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Study on sugar profile of rice during ageing by capillary electrophoresis with electrochemical detection

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Abstract

A simple, reliable and reproducible method based on capillary electrophoresis with electrochemical detection, for the determination of sucrose, maltose, glucose and fructose in rice flour was described in this work. Operated in a wall-jet configuration, a 140 μm diameter copper-disk electrode was used as working electrode, which exhibits good response at +650 mV (vs. SCE) for sucrose, maltose, glucose and fructose. Under the optimum conditions, four analytes in 50 mmol/l sodium hydroxide buffer were base-line separated within 15 min. The response was linear over two orders of magnitude, and the detection limit ($S/N = 3$) is 9×10^{-7} g/l, 1.4×10^{-6} g/l, 6×10^{-7} g/l and 1.3×10^{-7} g/l for sucrose, maltose, glucose and fructose, respectively. With this method, a sugar profile study of rice flour was conducted to determine changes produced during ageing. It is observed that there were decreases in the sucrose and maltose contents, and increases in glucose and fructose contents in rice during storage.

Keywords: Capillary electrophoresis; Sugar; Rice ageing

1. Introduction

Cultivated rice at present sustains two-thirds of the world's population. A small amount of the rice crop is used as ingredients in processed foods and as feed, but the bulk is consumed as cooked rice. This pattern of usage results in the need to store rice over varying periods. During storage, a number of physico-chemical and physiological changes occur, this is usually termed ageing. Although the mechanism of rice ageing is not fully understood, the changes of pasting properties, colour, flavour, and composition of rice can be observed (Chrastil, 1992), which affects milling, cooking and eating quality (Perdon, Marks, Siebenmorgen, & Reid, 1997). At present, researches into the cause of ageing have focused on the rice components, such as starch, protein, and lipid, and the interactions between them during storage (Chrastil, 1994). The activity of enzyme including α-amylase, β-amylase (Dhaliwal, Sekhon, & Nagi, 1991), peroxidase, proteases, lipases and lipoxygenase (Desikachar & Subrahmanyan, 1960) and the contents of free phenolic acids (Osawa, 1999) that exert a significant effect on property of cell wall have also been investigated for this purpose. As for the changes of sugar during storage, it is merely observed that there is a significant proportional increase in reducing sugars and a decrease in non-reducing sugar (Pushpamma & Reddy, 1979). So far, there is a few, if any, report on changes of sugar profile during ageing. In fact, study on changes of sugar profile is extremely helpful to understand the metabolism of starch, the activity of some kinds of enzyme, and even the mechanism of rice ageing.

In order to estimate the changes of sugar profile during rice ageing, it is necessary to develop a method that can be used to assay all the constituents of saccharides in rice, yet is dependable and convenient. At present, high-performance liquid chromatography (HPLC) (Cataldi, Margiotta, & Zambonin, 1998) and high-performance anion-exchange chromatography (Yu, Ding, Mou, Jandik, & Cheng, 2002) with pulsed-amperometric detection

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or HPLC with refractive index detection serves as a common technique for underivatized carbohydrate. For derivatized carbohydrates, HPLC with fluorescence and spectrophotometric (UV) detection (Rassi, 1996) or gas chromatography (GC) with mass spectrometry (Fox, Wunschel, Fox, & Stewart, 1998) was used. Capillary electrophoresis (CE) is becoming increasingly recognized as an important analytical separation technique because of its speed, efficiency, reproducibility, ultra-small sample volume, and little consumption of solvent. CE has been successfully applied to carbohydrate analysis (Rassi, 1995). However, carbohydrates are in general difficult to separate and detect due to their lack of readily ionizable functional groups and chromophores. Precolumn derivatization is the most widely used means, and most of the methods are based on reductive amination (Larsson, Sundberg, & Folestad, 2001). Although this method gives excellent results, it is generally time-consuming and may result in the cleavage of some important sugar residues. Direct detection with indirect UV (Soga & Serwe, 2000) or refractive index (Ivanov, Nazimov, Lobazov, & Popkovich, 2000) has been used with CE, but is limited by poor sensitivity and specificity. On the other hand, the carbohydrates can be oxidized at copper electrode (Luo & Baldwin, 1995), and can be detected directly without derivatization. Moreover, with electrochemical detection (ED), CE–ED offers high sensitivity and good selectivity for the determination of carbohydrate (Baldwin, 1999; Ye & Baldwin, 1994a, 1994b). So, in this work, we developed a simple and rapid method to determine four carbohydrates in rice flour by CE–ED, and the changes of sugar profile during rice ageing were studied by this method.

