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## Original Article

# Effective extraction method through alkaline hydrolysis for the detection of starch maleate in foods



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## ABSTRACT

A high-performance liquid chromatography (HPLC) method was developed for the determination of maleic acid which was released from starch maleate (SM) through the alkaline hydrolysis reaction. The proper alkaline hydrolysis conditions and LC separation are reported in this study. The starch samples were treated with 50% methanol for 30 minutes, and then hydrolyzed by 0.5N KOH for 2 hours to release maleic acid. A C18 column and gradient mobile phase consisting of 0.1% phosphoric acid and methanol at a flow rate of 1.0 mL/minute were used for separation. The method showed a good linearity in the range of 0.01–1.0 µg/mL, with a limit of quantification (LOQ) at 10 mg/kg in starch. The recoveries in corn starch, noodle, and fish balls were between 93.9% and 108.4%. The relative standard deviation (RSD) of precision was <4.9% ( $n = 3$ ). This valid method was rapid, sensitive, precise, and suitable for routine monitoring of the illegal adulteration of SM in foods.

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## 1. Introduction

Starch maleate (SM) is modified starch made of starch and maleic anhydride through the esterification process. It has been used in the paper industry as sealing adhesive [1] and evaluated as a potential drug delivery carrier [2]. Modified starches, in Taiwan, by law are listed in the “Standards for

Specification, Scope, Application and Limitation of Food Additives” [3] such as, acid-modified starch, alkaline-treated starch, oxidized hydroxypropyl starch, and starch sodium octenyl succinate allowed to be used in foods.

Although SM is widely manufactured and used in industries, it is not a permitted raw material for foods. However, it has been reported that SM was illegally adulterated into starch and starch food products in Asia to enhance starch

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properties for the purposes of water keeping, anti-retrogradation, and chewy texture. Hence, there are needs to establish a quantitative and qualitative method for monitoring the prohibited SM in foods.

The monitoring of maleic acid in food products was one of the means for the prevention of SM adulteration in food. Gas chromatography (GC), high-performance liquid chromatography (HPLC), capillary electrophoresis (CE) and LC with tandem-mass spectrometry (LC-MS/MS) [4–6] were reported for the determination of organic acids and/or maleic acid in a wide variety of samples. Among these methods, however, they were limited on the detection of free maleic acid in sample solutions. In SM, maleic acid linked to the hydroxyl group of starch through ester bonding. Therefore, a proper alkaline hydrolysis treatment was necessary in order to release maleic from modified starch. In this study, the proper alkaline hydrolysis conditions, extraction, and LC separation for the determination of maleic in SM and starch products were investigated and are described.

## 2. Methods

### 2.1. Materials

Twenty three commercial food starches (1 corn starch, 3 tapioca starches, 3 potato starches, 2 sweet potato starches, and 14 rice flours) and 51 starch foods (4 tapioca starch balls, 15 noodles, 16 fish products, 2 glass vermicelli, 11 rice flour cakes, 2 rice balls, 1 sweet potato, and 1 taros ball) were collected from the retailers and supermarkets in Taipei. These samples were homogenized and stored below  $-18^{\circ}\text{C}$  until analysis.

### 2.2. Chemicals and reagents

Maleic acid (99%), oxalic acid, malic acid, succinic acid, and maleic anhydride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Citric acid was purchased from JT Baker Chemical Co. (Phillipsburg, NJ, USA). Fumaric acid was from Nacalai Tesque Inc. (Kyoto, Japan). HPLC grade methanol, glacial acetic acid (100%), ascorbic acid, potassium hydroxide, phosphoric acid (85%), and hydrochloric acid were from E. Merck (Darmstadt, Germany).

### 2.3. Preparation of SM

A mixture of corn starch (10 g) and glacial acetic acid (10 mL) was stirred at  $95^{\circ}\text{C}$ , and then maleic anhydride (10 g) was added. This dispersed starch slurry gradually turned a pale

yellow color while stirring and heating. Sulfuric acid (0.98 mmole) was slowly added to the mixture, and it was stirred continuously for a further 30 minutes at the same temperature. Upon completion of the reaction, the mixture was cooled to room temperature, and then the SM product was precipitated by absolute ethanol (50 mL) at room temperature. MS was collected by vacuum filtration, and then rinsed three times with 95% ethanol to remove excess maleic anhydride. The product was then dried overnight at  $45^{\circ}\text{C}$  in a conventional oven. The major pathway of the esterification is shown in Fig. 1 [7]. The bound maleic acid can be released form SM through alkaline hydrolysis reaction (Fig. 1).

