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Determination of the botanical origin of starch using direct potentiometry and PCA

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ABSTRACT

A new approach for the determination of the botanical origin of starch is presented based on the formation of starch–triiodide complexes. The starch samples were extracted from wheat (Srpanjka), potato, maize, rye (Barun), barley (Conduct), rice, tapioca and a commercial modified starch. The amylose/amylopectin ratios of starches, among various other properties, differ between starches of different botanical origins. Triiodide ions bind characteristically to the amylose and amylopectin of the starch depending on the starch's origin. The new technique includes direct potentiometric measurements of the response of free triiodide ions in starch–triiodide solutions where the data is analysed by principal component analysis (PCA). PCA gave graphical results for statistical differentiation between starches of different botanical origins.

1. Introduction

Starch is a semicrystalline biopolymer and is stored in various plant locations, such as in cereal grains, roots, tubers, stempiths, leaves, seed, fruit and pollen. Starch, as a low-cost polysaccharide, is the most widely used thickening and gelling agent in the food industry.

Starch granules in plant storage tissues can vary in shape, size and composition. Starch granules in higher plants contain two principal types of polysaccharides: amylose and amylopectin. Amylose is a predominantly linear polymer that contains 99% α (1–4) and 1.0% α (1–6) linkages. Amylopectin contains 95% α (1–4) and 5.0% α (1–6) linkages (Biliaderis, 1998; Buléon, Colonna, Planchot, & Ball, 1998). The starch granules from different botanical sources also vary in size, shape, and content of amylose and amylopectin, which affects their chemical and physical properties (Gebre-Mariam & Schmidt, 1996; Smith, 2001).

The typical origin analysis of starch includes indirect techniques that measure the differences in the physical and chemical properties of the starches (Chatel, Voirin, Luciani, & Artaud, 1996). The tools often used to identify starch origin include optical and electronic microscopy, enzymology, rheology, chromatography, NMR, X-ray diffraction, viscometer profiles and FTIR spectroscopy (Bernetti, Kochan, Trost, & Young, 1990).

Recently, some authors (Chough, 2006) have reported using a glucose biosensor based on glucose oxidase and free amyloglucosidase to identify the botanical origin of starch. The identification was based on variations in the shapes and sizes of granules among starches of different origins, and when combined with enzymatic hydrolysis yielded comprehensive information about the botanical source of starches.

The interaction of starch and iodine results in the formation of complexes (Teitelbaum, Ruby, & Marks, 1978; Teitelbaum, Ruby, & Marks, 1980) with characteristic colors. The color of the starch–triiodide complex has been shown to vary with starch chain length (Bailey & Whelan, 1961). Because starch assumes a helical structure, iodine molecules occupy the central cavity of the helical molecule in the complex (Hinrichs et al., 1987). Many physicochemical properties of starch, such as its iodine binding capacity and degree of polymerization (DP), depend on the starch's botanical origin.

Based on our previous work (Sakač et al., 2011) in this paper, we report our attempt to identify the botanical origin of starch using a direct potentiometric technique based on starch–triiodide complex formation and statistical analysis using PCA.

2. Experimental

2.1. Reagents and solutions

Starch samples were isolated from wheat (Srpanjka), potato, maize, rye (Barun), barley (Conduct), rice, and tapioca, which were

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obtained at the local market store in Croatia, the commercial model starch was obtained from Kemika (Croatia).

Potassium triiodide solution was prepared using iodine (I_2) and potassium iodide (KI), both purchased from Sigma–Aldrich (Germany). Glacial acetic acid (CH_3COOH) was purchased from Panreac (Spain), and sodium acetate trihydrate ($CH_3COONa{\cdot}3H_2O$) was purchased from J.T. Baker (The Netherlands).

2.1.1. Starch sample preparation

The samples' seed coats were peeled off, and an alkali steeping method (Baik, Kim, Cheon, Ha, & Kim, 1997; Kim, Wiesenborn, & Grant, 1997) was used to separate the starches. Each sample was ground in three volumes of 0.2% NaOH solution with a Philips blender for 5 min and passed sequentially through 70 and 100 mesh sieves. The suspension was allowed to settle for 5 h at 4 °C and was then decanted. The sediment was mixed with three volumes of 0.2% NaOH, allowed to settle at 4 °C and again 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 with respect to humidity at 20 °C and stored at 4 °C.

