

嘉南藥理學院專題研究計畫成果報告

計畫名稱：蔬果酪胺酸酶抑制劑之純化及其應用

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ABSTRACT

The citrus peel extract exhibited high tyrosinase inhibitor activity, IC_{50} was 1.67 mg/ml. The apparent molecular weight was estimated to be 2.5 KDa. From the studies of the reaction with ninhydrin, dye-binding reagent or silver staining on PAGE, the tyrosinase inhibitor from citrus peel was probably a peptide compound. The inhibitor exhibited a noncompetitive type for the oxidation of L-DOPA. The inhibitory mechanism of tyrosinase inhibitor from citrus peel was probably derived from the reduction of Cu^{2+} to Cu^{+} or reduction of O-quinone to L-DOPA.

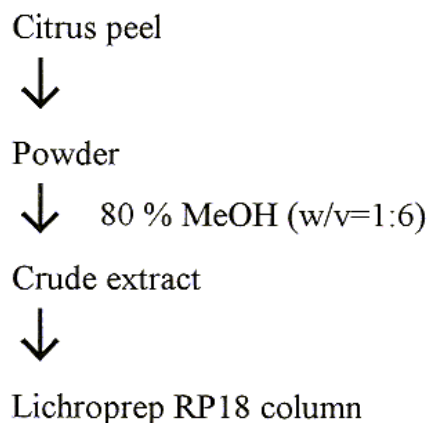
Key words: Tyrosinase inhibitor, Citrus peel, Browning reaction

INTRODUCTION

Tyrosinase(EC 1.14.18.1) play important role in the browning reaction of fruit and vegetable, and such enzymatic browning might further result in discoloration and reducing the nutritional value. In order to prevent the browning reaction, use of food additives including reducing agent, acidulants, and enzyme inhibitor (such as sulfite) had been developed. Among them, enzyme inhibitor from natural sources become more important in the recent approach. In this study, we found high tyrosinase inhibitor activity in the citrus peel (*Citrus poonensis* Hort.). Thus, the objective of present work is to purify and characterize the tyrosinase inhibitor from citrus peel and to correlate the tyrosinase inhibitor with browning reaction.

METHODS

1. Partial purification of tyrosinase inhibitor from citrus peel:



↓ H₂O
 ↓ 20% MeOH
 ↓ 80% MeOH
 active fraction in 80% MeOH elution
 ↓
 Sephadex LH-20 column
 ↓
 partially purified tyrosinase inhibitor

2. Assay of tyrosinase inhibitor activity

The activity of tyrosinase inhibitor was determined as the degree of inhibition on the tyrosinase-catalyzed oxidation of L-DOPA. The reaction mixture consist of the partially purified tyrosinase inhibitor (0.1 ml), mushroom tyrosinase (125 units, *Agaricus bisporus* from Sigma) 25 mM phosphate buffer (0.8ml,pH 6.8) and L-DOPA (3.8 mM) . The reaction was performed at 25°C, and the absorbance was assay at 475nm for 5 min.

The relative inhibition (%) = $(1 - \Delta OD_{475} \text{ in sample} / \Delta OD_{475} \text{ in blank}) * 100\%$

CONCLUSION

Table 1. Some properties of tyrosinase inhibitor from citrus (*Citrus poonensis* Hort.) peel.

Characteristics	
*IC ₅₀ (crude extract)	1.67 mg/ml
Optimal preincubation time	30 min
Optimal preincubation temperature	37°C
Optimal preincubation pH	5.5
Molecular weight	2.5KD
Constitute	peptide compound

* IC₅₀, inhibitory concentration caused 50% inhibition of the tyrosinase catalyzed oxidation of L-DOPA

Table 2. Inhibitory mechanism of tyrosinase inhibitor from citrus peel.

