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Enzymatic Method for Detecting Sucrose in Ancient Chinese Mortars

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Saccharides were used worldwide among many organic additives in masonry mortars. In China, one of the most widely used saccharides was sucrose (brown sugar). According to ancient literature records, brown sugar was usually added in lime, earth and "tabia" to build forts and vernacular dwellings in eastern and southeastern China. Common methods used to detect sugars in mortars were mainly chemical methods, FTIR, *etc*. In this work, an enzymatic method was tested and performed on simulated mortars and ancient mortar samples. The results suggested that this method was sensitive and exclusive, with low detection limits and simple operations and is recommended for sucrose detection in ancient mortars.

Keywords: Sucrose, Chinese mortar, Analyses, Benedict's reaction, FTIR, Enzymatic method.

INTRODUCTION

Mortar is an important paste used to bind construction blocks (stone, brick, cinder blocks, *etc.*) together and fill the gaps between them. At the beginning of human civilization (12000-7000 B.C.), our ancestors constructed buildings primarily with earth and clay[1]. Shortly after, lime was used in many parts of the world[2-4]. During the Hellenistic period (323-146 B.C.), the Romans invented pozzolanic mortar by adding volcanic ash or brick dust to lime plasters, thus enhanced the hydraulic properties of lime mortars[5]. Later people began to put natural organic admixtures in mortars, such as proteins (*e.g.* animal blood[6], egg white[7], milk[8]), saccharides (*e.g.* starch[9], cactus juice[10]), oils (*e.g.* olive oil[11], egg yolk[12]), *etc*. Unlike pozzolanic mortar, which was hydraulic, traditional Chinese mortars were air-hardened, possibly due to the scarcity of volcanic ash. Chinese people began to calcine lime from the middle and late Neolithic Age (5000-2000 B.C.)[13] and invented "tabia" (mixture of sand, lime and clay) in no later than Western Zhou dynasty (1046 -771 B.C.)[14]. Organic matters such as sticky rice was first discovered in a tomb of the Northern and Southern dynasty (420-589 A.D.)[15]. Natural organic admixtures in ancient Chinese mortars comprised mainly of proteins (*e.g.* pig blood[16], egg white[17]), saccharides (*e.g.* sticky rice[18], juices of kiwifruit canes[19] and camphor tree leaves[20], brown sugar[21]), oils (*e.g.* resin[22], tung oil[23]) and *etc*. Since the 18th century, the appearance of Portland cement has enjoyed so much popularity that it eventually replaced most traditional mortars, due to the fact that shorter setting times contributed to quicker constructions. However, the use of cement in fields of protection and restoration of historic buildings was repeatedly denied because of unsatisfying compatibility problems. Therefore, studies on the original architectural materials became increasingly important. Our research group has done many researches on several categories of traditional Chinese mortars, such as sticky rice mortar[24-29], tung oil mortar[30] as well as pig blood mortar[31]. In continuation of our research, this article intends to focus on the identification of sucrose in ancient Chinese mortars. The aim of this paper is to develop an enzymatic method for sucrose detection in ancient mortars.

Brown sugar mortar was widely used in eastern and southeastern China (Fig. 1). A literature from Ming dynasty recorded that forts can be strengthened by combining bricks with tabia and sticky rice porridge as well as brown sugar syrup[32]. Similarly, oyster shell ash (main composition CaO), cooked sticky rice and brown sugar were used to adhere stones when building Qilu Fort (Guangdong province)[33]. In a literature of Qing dynasty, stone bands of a river dam were combined by brown sugar mortar[34]. Vernacular dwellings in Nantong (Jiangsu province) use egg white, sticky rice porridge and brown sugar to bind bricks[35] and similar formulas are also present in Hakka earth buildings in southern Fujian province[36].

Some earth buildings in the Pearl River Delta area (Guangdong province) are built by rammed earth, in which brown sugar syrup and sticky rice porridge are sometimes added[37]. For instance, Kaiping Diaolou, a type of vernacular building in Guangdong province, was built by tabia and brown sugar syrup[38].

