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Short communication

Analysis of enzymatically glucosylated flavonoids by capillary electrophoresis

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Abstract

HPCE with UV detection was applied to the analyses of enzymatically glucosylated flavonoids, which are used as natural food additives in Japan. Four items, which have flavonol or flavanone as aglycone, were analyzed. Each of these items is a mixture of glycosides with various lengths of maltooligosaccharide chain. On capillary zone electrophoresis with an untreated fused-silica capillary at alkaline pH, glycosides with longer sugar chains migrated more rapidly. Flavonol glycosides with 1–13 glucose units were distinguished with the borate buffer (pH 10.0). Flavanone glycosides needed higher pH values for good separation than flavonol glycosides

Keywords: Buffer composition; Flavonoids; Aglycones; Glycosides

1. Introduction

Flavonoids from natural sources have various chemical and biological activities, and their use as natural food additives is expected. However, their use is limited because of their poor solubility in water. Enzymatically modified (EM) flavonoids are water-soluble modifications, which are manufactured by transglycosylation with cyclodextrin glucanotransferase (CGTase) [1–3]. Several items are authorized for use as natural food additives in Japan. Each of them is a mixture of glycosides with the same aglycone and various lengths of maltooligosaccharide chain. Structures and names of the constituents are depicted in Fig. 1. The sizes and the respective contents of these glycosides should be different, depending on the manufacturing process. To evaluate the quality of individual item, its chemical composition must be clarified. We reported the analyses of EM quercetin glycosides by high-performance liquid chromatography (HPLC), but some constituents could not be separated completely [4].

Applications of high-performance capillary electrophoresis (HPCE) to the separation of flavonoid glycosides were reported by several authors. Some of those reports dealt with glycosides with the same aglycone and different sugar moieties [5–7]. HPCE of derivatized maltooligosaccharides was also studied [8,9]. In both cases, fairly good separations were achieved by capillary zone electrophoresis (CZE). Consequently, it is expected that the constituents of EM flavonoids, which have flavonoid aglycones and maltooligosaccharide chains, are also separated by CZE. In this work, therefore, four items, i.e., EM rutin, EM isoquercitrin, EM hesperidin and EM naringin (Fig. 1), were analyzed by CZE with UV detection. Constituents were resolved by CZE with an untreated fused-silica capillary at

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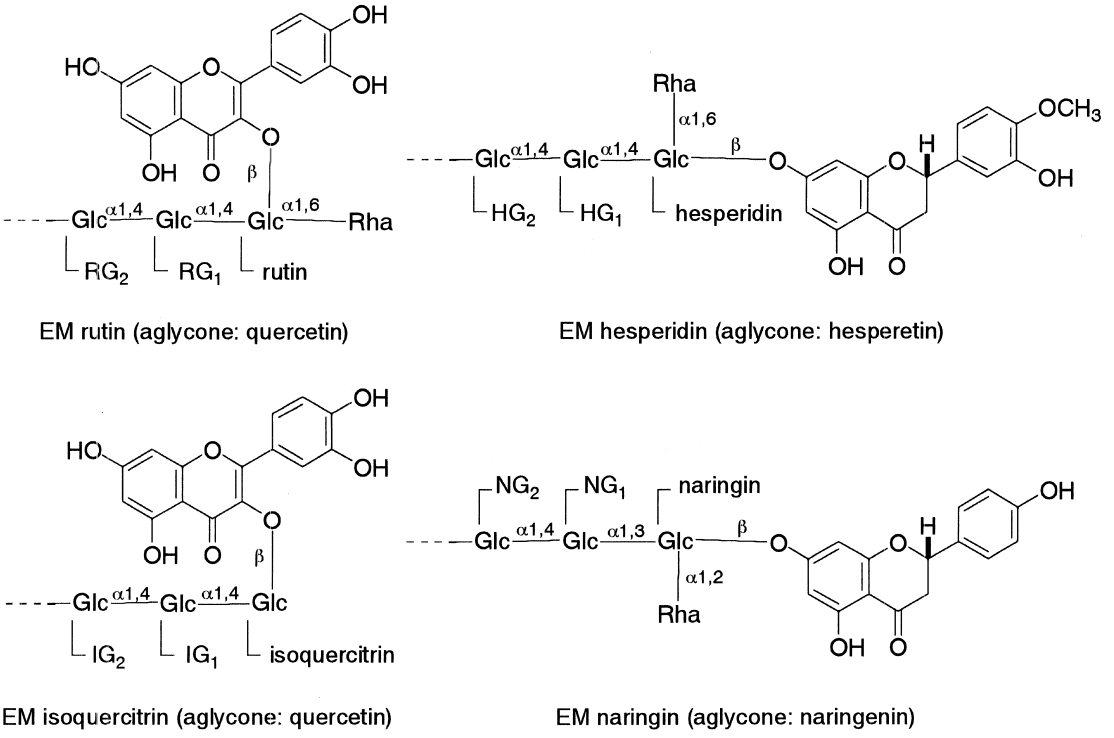

