Partial Purification and Characterization of Pectin Methylesterase from Green Beans (*Phaseolus vulgaris* L.)

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Pectin methylesterase (PE) is present in green beans and may have an influence on the texture of green beans after commercial sterilization. Two fractions of PE were partially purified from the pods and the seed hulls of green beans (cv. Odessa). PE-1 and PE-2 had molecular weights of 45 and 29 kD, respectively, under native conditions and of 42 and 33.5 kD under denaturating conditions. PE-1 and PE-2 both contained two isoforms of PE activity with isoelectric points of 8.4 and 9.8 and 10.5 and >11, respectively. The active fractions showed a difference in salt dependency, and PE-2 was less heat stable than PE-1.

Keywords: *Isoforms; pectin methylesterase; Phaseolus vulgaris; purification; texture*

INTRODUCTION

The texture of fresh and processed vegetables is an important quality aspect. In processed foods, due to the lack of turgor, the perception of texture is largely determined by the structure of the cell wall and the middle lamella. The composition of the cell wall can alter due to the activation of specific cell wall enzymes. Processing dramatically changes the structure of the pectic fraction. Two major enzymes involved in the degradation of pectin are pectin methylesterase (PE) (EC 3.1.1.11) and polygalacturonase (PG) (endoPG, EC 3.2.1.15; exoPG, EC 3.2.1.67). PE is able to catalyze the de-esterification of pectic compounds in the plant cell wall. Demethylated pectin can be enzymatically hydrolyzed by PG, resulting in a decrease in degree of polymerization of the pectin chains and a loss of firmness of the tissue. Oppositely, demethylated pectin may cross-link with divalent ions to form pectate gels and it is less sensitive to β-eliminative breakdown during sterilization than is highly methylated pectin, both features increasing the firmness of the cell wall. Blanching at 70 °C activates PE and therefore is supposed to improve the firmness of vegetables after "commercial" sterilization. Summers (1989) did not find any correlation between the levels of PE and PG and the firmness of green beans after processing. By expression of an antisense PE gene in tomato a more than 10-fold reduction in PE activity was achieved. These reduced levels of PE activity caused a shift from bound to soluble calcium in the pericarp of tomato. This observation might be explained by a possible reduced calcium binding ability of carboxylic moieties resulting from low PE activity (Tieman and Handa, 1994).

PE has been purified from several plant sources such as peach (Glover and Brady, 1994), papaya (Fayyaz et al., 1994), tomato fruits (Zhang, 1994; Pressey and Woods, 1992), flax callus (Gaffe et al., 1992), Marsh white grapefruit (Seymour et al., 1991), apple (Castaldo et al., 1989), soy bean culture (Moustacas et al., 1986), and orange (Versteeg et al., 1978). A large variability with respect to almost any characteristic (molecular weight (M_r), isoelectric point (pI), kinetic properties, and thermostability)) is reported (Wicker et al., 1988; Versteeg et al., 1978; Seymour et al., 1991a; Giovane et al., 1990).

In this paper, we describe the partial purification, some physicochemical properties, and the tissue distribution of PE isoforms from green beans.

MATERIALS AND METHODS

Plant Material. Pods of *Phaseolus vulgaris* L. cv. Odessa were grown in the green house and harvested at a seed percentage of approximately 23% (w/w). The pods were picked and deseeded by hand.

Reagents. CM Sephadex C-50, CM Sepharose CL-6B, Heparin Sepharose CL-6B, and Sephadex G-100 superfine were obtained from Pharmacia LKB Biotechnology, Uppsala, Sweden. Citrus pectin and bromothymolblue were purchased from Sigma (Chemical Co., St. Louis, MO). Coomassie Plus protein assay reagent and bovine serum albumin were obtained from Pierce (Rockford, U.S.).