Researches have revealed that sucrose can improve many properties of mortars. For example, it can be a good superplasticizer[39], enhance the strength[40,41] and aggregation[42] and adjust the setting time[43,44]. Methods of detecting sucrose (or reducing sugars) include Benedict's reaction[45], sulfuric acid-phenol method[46], DNS method[47], anthrone method[48], Nelson-Somogyi method[49], molybdenum blue method[50], near-IR method[51], LC-MS method[52,53], HPLC method[54], enzymatic method[55-59], *etc*. Although methods like HPLC and LC-MS have higher sensitivity and precision in sucrose detection, both these methods are labor intensive and time consuming due to their complicated procedures. The enzymatic method is easy to operate and fast to produce results and also possesses the required sensitivity and precision for mortar studies in the archaeological field.

An enzymatic fluid sucrose assay (R-biopharm) was used for sucrose detection. A glucose-dependent secondary reaction was involved and the content of total sucrose was calculated according to absorption value difference of NADH at 340 nm. To avoid the interference of glucose which had been already present in the sample, the samples were then measured in an additional run with enzytec fluid D-glucose assay (R-riopharm). The corresponding glucose response was subtracted from the total sucrose response, leaving only the sucrose response. Principles of total sucrose detection and glucose detection are shown below.

(a) Principle of total sucrose detection

$$\text{Sucrose} + H_2O \xrightarrow{\beta\text{–Fructosidase}} \text{D-glucose} + \text{D-fructose}$$

$$\text{D-glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{glucose-6-phosphate} + \text{ADP}$$

$$\text{Glucose-6-phosphate} + \text{NAD} + \xrightarrow{\text{Glucose-6-phosphate dehydrogenase}} \text{glucose-6-phosphate} + \text{NADH} + H^+$$

(b) Principle of glucose detection

$$\text{D-glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{glucose-6-phosphate} + \text{ADP}$$

$$\text{Glucose-6-phosphate} + \text{NAD} + \xrightarrow{\text{Glucose-6-phosphate dehydrogenase}} \text{glucose-6-phosphate} + \text{NADH} + H^+$$

EXPERIMENTAL

Reagents for chemical analyses on reducing sugar were sodium citrate, sodium carbonate, deionized water and cupric sulfate. All reagents were of analytical-reagent grade and Benedict's solution was prepared by method in Fang *et al.*[45].

Reagents for enzymatic analyses on sucrose were detection kits of R-biopharm enzytec fluid sucrose (*via* glucose) (E5180) and enzytec fluid D-glucose (E5140), deionized water (H_2O), hydrochloric acid (HCl) and sodium hydroxide (NaOH).

Enzymatic analyses were performed by UV-visible spectrophotometer (UV-1800PC, produced by Mapada). The infrared spectrums were acquired by Fourier transform infrared spectroscopy (Nicolet iS10, produced by Thermo Fisher Scientific).

Preparation of simulated brown sugar mortars: To test the limit as well as credibility of all methods, simulated brown sugar mortars were prepared. According to literature records, brown sugar was added in lime[33], earth[38] as well as in tabia[21], therefore three series of mortars were prepared, using $Ca(OH)_2$, earth, $Ca(OH)_2$ + earth (m/m = 1:1, to simulate simple tabia) respectively as inorganic binders. To prepare the simulated mortars, brown sugar solutions of six different concentrations were added in each series and the water/inorganic binder ratio was 0.8. Detailed information of simulated brown mortars is given in Table-1. The mortars were prepared on October 23rd, 2012.