Fig. 1. Constituents of EM flavonoids.

alkaline pH. Analytical conditions were optimized for each item.

2. Experimental

2.1. Instrumental

A P/ACE 5500 system with System Gold software (Beckman, Fullerton, CA, USA) was used. An untreated fused-silica capillary of 57 cm (detection window at 50 cm)×75 μm I.D. (Beckman) was housed in a cartridge that allowed liquid cooling. The capillary was prepared by flushing the column for 5 min each with 0.1 *M* HCl and 0.1 *M* NaOH solutions alternately twice, and water for 1 min, and then rinsing with the buffer solution for 1 min. Between the sample analyses, the capillary was successively rinsed with 0.1 *M* NaOH (5 min), water (1 min), and the buffer (1 min). Pressure (0.5 p.s.i.) injection for 5 s was used (1 p.s.i.=6894.76 Pa). An HPCE run was performed at 25°C, and the glycosides were detected at 254 nm with a UV detector (Beckman).

2.2. Samples and reagents

Commercial EM rutin, EM isoquercitrin, EM hesperidin and EM naringin were obtained through the Japan Food Additive Association. They were dissolved in water at the concentrations of 4, 2, 10 and 10 mg ml^{-1}, respectively. All chemicals were of reagent grade. Ultrapure water (>18 MΩ cm) prepared with a Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA) was used throughout the experiment.

The running buffer was either 0.02, 0.04 or 0.1 *M* H_3BO_3–NaOH (pH 8.5, 9.0, 9.5 or 10.0), 50 m*M* Na_2HPO_4–NaOH (pH 12.0) or 30 m*M* $Na_2B_4O_7$–50 m*M* NaH_2PO_4 (pH 10.5). For each running buffer, pH was adjusted with 1 *M* NaOH.