Determination of PE Activity. PE activities in column chromatography fractions, in kinetic studies, and in the salt and temperature dependency experiments were determined spectrophotometrically by following the method of Hagerman (1986) as modified by Seymour et al. (1991b) with the following adjustments: a 0.2% (w/v) solution of orange peel pectin (Sigma) was used, and the activity was measured at 25 °C. K_m determinations were made at pectin concentrations of 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.8, and 1.0 mg mL^{-1} according to the Lineweaver–Burk equation. Linear regression calculated from the determinations of two determinations for each point gave a straight line with regression coefficients of 1.0 and 0.97 for P1 and S2, respectively. P2 and S1 did not give straight Lineweaver–Burk plots.

Activity was measured titrimetrically in pooled chromatography fractions and in the pH-optimum studies. The carboxyl groups liberated by PE from 0.2% citrus pectin (Sigma) in the presence of 0.1 M NaCl were titrated with 10 mM NaOH at 25 °C, the pH being maintained at pH 7.5 or chosen values ranging from 4 to 9 with an automatic titrator (VIT90 videotitrator, Radiometer, Copenhagen, Denmark). One

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nanokatal (nkat) of PE activity is defined as the amount of enzyme that liberates one nanomole (nmol) of methyl groups per second at standard conditions.

Protein content was measured with the micromethod of Bradford using Coomassie Plus protein assay reagent according to the instructions of the manufacturer (Pierce).

Purification of PE. The purification of pectin methylesterase from green beans was achieved by adapting a method developed by Giovane et al. (1990). PE was purified from both the pods and the seed hulls. Green beans were divided in pods (590 g), seeds (175 g), and seed hulls (105 g). Seed hulls were ground in liquid nitrogen and homogenized with cold milli Q water (1:2 w/v) with a blender mixer. The homogenate was stirred for 1 h at 4 °C and centrifuged for 20 min at 15000*g*. The supernatant was discarded, and the pellet was resuspended in 1 M NaCl pH 7.8 (1:1 w/v). After 2 h of stirring at 4 °C the homogenate was centrifuged for 20 min at 15000*g*. The supernatant was filtrated over four layers of cheese cloth and brought to 35% saturation with ammonium sulfate and stirred for 2 h at 4 °C. After removal of the precipitate by centrifugation (20 min at 15000*g*) solid ammonium sulfate was added to 90% saturation. After incubation overnight the precipitate was collected by centrifugation (20 min at 15000*g*). The precipitate was suspended in 20 mL of 10 mM potassium phosphate (pH 7.8), 20 mM NaCl, and 5 mM β-mercaptoethanol with the use of an ultrasonic bath and dialyzed against three changes of 25 volumes of the same buffer. The dialyzed sample was centrifuged (15000*g* for 15 min) before it was loaded onto a CM Sephadex C50 (weak cation exchanger) (35 $\times$ 1.6 cm), and the column was washed with the same buffer and eluted with a linear gradient from 20 to 1000 mM NaCl in 10 column volumes.

Fractions of 4 mL were collected and assayed for PE activity and protein content. The active fractions of each activity peak were pooled, dialyzed to remove NaCl, and loaded onto a Heparin Sepharose CL-6B column. The column was washed with 10 mM potassium phosphate (pH 7.8), 20 mM NaCl, and 5 mM β-mercaptoethanol and eluted with a salt gradient from 20 mM to 1.0 M NaCl in approximately 10 column volumes. The active fractions were pooled.

Native Molecular Weight Determination (M_r). M_r values of native PE's were determined with gel filtration using an FPLC equipped with a Sephadex G-100 (super fine) column (1.6 $\times$ 50 cm). The column was calibrated with bovine serum albumin (M_r 68 000), ovalbumin (M_r 43 000), chymotrypsinogen (M_r 25 000), and cytochrome *c* (M_r 12 500) purchased from Boehringer (Mannheim, Germany). The column was eluted with a buffer containing 10 mM Tris-HCl (pH 7.8), 100 mM NaCl, and 5 mM β-mercaptoethanol at a flow rate of 0.25 mL min^{-1}.