2. Experimental

2.1. Apparatus

The house-built CE–ED system (Ye & Baldwin, 1993) was employed in this work. A 30 kV high-voltage power supply (Shanghai Institute of Nuclear Research, China) provided a voltage between the ends of the capillary. The inlet end of the capillary was held at a positive potential and the outlet end was maintained at ground. A 60 cm length of 25 μm i.d. and 360 μm o.d. fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA) was used for the separation. A copper-disk electrode with 140 μm diameter was employed as the working electrodes as described previously (Ye & Baldwin, 1994a, 1994b). Before use, the surface of the working electrode was polished with emery sand paper, sonicated in deionized water, and then positioned carefully opposite the capillary outlet with the aid of a micropositioner (Shanghai Lianyi Instrument Factory). A three-electrode cell system consisting of a working electrode, a platinum auxiliary electrode and a SCE reference electrode was used in combination with a BAS LC-3D amperometric detector (Biochemical Scheme, West Lafayette, IN, USA). The electropherograms were recorded using a chart recorder (Shanghai Dahua Instrument factory, China).

2.2. Reagents and materials

Sucrose, maltose, glucose, and fructose were purchased from Shanghai Chemicals Reagent Co. (Shanghai, China). Other reagents were analytical reagent grade. All reagents were used without further purification. The samples of fresh and aged rice were obtained from a local granary (Wuxi, China). Stock solutions of four analytes (1.00×10^{-3} g/ml, each) were prepared in deionized water, and were diluted to desired concentration with the running buffer (sodium hydroxide running buffer with the concentrations from 25 to 100 mmol/l). Before use, all solutions were filtered through 0.22 μm nylon filters.

2.3. Sample preparation

Paddy samples stored for varying time were first shelled to brown rice by JLG4.5 rice hulling machine (Taizhou Liangyi Factory, Taizhou, China), then polished to grade A rice by JNMJ3 paddy pounder (Taizhou Liangyi Factory, Taizhou, China), finally milled to rice flour (40-mesh) by JFSD-100 pulverizer (Jiading Liangyou Instrument Co., Shanghai, China). Two grams of rice flour samples were weighed accurately, and extracted with 25 ml deionized water in an ultrasonic bath for 30 min. After centrifugation, extract solution was filtered through a 0.22 μm syringe filter. Next, 1.0 ml of this solution was diluted with 2.0 ml running buffer, then injected directly to capillary electrophoresis system electrokinetically.

3. Results and discussion

3.1. Effect of the potential applied to the working electrode

Because copper electrode can catalyze the oxidation of the carbohydrates in basic media, copper-disk electrode was used as working electrode for detection of sucrose, maltose, glucose and fructose. Hydrodynamic voltammetry (HDV) of sucrose, maltose, glucose and fructose at a copper-disk electrode were investigated to select the optimum potentials applied to working electrodes. The results are shown in Fig. 1. When applied potential at copper electrode exceeds +0.50 V, the oxidation currents of all four analytes increase rapidly. When the applied potential is greater than +0.70 V,

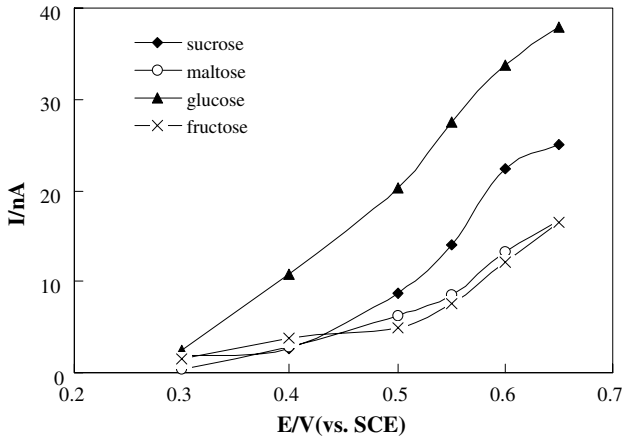

Fig. 1. Hydrodynamic voltammograms (HDVs) of sucrose, maltose, glucose and fructose. Fused-silica capillary: 25 m i.d. × 60 cm; concentration of eight analytes: 5.0×10^{-5} mg/l, each; working electrode: 140 μm diameter copper-disk electrode; running buffer: 50 mmol/l sodium hydroxide solution; separation voltage: 16 kV; injection time: 16 kV/6 s.

