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Research Article

Data Analysis on Properties of Polygalacturonase Purified and Separated From Kiwifruit

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Abstract: In this study, characteristics and enzyme activities of Polygalacturonase Purified and Separated from kiwifruit were studied. The study used chemical analysis and computer data analysis methods to make clear the best reaction temperature, best pH value to keep the Polygalacturonase activity and the inhibition activity of Polygalacturonase on certain metal ions were also investigated. The experiments results showed that the best temperature ranges for keeping enzyme activity were 30-50°C and the best pH value ranges were pH4.5-6.5. And the tests also showed that Polygalacturonase has obvious inhibition activities for many metal ions.

Keywords: Data analysis, kiwifruit, polygalacturonase

INTRODUCTION

Kiwifruit is native to northern China. Other species of Actinidia are native to India, Japan and southeastern Siberia. Cultivation of the fuzzy kiwifruit spread from China in the early 20th century to New Zealand where the first commercial plantings occurred. The fruit was called "yang tao" but was changed to "Chinese gooseberry" by the New Zealanders (Li, 2014). It proved popular with American servicemen in New Zealand during World War II. Because of this the fruit was exported to California using the names "Chinese gooseberry" and "melonette". Because the California based importer rejected these names the fruit was re-branded "kiwifruit" after the kiwi (bird) as the bird and the fruit share a similar appearance and both are associated with New Zealand. Kiwifruit has since become a common name for all commercially grown fruit from the family Actinidia (Brummell, 2006).

Polygalacturonase (EC 3.2.1.15, pectin depolymerase, pectinase, endopolygalacturonase, pectolase, pectin hydrolase, pectin polygalacturonase, endo-polygalacturonase, poly-alpha-1, 4-galacturonide glycanohydrolase, endogalacturonase, endo-D-galacturonase, poly (1, 4-alpha-D-galacturonide) glycanohydrolase) is an enzyme produced in plants which is involved in the ripening process and by some bacteria and fungi which are involved in the rotting process (Mao, 2007). PGs degrades polygalacturonan present in the cell walls of plants by hydrolysis of the glycosidic bonds that link galacturonic acid residues (Luo, 2007). Polygalacturonan is a significant carbohydrate component of the pectin network that comprises plant cell walls. The activity of the endogenous plant PGs work to soften and sweeten fruit during the ripening process. Similarly, phytopathogens use PGs as a means to weaken the pectin network, so that a host of digestive enzymes can be excreted into the plant host to acquire nutrients (Gayathri and Nair, 2014). Polygalacturonase plays an important role in the maturation and softening of fruit. Therefore, how to control the maturation and softening of fruit through controlling the activity of Polygalacturonase is of important significance (Li *et al*., 2014). This study is for the nature of Kiwifruit's Polygalacturonase, further knowing its Properties and inhibiting/slowing down the increase of Polygalacturonase activity in its pulp tissue can help enhance the ability and storability to resist external adverse factors and also extend its shelf life.

MATERIALS AND METHODS

Materials: Kiwifruit was picked in Qianshan mountain of Anshan in China's Liaoning Province and stored in refrigerator at 4°C.The physiological indicators of the test Kiwifruit material selected in the experiment were as shown in Table 1.

Enzyme separating and purifying: Fresh Kiwifruit was taken and washed. Then, it was grinded into powder with liquid nitrogen together in a pre-cooling mortar. 50 mmol/L pH5.5 acet-sodium acetate buffer solution (including NaCl and DTT) was added into the powder and placed at 4°C, then extracted for 1h, centrifuged for 20 min at 4°C. The collected liquid supernatant was crude enzyme. Crude enzyme fluid was purified by ammonium sulfate, CM Sepharose Fast Flow and Sephadex G-75. Then, high Polygalacturonase activity was collected and dialyzed, frozen and concentrated.

Table 1: Physiological indicators of Kiwifruit

Picking date	Hardness (kg/cm^2)	Soluble solids (%)
2012.9.4	2.44±0.02	9.72±0.03

Polygalacturonase activity measurement: That is, DNS Colorimetry was applied. The optimum reaction temperature of Polygalacturonase. Polygalacturonase was added in polygalacturonic acid sodium salt reaction system for 90 min at 30, 35, 40, 45, 50, 55, 60, 65, 65°C, respectively. The effect of different temperature on the relative Polygalacturonase activity was studied (Xu and Su, 2013).

Thermal stability of Polygalacturonase: In order to study thermal stability of the enzyme, the purified enzyme were placed in five thermostat water baths, respectively for 90 min which are set at 30°C, 40°C, 50°C, 60°C and 70°C and the enzyme activity was measured every 15 min. The result was shown by a percentage of the remaining enzyme activity to initiate activity.

