

Modulation of external factors to improve the efficiency conversion of isoquercitrin by amylosucrase from *Deinococcus* geothermal conversion of isoquercitrin



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Introduction

In addition to nutrients such as proteins, lipids, and carbohydrates, a rich variety of secondary metabolites, such as flavonoids, terpenoids, alkaloids, and phenylpropanoids, are also present in mulberry, of which mulberry flavonoids have attracted much attention as important active substances and have become one of the hotspots in the field of research on the utilization of sericulture resources today. One of the typical flavonoids, quercetin, has a variety of biological functions such as anti-inflammatory and antioxidant. However, quercetin has low solubility in water, low bioavailability, and limitations in its use. Isoquercitrin (quercetin 3-O- β -D-glucoside) is a representative glycoside of quercetin. However, the inherent physicochemical properties of isoquercitrin have limitations in the food and pharmaceutical industries. Studies have shown that enzymatically prepared glucosyl isoquercitrin (EMIQ) with an α -1,4 glycosidic linkage has better solubility and bioavailability than quercetin and isoquercitrin.

Methods

EMIQ produced by ASase from *Deinococcus* geo-thermalis (DGAS) has the advantages of high yield, simple process and low by-products. The preparation of EMIQ using DGAS eliminates the starch glycation and secondary enzyme processing steps normally required in CGTase-related processes. DGAS can link donor glucose molecules to various acceptor molecules by forming α -1,4-glycosidic bonds, resulting in a variety of bioactive compounds.

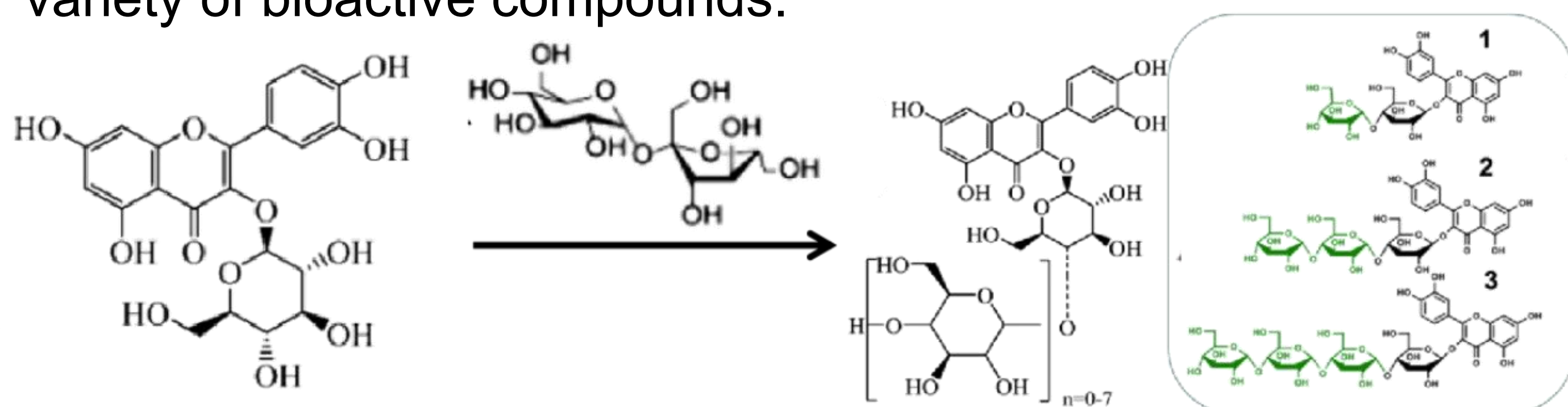


Fig.1 Reaction diagram of EMIQ

Results & Discussion

In this study, DGAS was clonally expressed in *E. coli* BL21, and the enzyme solution was purified by Ni-TED, and the enzymatic activity of DGAS was measured by the dinitrosalicylic acid method (DNS). Figure 2A illustrates the enzymatic properties of DGAS measured under standard conditions and shows that the enzyme has the highest activity at 50°C, while the enzymatic activity is above 65% in the range of 40°C-55°C. Figure 2B shows the optimum pH for DGAS, showing the highest enzyme activity at pH 8, while the enzyme activity was around 80% in the pH range of 7.0-9.0. Therefore, the optimum conditions, a temperature of 50°C and a pH of 8.0, were chosen for the catalytic reaction.