### 2.4. Instrumentation and HPLC-DAD, LC-MS/MS analytical conditions

The separation of target analytes was carried out using an UltiMate3000 Standard LC System with a detector DAD-3000RS (diode array detector, Dionex Corporation Sunnyvale, CA, USA) which equipped with an InertSustain C18 (4.6 mm  $\times$  250 mm, 5  $\mu\text{m}$ ) column (GL Sciences, Tokyo, Japan). The mobile phase was a mixed solution of 0.1% phosphoric acid/methanol (98:2, v/v). Isocratic elution chromatography was at a flow rate of 1 mL/minute. The target analytes were detected at the wavelength of 214 nm. The injection volume was 20  $\mu\text{L}$ .

LC-MS/MS chromatographic separation was performed using an HPLC system including an ekspert ultraLC 100 system (Eksigent Technologies, Livermore, CA, USA), a triple quadrupole mass spectrometer QTRAP 5500 LC-MS/MS system (Applied Biosystems, Foster City, CA, USA), a Turbo V ion source, and a GL Sciences InertSustain C18 column (2.1 mm  $\times$  150 mm, 3  $\mu\text{m}$ ). Gas nitrogen was supplied by a nitrogen generator (Peak Scientific Instruments Ltd., Chicago, IL, USA). Nitrogen was employed as curtain gas, nebulizer gas, and collision gas on the MS. The mobile phase consisted of 0.1% formic acid/methanol (98:2, v/v) at a flow rate of 0.3 mL/minute. The electrospray ionization mass spectra (ESI-MS) were acquired in the negative ion (ESI<sup>-</sup>) mode. The other MS parameters were: ion spray voltage:  $-4.5$  kV, curtain gas: 20 psi, collision gas: high, turbo gas: 55 psi, nebulizer gas: 55 psi, declustering potential: 15V, collision energy: 11 and 30V for  $m/z$  115 > 71 and  $m/z$  115 > 27, respectively.

### 2.5. Fourier transform infrared spectrometer

Infrared spectra of SM and starches were recorded using Smiths Identify IR (Smiths Detection Inc., Watford, UK) with attenuated total reflectance (ATR) accessory (resolution = 4  $\text{cm}^{-1}$ , 32 scans/

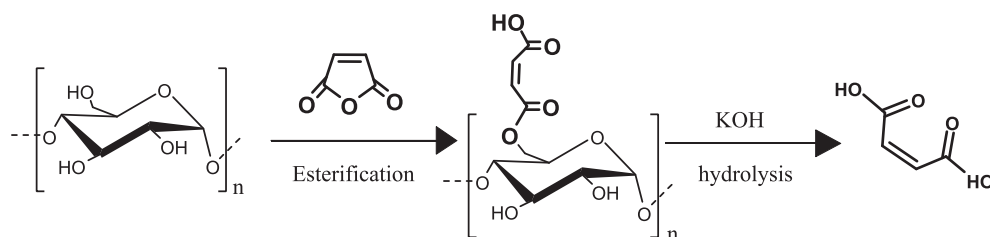


Fig. 1 – Pathway of the esterification and hydrolysis of starch maleate.

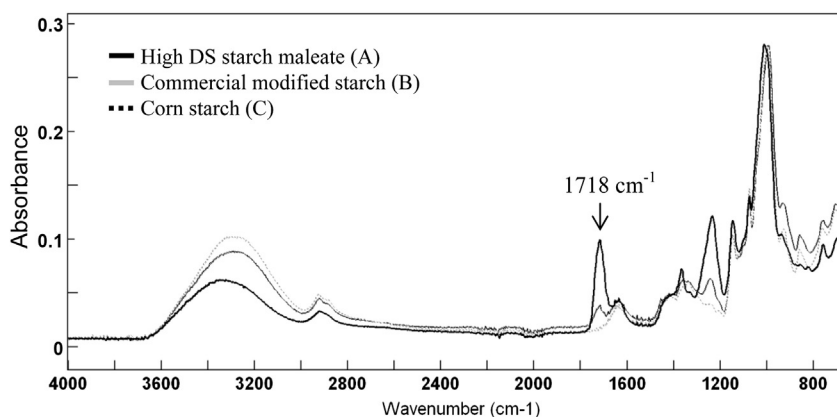


Fig. 2 – Infrared (IR) spectra of (A) high degree of substitution (DS) starch maleate, (B) commercial modified starch, and (C) corn starch.

spectra, range = 650–4000  $\text{cm}^{-1}$ ). Samples (10 mg) were applied directly to the diamond ATR top-plate.

