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PREPARATION OF GLUCONIC ACID BY OXIDATION OF GLUCOSE WITH HYDROGEN PEROXIDE

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ABSTRACT

Hydrogen peroxide (H_2O_2) is a strong oxidant that oxidizes the hemiacetal hydroxyl to carboxyl group in glucose. In this study, gluconic acid was prepared by oxidation of glucose with H_2O_2, and the pH of the mixture was monitored during the preparation. The pH of the mixture was affected by the reaction time, temperature and H_2O_2 concentration. Under the optimal conditions, i.e. reaction time of 70 min, reaction temperature of 80C and 12% H_2O_2, the minimum pH of the reaction mixture was 3.28. Extended reaction times, high temperatures and high H_2O_2 concentrations did not further decrease the pH of the mixture. High-performance liquid chromatography and liquid chromatography-mass spectrometry indicated that the resulting product contained 79.02% gluconic acid and 14.48% glucose. Results demonstrated that the oxidation of glucose with H_2O_2 could be a promising method for the preparation of gluconic acid.

PRACTICAL APPLICATIONS

Gluconic acid can be prepared by oxidation of glucose with H_2O_2 and could be of practical food and medicine applications.

INTRODUCTION

Gluconic acid and its salts are important compounds that are extensively used in pharmaceutical, food and chemical industries. For the production of gluconic acid, biocatalytic, stoichiometric chemical, homogeneous and heterogeneous catalytic and fermentation methods have been applied (Witońska *et al.* 2011; Mafra *et al.* 2015; Qi *et al.* 2015). At present, biochemical processes are the dominant methods for gluconic acid production. However, biochemical processes have many disadvantages, i.e. difficulty in product purification, waste removal and low yield. Thus, new methods for gluconic acid preparation to eliminate the drawbacks are of urgent need.

Hydrogen peroxide (H_2O_2) is used to hydrolyze polysaccharides, such as cellulose, starch and hemicellulose, because it is easy to handle, readily available and environmentally friendly (Chang *et al.* 2001; Qin *et al.* 2002; Shao *et al.* 2003). This technique is based on the formation of free radicals, which can attack the glucosidic linkages of the polysaccharides. However, whether H_2O_2 can oxidize the hemiacetal hydroxyl to the carboxyl group in glucose remains uncertain.

In the present study, gluconic acid was prepared by hydrolysis of glucose using H_2O_2, in which the reaction conditions were optimized and products were characterized.

MATERIALS AND METHODS

Materials

H_2O_2 (30%, vol/vol) was purchased from Laiyang Kant Chemical Co., Ltd. (Laiyang, China). Glucose was purchased from Fuchen Chemical Reagents Co. (Tianjin, China). All other chemicals used were of reagent grade.

Preparation of Gluconic Acid

Glucose was dissolved in distilled water to yield a solution with a concentration of 2% (wt/vol). Varying concentrations of H_2O_2 (3%, 6%, 9%, 12%, 15% and 18%) were added to the solution, and the reactor was incubated in a thermostatic water bath at different temperatures (70C, 75C, 80C, 85C, 90C and 95C) for designated time periods