2.1.2. Starch solution preparation

The starch solution was prepared in a concentration range of 1–5 g/L by the addition of 0.1–0.5 g, respectively, of dried starch to 25 mL of 0.1 M acetate buffer solution (pH 6.0) in a closed 100 mL volumetric flask. After the solution was heated to the boiling point and stirred for 10 min, it was allowed to cool to room temperature and was then diluted to 100 mL with deionized water in a volumetric flask. These solutions were then used for further investigations. Starch solutions were prepared fresh each day to avoid microbial degradation. Solutions with starch concentrations greater than 5 g/L are difficult to manipulate.

2.1.3. Triiodide solution preparation

The potassium triiodide solution was prepared by the dissolution of solid iodine (100 μM) in 500 mL of 0.05 M potassium iodide solution. Because iodine exhibits low solubility in water but a high solubility in potassium iodide solution, solid iodine and iodide were first added to a flask that contained a small volume of water. After the iodine/iodide solution was intensively stirred, the iodine was completely dissolved, and the volumetric flask was filled to the mark.

2.2. Apparatus

Direct potentiometric response measurements were performed on a Metrohm 780 pH meter, 728 Stirrer, titration vessel, 765 Dosimat (all from Metrohm, Switzerland) with custom homemade software and a platinum redox electrode IJ64 (Ionode, Australia). A silver/silver(I) chloride electrode (Metrohm, Switzerland) served as the reference electrode. Statistica (StatSoft, USA) software was used for PCA analysis. Before computation the data were standardized (mean centered and divided by the relevant standard deviations).

2.3. Procedure

Eight milliliters of triiodide solution was transferred to the titration vessel. The responses of the platinum redox electrode were measured by accurate, incremental additions of the prepared starch solutions. The solutions were continuously stirred during the addition of starch and during the measurements.

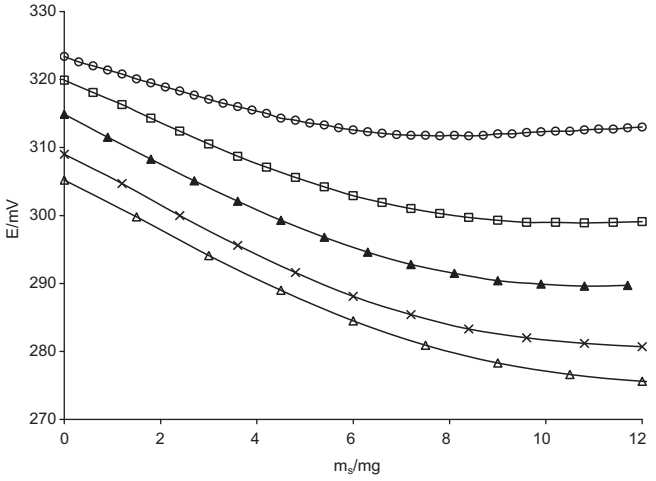

Fig. 1. Platinum redox sensor response for different starch concentrations (model starch, 1 g/L (○), 2 g/L (□), 3 g/L (▲), 4 g/L (×), 5 g/L (△)). Here and in later figure mass of added starch, m_s, is calculated from the added starch solution volumes. Some curves presented are displaced vertically for clarity.

3. Results and discussion

3.1. Sensor response mechanism

Potassium triiodide, I_3^-, is obtained by the dissolution of iodine in potassium iodide solution. A large excess of iodide is necessary to keep the triodide concentration constant because of the low equilibrium constant for triiodide formation.

For the reaction

$$[I_3^-] + 2e^- \rightleftharpoons 3[I^-] \quad (1)$$

the corresponding redox potential can be described by the Nernst equation:

$$E = E^\circ + \frac{RT}{2F} \ln \frac{[I_3^-]}{[I^-]^3} = E^\circ + S \log \frac{[I_3^-]}{[I^-]^3} \quad (2)$$

Eq. (2) represents the response of the redox sensor toward the iodide/triiodide couple.

The formation of the complex between starch and triiodide can be described by the following equation:

$$[KI_3]_0 + \text{starch} \rightleftharpoons [KI_3\text{–starch}] + [KI_3]_f \quad (3)$$

Because a large excess of iodide is present, its concentration is assumed to be constant.