TABLE-1
FORMULAS OF SIMULATED BROWN SUGAR MORTARS

Inorganic binders	Concentration no.	Brown sugar in solution (w %)	Water/inorganic binder ratio (w/w)	Brown sugar in mortar (w %)
$Ca(OH)_2$, earth[a], $Ca(OH)_2$ + earth (m/m = 1:1)	1	4.000	0.8	2.222
	2	1.000		0.556
	3	0.250		0.139
	4	0.060		0.033
	5	0.015		0.008
	6	0.004		0.002

[a]The earth was taken from the subsoil layer in Zhejiang province eastern China). It was first crushed and sifted by 100 mesh sieve, then dried under 100 °C in baking oven for 48 h

Description of ancient mortar samples: Fifteen ancient mortar samples with different inorganic binders (lime, earth and tabia) and from different provinces (Zhejiang, Jiangsu, Fujian and Gansu, Fig. 1) were analyzed. The samples were taken from dwellings, forts, temples and pagodas, which types of constructions are likely to contain brown sugar mortar according to literature records[32,35,36].

Fig. 1

Method of chemical analyses: Benedict's reaction was used to detect reducing sugars in mortars, according to procedures of Fang *et al.*[45].

Method of enzymatic analyses: (a) Sample preparation: for mortars with inorganic binders of $Ca(OH)_2$ and $Ca(OH)_2$ + earth, the samples should be ground to powders and dissolve $Ca(OH)_2$ in hydrochloric acid. Adjust pH to 7 and take the supernatant for enzymatic analyses. For mortars with earth as inorganic binder add water to ground samples, adjust pH to 7 and put the mixtures in 60 °C water bath for 15 min to dissolve as much sucrose as possible, then take the supernatant for analyses.

(b) Analyses: Enzymatic analyses procedures of total sucrose and glucose are illustrated in Tables 2 and 3.

TABLE-2
ENZYMATIC ANALYSES PROCEDURES OF TOTAL SUCROSE[a]

Step	Content	Sample (µL)	Reagent blank (µL)
1	Sample solution	100	-
	Deionized water	-	100 µL
2	Reagent 1	2000	2000 µL
3	Incubate for 15 min at 20-25 °C, read absorbance A1		
4	Reagent 2	500	500 µL
	Reagent 3	500	500 µL
5	Incubate for 15 min at 20-25 °C, read absorbance A2		

[a]Wavelength: 340 nm, optical path: 1 cm, measurement: against water. Reagent blank must be performed for every run and subtracted during calculation of results

TABLE-3
ENZYMATIC ANALYSES PROCEDURES OF GLUCOSE[a]

Step	Content	Sample (µL)	Reagent blank (µL)
1	Sample solution	100	-
	Deionized water	-	100
2	Reagent 1	2000	2000
3	Incubate for 3 min at 20-25 °C, read absorbance A1		
4	Reagent 2	500	500
5	Incubate for 15 min at 20-25 °C, read absorbance A2		

[a]Wavelength: 340 nm, optical path: 1 cm, measurement: against water. Reagent blank must be performed for every run, and subtracted during calculation of results

(c) Calculation and determination of sucrose: First the content of total sucrose and glucose are to be calculated, respectively, then content of glucose is subtracted from content of total sucrose to get the content of real sucrose in the samples. Calculation steps are shown below.

$$\Delta A_{total\text{-}sucrose} = (A2 - df \times A1)_{sample} - (A2 - df \times A1)_{RB}$$
$$\left(df = \frac{V_{sample} + V_{reagent\,1}}{V_{sample} + V_{reagent\,1} + V_{reagent\,2} + V_{reagent\,3}} = 0.677 \right) \quad (1)$$

$$Content_{total\text{-}sucrose}\left[\frac{g}{1}\text{ sample solution}\right] = \frac{V \times MW \times \Delta A_{total\text{-}sucrose}}{s \times d \times v \times 1000} \quad (2)$$

V (volume) = 3100 [µL], MW (molecular weight) = 342.3 [g/mol], ε (extinction coefficient NADH) = 6.3 [L × $mmol^{-1}$ × cm^{-1}], d (optical path)=1 [cm], v (sample volume) = 100 [µL]).