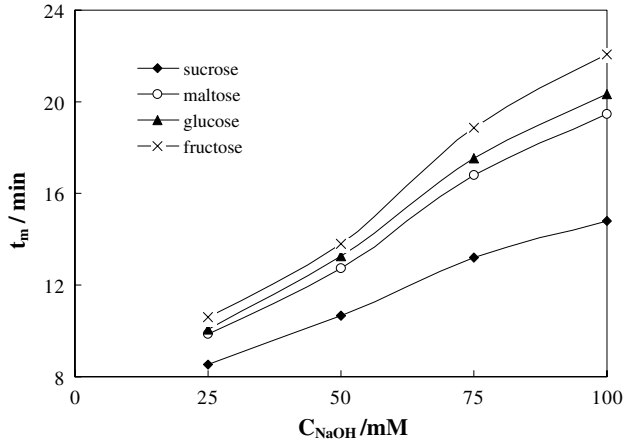

Fig. 2. Effect of running buffer concentration on migration time. Working electrode potential is 0.65 V (vs. SCE). Other conditions are the same as in Fig. 1.

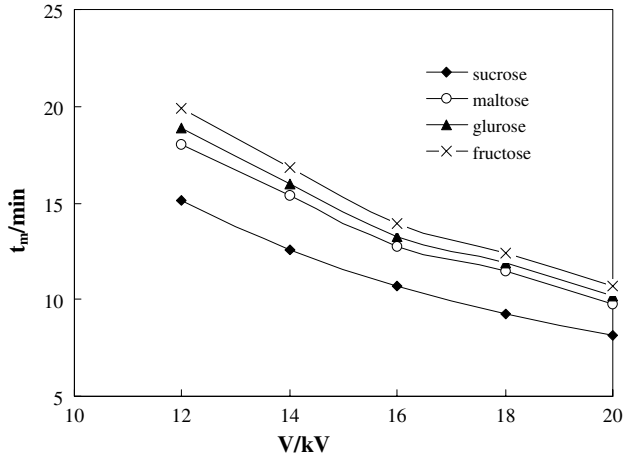

Fig. 3. Effect of separation voltage on migration time. Working potential is 0.65 V (vs. SCE). Other conditions are the same as in Fig. 1.

though the peak currents of all four analytes still increase, the baseline noise also increases very strongly. Therefore, the applied potential of +0.65 V (vs. SCE) was selected, where the background current is not too high and the S/N ratio is the highest.

3.2. Effect of the concentration of the running buffer

Sodium hydroxide solution was used as the running buffer because only in basic media, can carbohydrates separate completely, and has good response at copper electrode. The concentration of sodium hydroxide directly affects electro-osmotic flow (EOF) and the migration velocity of the analytes. As shown in Fig. 2,

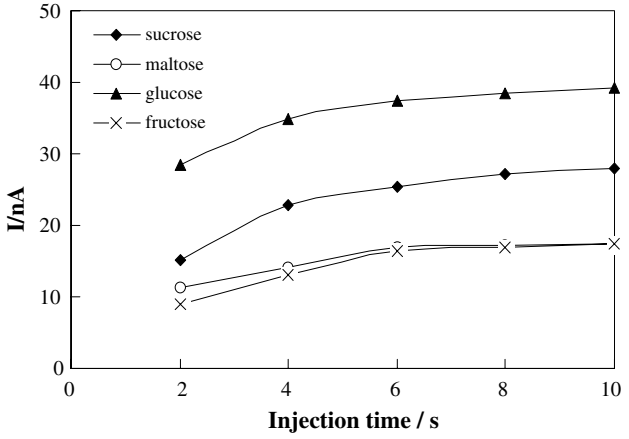

Fig. 4. Effect of injection time on peak current. Working potential is 0.65 V (vs. SCE). Other conditions are the same as in Fig. 1.

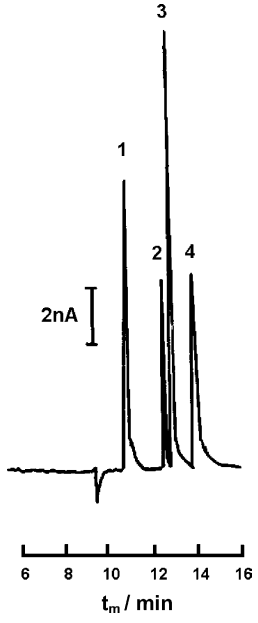

Fig. 5. The electropherogram of standard solution containing sucrose, maltose, glucose and fructose (2.00×10^{-5} g/ml, each). Peak identification: (1) sucrose, (2) maltose, (3) glucose, (4) fructose. Working potential is 0.65 V (vs. SCE). Other conditions are the same as in Fig. 1.

when the concentration of sodium hydroxide is 25 mmol/l, neither solvent peak and sucrose, nor maltose and glucose can be separated. When the concentration is greater than 50 mmol/l, all analytes can be baseline separated, but the migration time of the analytes also increases due to reduced EOF. In order to obtain satisfactory separation in relative short analysis time, 50 mmol/l sodium hydroxide was selected as the running buffer.