After the addition of a known amount of the starch solution to the iodide–triiodide solution, the following equilibrium is established:

$$[I_3^-]_f = [I_3^-]_0 - [I_3^-]_b \quad (4)$$

where $[I_3^-]_f$ is the free triiodide concentration, $[I_3^-]_0$ is the initial triiodide concentration, and $[I_3^-]_b$ is the concentration of triiodide bound to starch.

The difference between the initial and free triiodide-ion concentration directly depends on the amount of starch added. The redox potential of the investigated solution changes when the starch sample is incrementally added to the triiodide solution because of the changes in the free triiodide concentration (Eq. (4)). This situation is described in Eq. (5), which is obtained by insertion of Eq. (4) into Eq. (2)

$$E = E^\circ + S \log \frac{[I_3^-]_0 - [I_3^-]_b}{[I^-]^3} = E^\circ + S \log \frac{[I_3^-]_f}{[I^-]^3} \quad (5)$$

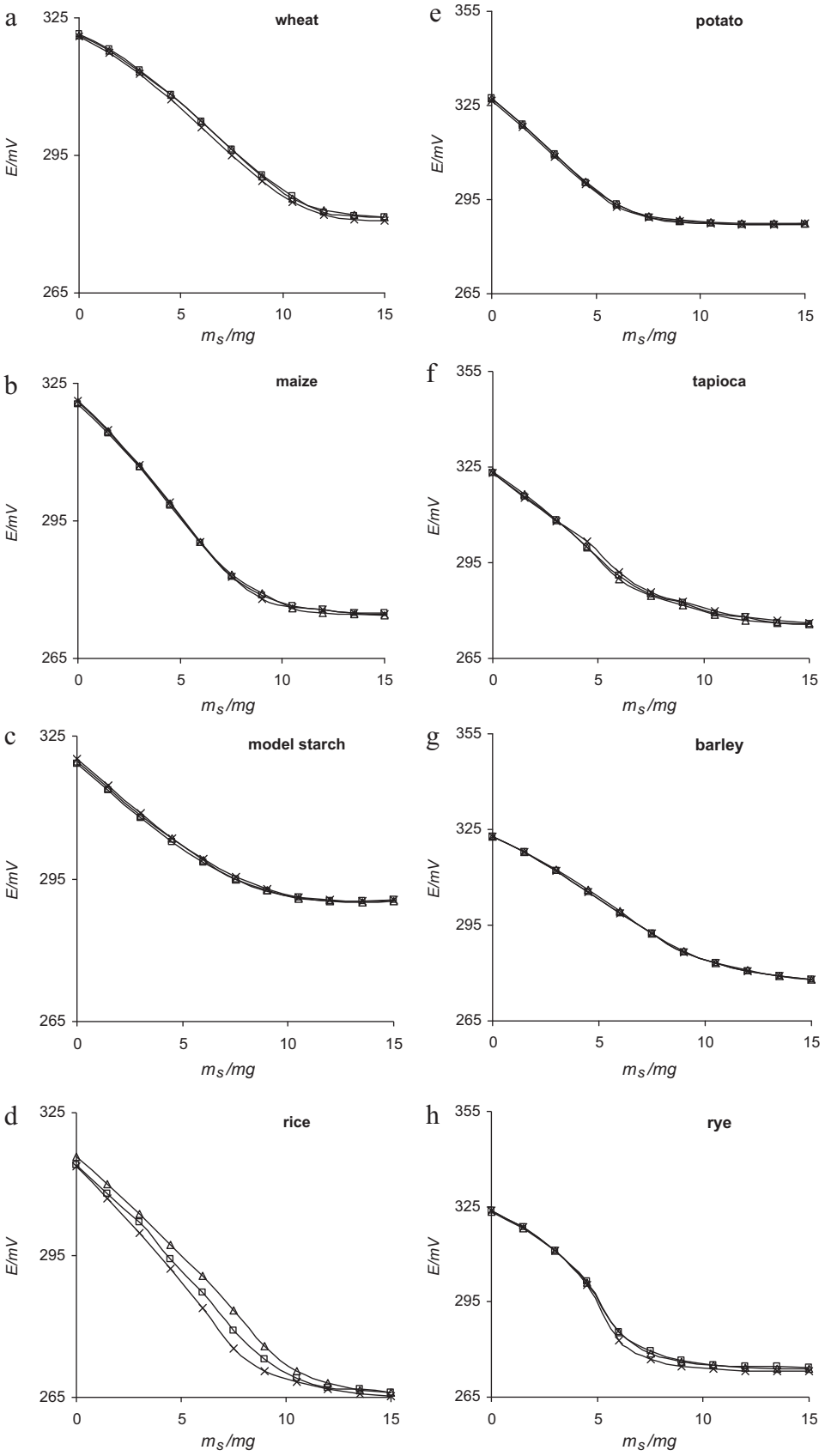

Fig. 2. Platinum redox sensor response curves and their reproducibility for starch samples (5 g/L) from different botanical origins.

Thus, the redox potential change is directly dependent on free triiodide concentration, $[I_3^-]_f$.

3.2. Starch–triiodide complex response

The redox potential change was measured after incremental additions of the prepared starch samples to the potassium triiodide solution. A stable sensor response was obtained within a few seconds because of the high ionic strength (I = 0.05 M) of the investigated solutions. The results for the responses of the model starch over the concentration range 1–5 g/L are shown in Fig. 1 (some curves presented are displaced vertically for clarity). For further investigations, a starch concentration of 5 g/L was chosen because the starch solution with this concentration exhibited the greatest change in potential as the amount of added starch was increased. This characteristic is used for further analysis.

After an optimal starch concentration of 5 g/L was chosen, the solutions of wheat, potato, maize, rye, barley, rice and tapioca starch were measured. The measurements for each starch sample were performed in triplicate. According to Eq. (5), the platinum redox sensor showed a decrease in the response potential for each starch sample, but the curve shapes and slopes differed between starch samples. The individual response curves for the starch samples of different botanical origins are given in Fig. 2. The results exhibited reproducible responses, except that of rice starch, which