TEMPERATURE PROFILES OF SOLUBLE AND BOUND PECTIN METHYLESTERASE FROM APPLE PEEL

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ABSTRACT

Soluble (SPME) and bound pectin methylesterase (BPME) from Golden Delicious apple peel were separated and partially purified by 0-95% $(NH_4)_2SO_4$ precipitation, dialysis and centrifugation. The specific activities of SPME and BPME were 4.2 and 3.5 folds higher than that of the crude extract. The temperatures for the 50% loss of SPME and BPME in 5 min were 70° for SPME and 67.5°C for BPME, respectively. Both fraction lost almost 90% of their activities in 5 min at 80°C

Key words: Apple, pectin methylesterase, purification, temperature profile

ELMA KABUĞUNDA BULUNAN ÇÖZÜNÜR VE BAĞLI PEKTİN METİLESTERAZ ENZİMLERİNİN SICAKLIK DERECESİ PROFİLLERİNİN BELİRLENMESİ

ÖZET

Golden Delicious elmalarında bulanan çözünür (SPME) ve bağlı pektin metilesteraz (BPME) enzimleri birbirinden ayrılmış ve %0-90 $(NH_4)_2SO_4$ ile çöktürülerek, dializ ve santrifüj uygulanarak kısmi saflaştırma sağlanmıştır. Bu şekilde ayrılmış olan SPME ve BPME enzimlerinin spesifik aktiviteleri, ham ekstraktın aktivitesine göre sırasıyla 4.2 ve 3.5 katına ulaşmıştır. SPME ve BPME enzimlerinin 5 dakika içerisinde aktivitelerinin %50'sini yitirdikleri sıcaklık dereceleri sırasıyla; 70° ve 67.5°C dir. 80°C de 5 dakikalık bir ısıtma, her iki fraksiyonun aktivitelerinin %90'ını yitirmesine neden olmaktadır.

Anahtar kelimeler: Elma, pektin metilesteraz, saflaştırma, sıcaklık derecesi profili

INTRODUCTION

Hot water immersion (HWI) of fruits is a quarantine treatment for the disinfection of fruit flies and prevention of fungal activity (1, 2). The temperature of the HWI treatment should be chosen very carefully to prevent heat damage (3). The suitable temperatures of HWI treatment for some fruits were reported as 45°C (30-60 min) and 46°C (30-50 min) for cucumbers (4), 42-49°C for papaya fruits (2) and 45°C for some

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apple cultivars (1). HWI was also reported to improve the texture of some apple cultivars, i.e. Golden Delicious and Delicious (1).

The increase in firmness by heat treatment is attributed to the accelerated actian of Pectin Methylesterase (PME, E.C.3.1.11) which demethylates pectic compounds and causes the increase of their interaction with divalent ions, i.e. Ca^{++} and Mg^{++} (5, 6, 7). This interaction creates a rigid network, called the egg-box model and improves the texture (8, 9).

The thermal characteristics of in situ PME activity of apple waste (10) and PME from whole apple fruits (11) were investigated. However, no studies have been found raelated to the PME from apple peel. In this work the temperature profiles of soluble and cell wall bound PME from Golden delicious apple peel were determined for the better understanding of the thermal characteristics of enzyme PME.

MATERIAL AND METHODS

Material

Golden Delicious apples were obtained from the experimental orchards of Ankara University.

Methods

Extraction of enzymes

Acetone powder, obtained according to the method of Yemenicioğlu et al (12), was used as enzyme source in this study. All extraction and inactivation studies were carried out in 0.01M Tris-HCI buffer at pH 7.5 unless otherwise stated.

Buffer extraction

3-5g acetone powder was suspended in 200 mL cold buffer (+4°C) containing 1M NaCI. The extraction was carried out 24h at +4°. The slurry was then filtered through a muslin cloth and centrifuged at 7000g for 1h.

$(NH_4)_2SO_4$ precipitation and dialysis

For further purification the supernatant was brought to 0-95% $(NH_4)_2SO_4$ saturation and stirred 3.5h at +4°C. The formed precipitate was separated by centrifugation at 7000g for 1.5h, dissolved in minimum amount of buffer and dialysed 48h against the same buffer by two changes (2 x 2L). After dialysis, the formed precipitate was collected by centrifugation and the clear supernatant was named as Soluble PME (SPME).