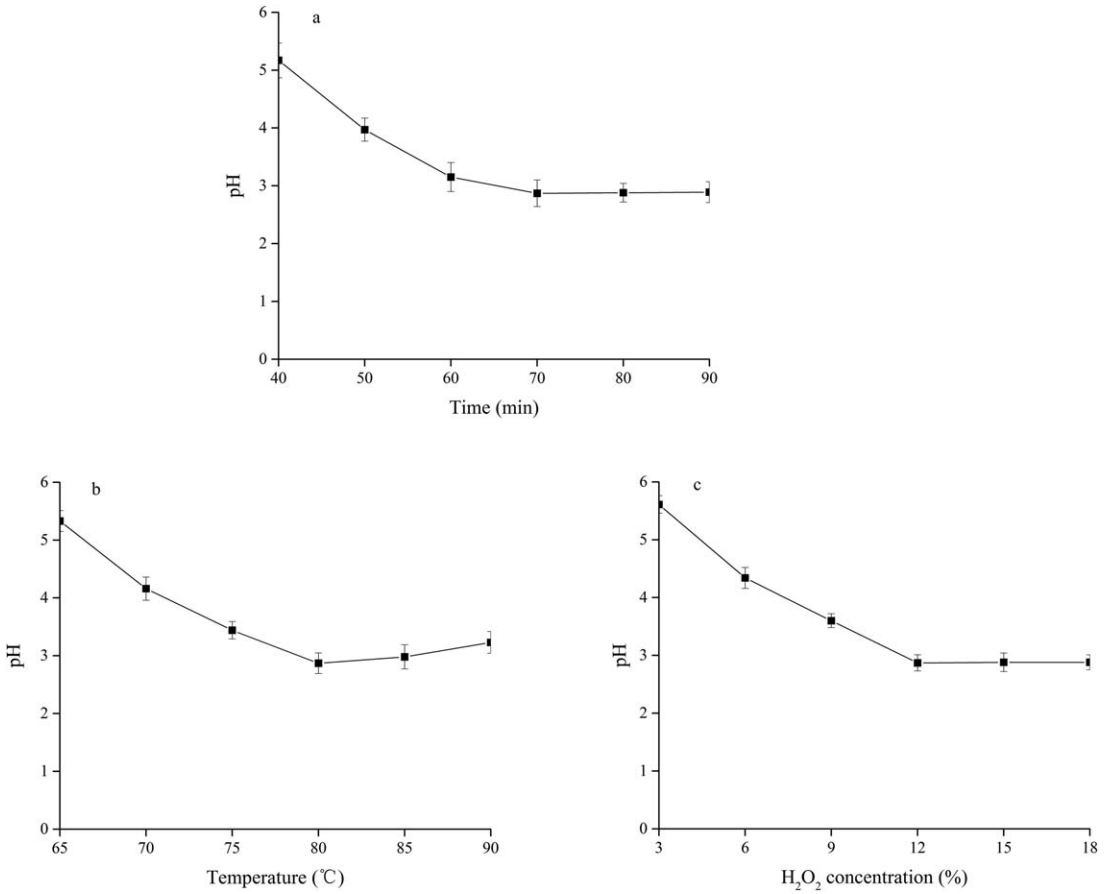

FIG. 1. EFFECT OF TIME, TEMPERATURE AND H_2O_2 On pH. BARS REPRESENT THE STANDARD DEVIATION (SD). DATA ARE SHOWN AS THE MEAN ± SD ($N = 3$)

(40, 50, 60, 70, 80 and 90 min). Aliquots of the suspension were periodically withdrawn and cooled below 10C to terminate the reaction.

The reactants were concentrated to ∼15% and freeze dried.

Analytical Methods

The pH of the solution was recorded using a digital pH meter (Model: PHS-3C, CD Instruments, China). Ash, moisture, total sugar and protein contents of the samples were determined according to standard methods (Hou 2004). The reducing sugars were estimated by the Somogyi method (Nelson 1944).

The composition of sugar in the resulting products was analyzed by Water600 high-performance liquid chromatography (HPLC) equipped with a double-column system (LC-10A, Shimadzu, Japan). The first column (Sugarpark 1, 6.5 mm id × 300 mm) used pure water as mobile phase at a flow rate of 0.5 mL/min, and the column temperature was maintained at 85C. The second column (SpherisorbNH_2, 4.6 mm id × 250 mm) used acetonitrile/water (70/30, vol/vol) as mobile phase at a flow rate of 1 mL/min, and the column temperature was 30C. The detector sensitivity was 4 μRIU, and the injection volume was 10 μL.

Liquid chromatography-mass spectrometry (LC-MS, WATERS ACQUITY UPLC, USA) with a detector (WATERS ACQUITY PDA, USA) and column (BEHC18 2.1 × 100 mm, 1.7 μm) was used to determine gluconic acid. The mobile phase consisted of acetonitrile (Solvent A) and formic acid (Solvent B) at a flow rate of 0.3 mL/min. The column temperature was maintained at 45C, and the injection volume was 1 μL. The gradient profile was conducted as follows: 5% A and 95% B for 20 min, 30% A for 25 min, 100% A for 26 min and then 5% A and 95% B for 30 min. WATERS MALDI SYNAPT Q-TOF MS with an electrospray ionization interface was used to acquire total ion chromatogram and fragmentation information. Running parameters were set as follows: capillary voltage, 3,000 V; cone voltage, 30 V; source block temperature, 100C; desolvation temperature, 400C; desolvation gas flow, 500 L/h; cone gas flow, 50 L/h; collision energy, 6 eV; mass range, 50–1,000 m/z; and detector, 1,800 voltage. The system operation, data acquisition and analysis were controlled and processed by

