starch suspension at 55 and 60 °C for 1 h.

These methods are using the principal component analysis and the direct plot on the new coordinate frame developed with new two variables, $X = (R_{60} - R_{55})$ and $Y = (R_{60}/R_{55})$, calculated from glucose product ratio (R), where R is the amperometric response ratio of glucose produced by enzymatic starch hydrolysis to that from a 50 μM glucose standard.

Keywords: Botanical origin of starch; Principal component analysis; Glucose biosensor; Enzymatic hydrolysis; Amyloglucosidase

1. Introduction

The effective identification of the botanical origins of starches is an important problem to control the quality in the food processing industries that use starches. Identification usually depends on the microscopic shape character of the granules because their chemical compositions are very similar [1,2]. Even though the microscopic characteristics are distinctive in the identification, it requires an expert trainee in the field. Thus, a new method for the identification using an objective observation like a sensory response signal would be more convenient.

Starch is a major carbohydrate component isolated from green plants. This carbohydrate component consists of a mixture of amylopectin and amylose. Amylose is a linear polymer made of α-D-glucopyranose units linked through α (1–4) linkage; amylopectin is a large branched molecule with side chains grafted to the linear α (1–4) polymer by a single α (1–6) junction. The starch granules from different botanical sources vary in size, shape, and content of amylose and amylopectin which affects their chemical and physical properties [3]. The granules are semi-crystalline materials varying the crystallinity and structure depending on the origins. Thus, the general properties of a starch such as gelatinization, solubilization, swelling, granule size, chemical constitution, crystal type, and enzymatic degradation differ as a result of the difference on the origins.

Enzyme digestibility of starches is dependent not only on the starch origins but also on the character of the enzyme that catalyzes the hydrolysis of glucopyranose linkages. Enzymatic hydrolysis generally occurs predominantly in a swelled amorphous region rather than in the crystallites. The crystallites are also disrupted and gelatinized in water by heat

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treatment. Most starch analyses using enzymatic degradation have focused on the determination of the degree of gelatinization as a function of temperature and measure the total starch in a granule using the total glucose released after complete hydrolysis [4–6].

There are several enzymes that hydrolyze starches. Each of these yields a different number of products depending on their mode of action. Among them, amyloglucosidase is an exo-enzyme that liberates glucose units from a non-reducing end of starch, amylose, amylopectin, and amylodextrin chains [7,8]. Since this enzymatic reaction occurs only at the end of the molecular chains exposed on the outer surface of the starch particle, the reaction for the glucose production is modulated by the physical and chemical structure of starch. Consequently, careful examination of this enzymatic hydrolysis could yield comprehensive information about the botanical source of starches.

In this paper, we report a new attempt to identify the botanical origin of starch using a glucose biosensor based on glucose oxidase and free amyloglucosidase.

2. Experimental

2.1. Starch samples

Rice (Aggibare), potato (Irish Cobbler), sweet potato (Ulmi), corn, wheat, banana, chestnut, and tapioca were obtained in a rural market in Korea.

2.2. Chemicals and enzymes

Glucose oxidase (GOD, EC 1.1.3.4, 187 U/mg from *Aspergillus niger*), amyloglucosidase (EC 3.2.1.3, 22.5 U/mg from *Rhizopus* mold), glucose and glutaraldehyde were purchased from Sigma Co., and sodium hydrogen phosphate and sodium dihydrogen phosphate were obtained from Aldrich. Polyvinyl alcohol (PVA, 88% hydrolyzed, MW 20,000) was from Janssen Chimica (Belgium). All solutions were prepared using nanopure water.

2.3. Starch preparation

After the seed coat of each sample was peeled off, the starch was separated by the alkali steeping method [9,10] with minor modification. Each sample was ground in three volumes of 0.2% NaOH solution with a Waring blender for 3 min, and passed sequentially through 70 and 100 mesh sieves. The suspension was allowed to settle for 5 h at 4 °C and decanted. The sediment was mixed with three volumes of 0.2% NaOH and allowed to settle at 4 °C and decanted. This process was repeated until protein was not detected by the biuret reaction. The final sediment was rinsed with deionized water until it was at neutral pH. The recovered starch was air-dried at room temperature and ground to pass through a 100 mesh sieve. The starches were equilibrated humidity at 20 °C and stored at 4 °C. The amylose contents in the starches used here were in the range of 20–30%, measured by the bound iodine [11].

2.4. Glucose sensor

Glucose oxidase (3 units) was immobilized with PVA on 3 mm diameter pieces of tracing paper. Aliquots of 10 ml of 20 wt% PVA were mixed with 0.5 ml of glutaraldehyde (25%), and reacted at 60 °C for 4 h to prepare partially crosslinked PVA. The PVA solution (8 μl) was added on the tracing membrane and mixed together with 3 units of GOD. After drying at room temperature, the GOD membrane was exposed to glutaraldehyde vapor for 60 min. This GOD membrane was attached to a Pt electrode. Glucose was measured amperometrically at the potential of +700 mV versus an Ag/AgCl reference electrode using a hand-made potentiostat.

2.5. Experimental procedure

Starch (25 mg) was suspended in 30 ml of deionized water. The suspension was added to a three-mouth round bottom flask with a reflux condenser in a water bath, and stirred with a magnetic bar for 1 h at a constant temperature at 5 °C intervals from 50 to 65 °C. This suspension (1 ml) and 2 units of amyloglucosidase were mixed in 2 ml tube and incubated it for 5 min at 37 °C. Then, 100 μl of this incubated solution was added to 10 ml of 0.6 M phosphate buffer solution (pH 6.5) in an electrochemical cell equipped with a glucose biosensor and an Ag/AgCl reference to measure the glucose liberated from the starch. The electrochemical cell was maintained constantly at 37 °C throughout all experiments. Because the response of the glucose biosensor might be changed in different starch, the biosensor was calibrated after each measurement of a sample starch. The calibration was accomplished with the standard glucose solution (50 μM). The dimensionless normalized response is represented as the glucose product ratio (R) calculated as follows: $R = I_p/I_s$ where I_p is an amperometric response of glucose produced from the sample starch and I_s is the response from the standard glucose (50 μM).

3. Results and discussion

3.1. Characteristics of glucose biosensor

The glucose biosensor was characterized using standard glucose solution to obtain the calibration curve. The biosensor response to glucose was linear from 5 μM to 1.5 mM, but the glucose concentration range used in this experiment was below 100 μM. The linear equation up to 300 μM was $A = 0.475C - 0.421$ with $r = 0.998$ where A is a measured current (nA) and C is a concentration of glucose (μM). The sensor signal was very stable and reached rapidly to a steady state within one minute. However, the responses of a sensor