Electrophoretic Procedures and Activity Staining. M_r values were estimated using SDS polyacrylamide gel electrophoresis (SDS-PAGE) under denaturating and reducing conditions on a 10−15% gel (Phast System, Pharmacia) or according to Laemmli (1970) on a 12% gel using a Bio-Rad Protean II vertical slab unit (Bio-Rad, Veenendaal, The Netherlands). The 10−15% gels were stained with silvernitrate according to the manufacturer's instructions (Pharmacia) and the 12% gels were stained with silver nitrate according to Morrisey (1981).

Isoelectric points of the isoforms were determined using Phastgel dry IEF gels (Pharmacia) equilibrated with Pharmalyte 8-10.5 using a high-pI kit (Pharmacia) for calibration. Staining for PE activity after isoelectric focusing was carried out by means of the agar−pectin sandwich technique (Bertheau et al., 1984) using ruthenium red. After being run , the gel was incubated for 15 min in reaction buffer (0.05 M citrate−phosphate (pH 7.8), 0.2 M NaCl) at 37 °C. IEF gels were placed on the agar−pectin gels (1.5% agar, 0.5% pectin) and were incubated at 37 °C for 2 h. After separation, both gels were incubated in 0.1 M malic acid for 30 min. Then the two gels were incubated in 0.02% Ruthenium Red for minimally 30 min and washed with milli Q water.

Immunoblot Assays. Proteins separated by SDS-PAGE were electrophoretically transferred to a PVDF membrane. The cathode buffer contained 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) (pH 11.0) and 0.03% SDS, and the anode buffer contained 10 mM CAPS (pH 11.0) and 20% methanol. Bovine serum albumin (3% in TBS:0.1 M Tris-HCl (pH 7.5), 150 mM NaCl) was used as a blocking reagent. Polyclonal PE antisera prepared against tomato fruit PE were kindly provided by Dr. Tucker (University of Nottingham). Antisera were diluted (1:1000) in TTBS (TTBS: TBS + 0.05% Tween-20) for immunostaining of the blots. Antibody binding was visualized using alkaline phosphatase-conjugated anti-rabbit IgG antibodies (1:1000 in TTBS + 3% BSA). The membrane was washed with alkaline phosphatase buffer (AP-buffer: 100 mM Tris-HCl (pH 9), 100 mM NaCl, 50 mM $MgCl_2$). The staining was carried out by immersion of the membrane in 10 mL of AP-buffer containing 45 μL of NBT (4-nitroblue tetrazolium chloride (30 mg/mL of 70% dimethylformamide)) + 35 μL of BCIP (5-bromo-4-chloro-3-indolyl phosphate (15 mg/mL of 100% dimethylformamide)).

Table 1. Tissue Distribution of PE Activity in Green Beans (cv. Odessa)

bean part	weight (g)	PE activity (nkat/g of FW)	total PE activity (μkat)	protein content (mg/ g of FW)	specific activity (nkat/mg of protein)
pod	433	13.6	5.9	0.44	30.9
endosperm	52	33.7	1.8	5.18	6.50
seed hull	80	983	78.6	1.62	607

Thermal Inactivation. Eppendorf tubes with 100 μL of PE solution in 10 mM potassium phosphate buffer (pH 7.8) were placed in a waterbath at different temperatures. To establish the thermal stability of the PE isoforms, the tubes were incubated for 0−30 min at 30, 40, 50, 60, 70, and 80 °C. After the heat treatment the samples were immediately frozen in liquid nitrogen. The residual activity was measured at 25 °C according to the method of Hagerman (1986).

PE isoforms were inactivated by a first-order reaction with denaturation rate constant k_d and energy for denaturation E_d. The following equations are used to determine the thermostability of the different isoforms:

$$PE \xrightarrow{k_d} PE_{denat} \quad (1)$$

$$PE = PE_0 e^{-k_d t}$$

$$k_d = k_{d\,ref} {}^* e^{E_d/R(1/T_{ref} - 1/T_{abs})} \quad (2)$$

$$T_{ref} = 60\ °C$$

Linear regressions were calculated from the mean of four determinations for each point.

