Strawberry Pectin Methylesterase (PME): Purification, Characterization, Thermal and High-Pressure Inactivation

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Pectin methylesterase (PME) was extracted from strawberries (Fragaria ananassa, cv Elsanta) and purified by affinity chromatography on a CNBr-Sepharose 4B—PME-inhibitor column. A single protein and PME activity peak was obtained. A biochemical characterization in terms of molecular mass, pl, and kinetic parameters of strawberry PME was performed. In a second step, the thermal and high-pressure stability of the enzyme was studied. Isothermal and combined isothermal—isobaric inactivation of purified strawberry PME could be described by a fractional-conversion model. Purified strawberry PME is much more stable toward high-pressure treatments in comparison to those from oranges and bananas.

Introduction

Pectin methylesterase (pectinesterase, PME, EC 3.1.1.11) is widely distributed in plants and microorganisms. In plants, PME is bound to the cell wall by electrostatic interaction. It catalyzes the de-esterification of methyl esters of polygalacturonic acid polymers to form pectic acids/pectic acids and methanol. Detrimental effects of PME activity on cloud stability of juices and nectars have been reported in detail. In contrast, beneficial effects of PME, including (i) enhancement of firmness of processed fruit and vegetable products, (ii) effective increment of extracting yield of juices by conventional approaches, and (iii) promotion of water removal from tissues upon drying, have also been reported. PME has been extracted and purified from different plant sources and characterized in terms of biochemical properties and thermal stability. In this paper, PME was extracted from strawberries and purified using a single affinity chromatography step. Purified strawberry PME was characterized in terms of biochemical properties and subjected to thermal- and high-pressure inactivation experiments.

Materials and Methods

Strawberries (Fragaria ananassa, cv Elsanta) were purchased from a local auction (Antwerp, Belgium). Apple pectin (degree of esterification 70–75%) was obtained from Fluka Chemical Co. (Switzerland). CNBr-Sepharose 4B resin was purchased from Sigma (USA). All other chemicals were of analytical grade.

PME was extracted from strawberries and purified by affinity chromatography on a CNBr-Sepharose 4B—PME-inhibitor column and finally stored in 20 mM Tris-HCl buffer (pH 7.0) at –80 °C using the same procedures described by Ly-Nguyen et al. (9).

Routine PME Assay. PME activity was measured by continuous recording of the titration of carboxyl groups released from a pectin solution using an automatic pH-STAT (Metrohm, Switzerland) and 0.01 N NaOH. Assays were performed with a 3.5 mg/mL apple pectin solution (30 mL) containing 0.117 M NaCl at pH 7.0 and 22.5 °C. The activity unit (U) of PME is defined as the amount of enzyme required to release 1 mmol of carboxyl group per min, under the aforementioned assay conditions.

Protein Determination. Protein concentration of samples was determined using Sigma procedure No. TPRO-562 (for Kit No. BCA-1 and Product No. B-9643).

Gel Electrophoresis. A PhastSystem (Amersham Biosciences, Sweden) was used for both SDS/PAGE and IEF experiments. SDS/PAGE was performed using PhastGel homogeneous 20% and PhastGel Tris–tricine SDS buffer strips. Samples were boiled for 5 min at 100 °C in a buffer containing SDS (2.5%) and β-mercaptoethanol (5%). For IEF, PhastGel IEF media (polyacrylamide gels) with a pH range of 3.0–9.0 were used. Gel staining was performed with silver nitrate according to Heukeshoven and Dernick (10) using equipment from Amersham Biosciences.

Thermal Inactivation of Purified Strawberry PME. Thermal inactivation of purified strawberry PME was investigated within a temperature range from 54 to 63 °C. Isothermal treatments were performed in a temperature-controlled water bath using 200 μL micropipets (Blaubrand, Germany) to enclose the enzyme solution. After treatments, micropipets were immediately cooled in ice water. Residual activities of PME were measured within 60 min of storage in ice water.

High-Pressure Inactivation of Purified Strawberry PME. All pressure experiments were conducted in a multivessel high-pressure apparatus (eight vessels of 8 mL) (Resato, Roden, The Netherlands). Enzyme samples in 0.3 mL flexible microtubes (Elkay, Leuven, Belgium) were enclosed in the pressure vessels, already equilibrated to a certain temperature. Pressure was built up slowly (100 MPa/min) to minimize temperature increases due to adiabatic heating. After pressure buildup,
Pressure (MPa), respectively; an equilibration period of 2 min to allow temperature to evolve to its desired value was taken into account. After pressure release, samples were immediately cooled in ice water and the residual PME activity was measured within 60 min of storage time in ice water. The pressure range studied varied from 850 to 1000 MPa at 10 °C.