Inhibition mechanism	Properties
Type of inhibition	noncompetitive
Reduction of Cu^{2+} and Cu^+	positive
Reduction of O-quinone to L-DOPA	positive



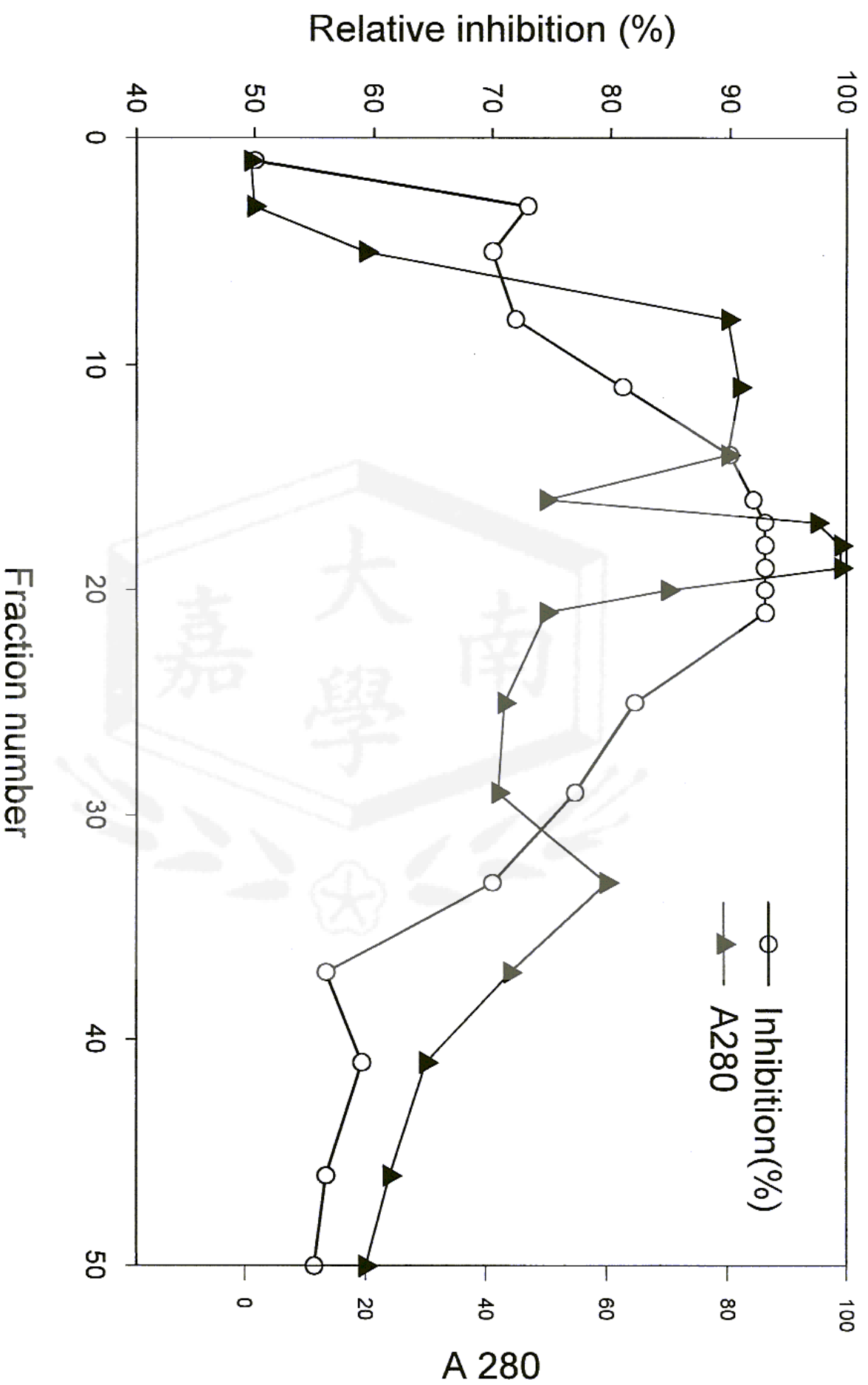


Fig. 1. Elution profile of the tyrosinase inhibitor from citrus peel on Lichroprep RP-18 column(3.8 x 47.0 cm) chromatography.