The optimum reaction pH of Polygalacturonase: Disodium hydrogen phosphate- citrate buffer solution was prepared with interval 0.5 pH in the pH range of 3.0-8.0. The different pH buffer was used to study the effect of different pH on the Polygalacturonase activity.

pH stability of Polygalacturonase: Disodium hydrogen phosphate-citrate buffer solution was prepared with interval 0.5pH in the pH range of 3.0-8.0. The purified enzyme solution mixed with buffer solution at a ratio to 1:1 (V: V) at 4°C for 12 h and enzyme activity was measured with Untreated POLYGALACTURONASE enzyme activity set to 100%:

Polygalacturonase Km. Line weave-Burk [9]

Effect of different concentration of EDTA on Polygalacturonase activity: The purified enzyme was placed in 1, 2, 4, 6, 8, 10 mmol/ L of acetic acid-sodium acetate buffer (50 mmol/L, pH5.5), respectively at the room temperature for 30 min and Polygalacturonase relative enzyme activity was measured. Polygalacturonase enzyme activity not added EDTA was as 100%.

Effect of different metal ions of EDTA on Polygalacturonase activity: The purified enzyme was placed in 50 mmol/L of acetic acid-sodium acetate buffer (pH5.5) including K+, Ca2+, Cu2+, Zn2+, Mn2+, Fe2+, Fe3+, Ba2+, Mg2+, Al3+, respectively at the room temperature for 30min and relative enzyme activity was measured (enzyme activity not added EDTA was as 100%).

RESULTS AND DISCUSSION

The optimum reaction temperature of Polygalacturonase: Enzymes have their own optimum reaction temperature. The protein conformation of enzyme will make irreversible change which result in denaturation when temperature is too high and enzyme does not play a role when temperature is too low. Effect of temperature on Polygalacturonase activity is shown as Fig. 1, enzyme activity maintains at high level when temperature is around 30-50°C, achieve the highest level when temperature is to 40°C, then declines quickly. When the temperature is up to 70°C, Polygalacturonase activity only reaches 20% of the highest enzyme activity. So the optimal reaction temperature is 40°C.

Thermal stability of Polygalacturonase: As shown in Fig. 2, Polygalacturonase is quickly inactivated along with the increase of the temperature and reaction time. Enzyme activity could be maintained at more than 75% at 30-40°C, but reduced to around 48% at 50°C for 1h and almost lost activity at 60°C-70°C for 1 h. This shows that Polygalacturonase has poor stability at high temperatures.

The optimum reaction pH of Polygalacturonase: Enzymes which are composed of amino acids, have a certain amount of positive or negative charges under a

