Supplementary information

Colorimetric detection of hydrogen peroxide and glucose by exploiting the peroxidase-like activity of papain

Yuye Chen [a], Qingmei Zhong [a], Yilin Wang*[a, b], Chunling Yuan [a], Xiu Qin [a], and Yuanjin Xu [a]

([a] School of Chemistry and Chemical Engineering, Guangxi University, Guangxi Key Laboratory of Biorefinery, Nanning 530004, China

[b] Guangxi Key Laboratory for Agro-Environment and Agro-Product Safety, Nanning 530004, China)

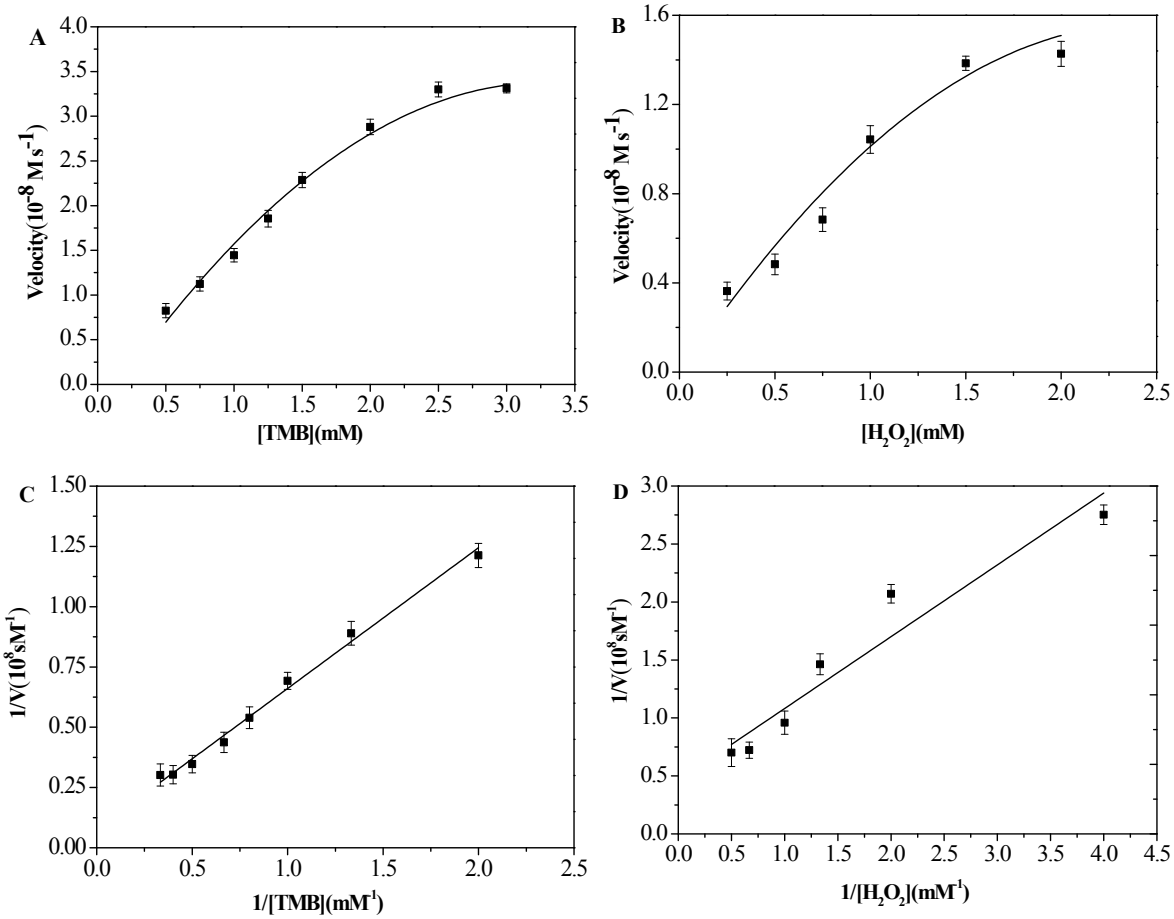

Fig. S1. Steady-state kinetic assays of papain using Michaelis–Menten model, (A) at a constant concentration of H_2O_2 [5.0 mM] while TMB concentration was varied from 0.5 to 3.0 mM and (B) at a constant concentration of TMB [1.0 mM] while H_2O_2 concentration was varied from 0.25 to 2.0 mM. (C) Double-reciprocal plots of initial velocity versus TMB concentration and (D) Double-reciprocal plots of initial velocity versus H_2O_2 concentration.