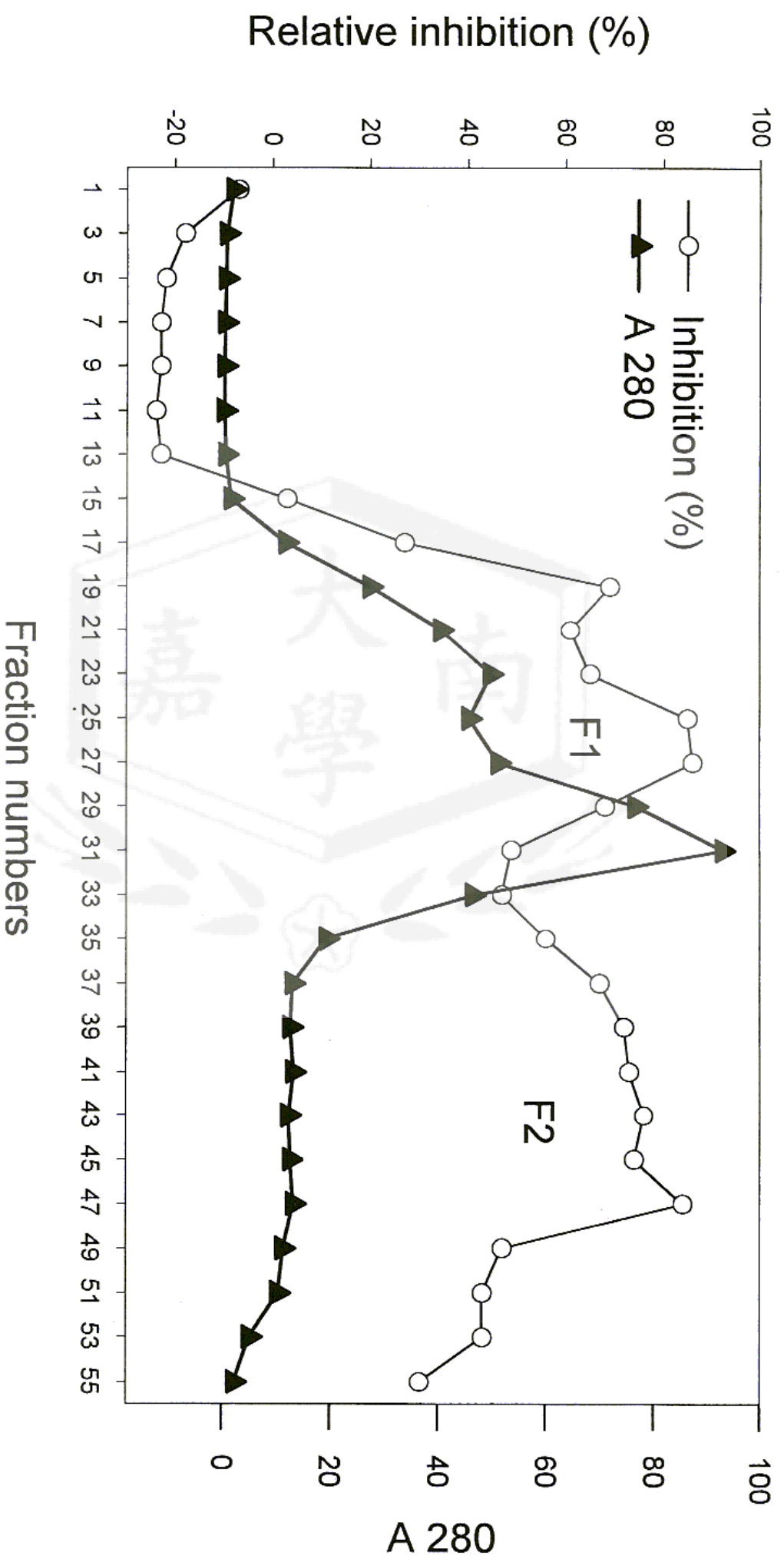


Fig. 2. Elution profile of the tyrosinase inhibitor from citrus peel on Sephadex LH-20 column (2.7 x 67.0 cm) chromatography.