$$\Delta A_{glucose} = (A2 - df \times A1)_{sample} - (A2 - df \times A1)_{RB}$$
$$\left(df = \frac{V_{sample} + V_{reagent\,1}}{V_{sample} + V_{rreagent\,1} + V_{reagent\,2}} = 0.808 \right) \quad (3)$$

$$Content_{glucose}\left[\frac{g}{1}\text{ sample solution}\right] = \frac{V \times MW \times \Delta A_{glucose}}{s \times d \times v \times 1000} \quad (4)$$

V (total volume) = 2600 [µL], MW (molecular weight) = 180.16 [g/mol], ε (extinction coefficient NADH) = 6.3 [L × $mmol^{-1}$ × cm^{-1}], d (optical path) = 1 [cm], v (sample volume) = 100 [µL]).

$$Content_{sucrose}\left[\frac{g}{1}\right] = content_{total\text{-}sucrose} - content_{glucose} \times 1.90 \quad (5)$$

RESULTS AND DISCUSSION

Chemical analyses: Results of Benedict's reaction on simulated mortars are shown in Fig. 2. It can be observed that the detection limit of chemical analyses on simulated brown sugar mortars with inorganic binders of $Ca(OH)_2$ and $Ca(OH)_2$ + earth was 0.556 %, but the reaction of the latter was not as strong as the former one. The detection limit of simulated with earth as the inorganic binder, however, was 2.222 %. Overall speaking, the detection limit of Benedict's reaction was relatively high.

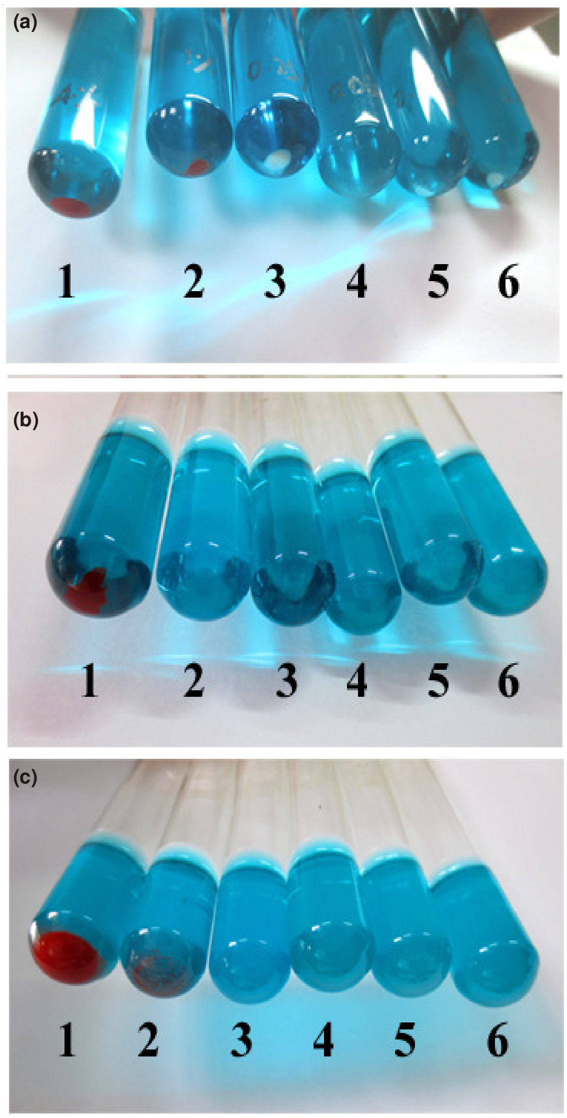

Fig. 2

Chemical analyses were then performed on ancient mortar samples and 5 out of 15 ancient mortar samples gave positive results. Information and images of the 5 samples are shown in Table-4 and Fig. 3. One problem was that Benedict's reaction could only identify reducing sugars, so the positive result of Tabia from Tiger Hill Pagoda may have been brought by the plant stems in the mortar (Fig. 3c). Further studies are needed to distinguish polysaccharides and sucrose in mortars.