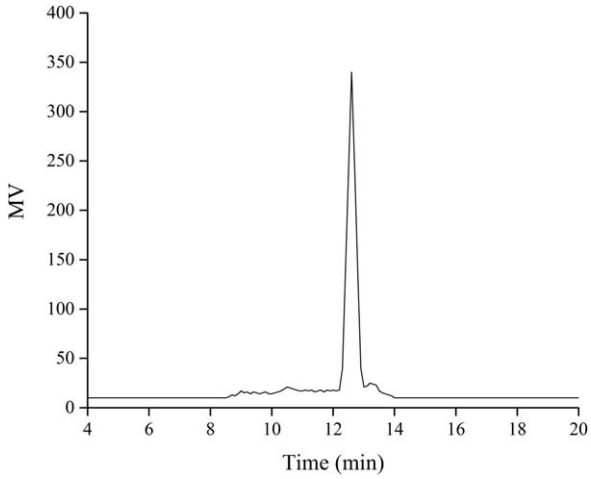

FIG. 2. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY SPECTRA OF SAMPLE SUGAR COMPOSITION

MassHunter software. The percentage yield of gluconic acid obtained was calculated using Eq. (1).

$$\text{Yield} = 100\ W_2/W_1 \quad (1)$$

where W_1 and W_2 represent the weights of the recovered gluconic acid and the original glucose, respectively.

Statistical Analysis

All experiments were carried out in triplicate. All data are presented as the mean ± standard deviation (SD). ANOVA was used to compare data between groups. A P value of <0.05 was considered statistically significant.

RESULTS AND DICUSION

Effect of Time, Temperature and H_2O_2 Concentration on pH

Glucose can be converted into gluconic acid by oxidizing the hemiacetal hydroxyl in glucose to the carboxyl group in gluconic acid with H_2O_2. The carboxyl group in gluconic acid in water partly dissociated to provide hydrogen proton, and the pH of the reaction mixture decreased. Thus, the pH of the reaction mixture could be used as an index of the reaction. The reaction time, temperature and H_2O_2 concentration are important parameters to ensure efficient oxidation of the hemiacetal hydroxyl in glucose. Therefore, the effects of different reaction times, temperatures and H_2O_2 concentrations on pH were investigated. The reaction was found to be optimal at a reaction time of 70 min (Fig. 1), temperature of 80C (Fig. 2) and H_2O_2 concentration of 12% (Fig. 3). Prolonged time and excessive H_2O_2 amount did