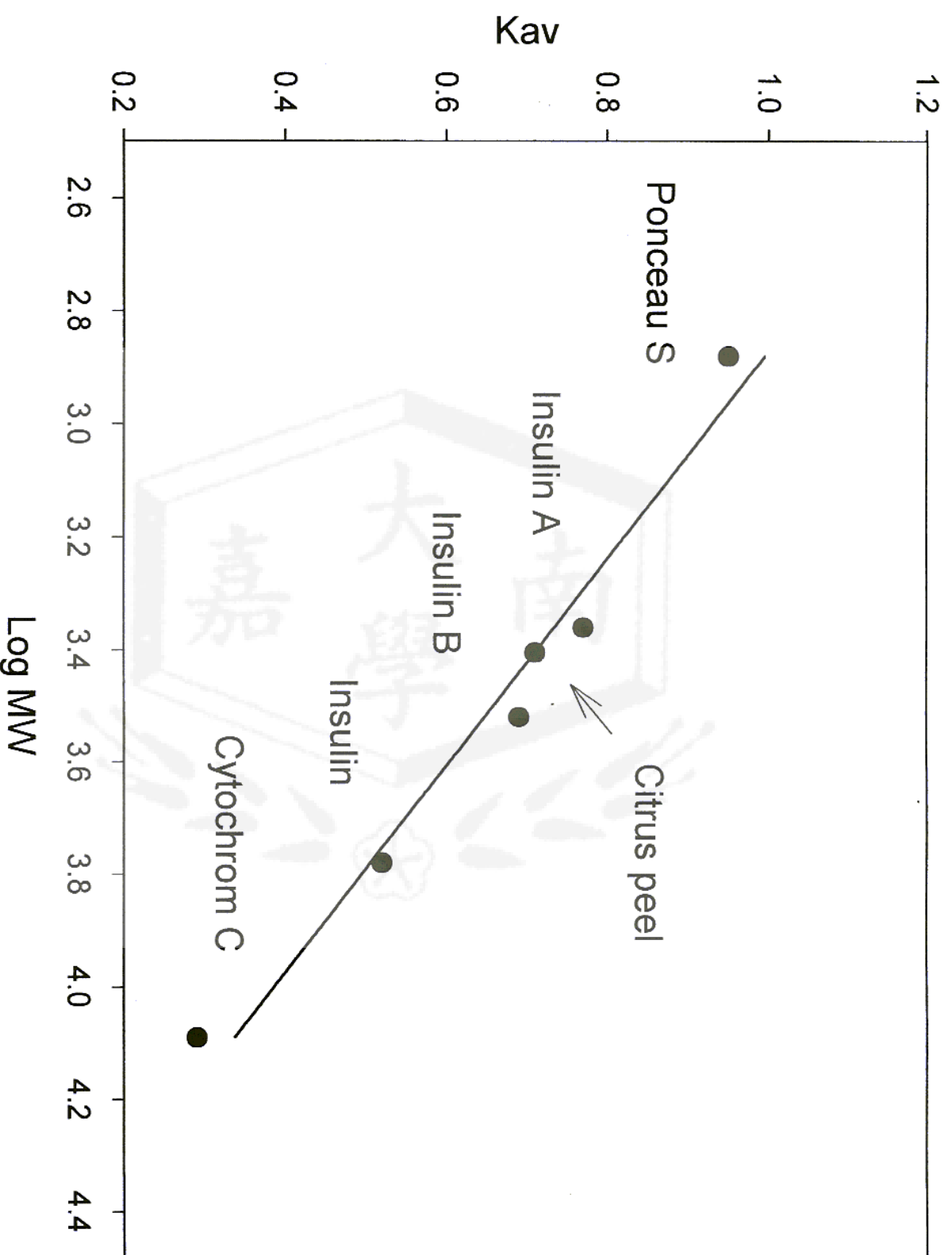


Fig. 3. Determination the molecular weight of tyrosinase inhibitor from citrus peel by Sephadex G-50 column chromatography.