### 2.6. Preparation of standard solutions

The stock standard solutions were prepared by dissolving organic acids (100.0 mg) individually in 100 mL water. Specific amounts of stock standard solutions were then mixed to obtain the combined solution at a concentration of 1  $\mu\text{g}/\text{mL}$  for oxalic acid, ascorbic acid, maleic acid, fumaric acid, and 50  $\mu\text{g}/\text{mL}$  for malic acid, acetic acid, citric acid, and succinic acid. The stock solution of maleic acid was diluted with water to obtain a series of standard solutions (0.01–1.0  $\mu\text{g}/\text{mL}$ ).

### 2.7. Preparation of sample solutions

A homogenized starch sample (1 g) was accurately weighed and then transferred into a centrifuge tube; 50% methanol (50 mL) was added, and then the tube was shaken for 30 minutes. To this mixture was added 0.5N potassium hydroxide (20 mL) and the mixture was stored at room temperature for 2 hours prior to acidifying with hydrochloric acid (~3 mL, 5N). The solution was diluted with water to 50 mL, and then 100  $\mu\text{L}$  of the solution was further diluted with water to 1000  $\mu\text{L}$ . This diluted solution was filtered through a 0.22  $\mu\text{m}$  membrane filter prior to HPLC analysis.

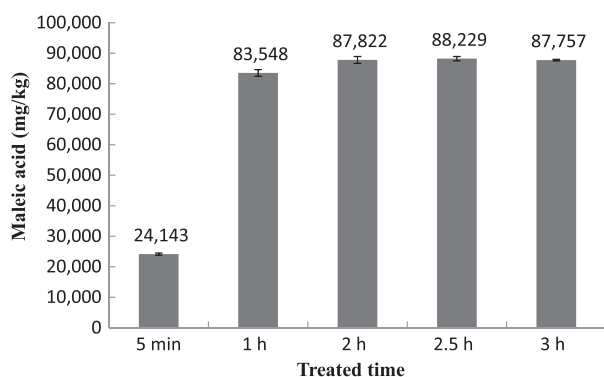


Fig. 3 – The effect of the treating time for starch maleate alkaline hydrolysis.

## 3. Results and discussion

### 3.1. Preparation of maleic anhydride modified starch

SM may be prepared in various ways, such as through esterification of starch with maleic anhydride in NaOH water solution, and the degree of substitution (DS) of SM obtained

Table 1 – Results of recoveries, precision and limit of quantification (LOQ).

| Matrix      | Spiked (mg/kg) | Detected (mg/kg) | Recovery (%) | Intra-day RSD (%) | LOQ (mg/kg) |
|-------------|----------------|------------------|--------------|-------------------|-------------|
| Corn starch | 10             | 10.1             | 101.1        | 0.5               | 10          |
|             | 50             | 51.3             | 102.6        | 4.9               |             |
| Noodle      | 10             | 10.8             | 108.4        | 1.21              | 10          |
|             | 50             | 48.0             | 95.9         | 3.52              |             |
| Fish ball   | 10             | 10.3             | 103.2        | 3.35              | 10          |
|             | 50             | 47.0             | 93.9         | 3.97              |             |

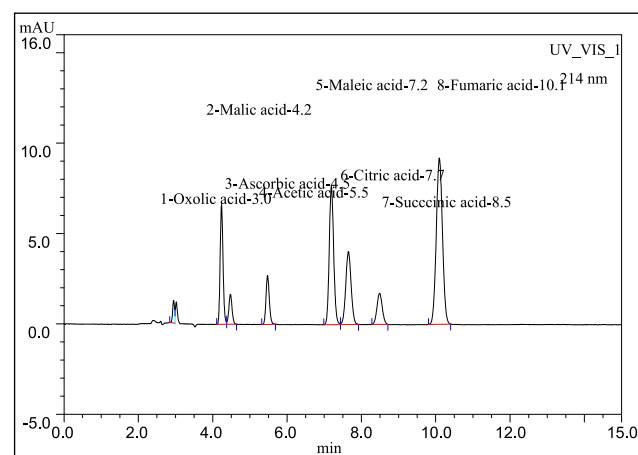


Fig. 4 – High-performance liquid chromatography (HPLC) chromatogram of maleic acid and seven organic acids standards. The concentrations of oxalic acid, maleic acid, ascorbic acid, and fumaric acid are 1  $\mu\text{g}/\text{mL}$ , and of malic acid, acetic acid, citric acid, and succinic acid are 50  $\mu\text{g}/\text{mL}$ .