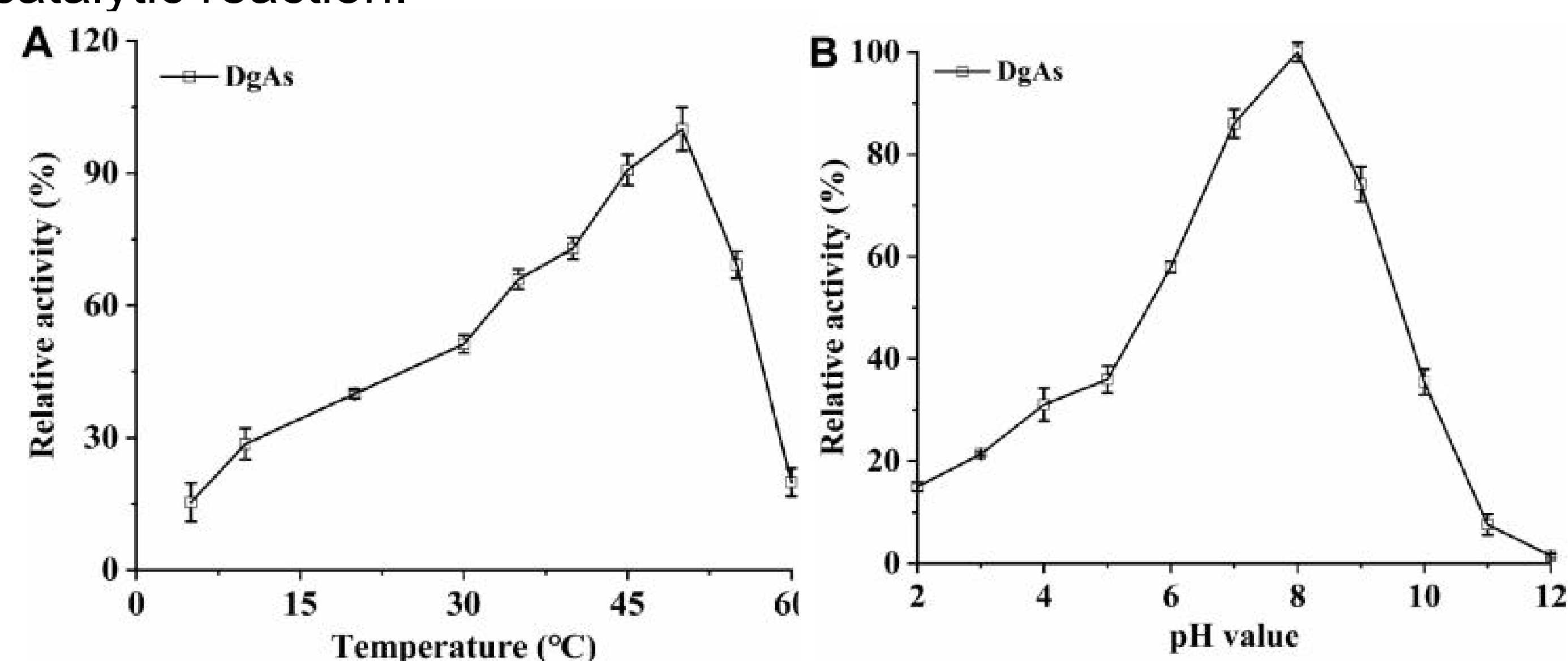


Figure.2 (A) Optimum temperature for DGAS; (B) Optimum pH for DGAS.

Under the same phosphate conditions of controlling low sucrose concentration and isoquercitrin concentration, common buffer solutions such as Citrate phosphate (CP), Sodium phosphate (SP) and Tris-HCl (TH) were chosen to control the pH of 8.0 and catalyze the reaction at 50°C for 24h. Figure 3A shows the results of reveal different products catalyzed under different buffer conditions. The catalytic efficiency of TH in the different buffers was much less than that of CP and SP. Under the conditions of SP, the conversion of isoquercitrin reached 48.84%; under the conditions of CP, the conversion of isoquercitrin reached 54.68%. As shown in Figure 3B, no IQ-G4 was produced under TH conditions, and the conversion rates of each substance were similar under SP and CP conditions. It is speculated that phosphate may inhibit enzyme catalysis to some extent during biocatalysis, and phosphate buffer has less buffering capacity above pH 7.5, hence low conversion relative to the buffer of CP. TH buffer is influenced by temperature, which is 50°C for this catalytic reaction, and high hydrogen ion concentration.