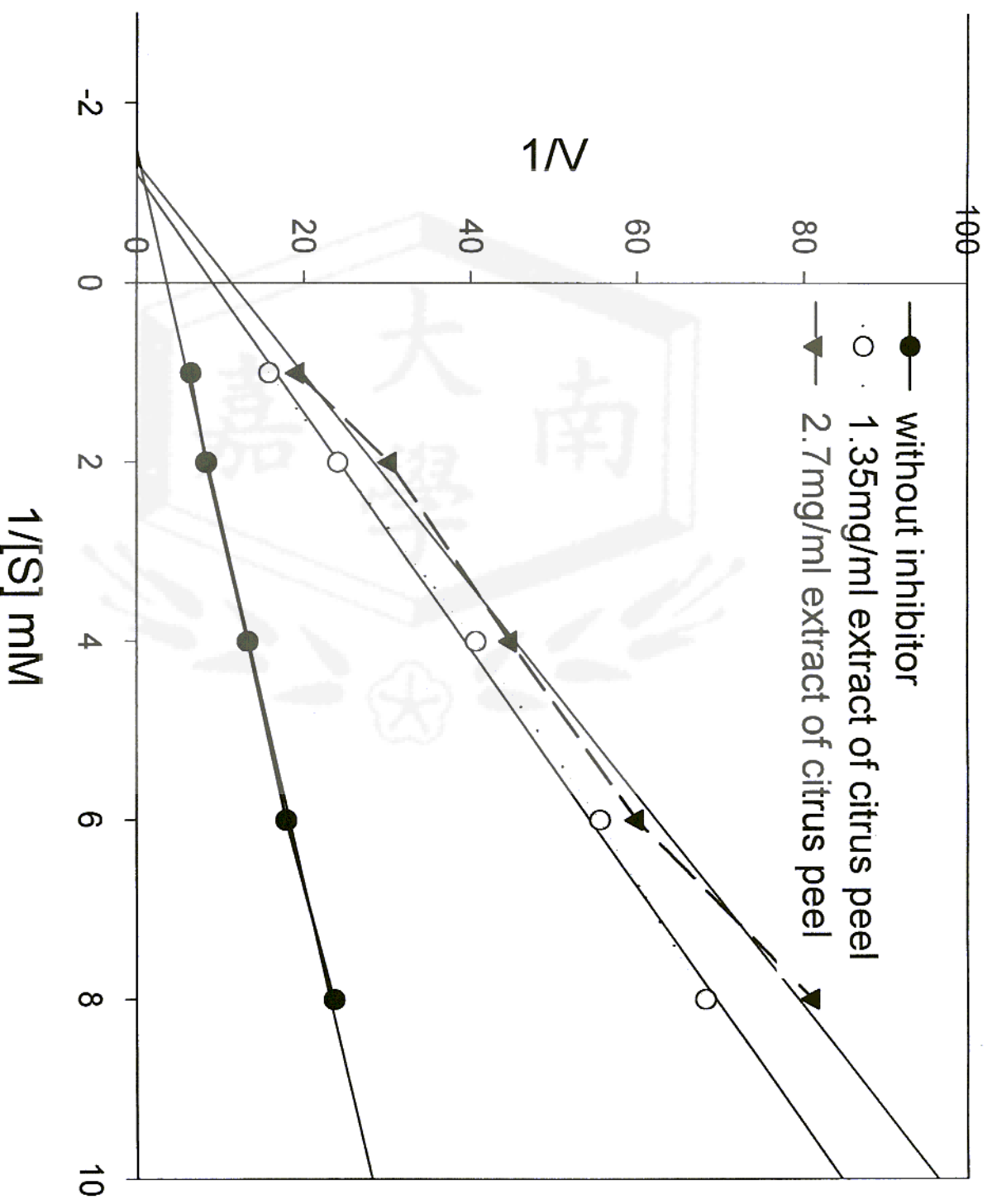


Fig. 4. Lineweaver-Burk plots of mushroom tyrosinase and L-DOPA without(●) or with (○)1.35mg/ml, (▼)2.7mg/ml tyrosinase inhibitor from citrus peel.

