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SOURSOP PECTINESTERASE: EXTRACTION, PURIFICATION, PROPERTIES AND EFFECT ON CLOUD STABILITY OF SOURSOP JUICE

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BY
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SOURSOP (*Anona muricata*) PECTINESTERASE : EXTRACTION, PURIFICATION, PROPERTIES AND EFFECT ON CLOUD STABILITY OF SOURSOP JUICE

By

SITI ARBAISAH MISMAN

May, 1996

Chairman : Assoc. Prof. Dr Asbi Ali

Faculty : Food Science and Biotechnology

The presence of pectinesterase in citrus fruit particularly in cloud loss of citrus juice is one of the most intensively studied problems in food technology. However, current reports concerning this enzyme in tropical fruit such as soursop fruit are limited. This project was carried out to study the soursop pulp pectinesterase and its effect on cloud stability of soursop juice. Statistical methods namely fractional factorial design and response surface methodology were applied in experimental design in order to establish optimum conditions for the pectinesterase extraction procedure. Optimum extraction of soursop pectinesterase was obtained using 1.92 M NaCl solution of pH 8.4. Two forms of pectinesterases called PE I and PE II were purified to a single band of protein on SDS PAGE using the techniques of ammonium sulphate fractionation, ion exchange chromatography and gel filtration. PE I had a specific activity of approximately 4 units/mg achieving of purification 43 fold and that of PE II was 6.4 units/mg (229 fold of purification) .

These pectinesterases PE I and PE II had an approximate molecular weights of 29,100 and 24,100 Dalton, respectively as estimated by gel filtration. Comparing their electrophoretic mobilities with those of standard proteins using denaturing electrophoresis, a molecular weight of 31,000 and 28,000 Dalton for PE I and PE II were obtained, respectively. The optimum temperature for enzymic activity was 60°C for both PE I and PE II. The activation energies of PE I and PE II were calculated as 36 kJ/mol°K and 42 kJ/mol°K respectively. The optimum pH for both pectinesterases lie within the range of pH 7.5-8.0. The K_m value for PE I was 0.52mg/ml of substrate and 0.0843 mg/ml of substrate for PE II. PE I had a maximum velocity (V_{max}) of 154 units/mg protein and PE II had a V_{max} value of 726 units/mg protein respectively. The logarithmic values of decimal reduction times plotted against temperature had a classic biphasic pattern featuring a sudden change in slope at a temperature exceeding 60°C. Thermal stability data showed that PE I was more thermostable than PE II in the buffer of pH 7.5 . D values at 65°C were approximately 5.8 min and 3.4 min for PE I and PE II, respectively. The PE I and PE II possesses Z value of 8.5°C and 8.6°C, respectively. There were less than 1% loss of activity of these enzymes after a year's storage in 0.02 M phosphate buffer pH 7.5 and storage temperature of 4°C.

Both enzymes were also tested positive for their ability to destabilize soursop juice cloud at 5°C and 30°C. Cloud destabilization by PE I occurred the fastest (large decrease in absorbance at 660nm) in the natural juice at 30°C.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

PEKTINESTERASE DURIAN BELANDA (*Anona muricata*): PENGEKSTRAKAN, PENULENAN, PENCIRIAN DAN KESAN KE ATAS KESTABILAN MENDAKAN JUS DURIAN BELANDA

oleh:

SITI ARBAISAH MISMAN

May,1996

Pengerusi: Prof Madya Dr Asbi Bin Ali

Fakulti : Fakulti Sains Makanan dan Bioteknologi

Kehadiran pektinesterase di dalam buah sitrus terutamanya pemendakan mendakan dalam jus sitrus adalah salah satu daripada kajian yang dijalankan secara terperinci. Walaubagaimanapun kajian berkaitan enzim ini dalam buah-buahan tropika terutamanya buah durian belanda adalah terhad. Projek ini dijalankan untuk mengkaji pektinesterase daripada buah durian belanda dan kesannya terhadap pemendakan jus durian belanda. Kaedah statistik iaitu `fractional factorial design' dan `response surface methodology' telah digunakan bagi memudahkan penganalisaan ke atas kaedah pengekstrakan yang optimum. Pengekstrakan pektinesterase daripada pulpa buah durian belanda menggunakan larutan NaCl 1.92 M pada pH 8.4 telah menghasilkan jumlah pektinesterase yang optimum. Penulenan ke atas pektinesterase ini telah dijalankan menggunakan kaedah pemendakan berperingkat ammonium sulfat, kromatografi turus pertukaran ion dan juga penurasan gel. Ini telah menghasilkan dua isoenzim PE I dan PE II. Enzim PE I ini didapati mempunyai aktiviti spesifik 4 unit/mg bagi PE I dan PE II 6.4 unit/mg.