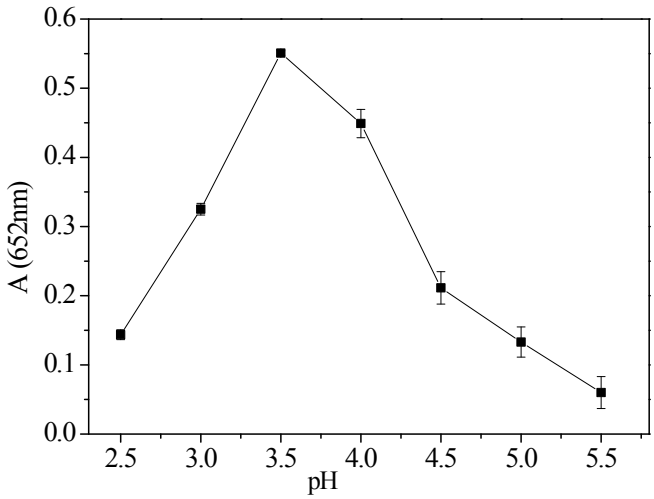

Fig. S2. Effect of pH on the absorbance of the reaction system. (other conditions: 100 μL of 100 μg/mL papain; 1.0 mL of 5.0 mM TMB; 1.0 mL of 50μM H_2O_2; reaction temperature is 40℃; reaction time is 20 min.)

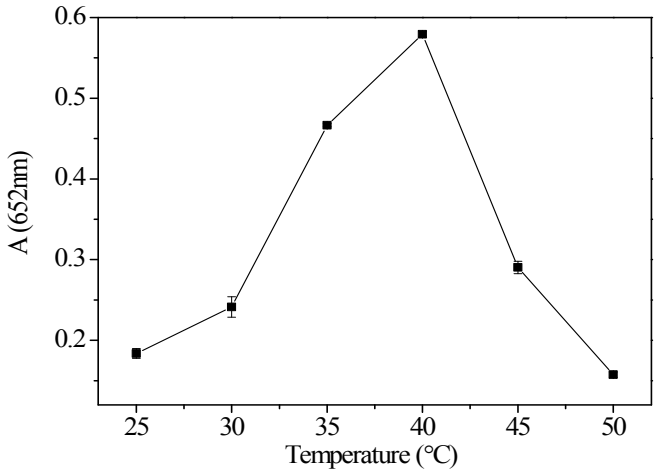

Fig. S3. Effect of temperature on the absorbance of the reaction system. (other conditions: 100 μL of 100 μg/mL papain; 1.0 mL of 5.0 mM TMB; 1.0 mL of 50 μM H_2O_2; pH=3.5; reaction time is 20 min.)

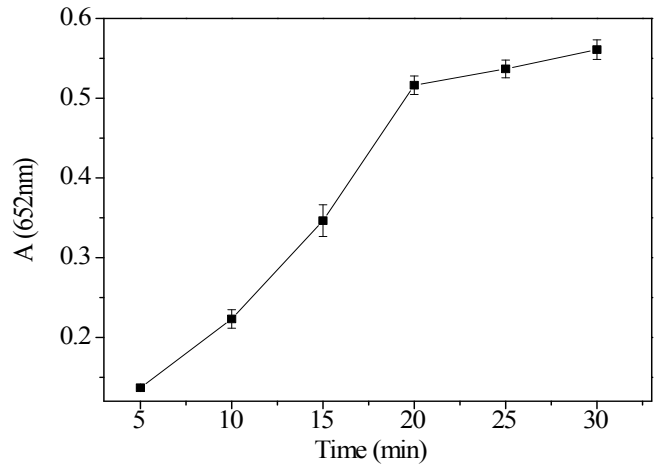

Fig. S4. Effect of reaction time on the absorbance of the reaction system. (other conditions: 100 μL of 100 μg/mL papain; 1.0 mL of 5.0 mM TMB; 1.0 mL of 50 μM H_2O_2; pH=3.5; reaction temperature is 40℃.)

Table S1. Comparison of kinetic parameters between papain and other catalysts

Catalysts	[*E*] (M)	K_m(mM)		V_{max}(10^{-8}M·s^{-1})		K_{cat} (s^{-1})		Reference
		TMB	H_2O_2	TMB	H_2O_2	TMB	H_2O_2	
HRP	2.27×10^{-11}	0.15	0.61	4.53	2.53	2.00×10^{3}	1.04×10^{3}	[37]
Ficin	4.20×10^{-8}	0.19	0.35	4.69	3.42	1.12	0.81	[37]
Papain	4.27×10^{-8}	6.94	1.29	12.03	2.11	2.82	0.49	This work

[*E*] is the enzyme concentration, K_m is the Michaelis constant, V_{max} is the maximum reaction rate, K_{cat} is the catalytic constant, where $K_{cat} = V_{max}/[E]$