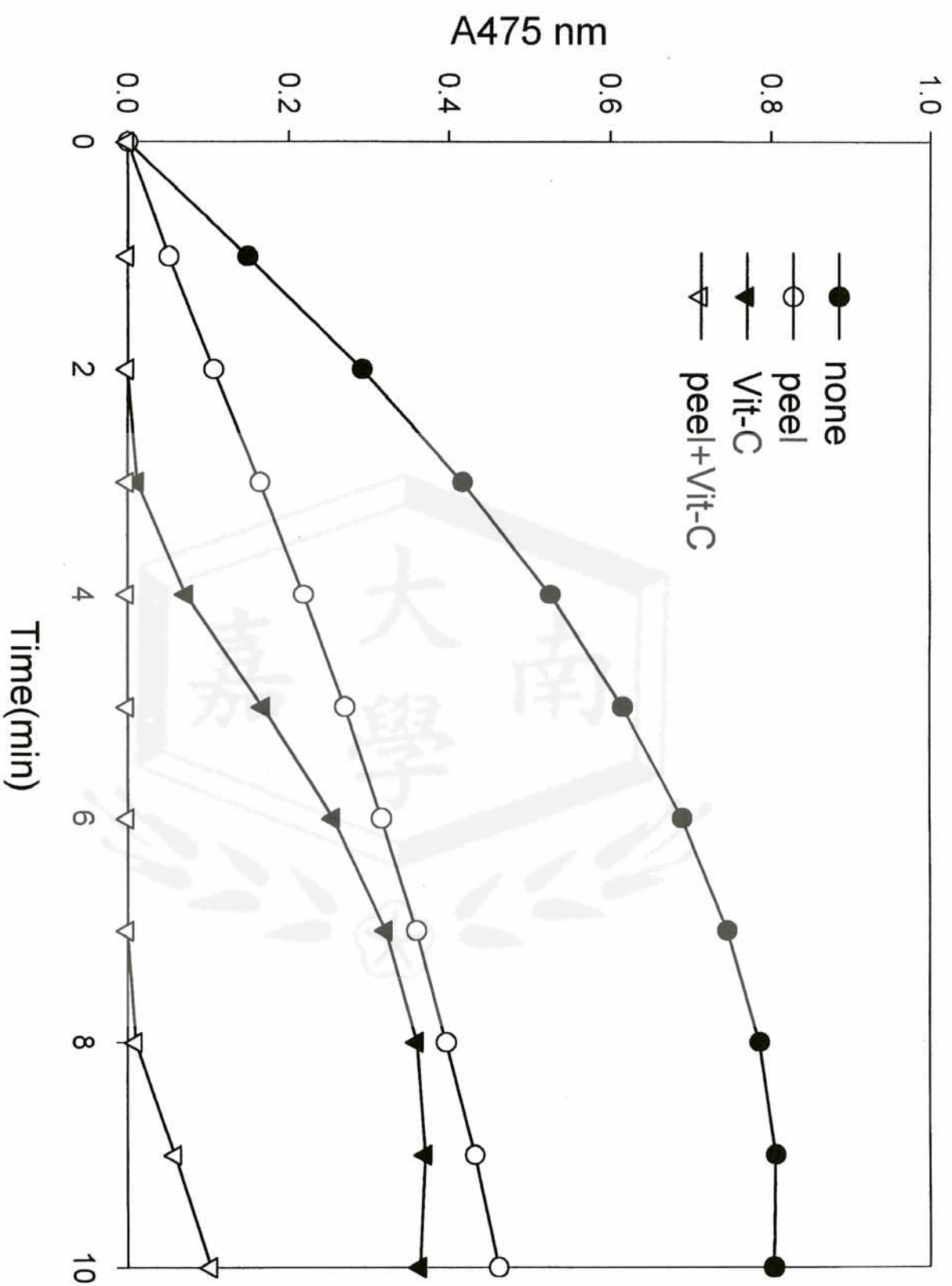


Fig. 5. Effect of L-ascorbic acid (0.3 mM) and tyrosinase inhibitor from citrus peel.