Preparation of bound PME extract

On this purpose the precipitate separated from the centrifugation of dialyzate was washed with 3 mL of distilled water (+4°C) and dissolved in 3 mL of buffer. This extract was named as bound PME extract (BPME).

Determination of protein

The protein was determined according to the method of Lowry (13).

PME activity

PME activity was determined titrimetrically by the method of Alonso et al (8) with modifications. The reaction mixture consisted of 0.2-0.5 mL of PME extract and 20 mL of 0.5% citrus peçtin (Sigma Chem. Co. St Louis, MO) solution in 0.1N NaCI. The pH of the mixture was immediately adjusted to 7.5 by adding 0.1N NaOH and maintained at this value for 5 min by titrating slowly with 0.01N NaOH. The titration was carried out in a cell maintained at 30°C constant temperature and the activity was expressed as

units (U). One unit of PME activity was defined as the μM of carboxyl groups released by 1 mL of enzyme extract in 5 min.

Temperature profiles

For the determination of temperature profiles, 0.3 mLs of PME extracts were incubated in thermal inactivation time (TIT) tubes (i.d. 9 mm, wall thickness 1 mm) for 5 min at various temperatures (50-70°C). The percent PME activity remaining after each heat treatment was calculated from the initial activity.

RESULTS AND DISCUSSION

Partial purification

The crude extract of acetone powder from golden Delicious apples contained very low PME activity (90110U. mL^{-1}). The total activity of SPME was almost two-fold higher than that of BPME (Tab. 1). The respective specific activities of SPME and BPME were 4.2 and 3.5 folds higher compared to that of crude extract.

Temperature profiles

The temperature profiles of SPME and BPME are given in Fig. 1. A linear temperature profile was obtained for BPME whereas, SPME showed gradual increase in activity from 50° to 60°C and a sharp decrease above 60°C. Comparing to the temperature profile of BPME, the SPME inactivation was found to be more affected by the increase of temperature.

The temperatures for the 50% loss of PME activity in 5 min were 67.5°C for BPME and 70°C for SPME, respectively. Both BPME and SPME lost almost 90% of their activities in 5 min at 80°C.

Comparison of our heat inactivation data revealed that PME from apple peel was more heat stable than PME from Bramley apple [$t_{1/2} \cong 5$ min at 60°C] (10), Purified PME from mandarin orange [$t_{1/2} \cong 1$ min at 65°C] (14) and PME from Valencia orange [$t_{1/2} \cong 0.5$ min at 70°C] (15). Therefore, temperatures applied in quarantine treatment [40-50°C (1)] is not likely to be very affective on the inactivation of PME from G. Delicious apple peel.

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Table 1– Partial purification and separation of soluble and bound PME from apple peel

Fraction	Volume (mL)	T. Activity (Unit. 10^{-4})	T. Protein (mg)	S. Activity (U. mg^{-1}).10^{-4}	Purity (Fold)	Recovery (%)
Crude extract:						
	182	1640	23.7	69.2	1.0	100
0-90% $(NH_3)_2SO_4$ precipitation and dialysis:						
Soluble PME						
	5.9	243	0.832	292	4.2	15
Bound PME						
	3.0	131	0.535	245	3.5	8.0

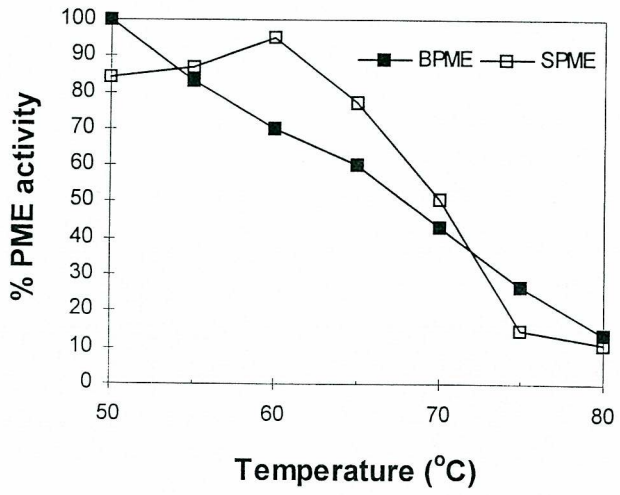

Figure 1- Temperature profiles of soluble and bound pectin methylesterase from apple peel.