Statistical Analyses. The data obtained from the thermal inactivation studies were analyzed using nonlinear regression (statistical package GENSTAT) with heating time and temperature simultaneously as explaining variables. No data transformation was applied to avoid error induction.

RESULTS

Tissue Distribution of PE Activities in Green Beans. The PE activity was measured in crude extracts of different parts of the beans (cv. Odessa). It appeared that the highest specific activity was found in the seeds of beans (Table 1). Therefore seed hulls were used as a source for PE purification. PE was also purified from the pods of the bean because the pods are of high importance with respect to the sensory perception of the texture. The specific activity of PE in the seed hull was approximately 20 times higher as compared to pods.

Purification of PE from Seed Hull. Cell wall bound PE was extracted from the wall matrix using a high salt concentration (1 M NaCl). The ammonium sulfate precipitation was used to remove high molecular weight proteins and sugars and to concentrate the

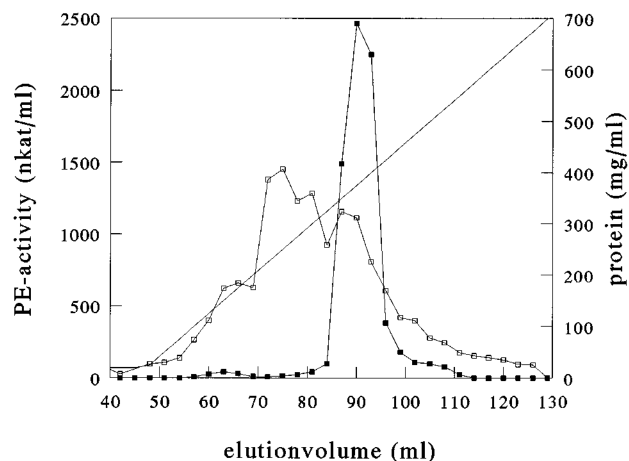

Figure 1. Purification of PE from seed hulls of green beans (cv. Odessa) by chromatography on CM-sephadex C50 column material. PE activity is shown as closed squares, and protein content measured by the Bradford method is shown as open squares. The NaCl concentration (no symbols) is drawn on the right *Y* axis.

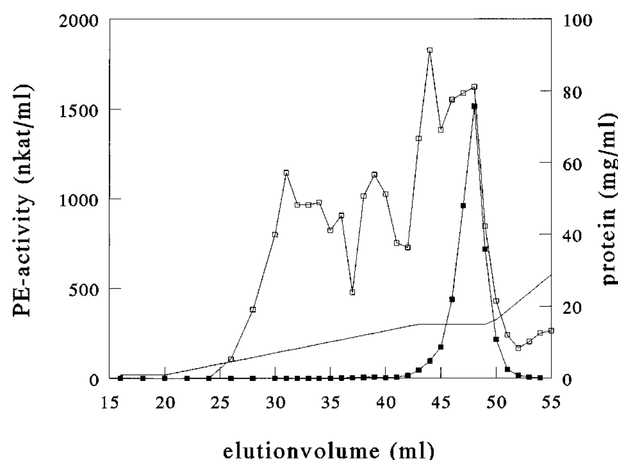

Figure 2. Purification of S2 from seed hulls of green beans (cv. Odessa) by chromatography on heparin sepharose column material. PE activity is shown as closed squares, and protein content measured by the Bradford method is shown as open squares. The NaCl concentration (no symbols) is drawn on the right *Y* axis.

sample. After fractionation on the CM Sephadex C50 column, two peaks of PE activity were found (Figure 1). A minor peak (S1) with approximately 3% of the PE activity eluted at an NaCl concentration of approximately 0.15 M. A major peak (S2) of PE activity eluted at approximately 400 mM NaCl. The pooled fractions of S1 and S2 were dialyzed against 10 mM potassium phosphate, 20 mM NaCl, and 5 mM β-mercaptoethanol and loaded onto a Heparin Sepharose column. Figure 2 shows the elution profile of fraction S2. Heparin sepharose chromatography resulted in a further purification of PE-pool S2, as reflected by a 2-fold increase in specific activity (Table 2). PE-pool S2 eluted as a single peak at 0.3 M NaCl. The active fractions were pooled and dialyzed against 0.05 M ammonium carbonate and subsequently lyophilized. The specific activity of the partly purified S2 fraction was approximately 12 μkat/mg of protein.