TABLE-4
ANCIENT MORTAR SAMPLES
SUSPECTED TO CONTAIN SUCROSE

Description	Province	Year of construction	Result
Lime mortar from Tiger Hill Pagoda	Jiangsu	959 A.D.	+
Earth from Wuyun building-a	Fujian	1567-1572 A.D.	+
Tabia from Tiger Hill Pagoda	Jiangsu	959 A.D.	+++
Tabia from Qingshan temple-a	Gansu	420-589 A.D.	+
Tabia from Anyuan fort	Zhejiang	1884 A.D.	+

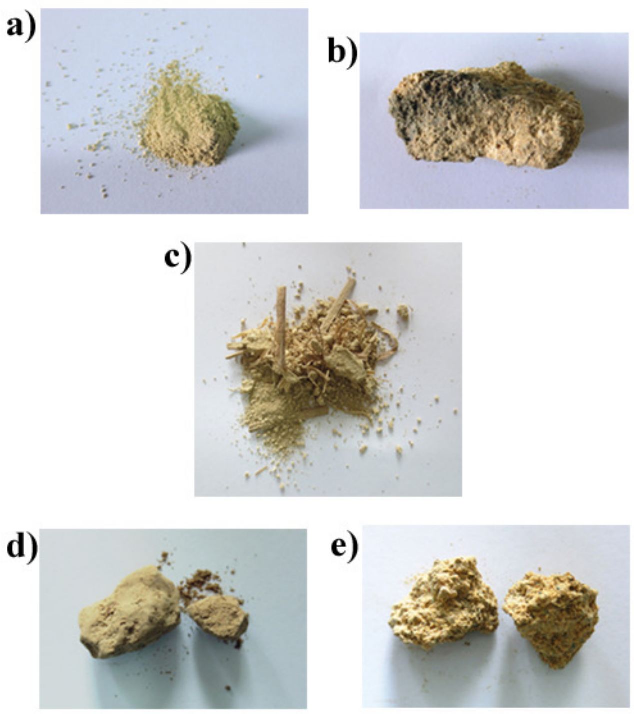

Fig. 3

FTIR analyses: To verify the results of chemical analyses, simulated mortars along with the suspected samples were analyzed by FTIR. It can be seen that the absorption bands at 990, 1050 and 1070 cm^{-1} in the curve of brown sugar belong to stretching vibration of C-O (Fig. 4a-c). When brown sugar was added in lime, one broad band was shown at 950-1150 cm^{-1} (Fig. 4a). The broad peak was also present in the sample lime mortar from Tiger Hill Pagoda, which confirmed the presence of sugars, but the exact type of sugar still could not be distinguished in the spectrums. When brown sugar was added in earth and $Ca(OH)_2$ + earth, however, since the SiO_2 in earth also has absorption bands at 1050-1100 cm^{-1}, the peaks of brown sugar and SiO_2 overlapped (Fig. 4b-d), thus made the identification of sugars in mortars impossible since all samples showed one broad peak between 1150-950 cm^{-1}.