3.3. Effect of separation voltage and injection time

For a given capillary length, the separation voltage determines the electric field strength, which affects both the velocity of EOF and the migration velocity of the analytes, which in turn determine the migration time of the analytes. Fig. 3 indicates the influence of separation voltage on migration time in the selected running buffer. As expected, higher separation voltage gives shorter migration time for the analytes. However when the separation voltage exceeds 16 kV, baseline separation of maltose and glucose cannot be achieved. Besides, with the separation voltage increase, baseline noise becomes larger. In order to obtain high separation efficiency and short analysis time, 16 kV was the optimum selected separation voltages.

Injection time influences sensitivity and resolution. As injection time is long, sensitivity is high, but peak may broaden. Therefore, it is important to choose appropriate injection time. Fig. 4 shows the effect of injection time on peak currents of the analytes. As we can see, from 2 to 6 s, peak currents increase accordingly; as the injection time becomes greater than 6 s, peak currents becomes almost constant, while peak broadening becomes severe. 6 s at 16 kV was selected as optimum injection time.

Fig. 5 shows the electropherogram of a standard solution of the four analytes obtained under the selected optimum conditions; all analytes were fully separated within 15 min.

Table 1
The regression equations and detection limits[a]

Compound	Regression equation[b]	Correlation coefficient	Linear range (g/ml)	Detection limit (10^{-6} g/l)
Sucrose	$y = 5.03 \times 10^5 x - 0.51$	0.9996	2×10^{-6}–5×10^{-4}	0.9
Maltose	$y = 3.30 \times 10^5 x + 0.56$	0.9998	2×10^{-6}–5×10^{-4}	1.4
Glucose	$y = 7.48 \times 10^5 x + 0.32$	0.9994	1×10^{-6}–5×10^{-4}	0.6
Fructose	$y = 3.29 \times 10^5 x + 0.07$	0.9981	2×10^{-6}–5×10^{-4}	1.3

[a] CE–ED condition are the same as Fig. 5.
[b] In the regression equation, the x value is the concentration of analytes (g/ml), the y value is the peak current (mA).

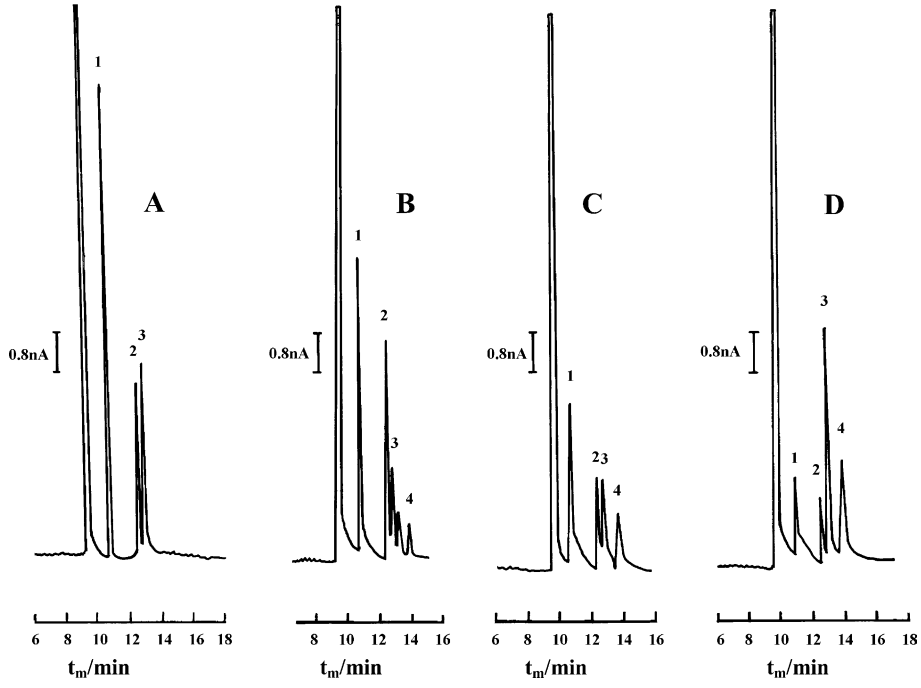

Fig. 6. The electropherograms of rice flour samples stored for varying periods: 6 months (A), 30 months (B), 54 months (C) and 78 months (D). Peak identifications and determination conditions are the same as in Fig. 5.