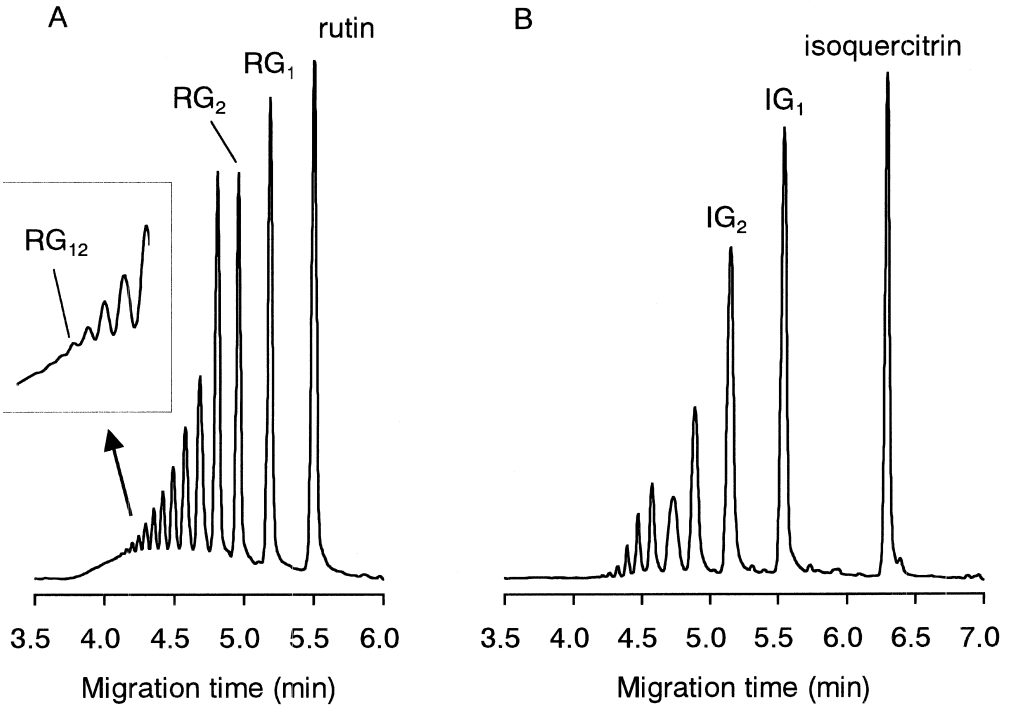

Fig. 2. Separation of EM rutin and EM isoquercitrin with H_3BO_3–NaOH buffer. (A) EM rutin. (B) EM isoquercitrin. Capillary, untreated fused-silica 57 cm×75 μm I.D.; buffer, 0.1 *M* H_3BO_3–NaOH, pH 10.0; voltage, 30 kV; temperature, 25°C; detection, 254 nm.

3. Results and discussion

3.1. H_3BO_3–NaOH buffer

EM flavonoids were analyzed by CZE using an untreated fused-silica capillary and the borate buffer. Fig. 2 shows the results of EM rutin and EM isoquercitrin. Glycosides with longer sugar chains migrated more rapidly. This suggests that every quercetin glycoside was separated as a singly charged anion, which may be due to the formation of borate complex on the ring B (Fig. 3) [6]. These anions are attracted to the anode, although they are forced to migrate towards the cathode because of the large electroosmotic flow. Glycosides with longer sugar chains have lower electrophoretic mobility due to their smaller charge/size ratio, which then causes shorter migration time.

Different pH (8.5–10.5) and different borate concentration (0.02–0.1 *M*) were tested for EM rutin. Higher pH and higher concentration gave better resolution (Fig. 4). Under the optimum conditions, i.e., 0.1 *M* borate at pH 10.0, each of the RG_ns ($n<13$) was separated completely from adjacent peaks (Fig. 2).

CZE of EM hesperidin and EM naringin gave similar electropherograms to that of EM rutin at pH 10.0. Glycosides with longer sugar chains have higher mobilities. However, these flavanone glycosides showed poor resolution at lower pH. It was suggested that the ionization took place by the proton

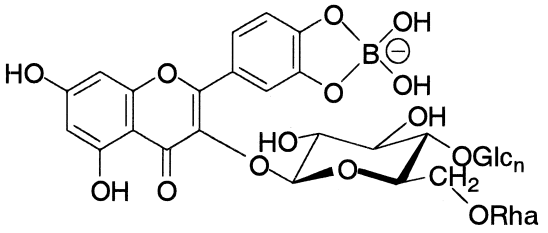

Fig. 3. The probable structure of the borate complex of RG_n.