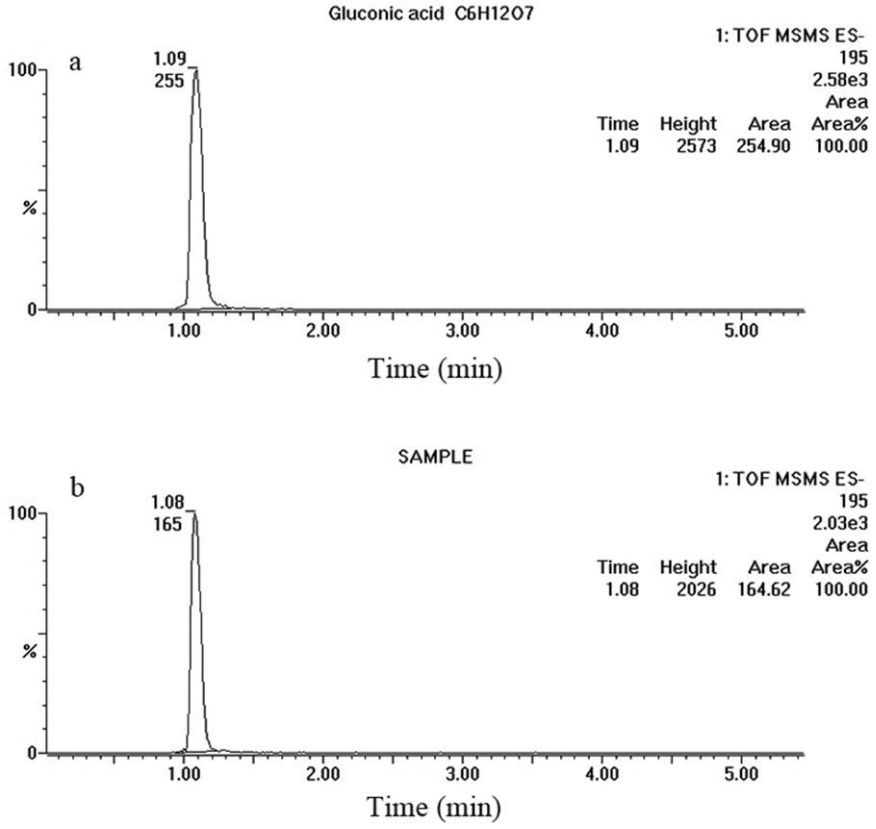

FIG. 3. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY SPECTRA OF SAMPLE AND STANDARD GLUCONIC ACID

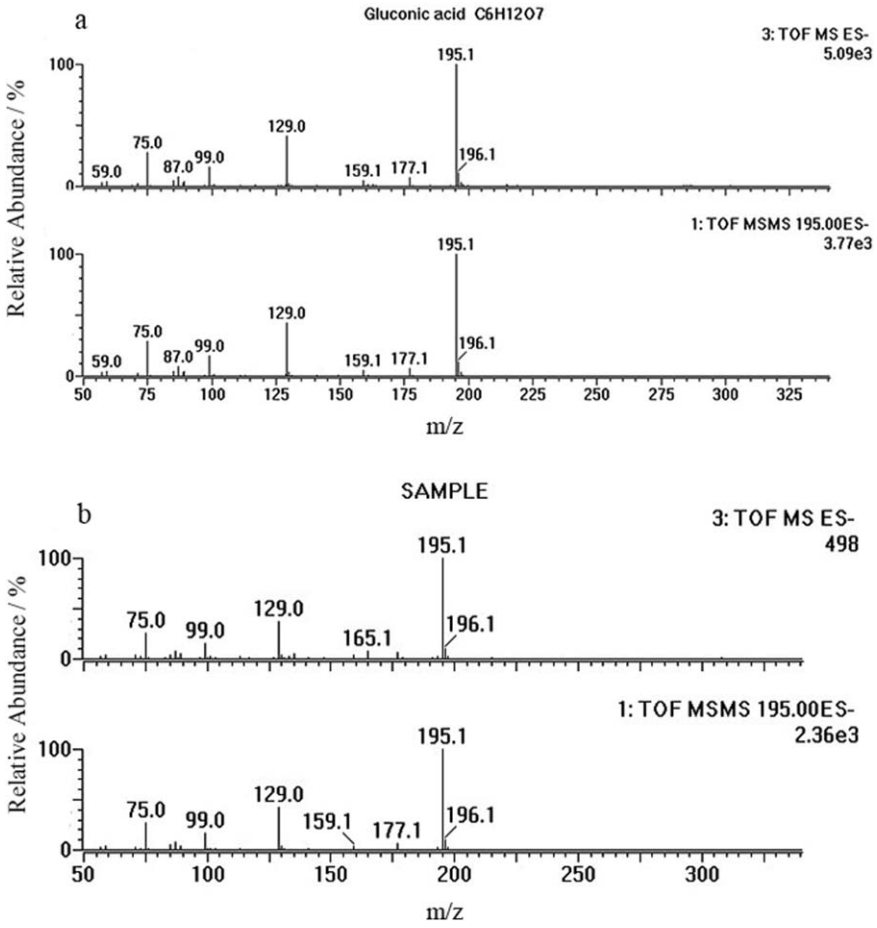

FIG. 4. LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY SPECTRA OF SAMPLE AND STANDARD GLUCONIC ACID

not decrease pH further. Simultaneously, high temperature increased the pH of the reaction mixture. This result could be attributed to the excessive oxidation of H_2O_2, which damaged the carboxyl group in gluconic acid. In contrast to these results, the optimal reaction time, temperature and H_2O_2 concentration for the hydrolysis of *Rhizoma Phragmites* polysaccharides using H_2O_2 are 40 h, 75C and 3.5% H_2O_2, respectively (Qian 2014). Differences in the reported optimal reaction times, temperatures and H_2O_2 concentrations could be attributed to the differences in the substrate source and reaction type.

Product Characterization

The ash, moisture and total sugar contents in the product were 2.79%, 2.11% and 4.51%, respectively, indicating that the main compositions of the products were not ash, moisture and sugar. The reducing sugar content in the product was 14.48%. Based on the reaction substrate and properties, we speculated that the main composition of the product could be gluconic acid, and glucose could be the main sugar. The composition of sugar in the resulting products was analyzed by Water600 HPLC, and the results are shown in Fig. 2. The HPLC spectrum peaked at 12.57 min, similar to the retention time of glucose, suggesting that glucose was the sugar composition of the product, which was consistent with the reaction substrate. The presence of gluconic acid in the product was determined by LC-MS, and the results are shown in Figs. 3 and 4. Both the standard gluconic acid and the product sample showed only one peak at approximately 1.05 min (Fig. 3), indicating that gluconic acid could be a composition of the product. Both the standard gluconic acid and product sample showed identical peak characteristics, which verified that gluconic acid was a composition of the product. Based on the concentrations and peak areas of the standard gluconic acid and product sample, gluconic acid in the product sample was 79.02%.

CONCLUSIONS

Gluconic acid was prepared by oxidation with H_2O_2, and the yield was affected by the reaction time, temperature and H_2O_2 concentration. Under the optimal reaction conditions, the yield of gluconic acid reached 79.02%, indicating

that oxidation of glucose with H_2O_2 could be a promising method for the preparation of gluconic acid. However, further studies should be performed to increase the yield of gluconic acid.

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