After Heparin sepharose chromatography none of the fractions were pure. On SDS-PAGE fractions S1 and S2 contained respectively 8 and 3 protein bands. Subsequent gel filtration chromatography of fraction S2 did not further increase the specific activity.

For determination of the characteristics of S1 and S2 a partial purification was performed with seed hull extracts from the same batch of beans on a CM Sepharose CL-6B column. S1 and S2 were eluted from this column at the same NaCl concentrations that afforded elution from CM Sephadex C50.

Purification of PE from the Pod. PE was extracted from homogenized pod tissue with 1.25 M NaCl. The extract was concentrated with ammonium sulfate precipitation (35−90%) and dialyzed against 10 mM potassium phosphate with 20 mM NaCl and applied onto a column of CM-Sepharose CL-6B. A minor part of the PE activity (2.5%) was not bound to the column. There was a large activity peak (P1) eluting at approximately 150 mM NaCl (75%) and a minor activity peak (P2) eluting at 600 mM NaCl. The purification procedure of PE from the pod is summarized in Table 3.

Molecular Weight Determinations. The molecular weights of the different PE isoforms were determined by FPLC gelfiltration on Sephadex G-100. Fractions P1 and S1 appeared to have a native molecular weight of approximately 45 kD, and fractions S2 and P2 appeared to have a native molecular weight of approximately 29 kD.

After gel filtration the active fractions were analyzed with SDS-PAGE electrophoresis under denaturating and reducing conditions. The proteins were stained with either silver nitrate or after blotting with antibodies directed against tomato PE. The cross-reacting proteins bands from P1 and S1 showed estimated M_r values of approximately 42 kD and a minor band of approximately 38 kD. In fraction S2 a single band of 33.5 kD was observed on the immuno-stained blot. In fraction P2, next to the 33.5 kD protein, also 42 and 38 kD proteins were detected with antibodies directed against tomato PE. The characteristics are summarized in Table 4.

Influence of Salt Concentration on PE Activity. For the fractions P1, P2, and S2 the influence of NaCl concentration on the PE activity was determined. The results are presented in Figure 3. The optimum salt concentration for fractions P2 and S2 appeared to be approximately 140 mM NaCl. The optimum for fraction P1 is slightly lower. The fractions P2 and S2 exhibited a strong salt dependency as they were not active in the absence of NaCl. For fraction P1 80% of its maximal activity was detected under these conditions. The fractions P1 and P2 had a broader NaCl optimum than fraction S2.

pH Dependency. The influence of pH was analyzed only for fractions P1 and S2. Fraction P1 of the pod had a pH optimum in the range 6.5−9. At pH 5 no PE activity was found. Fraction S2 had a slightly narrower pH optimum, from 7 to 9. At pH 6 only 2% of the maximal activity was detected.

Kinetic Parameters. The K_m values of P1 and S2, determined after cation exchange, were 0.104 and 0.049 mg of pectin/mL, respectively.

Estimation of Isoelectric Point. The isoelectric points of the different fractions with PE activity were estimated after gel filtration chromatography. Isoelectric focusing of fractions P1 and S1 revealed two protein bands possessing PE activity. A major band was observed at pI 9.8, and a minor band occurred at pI 8.4 for fraction P1. In fractions P2 and S2 a major band of activity with a pI of >11.5 was detected and as well as a minor band with pH 10.5.