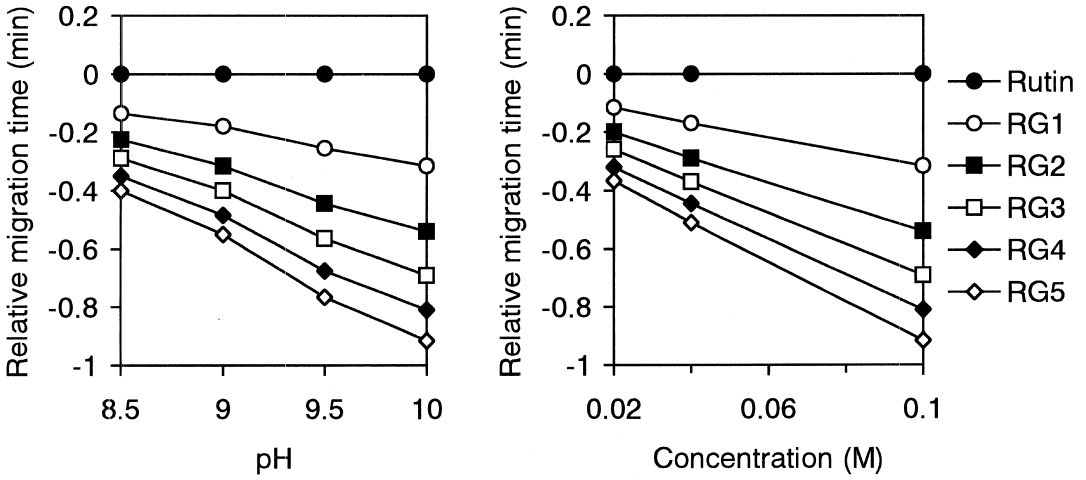

Fig. 4. Influence of pH and borate concentration in CZE of EM Rutin with H_3BO_3–NaOH buffer. Conditions are the same as those in Fig. 2, except for the running buffer, 0.02–0.1 *M* H_3BO_3–NaOH, pH 8.5–10.0.

