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

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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 (Dabili et al., 2019), 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. Mulberry flavonoids have important applications in the prevention and treatment of diabetes, cardiovascular diseases, tumors, and other diseases. 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 (Makino et al., 2009, Rogerio et al., 2007). Isoquercitrin (quercetin 3-*O* -*β*-*D*-glucothioside) 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.

Amylose sucrase (ASase, EC.4.2.1.4) is a multifunctional glycosyltransferase belonging to the glycoside hydrolase family 13 (GH13). (Skov et al., 2001) In the GH13 family, ASases not only use sucrose as a glucosyl donor for the synthesis of straight-chain amyloid polymers, but also specifically target a variety of acceptor molecules in response to transglycosylation. Compared to cyclodextrin glucanotransferase (CGTase), which is often used in traditional 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. DGAcanto link donor glucose molecules to various acceptor molecules by forming alpha-1,4-glycosidic bonds, resulting in a variety of bioactive compounds (Owczarek-Januszkiewicz et al., 2022). As a result of these reactions, DGAS produces a variety of trans-glycosylation products, including biologically active flavonoids. The length of the transferred glucose units may vary depending on chemical and physical properties such as solubility, location of free -OH and pKa of the free -OH of the glucose acceptor.

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 1A 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 1B 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.

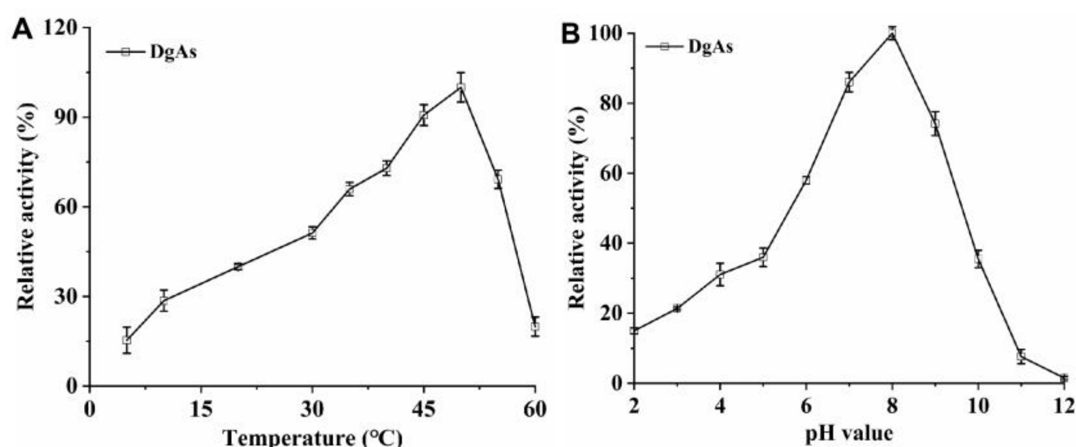

Figure.1 (A) Optimum temperature for DGAS; (B) Optimum pH for DGAS.

Under the same 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 2A shows the analysis by HPLC-UV spectroscopy at 254 nm, the results show 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 2B, 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. During the catalytic process of DGAS, sucrose is hydrolyzed into glucosyl and fructose fractions (Rha et al., 2021), while H^+ may inhibit glucose and isoquercitrin binding. In conclusion, it is hypothesized that DGAS catalyzes isoquercitrin best under the conditions of Citrate phosphate.

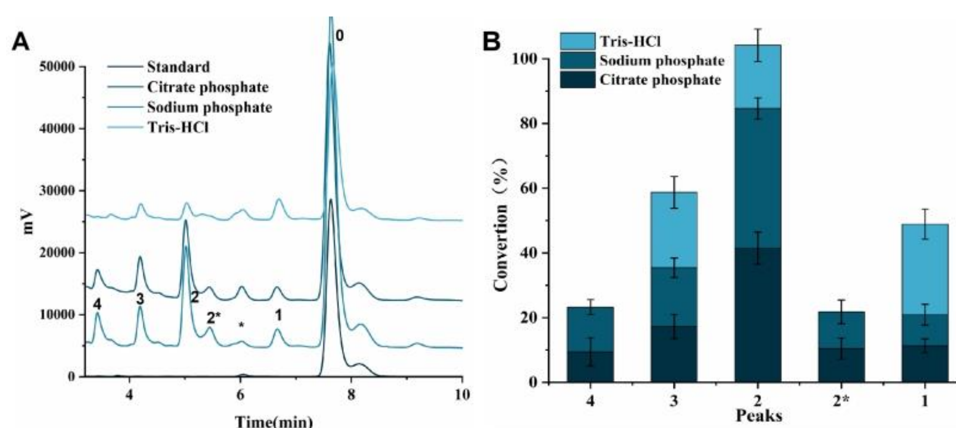

Figure.2 (A) HPLC was used to analyze the products DGAS catalytic reaction; (B) Conversion rates of the products after using different buffers; Peaks: *. Rutin; 0. IQ (isoquercitrin); 1. IQ-G1; 2 and 2*. IQ-G2 (quercetin-3-O-triglucosides); 3.IQ-G3(quercetin-3-O-tetraglucosides); and 4. IQ-G4(quercetin-3-O-pentaglucosides).

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