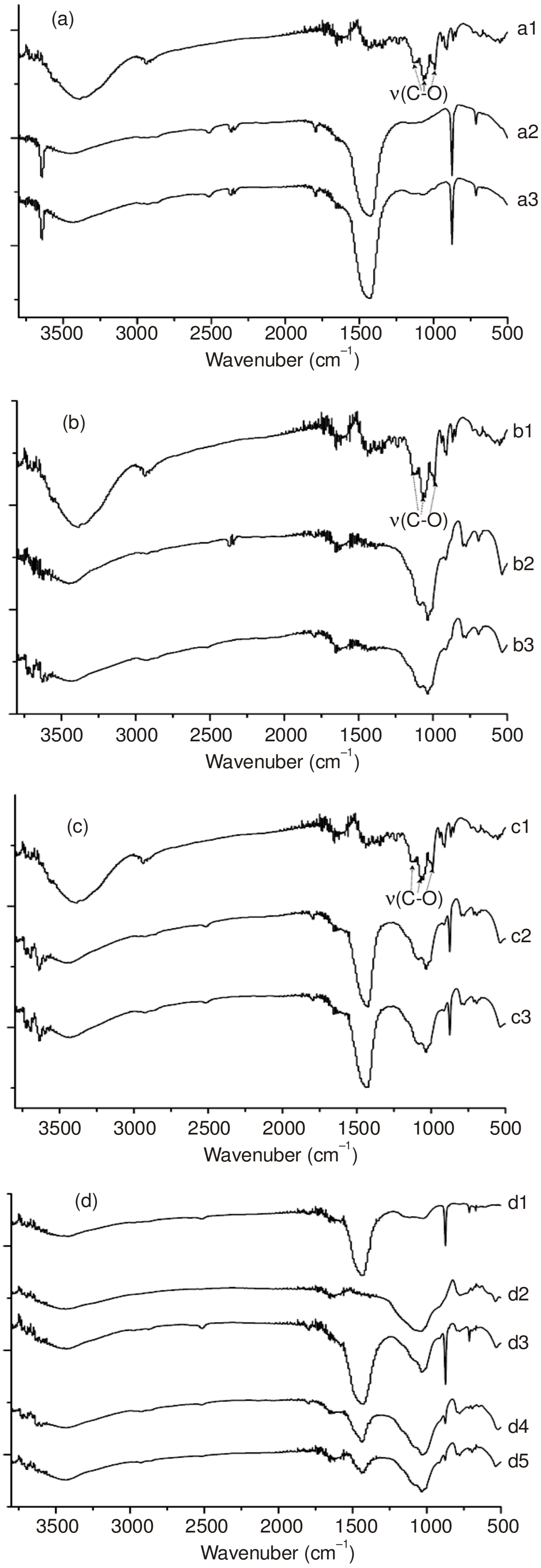

Fig. 4

Enzymatic analyses: A small modification was made to reagent blank (RB) in the tests on simulated mortars, by replacing deionized water with blank inorganic binder solutions and this "RB" was renamed "Concentration No. 0". For example, for simulated mortars with $Ca(OH)_2$ as inorganic binder, "Concentration No. 0" was $Ca(OH)_2$ + 0.000 % brown sugar and "Concentration No. 0" for the other two types of mortars were earth + 0.000 % brown sugar and $Ca(OH)_2$ + earth + 0.000 % brown sugar, respectively. In this case, the calculation formula was also changed to

$$\Delta A = (A2 - df \times A1)_{sample} - (A2 - df \times A1)_{concentration\ No.\ 0}$$

Results of enzymatic analyses on simulated brown sugar mortars are shown in Table-5. The detection limit of enzymatic analyses on simulated brown sugar mortars with inorganic binder of $Ca(OH)_2$ was 0.002 %. For simulated mortars with earth and $Ca(OH)_2$ + earth as inorganic binders, the detection limits were 0.556 and 0.033 %, respectively. Despite differences in the results, detection limits of all mortars were lowered. Moreover, chemical analyses could only identify reducing sugars, while enzymatic tests had exclusive reactions with sucrose. Therefore, the use of enzymatic method to detect sucrose in mortars yielded satisfying results.

Enzymatic analyses were then performed on all 15 ancient mortar samples and the results are shown in Table-6. It can be seen from the table that only 1 sample, Tabia from Wuyun Building, turned out to contain sucrose, which was in accordance with the literature records. Compared with the results on simulated brown sugar mortars with earth as the inorganic binder (Table-5), we believe that the percentage of sucrose present in the sample was around 2.222 %. However, if we take the construction year (1567-1572 A.D.) of Wuyun Building and the rapidness of sucrose decomposition in earth into consideration, it can be inferred that the original percentage of sucrose in the building material was much higher than 2.222 %. The results of enzymatic analyses also denied the presence of sucrose in Tabia from Tiger Hill Pagoda, suggesting that the positive result of chemical analysis was probably indeed brought by plant stems in the mortar (Fig. 3c).