3.4. Reproducibility, linearity, detection limit of all analytes

The reproducibility of responses is estimated by making repetitive injections of a standard mixture solution (5.0×10^{-5} g/ml for each analyte) under the selected optimum conditions. The relative standard derivation (RSD) of the peak current is 3.9%, 1.9%, 1.7% and 2.1% for sucrose, maltose, glucose and fructose, respectively ($n = 7$).

To determinate the linearity of all analytes, a series of mixed standard solutions of four analytes, from 5.0×10^{-7} g/ml to 5.0×10^{-4} g/l, were tested. The detection limit was evaluated on the basis a signal-to-noise ratio of 3. The data of regression analysis and detection limit were summarized in Table 1.

Table 2
Assay results for rice flour samples ($n = 3$)[a]

Storage time of paddy (months)	Ingredients	Found (mg/g)	RSD (%)
6	Sucrose	0.750	2.9
	Maltose	0.401	3.0
	Glucose	0.192	3.7
	Fructose	N.F.	
	Sum	1.343	
30	Sucrose	0.488	3.5
	Maltose	0.488	3.8
	Glucose	0.094	2.0
	Fructose	0.079	4.1
	Sum	1.149	
54	Sucrose	0.361	3.2
	Maltose	0.227	3.3
	Glucose	0.098	2.8
	Fructose	0.146	3.9
	Sum	0.832	
78	Sucrose	0.176	3.7
	Maltose	0.163	2.8
	Glucose	0.249	3.3
	Fructose	0.246	3.7
	Sum	0.834	

[a] CE–ED condition are the same as Fig. 4.

3.5. Application and recovery

Determination of sucrose, maltose, glucose and fructose was achieved under the selected optimum conditions. Typical electropherograms of samples were shown in Fig. 6. By adding of standards, sucrose (peak 1), maltose (peak 2), glucose (peak 3) and fructose (peak 4) in rice flour samples can be determined. The assay results are listed in Table 2. The recovery and reproducibility experiments under the optimum conditions were also conducted to evaluate the precision and accuracy of the method. Recovery was determined by standard addition method, and the results are listed in Table 3. The above assay results indicate that this method is accurate, sensitive and reproducible, providing a useful quantitative method for the analyses of actual samples.

3.6. The changes of sugar profiles during ageing

The changes of sugar profile during rice ageing were shown in Fig. 7. We can see that the content of sucrose decreases during storage of rice, which accords with Pushpamma and Reddy's experiment that there is a decrease in non-reducing sugars. Though, increases of fructose and glucose occurred, an increase in reducing sugars cannot be observed in this experiment, owing to decrease of the content of maltose. We also notice that there is decrease in disaccharide and increase in

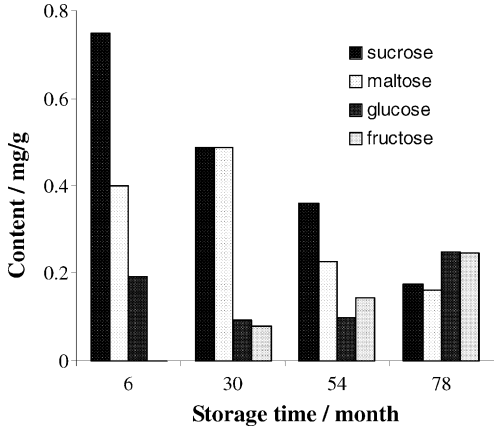

Fig. 7. The changes of sucrose, maltose, glucose and fructose during rice ageing.

Table 3
Results of recovery of this method with paddy sample (stored for 78 months) ($n = 3$)

Ingredient	Original amount (g/ml)	Added amount (g/ml)	Found (g/ml)	Recovery (%)	RSD (%)
Sucrose	4.70×10^{-6}	5.00×10^{-6}	10.0×10^{-6}	103.0	2.5
Maltose	4.36×10^{-6}	5.00×10^{-6}	9.26×10^{-6}	98.9	3.4
Glucose	6.63×10^{-6}	5.00×10^{-6}	12.1×10^{-6}	104.1	3.4
Fructose	7.05×10^{-6}	5.00×10^{-6}	11.6×10^{-6}	96.2	3.8

monosaccharides, which indicates that hydrolysis of disaccharides have continued during ageing. On the contrary, the gross of monosaccharides and disaccharides have downtrends shown in Table 2, it seems that these molecules, in reverse, bond together to form macromolecules. Of course, it needs further researches to confirm the presumption above-mentioned.

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