exhibited small fluctuations in response. Different starch samples possess different chemical and physical properties, and the amylase/amylopectin ratio varies depending on the starch's botanical origin. The amylopectin–triiodide complex is less stable than the amylose–triiodide complex but is still a significant factor in the total amount of starch–triiodide complex formed. We conclude that starches of various botanical origins exhibit different redox potential changes.

The response curve profile characteristics reflect the differences in the origins of the starches and provide sufficient information to allow the determination of the botanical origin for each starch type.

3.3. Principal component analysis (PCA)

Principal component analysis (PCA) is a multivariate analysis technique for transforming the original measurement variables into new variables called principal components (PCs). In general, the slopes and curve shapes presented in Fig. 2 show differences between starch samples for the same amount of added starch. To determine differences and starch origins, PCA was performed using all data sets provided by the measurements. PCA on the basis of the correlation matrix of the data provides the results given in Fig. 3 for the scores.

The Kaiser test revealed only two significant PCs (eigenvalue >1 rule, Kaiser criterion; Vandeginste et al., 1998, chap. 30). Since the response slope values are characteristically for each individual starch sample, these data were used for PCA calculation. If the first two PCs account for a substantial proportion of the total variation, as occurs with the starch data, then we can use the first two PC plots to visually identify possible clusters. Fig. 3 displays the result of the application of PCA. The horizontal and vertical axes of the score plot represent the first two PCs, which account for (70.64%) and (21.20%), respectively, of the variance in the data. As evident in the PC score plot, the different starch samples can be divided into eight groups, which would suggest that they have different origins. Furthermore, in Fig. 3, the starch sample from rice as well as those from the model starch and rye are clearly distinguished from the five other starches (tapioca, maize, wheat, potato and barley). The results of the PCA demonstrate that eight variables are sufficient for the discrimination of the different types of starches. The results of different data sets did not overlap at any positions, which indicates