Conclusion

Brown sugar was one of the most widely used saccharides in ancient Chinese mortars. It was usually added in lime, earth and tabia to build forts and vernacular dwellings in eastern and southeastern China.

To identify sucrose in mortars, chemical analyses, FTIR and enzymatic analyses were performed on both simulated mortars and ancient mortar samples in this work. The detection limits of chemical analyses on simulated brown sugar mortars with inorganic binders of $Ca(OH)_2$, earth and $Ca(OH)_2$ + earth were 0.556, 2.222 and 0.556 %, respectively. However, Benedict's reaction reacts with reducing sugars, but it cannot distinguish sucrose from other saccharides.

FTIR analyses confirmed the results from chemical analyses on ancient mortar samples, but sucrose cannot be identified either. Moreover, when earth was used in the inorganic binder, it was impossible to find absorption peaks of sugars since they overlapped with the peaks of SiO_2 in the earth.

Enzymatic analyses yielded satisfying results. The detection limits of enzymatic analyses on simulated brown sugar mortars with inorganic binders of $Ca(OH)_2$, earth and $Ca(OH)_2$ + earth were 0.002, 0.556 and 0.033 %, respectively. For ancient mortar samples, the enzymatic method successfully

TABLE-5
ENZYMATIC ANALYSES ON SIMULATED BROWN SUGAR MORTARS

Inorganic binder	Concentration no.	1	2	3	4	5	6	0
	Brown sugar (w %)	2.222	0.556	0.139	0.033	0.008	0.002	0.000
$Ca(OH)_2$	$Content_{Sucrose}$ (g/L)	0.330	0.335	0.121	0.142	0.115	0.091	0
	Result	+	+	+	+	+	+	-
Earth	$Content_{Sucrose}$ (g/L)	0.218	0.239	0	0	0	0	0
	Result	+	+	-	-	-	-	-
$Ca(OH)_2$ + Earth	$Content_{Sucrose}$ (g/L)	0.574	0.587	0.365	0.072	0	0	0
	Result	+	+	+	+	-	-	-

TABLE-6
ENZYMATIC ANALYSES ON ANCIENT MORTAR SAMPLES

Inorganic binder	Description			
$Ca(OH)_2$	Sample	Lime mortar from Liuhe Pagoda	Lime mortar from Tiger Hill Pagoda	$Ca(OH)_2$
	$Content_{Sucrose}$(g/L)	0	0	0
	Result	-	-	-
Earth	Sample	Earth from Wuyun Building-a	Earth from Wuyun Building-b	Earth
	$Content_{Sucrose}$(g/L)	0.216	0	0
	Result	+	-	-
$Ca(OH)_2$ + Earth	Sample	Tabia from Tianfeigong Fort	Tabia from Anyuan Fort	Tabia from Nanwan Fort-a
	$Content_{Sucrose}$(g/L)	0	0	0
	Result	-	-	-
	Sample	Tabia from Nanwan Fort-b	Tabia from Tiger Hill Pagoda	Tabia from Fuxin Building
	$Content_{Sucrose}$(g/L)	0	0	0
	Result	-	-	-
	Sample	Tabia from Chengqi Building	Tabia from Yude Building-a	Tabia from Yude Building-b
	$Content_{Sucrose}$(g/L)	0	0	0
	Result	-	-	-
	Sample	Tabia from Qingshan Temple-a	Tabia from Qingshan Temple-b	$Ca(OH)_2$+Earth
	$Content_{Sucrose}$(g/l)	0	0	0
	Result	-	-	-

identified sucrose in one sample, distinguished sucrose from polysaccharides in another sample and denied false-positive chemical results in the other three samples.

To sum up, enzymatic analyses have yielded satisfying results in that it was sensitive and exclusive, therefore we recommend this method to identify sucrose in ancient mortars due to low detection limits and simple operations.

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