**Table 2 – Maleic acid contents in the commercial starch and starch products.**

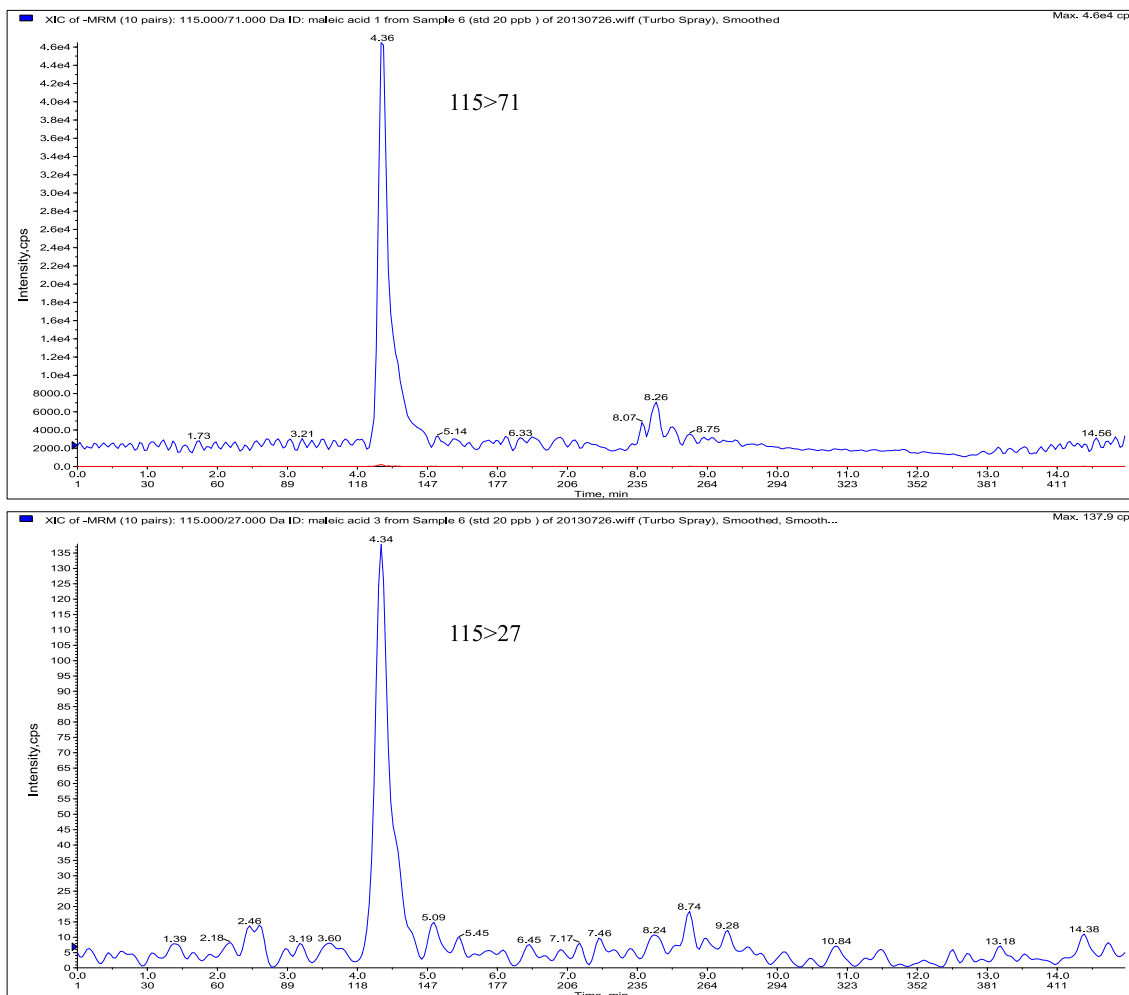
| Sample         | Number                      | Number detected | Content of maleic acid (mg/kg) |
|----------------|-----------------------------|-----------------|--------------------------------|
| Starch         | Corn starch                 | 1               | 0                              |
|                | Tapioca starch              | 3               | 0                              |
|                | Potato starch               | 3               | 0                              |
|                | Sweet potato starch         | 2               | 0                              |
|                | Rice flour                  | 14              | 0                              |
| Starch product | Tapioca starch ball         | 4               | 1                              |
|                | Noodle                      | 15              | 0                              |
|                | Glass vermicelli            | 2               | 0                              |
|                | Fish product                | 16              | 2                              |
|                | Rice flour cake             | 11              | 1                              |
|                | Rice ball                   | 2               | 0                              |
|                | Sweet potato and taros ball | 1               | 1                              |