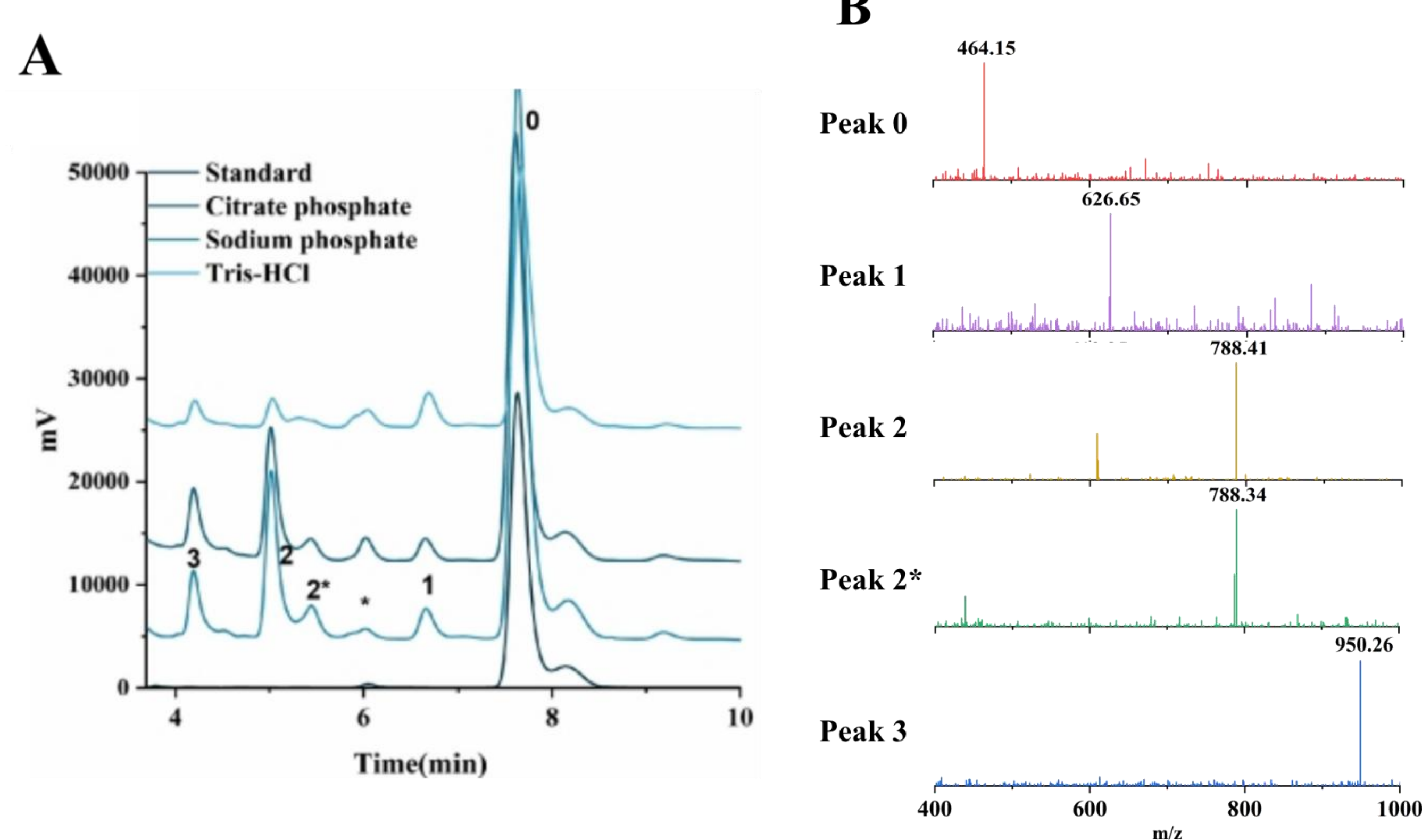


Figure.3 (A) HPLC was used to analyze the products DGAS catalytic reaction; (B) LC-MS products of DGAS catalytic reaction; Peaks: *. Rutin; 0. IQ (isoquercitrin); 1. IQ-G1; 2 and 2*. IQ-G2 (quercetin-3-O-triglucosides); 3. IQ-G3 (quercetin-3-O-tetraglucosides).

Conclusions

- As a result, it is hypothesized that DGAS catalyzes isoquercitrin best under the conditions of Citrate phosphate.

Acknowledgements

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