Melalui kaedah penurasan gel didapati PE I mempunyai berat molekul 29,100 dan 24,100 Dalton bagi PE II . Dengan menggunakan kaedah SDS PAGE didapati PE I mempunyai berat molekul 31,000 Dalton dan PE II 28,000 Dalton. Kajian kesan suhu ke atas enzim menunjukkan PE I dan PE II mempunyai suhu optimum bagi aktiviti enzim pada 60°C. Tenaga pengaktifan bagi PE I adalah 36 kJ/mol dan bagi PE II ialah 42 kJ/mol. pH optimum bagi kedua-dua isoenzim ini didapati sama iaitu pada julat pH 7.5 - 8.0. Nilai pemalar Michaelis (K_m) bagi PE I adalah 0.52 mg/ml substrat dan 0.0843 mg/ml substrat bagi PE II. Nilai V_{max} bagi PE I dan PE II adalah 154 unit/mg protein dan 726 unit/mg protein, masing-masing. Apabila disimpan dalam penimbal pH 7.5, PE I didapati lebih stabil terhadap haba berbanding dengan PE II. Nilai D bagi PE I dan PE II pada 65°C adalah 5.8 min dan 3.4 min masing-masing. Nilai Z bagi PE I ialah 8.5°C dan bagi PE II pula 8.6°C. Selepas satu tahun penyimpanan, aktiviti enzim didapati menurun kurang daripada 1 %.

Kedua-duanya memberikan kesan kepada kestabilan mendakan jus durian belanda pada suhu penyimpanan jus 4°C dan 30°C. Mendakan yang disebabkan oleh PE I pada suhu penyimpanan 30°C berlaku dengan kadar yang paling cepat.

CHAPTER 1

GENERAL INTRODUCTION

Pectinesterase (E.C.3.1.1.11) which belongs to the carboxylic ester hydrolase has also been referred to as pectase, pectin methoxylase, pectin demethoxylase, pectolipase and pectinmethylesterase (Whitaker, 1972).

The enzyme has been found in numerous higher plants and is especially active in fruits where it is generally accepted that it has an important role in softening of tissue during ripening. However, the specific role of this enzyme in the ripening process is not completely elucidated (Giovane *et al.*, 1994).

In the citrus juice industry, pectinesterase is responsible for the quality defects of juice cloud loss and concentrate gelation in frozen and unpasteurized product (Joslyn and Pilnik,1961; Krop,1974) which are undesirable. On the other hand, it also can be used for treatment of fruit juices and other beverages to facilitate filtration and clarification, and has been proposed for use in the preparation of low methoxyl pectin and galacturonic acid . The importance of pectic subtances and enzymes in nature and industry is well documented (Pilnik and Voragen,1991; Rombouts and Pilnik,1978), and more recently, its biological significance has received increased attention (Nari *et al.,1991*; Tieman *et al.*,1992).

Its substrate, pectin, is the main component of matrix material of the cell wall of higher plants. It acts as a cementing or binding agent and may also control the movement of soluble materials (Northcote,1972).

Due to its importance, pectinesterase characteristics have been widely investigated. It has been purified from many sources, either plant or microbial, and it is usually represented in multiple forms and different characteristics. Studies on pectinesterase of other fruits have been reported but there is still lack of information concerning pectinesterase from soursop fruit (*Anona muricata*).

Soursop is a tropical fruit, native and common in Malaysia, but is less popular compared to other local fruits. It is a member of the annonaceous fruits which are sometimes collectively known as `custard apples' from the custard like flavour of many soursop. In addition, it is rather more acid and less sweet than most other members of the group (Bueso,1980).This fruit is prized for its very pleasant, sub acid, aromatic and juicy flesh. However, this fruit softens very rapidly during ripening and becomes mushy and difficult to be consumed fresh.

In an evaluation of lesser known tropical fruits, scientists at the Research Laboratories of Nestle Products, Switzerland, recommended soursop as one of the most promising fruits because of its aromatic qualities and suitability for processing (Wuhrmann and Patron, 1965).