ranged from 0.03 to 0.25% [8]. In this study, a much sever esterification process was utilized to derive highly substituted SM (0.08 to 0.35%). The FT-IR (Fourier transform infrared spectroscopy) spectrum of SM synthesized in this study is shown in Fig. 2. The absorption band at 1718 cm<sup>-1</sup>, which was assigned to the carbonyl group, indicated the maleate moiety in the modified starch [8]. The commercial modified starch (starch acetate) also showed absorption at 1718 cm<sup>-1</sup>, which indicated the acetate ester bond, but the abundance was lower because of the lower DS. Compared to modified starches, this

band was absent in the unmodified corn starch. The other band at 3395 cm<sup>-1</sup> was broad and assigned to hydroxyl group vibration. This band showed decreased abundance when the DS was increased, which indicated the loss of free hydroxyl group and the formation of ester bonding.

### 3.2. Determination of the alkaline hydrolysis time

It was necessary to hydrolyze maleic acid from starch prior to analysis. The proper alkaline hydrolysis time for releasing



**Fig. 5 – The multiple reaction monitoring (MRM) chromatogram for maleic acid standard solution at 1 µg/mL.**

maleic acid from SM was determined by treating SM with 20 mL of 0.5N potassium hydroxide in 0.5 minutes to 3 hours. The results in Fig. 3 revealed that the highest enrichment factor can be obtained at 2 hours. The yield of maleic acid released from SM showed an increased trend. It was highest at 2 hours, and then remained constant.

### 3.3. Method precision

Maleic acid was spiked into samples in concentrations of 5 mg/kg, 10 mg/kg, and 50 mg/kg to evaluate method precision. The recoveries were 101.1–102.6%, 95.9–108.4%, and 93.9–103.2% in corn starch, noodles, and fish balls, respectively ( $n = 3$ , Table 1). The repeatability given as relative standard deviation (RSD) was <4.9%. The limit of quantification (LOQ) of this method was 10 mg/kg calculated by multiplying 10 times of the SD of the area of 10 mg/kg maleic acid spiked samples.

The LC chromatogram of eight acids, including maleic acid, is shown in Fig. 4. There was no significant interference in our optimized LC method. This reliable HPLC-DAD method can be applied to routine survey for the determination of the total amount of maleic acid and maleic anhydride in SM and products made of SM.

### 3.4. Determination of maleic acid in food samples

Twenty three commercial food starch and 51 starch food samples were hydrolyzed and analyzed. The concentration of maleic acid was calculated by an external calibration curve. Maleic acid was detected in five starch food samples (1 tapioca starch ball, 1 rice flour cake, 1 taros ball and 2 fish rolls), but not detected in all of the 23 starches (Table 2).

### 3.5. Confirmation with LC-MS/MS

The LC-MS/MS method was developed to provide confirmatory data for the analysis of food starch samples in which maleic acid was positively detected by HPLC-DAD. The MS/MS fragmentation conditions were optimized. For a method to be deemed confirmatory, one parent ion and two daughter ions must be monitored ( $m/z$  115 > 71 and  $m/z$  115 > 27). This yielded four identification points, which provided a suitable confirmatory method in accordance with 2002/657/EC [8]. Fig. 5 shows the multiple reaction monitoring chromatogram for maleic acid standard solution at 1  $\mu\text{g/mL}$ .

## 4. Conclusion

SM was an illegal food material. We have reported the detection of SM in food products. Maleic acid in SM was bonded with the hydroxyl group of starch through esterification. Therefore, a proper hydrolysis process was needed in order to release free maleic acid. In this study, the proper alkaline hydrolysis conditions and the simple, rapid HPLC method were developed for the determination of maleic acid. This method was useful for routine monitoring of the illegal adulteration of SM in foods.

## Conflicts of interest

All authors declared there is no conflict of interest.

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