Table 2. Purification of PE from the Seed Hull of Green Beans

	total volume (mL)	total activity (μkat)	total protein (mg)	specific activity (μkat/mg)	yield (%)	purification factor
water extract	198	6.8	208	0.023		
salt extract	298	103	170	0.61	100	1
ammonium sulfate (35%) (supernatant)	310	90	155	0.58	87	0.95
ammonium sulfate (90%) (pellet)	53	54.6	54.9	0.65	53	1.07
CM-sepharose C50						
S1	17	0.99				
S1 after dialysis	17	0.90	0.87	1.03	0.9	1.7
S2	84	28.3				
S2 after dialysis	89	23.9	4.11	5.8	23.1	9.5
heparin sepharose						
S1	9	0.59	nd[a]			
S2	47.5	16.8	1.37	12.2	16.3	20

[a] nd: not determined

Table 3. Purification of PE from the Pod of Green Beans

	volume (mL)	total PE activity (μkat)	protein content (mg)	specific activity (μkat/mg)	yield (%)	purification factor
crude extract	590	5.87	188	0.031	100	1
ammonium sulfate (35%) (supernatant)	640	4.61	184	0.025	79	0.81
ammonium sulfate (90%) (pellet)	47	4.45	92	0.048	76	1.6
P0	14	0.16	4.8	0.033	2.7	1.1
P1	24	2.07	5.6	0.37	35.2	11.9
P2	25	1.98	5.5	0.36	33.7	9.7

Table 4. Characteristics of PE Isoforms from Green Bean (*Phaseolus vulgaris*)

	MW[a] (kD)	MW[b] (kD)	IEP major	IEP minor	pH optimum
P1 (PE-1)	46	42/38	9.8	8.4	6.5−9
P2 (PE-2)	30	33.5	>11.5	10.5	
S1 (PE-1)	44	42/38	9.8		
S2 (PE-2)	28.5	33.5	>11.5	10.5	7−9

[a] Determined by gelfiltration (Sephadex G-100). [b] Determined by using antibody staining after gel electrophoresis.

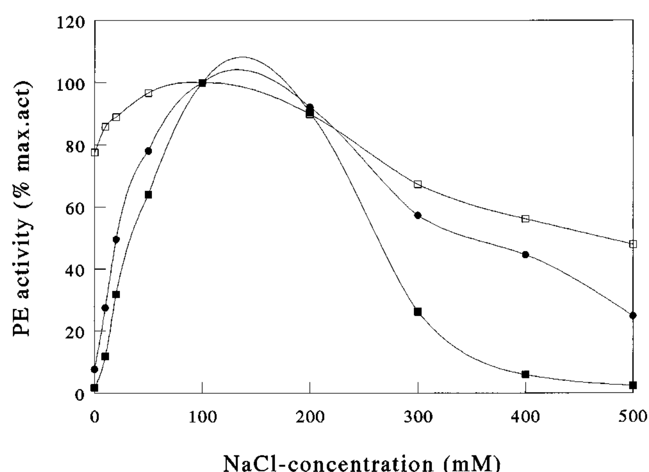

Figure 3. NaCl dependency of fractions P1 (open squares), P2 (closed circles), and S2 (closed squares). Assays were performed in 0.2% (w/v) orange peel pectin at pH 7.8.

Temperature Dependency. The PE isoforms from bean pods were subjected to a thermal treatment in order to investigate their resistance to temperature. The thermal resistance was tested at six different temperatures in the range 30−80 °C. P1 was more resistant to temperature than P2 (Figure 4). In Table 5 the calculated values heat denaturation parameters K_d and E_d for P1 and P2 are mentioned. S1 and S2 showed the same behaviors toward heat as P1 and P2, respectively.