In US and Europe, the fruit pulp was used to make drinks and sherbets and these products have excellent marketing possibilities (Bueso,1980). Moreover, the international fruit juice market has been growing rapidly over the past few years (Knight,1980). In fact, since the late 1980's, in Malaysia, big companies such as Guthrie, Golden Hope and government agencies have diversified into large scale planting and processing of tropical fruits (Arope ,1992). This has boosted contribution into the international export market of tropical fruits. The fruit industry are focussing on three main areas; export market for exotic tropical fruits, processing and domestic fresh market. Soursop appears to have good potential for juice (Salahuddin,1992)

An understanding of the soursop pectinesterase properties and the factors that affect the stability of this enzyme is important especially for processing purposes. Therefore, this research was conducted to:

1. establish a method for extracting pectinesterase from soursop .
2. study the properties of the purified enzyme
3. study the stability of pectinesterase and its direct role in soursop juice clarification.

CHAPTER 2

LITERATURE REVIEW

Classification of Pectic Enzymes

According to Rombouts and Pilnik (1978), pectic enzymes are classified based on their mode of attack on the galacturonan part of the pectin molecule (Rombouts and Pilnik,1978). From the scheme in Figure 1, one can distinguish between pectinesterase (PE; EC 3.1..11.1) which de-esterify pectins to low methoxyl pectins or pectics acids and pectin depolymerases which split the glycosidic linkages between galacturonosyl (methylester) residues. Polygalacturonases (PG;EC 3.2.1.15 and 3.2.1.67) split glycosidic linkages next to free carboxyl groups by hydrolysis.

Pectin

Pectin is a complex mixture of at least three polysaccharide components. The principal polysaccharide is polygalacturonic acid and this is normally combined with polygalactose and the highly branched polyarabinose. In addition, the carboxyl side groups of galacturonic acid are partly esterified with methanol residues. Pectin was found within and between the cell walls of higher plants, where they cement the cellulose fibres together to form rigid structure. In unripe fruit, pectins remains unsoluble and the juice is free to run.