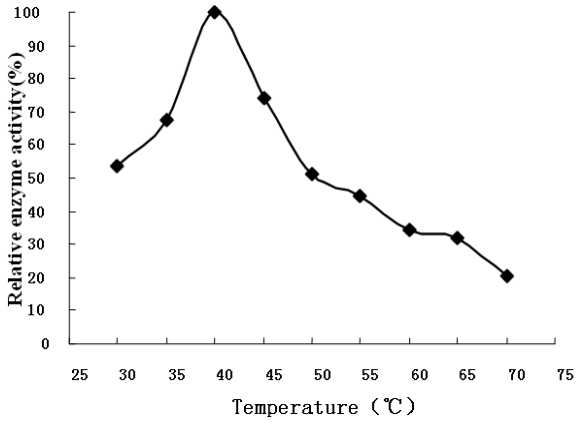

Fig. 1: Effect of temperature on Polygalacturonase activity

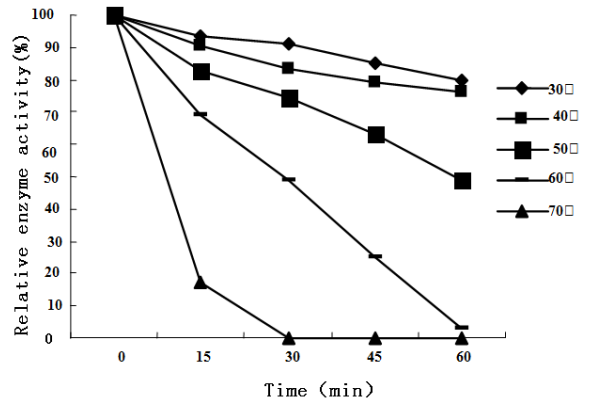

Fig. 2: Polygalacturonase activity heat stability determination

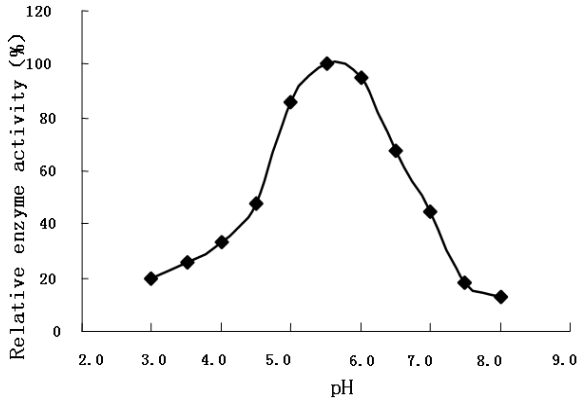

Fig. 3: Effect of pH on Polygalacturonase activity

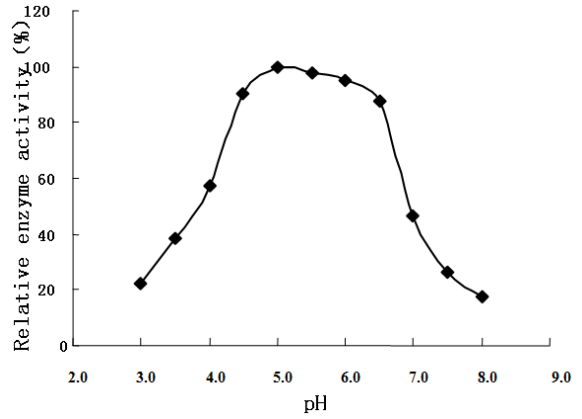

Fig. 4: Polygalacturonase activity pH stability determination

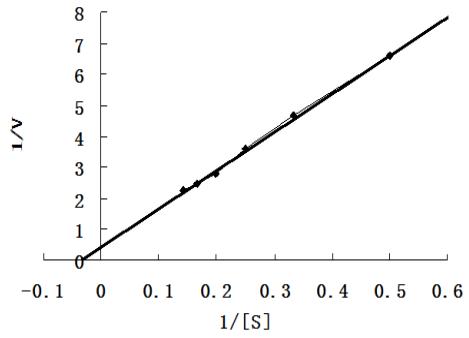

Fig. 5: Michaelis-Menten equation by Lineweave-Burk

Table 2: Effect of different metal ion on Polygalacturonase activity

Addition	Relative enzyme activity (%)
—	100
K^{+}	103.58
Ca^{2+}	100.93
Cu^{2+}	85.14
Zn^{2+}	96.82
Mn^{2+}	78.96
Fe^{2}+	102.75
Fe^{3+}	107.62
Ba^{2+}	52.17
Mg^{2+}	112.58
Al^{3+}	36.57

certain pH conditions. With pH changes, dissociative state of these charges will be changed, which lead to the change of enzyme activity. As shown in Fig. 3, the optimal reaction pH for Polygalacturonase is 5.5. When pH <5.5, the Polygalacturonase activity is increased along with the continuous increase of pH; when 6<pH<8, Polygalacturonase activity is decreased as the pH increased.

pH stability of Polygalacturonase As shown in Fig. 4, enzyme activity keeps in a relatively stable state under the condition of pH4.5-6.5 and decreased when the condition is above or below this range. Enzyme activity decreased obviously under acidic condition because polar amino acid residues in Polygalacturonase molecule are ionized, then like charges repel and peptide conformational change.

Polygalacturonase Km: Km is concentration of the substrate when the enzymatic reaction rate is half of the maximum rate and the enzyme can be identified by Km value. Different enzymes have different Km and a enzymes has different Km with different substrate in enzyme reaction. The Km parameter can be approximately expressed the enzyme affinity to substrate: Km is smaller, affinity is larger and vice versa. The equation was 1/V= 12.335×1/[S] +0.4516 by Lineweave-Burk method and the final results was Km ＝ 27.31 mg/mL.

Effect of different concentration of EDTA: Effect of different concentration of EDTA on Polygalacturonase activity was shown on Fig. 5. Polygalacturonase activity decreased with increased concentration of EDTA (Tijskens *et al*., 2008). EDTA has positive effect on Polygalacturonase activity when the concentration of EDTA was to 1 mmol/ and EDTA has an inhibitory effect on Polygalacturonase activity when the concentration of EDTA was higher than 2 mmol/L. Inhibition effect on Polygalacturonase activity was increased with increasing concentration of EDTA.

Effect of different metal ion on Polygalacturonase activity: According to details on Table 2, the metal ions of Cu2+, Zn2+, Mn2+, Ba2+, Al3+ can inhibit Polygalacturonase enzyme to some extent, the strength of inhibition: Al3+, Ba2+, Mn2+, Cu2+, Zn2+. The metal ions of K+, Ca2+, Fe2+, Fe3+, Mg2+ can activate the POLYGALACTURONASE enzyme to some extent, the strength of activation:

Mg2+, Fe3+, K+, Fe2+, Ca2+.

CONCLUSION

In this study, the test results show that the optimal reaction temperature is 40°C with a high enzyme activity around 30-50°C. Polygalacturonase gains a good thermo stability at the temperature ranging from

30-40°C, which decreases with increasing of temperature and Polygalacturonase will lose all its activity under 60-70°C for 1 h. The optimal reaction pH value for this enzyme is 5.5. As far as the stability in different pH values is concerned, Polygalacturonase is relatively stable for preservation at pH4.5-6.5. The michaelis constant for Polygalacturonase is 27.31. When the concentration for EDTA is higher than 2 mmol/L, it is inhibitive for POLYGALACTURO NASE. The metal ions of K+, Ca2+, Fe2+, Fe3+, Mg2+ can activate the Polygalacturonase enzyme to some extent, the strength of activation: Mg2+, Fe3+, K+, Fe2+, Ca2+; The metal ions of Cu2+, Zn2+, Mn2+, Ba2+, Al3+ can inhibit Polygalacturonase enzyme to some extent, the strength of inhibition: Al3+, Ba2+, Mn2+, Cu2+, Zn2+.

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