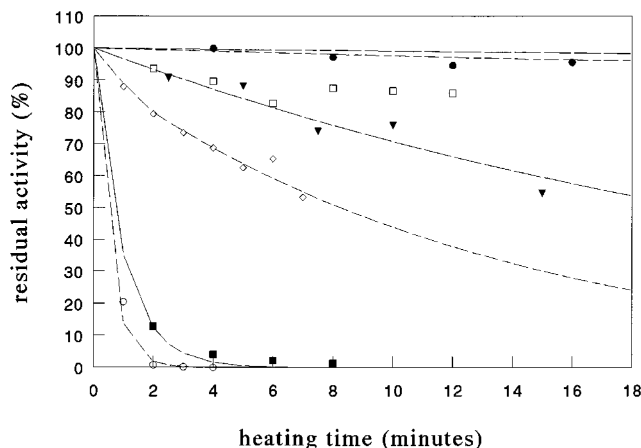

Figure 4. Thermostability of P1 (open symbols) and P2 (closed symbols) from the pods of green beans. The time-dependent enzyme inactivation was measured at 5 temperatures: 40 °(●), 50 °C (▼), 60 °C (■,□), 70 °C (◇) and 80 °C (○). The curves are fitted according to the calculated values for E_d and k_d for P1 (dashed lines) and P2 (straight lines).

Table 5. Heat-Inactivation Parameters of Two Isoforms of PE from Green Beans (cv. Odessa)

	PE-1	std	PE-2	std
% variance	99.1		96.4	
$K_{d\ ref}$	0.002325	0.000441	1.0413	0.0990
E_d/R (kJ/mol)	330	10	305	17

The energy necessary for deactivation (E_d) is approximately the same for both isoforms. This means that the increase in temperature necessary to observe, for example, a 10 times faster heat inactivation is the same for both isoforms. The rate of inactivation (k_d) is approximately 500 times lower for P1 than for P2.

DISCUSSION

In this investigation of cell wall modifying enzymes from green beans, only traces of PG activity were

detected (not published; Summers et al., 1989). This observation may be explained by a developmentally regulated expression of PG activity, resulting in virtually absence in the beans that were used in this study. The research is therefore focused on PE.

The specific activity of PE (31 nkat/mg of protein) in crude extracts from pods of green beans is low in comparison with PE from fruits, ranging from 105 nkat/mg of protein in apples to 3 μkat/mg of protein in tomato fruits and mandarin orange fruits (Castaldo, 1989; Zhang, 1994; Seymour, 1991b; Gaffe et al., 1994; Rillo et al., 1994).

Evaluation of Tables 2 and 3 indicated that ammonium sulfate precipitation and dialysis were critical steps in the purification procedure, normally resulting in largest activity losses. The PE activities of P1 and S1 appeared to be relatively unstable, as storage of a PE-containing solution at 4 °C for 24 h may result in a 20% activity loss and the freezing/thawing step may further decrease the activity with 50% (unpublished results). Tiznada and Handa (Gaffe et al., 1994) had observed that PE isoforms with pI 9.0 and higher tend to aggregate and become insoluble during freezing and thawing.

From two tissues of green beans, i.e., the pods and the seed hulls, PE isoforms were studied and characterized. PE isoforms from pods were included in this study because this tissue is, through its abundancy, of utmost relevance to sensory perception of texture. Seed hulls were included because they exhibit high specific activity and they represent, despite the relative low abundancy of this tissue (14% on fresh weight basis), over 91% of the total amount of PE activity calculated from Table 1.

In each tissue studied two isoforms of PE were detected, albeit at different relative proportions. Comparison of characteristics (M_r, chromatographic behavior, pI) leads to the conclusion that the low pI isoforms from pod and seed hull are identical (to be indicated with PE-1 from now on), as are the high pI isoforms (PE-2 from now on). Both isoforms appear to be present in pods in approximately equal amounts, whereas PE-2 is most abundant in seed hulls (>96% of total activity).

PE-2 exhibited a strong salt dependency for its activity, as at a salt concentration of 140 mM NaCl was maximal and PE activity in the absence of NaCl was negligible. The optimal activity of PE-1 was observed at approximately 100 mM NaCl, but in the absence of NaCl, still 80% of maximal activity was measured. Zhang (1994) and Warrilow and Jones (1995) also detected a difference in NaCl dependency for different isoforms in tomatoes. PE isolated from soybean also exhibited no activity in the absence of NaCl (Nari et al., 1991).