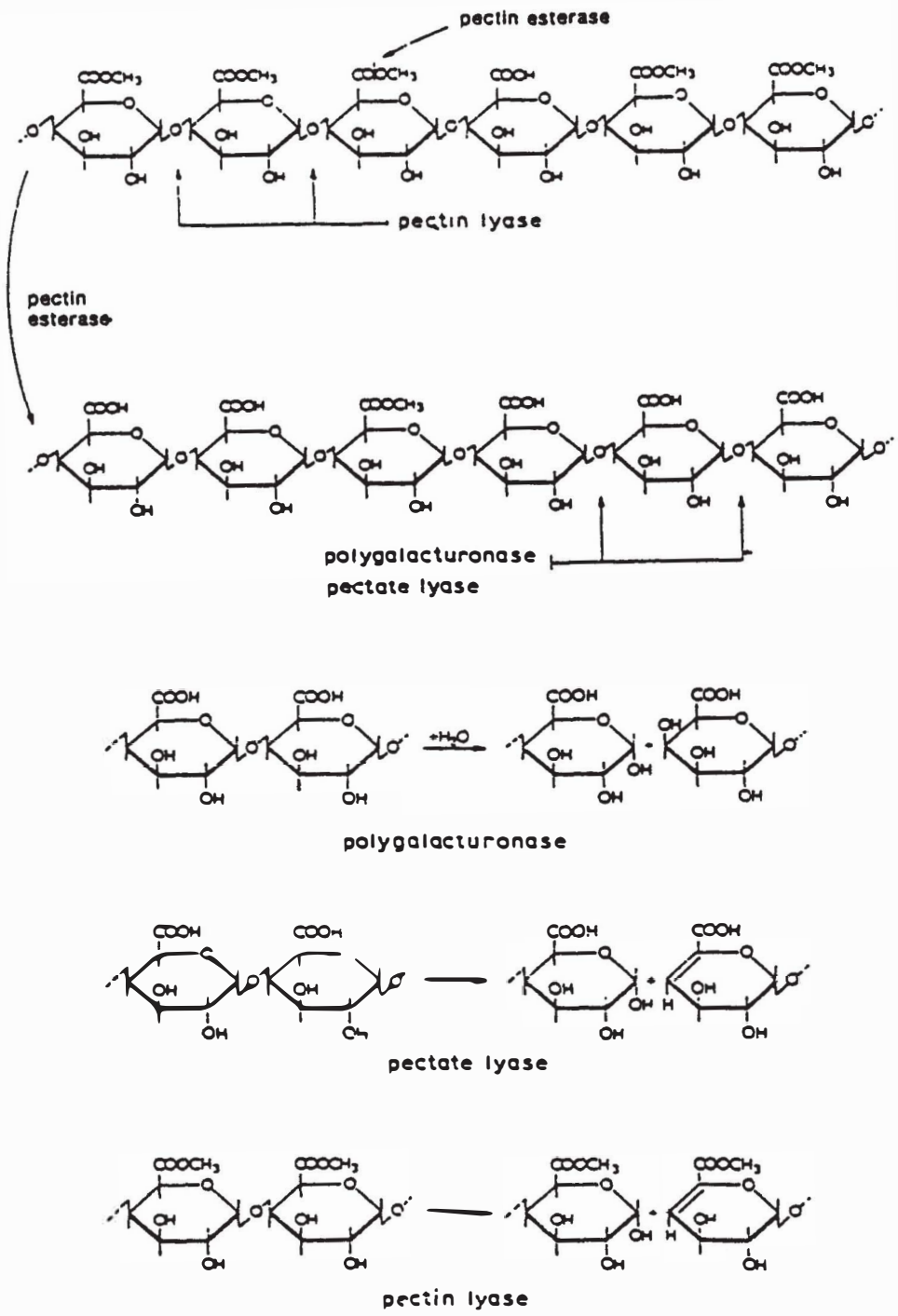

Figure 1. Fragment of a Pectin Molecule and Points of Attack of Pectic Enzymes
(Adopted from Rombouts and Pilnik, 1980)

However, as part of the natural ripening and softening of the fruit, pectin is partly degraded and solubilized by indigenous pectic enzymes. The soluble portion of the pectins extends into the juice while the insoluble end remains attached to the cellulose. The presence of this polysaccharide increases the viscosity of the juice, making it less free to run, and difficult to separate from the flesh of the fruit (Birch *et al.*, 1981)

Pectinesterase

The Occurrence of Pectinesterase in Plant

Numerous higher plants have been shown to contain pectinesterase (Table 1). In fact all higher plants seem to contain pectinesterase in all living tissue. For instance, pectinesterase was represented in seeds, leaves, stems, petioles, flowers, green and ripe fruit of cucumbers (Bell *et al.*, 1951). The level of activity varies with plant species, variety, part of the plant and stage of growth. Also in fruits there are zones which are relatively rich and poor in pectinesterase activity, like in citrus (Mc Donnel *et al.*, 1945; Rouse, 1953; Rothschild *et al.*, 1974; Tahir *et al.*, 1975) and persimmon (Nakayama and Iwasaki, 1966).

Pectinesterase is located in the free space at the site of its substrate between the cell walls as was shown for oat coleoptiles and tobacco pith (Glasziou, 1959) and the main part is bound by ionic interaction. In tomato cell walls and oat coleptiles it moreover demonstrated that there were binding sites specific for pectinesterase. The binding of pectinesterase was only partially or not all affected by prior saturation with other proteins or vice versa (Nakagawa *et al.*, 1971; Jansen *et al.*, 1960).

Table 1
The Presence of Pectinesterase in Some Higher Plants

Sources	References
Actinidia chinensis	Giovane *et al.* (1990)
Berries	Voragen and Pilnik (1989)
Bramley apple	King (1990)
Citrus	Wicker *et al.*(1991)
Cucumber	Bell *et al.* (1985) Mc Feeters *et al.* (1985)
Ficus awkeotsang	Komae *et al.* (1990) Lin *et al* ,(1989)
Guava	Fayyaz and Asbi (1993)
Leek	Voragen and Pilnik (1989)
Lemon fruits	Mac Donald *et* al.(1993)
Mung bean	Bordenave *et al.* (1993)
Mandarin orange fruits	Rillo et al. (1992)
Marsh white grape fruit	Seymour *et al.* (1991)
Passion fruits	Voragen and Pilnik (1989)
Papaya	Lim and Chung (1993) Fayyaz *et al.* (1993)
Persimmon	Awad (1985)
Peach	Javeri *et al.(*1991)
Pea	Pressey (1984)
Soy bean	Moustacas *et al.* (1986)
Tomato	Giovane *et al. (*1994)