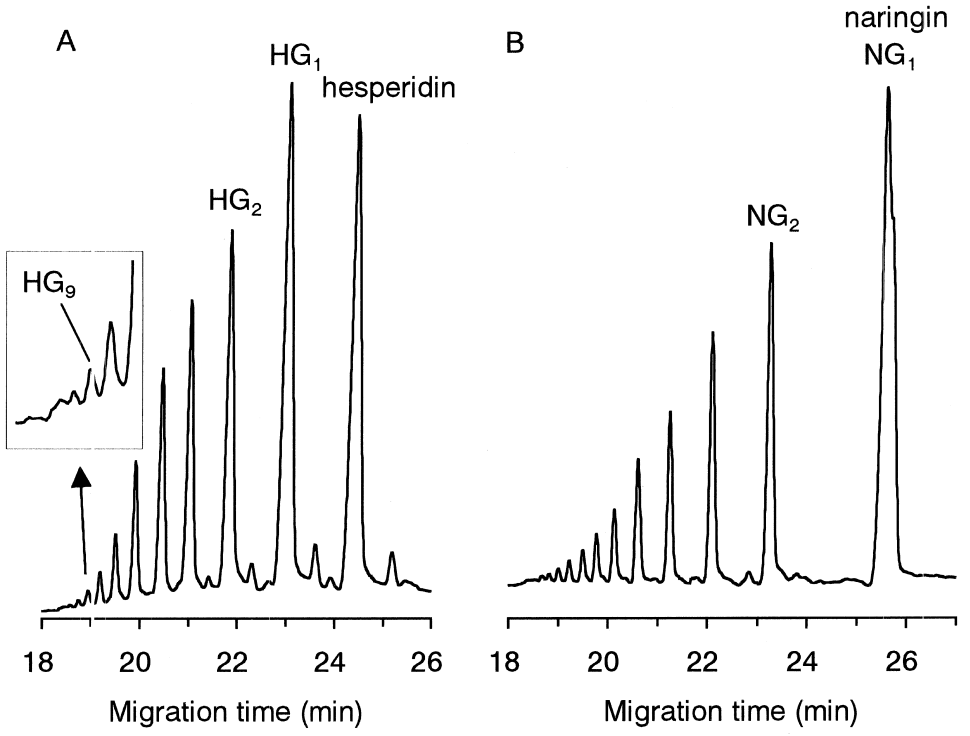

Fig. 5. Separation of EM hesperidin and EM naringin with Na_2HPO_4–NaOH buffer. (A) EM hesperidin. (B) EM naringin. Capillary, untreated fused-silica 57 cm×75 μm I.D.; buffer, 50 m*M* Na_2HPO_4–NaOH, pH 12.0; voltage, 10 kV; temperature, 25°C; detection, 254 nm.

abstraction from the phenolic hydroxyl groups of flavanone glycosides.

3.2. Na_2HPO_4–NaOH buffer

EM hesperidin and EM naringin were analyzed with Na_2HPO_4–NaOH buffer, which allows higher pH values. Major constituents of EM hesperidin, hesperidin and HG_ns ($n<10$), were separated from adjacent peaks with 50 m*M* buffer at pH 12.0 (Fig. 5A). On the other hand, the chemical composition of EM naringin could not be analyzed under these conditions, because naringin and NG_1 were not separated from each other (Fig. 5B).

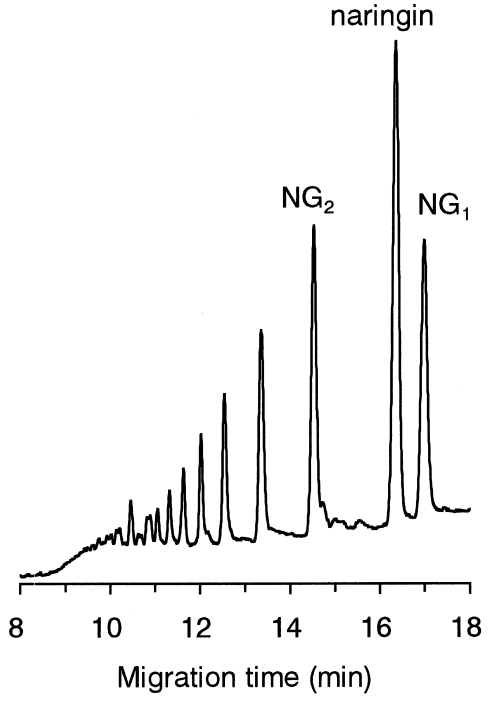

Fig. 6. Separation of EM naringin with $Na_2B_4O_7$–NaH_2PO_4 buffer. Capillary, untreated fused-silica 57 cm×75 μm I.D.; buffer, 30 m*M* $Na_2B_4O_7$–50 m*M* NaH_2PO_4, pH 10.5; voltage, 20 kV; temperature, 25°C; detection, 280 nm.

3.3. $Na_2B_4O_7$–NaH_2PO_4 buffer

EM naringin was also analyzed with the non-complexing phosphate–borate buffer. Using 30 m*M* $Na_2B_4O_7$–50 m*M* NaH_2PO_4 buffer (pH 10.5), the naringin peak was separated completely from the NG_1 peak (Fig. 6). Under these conditions, however, NG_1 migrated more slowly than naringin, although it has a larger molecular mass. The reason is not known.

4. Conclusions

CZE separated the constituents of four EM flavonoids, which have different length of maltooligosaccharide chains, with higher resolution than that in HPLC analysis. The optimal analytical conditions for each sample were: 0.1 *M* H_3BO_3–NaOH (pH 10.0) for EM rutin and EM isoquercitrin, 50 m*M* Na_2HPO_4–NaOH buffer (pH 12.0) for EM hesperidin and 30 m*M* $Na_2B_4O_7$–50 m*M* NaH_2PO_4 buffer (pH 10.5) for EM naringin. Further investigations will be carried out to apply CZE to other enzymatically glucosylated food additives.

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