The k_m values of PE from green beans are approximately 10-fold lower than values mentioned for most of the other sources (Seymour et al., 1991; Giovane et al., 1990; Rillo et al., 1994; Zhang, 1994). For PE from orange (0.083, 0.0046, and 0.041 mg/mL) (Versteeg et al., 1978) and flax callus (0.147 mg/mL) (Gaffe et al., 1992), k_m values of the same order of magnitude were found. However, it is difficult to compare the k_m values found in this study with other k_m values sighted in the literature because the k_m values are dependent on temperature, salt concentration, pectin source, and pH of the reaction medium (Goldberg et al., 1992).

M_r values of PE isoforms from different plant sources range from 23 to 110 kD under nondenaturating circumstances and from 12.5 to 71 kD under denaturating conditions. For PE-1 a native molecular weight of approximately 44 kD was determined. In orange and tomato, PE isoforms were reported with similar molecular weights (Wicker, 1988; Zhang, 1994). PE-2 had a M_r of approximately 33.5 kD. Moustacas et al. (1986) found a PE isoform with a similar M_r in soybean. Both enzymes occur in plants belonging to the *Fabaceae.* Measurements of M_r values under either native or under denaturating conditions revealed a discrepancy. Native M_r for PE-1 was slightly lower than "denaturated" M_r, whereas for PE-2, the opposite was observed. No definite explanation for these phenomena can be given as yet, but interactions between PE and column material may provide a partial explanation. Interactions between PE and Sephadex column material was reported before (Warrilow et al., 1994). Both PE-1 and PE-2 from green beans showed cross reactivity with polyclonal antibodies raised against tomato PE. Also orange PE cross reacted with these antibodies.

Incubating Phastgel dry IEF gels with Pharmalyte 8-10.5 performs a fast and easy way to determine the isoelectric point of basic proteins. pI could be measured more accurately, when more marker proteins for this pH range were available. High pI values were frequently reported for PE; for example, for grapefruit PE, a pI of >10 was reported (Seymour et al., 1991b), for orange PE 10, >11, and 10.2 (Versteeg et al., 1978), for mandarin fruit >9 (Rillo et al., 1992) , and for mung bean >10 (Bordenave and Goldberg, 1993). Also in this study relative high pI values were found; for example, in fractions containing the PE-1 isoform, pI values of 8.4 and 9.8 were detected, and in the PE-2 containing fractions, activity bands with pI values of 10.5 and >11.5 were observed. PE isoforms with pI values lower than 9 were observed in fast-growing tissues such as mung bean and flax callus (Bordenave and Goldberg, 1993; Gaffe et al., 1992) and in ripening fruits such as kiwi and tomato fruits (Giovane et al., 1990; Gaffe et al., 1994).

The thermostabilities of PE-1 and PE-2 are evidently different. Also PE isoforms in other fruits and vegetables show a difference in thermostability. In potato at least two isoforms with a different thermostability were detected. The E_d of the heat labile PE (300 kJ/mol) is approximately the same as the E_d of PE-1 (305 kJ/mol) (Andersson, 1994). Also, in tomatoes (Laratta et al., 1995), oranges (Versteeg et al., 1980), white grapefruit (Seymour et al., 1991a), and kiwi fruit (Giovane et al., 1989), different PE isoforms with different thermostabilities were detected. Van Buren (1962) showed that the rate of inactivation by heat is dependent on pH. The K_{ref} values of PE (in green beans) decrease from 0.01 at pH 5.5 to 0.001 at pH 7 and increase again to 0.0077 at pH 7.5. Differences in E_d values at various pH values in the medium are less evident.

In conclusion, PE may have an influence on the texture of green beans after sterilization. Blanching green beans at temperatures lower than 80 °C improves the firmness after sterilization (Stanley et al., 1995). However enzymes responsible for deterioration of the bean quality are also activated at temperatures lower than 80 °C (Steinbuch, 1986). Further research is necessary to determine the heat stability of the enzymes causing deterioration for optimalization of the blanching process.

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