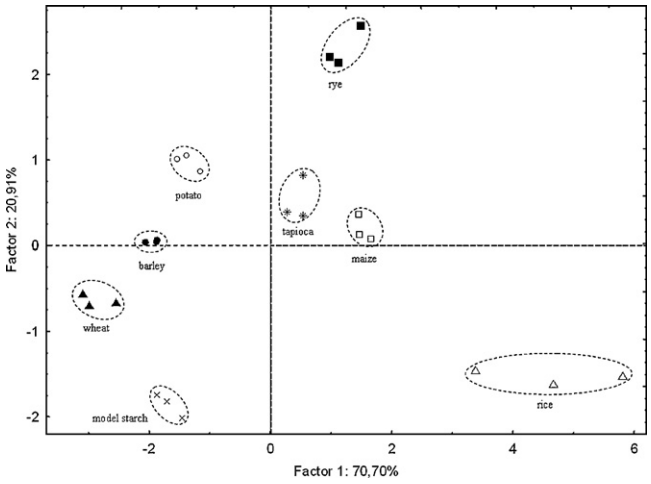

Fig. 3. The result of applying PCA for botanical starch origin determination using results obtained by direct potentiometric measurement of starch from various botanical starch sources.

the data are sufficient to distinguish different starch samples. Thus, PCA is an effective method for discriminating the different starch samples into groups based on their botanical origin.

4. Conclusions

We have developed a rapid and accurate method for the determination of a starch's origin based on direct potentiometric measurements of starch–triiodide complexes and the use of principal components data analysis (PCA). The platinum redox electrode was used to measure decreases in the free triiodide ion after its complexation into a starch–triiodide complex. The proposed method is more convenient, simpler, easier, less expensive and more objective for the determination of botanical starch origin than the usual microscopic methods.

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References

Baik, M. Y., Kim, K. J., Cheon, K. C., Ha, Y. C., & Kim, W. S. (1997). Recrystallization kinetics and glass transition of rice starch gel system. *Journal of Agricultural and Food Chemistry*, *45*, 4242–4248.

Bailey, J. M., & Whelan, W. J. (1961). Physical properties of starch. *Journal of Biological Chemistry*, *236*, 969–973.

Bernetti, R., Kochan, D. A., Trost, V. W., & Young, S. N. (1990). Modern methods of analysis of food starches. *Cereal Foods World*, *35*, 1100–1105.

Biliaderis, C. G. (1998). Structures and phase transition of polymers. In R. H. Walter (Ed.), *Polysaccharide association structures in foods* (pp. 57–168). New York: Marcel Dekker.

Buléon, A., Colonna, P., Planchot, V., & Ball, S. (1998). Starch granules: Structure and biosynthesis. *International Journal of Biological Macromolecules*, *23*, 85–112.

Chatel, S., Voirin, A., Luciani, A., & Artaud, J. (1996). Starch identification and determination in sweetened fruit preparations. *Journal of Agricultural and Food Chemistry*, *44*, 502–506.

Chough, S. H. (2006). Identification of botanical origins of starches using a glucose biosensor and amyloglucosidase. *Sensors and Actuators B-Chemical*, *114*, 573–577.

Gebre-Mariam, T., & Schmidt, P. C. (1996). Isolation and physico-chemical properties of enset starch. *Starch/Starke*, *48*, 208–214.

Hinrichs, W., Büttner, G., Steifa, M., Betzel, C. H., Zabel, V., Pfannemüller, B., & Saenger, W. (1987). An amylose antiparallel double helix at atomic resolution. *Science*, *238*, 205–208.

Kim, Y. S., Wiesenborn, D. P., & Grant, L. A. (1997). Pasting and thermal properties of potato and bean starches. *Starch/Starke*, *49*, 97–102.

Sakač, N., Sak-Bosnar, M., Horvat, M., Madunić-Čačić, D., Szechenyi, A., & Kovacs, B. (2011). A new potentiometric sensor for the determination of α-amylase activity. *Talanta*, *83*, 1606–1612.

Smith, A. M. (2001). The biosynthesis of the starch granule. *Biomacromolecules*, *2*, 335–341.

Teitelbaum, R. C., Ruby, S. L., & Marks, T. J. (1978). On the structure of starch–iodine. *Journal of the American Chemical Society*, *100*, 3215–3217.

Teitelbaum, R. C., Ruby, S. L., & Marks, T. J. (1980). A resonance Raman/iodine Moessbauer investigation of the starch–iodine structure. Aqueous solution and iodine vapor preparations. *Journal of the American Chemical Society*, *102*, 3322–3328.

Vandeginste, B. G. M., Massart, D. L., Buydens, L. M. C., De Jong, S., Lewi, P. J., & Smeyers-Verbeke, J. (1998). *Handbook of chemometrics and qualimetrics, part B* Amsterdam: Elsevier.