Kinetic Data Analysis. The inactivation of purified strawberry PME can be described by a fractional-conversion model (11):

\[ A_t = A_\infty + (A_0 - A_\infty) \exp(-kt) \]  

(1)

where \( A_0 \), \( A_t \), and \( A_\infty \) are the initial activity, the remaining activity at time \( t \) (min), and the remaining activity after prolonged treatment of PME (mL 0.01 N NaOH/min), respectively; \( k \) is the inactivation rate constant (min\(^{-1}\)).

The temperature or pressure dependence of inactivation rate constants can be estimated using the Arrhenius (eq 2) or the Eyring (eq 3) equations, respectively:

\[ \ln(k) = \ln(k_0) + \left[ \frac{E_a}{R_1(T_o - 1)} \right] \]  

(2)

\[ \ln(k) = \ln(k_0) - \left[ \frac{V_a}{R_0 P(T - P_o)} \right] \]  

(3)

where \( T \) and \( P \) are the absolute temperature (K) and pressure (MPa), respectively; \( T_0 \) and \( P_0 \) are the reference temperature (K) and pressure (MPa), respectively; \( k_0 \) is \( k \) at \( T_0 \) and \( P_0 \) (min\(^{-1}\)); \( E_a \) is the activation energy (kJ/mol); \( V_a \) is the activation volume (cm\(^3\)/mol); \( R_1 \) (8.314 J mol\(^{-1}\) K\(^{-1}\)) and \( R_0 \) (8.314 cm\(^3\) MPa mol\(^{-1}\) K\(^{-1}\)) are the universal gas constants.

Results and Discussion

Extraction and Purification of Strawberry PME. In Figure 1, the elution profile of strawberry PME shows that inert proteins were washed out using 2 mM KH\(_2\)PO\(_4\) buffer containing 0.5 M NaCl (pH 6.0). Purified strawberry PME was successfully eluted to give a single peak of proteins and PME activity. Each replicate purification of PME from the crude strawberry extract showed a single peak. Purified strawberry PME had a maximum activity of 176.04 U/mg protein corresponding to at least a 15.8-fold enrichment and an overall yield of at least 41.76%, based on the total enzymatic activity of 80% ammonium sulfate precipitate (Table 1).

Electrophoresis. Strawberry PME showed two bands on SDS/PAGE (Figure 2). A comparison using ImageMaster one-dimensional software (Amersham Biosciences, Sweden) based on standard proteins indicated apparent molecular masses of 33.5 and 43 kDa. The third thin band with a molecular mass of 73 kDa appearing on top of the gel is due to a contaminant in the sample buffer. On IEF gel, no protein band was found between pH 3.0 and 9.0. Therefore, we can conclude that strawberry PME pI is higher than 9.0, as reported for many plant PMEs.

Purified Strawberry PME Kinetic Parameters. Effect of Substrate Concentration. The kinetic parameter \( K_m \) for strawberry PME was determined by performing PME activity assays at different pectin concentrations. Activity assays were conducted at 35 °C using apple pectin solution up to 20 mg/mL containing 0.117 M NaCl. From the data observed, a \( K_m \) of 0.416 mg/mL pectin solution was obtained using the Michaelis–Menten model (Figure 3).

Effect of pH and Temperature. Optimal pH and temperature for strawberry PME activity were determined by performing PME activity assays in a temperature range from 50 to 65 °C and a pH range from 6.0 to 8.0 using a routine PME assay, taking into account the autohydrolysis of pectin at elevated temperatures and pH levels. Fitting a polynomial model to the experimental data, the effects of pH and temperature on strawberry PME activity were depicted in the response surface and contour plots (Figure 4), which show an optimum pH

Table 1. Extraction and Purification of Strawberry Pectin Methylesterase

<table>
<thead>
<tr>
<th></th>
<th>activity (U)</th>
<th>protein (mg)</th>
<th>specific activity (U/mg(^{-1}))</th>
<th>recovery (%)</th>
<th>purification factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>80% ammonium sulfate precipitate</td>
<td>1092</td>
<td>97.85</td>
<td>11.16</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>purified PME</td>
<td>456</td>
<td>2.59</td>
<td>176.04</td>
<td>41.76</td>
<td>15.8</td>
</tr>
</tbody>
</table>

Figure 1. Elution profile of strawberry PME on a CNBr–Sepharose 4B–PME–inhibitor column. Washing solution was 2 mM KH\(_2\)PO\(_4\) containing 0.5 M NaCl, pH 6.0. Elution buffer was 50 mM Na\(_2\)CO\(_3\) containing 1 M NaCl, pH 9.85. Also shown are UV absorbance (measured at \( \lambda = 280 \text{ nm} \) (○) and PME activity (●).

Figure 2. SDS/PAGE of strawberry PME. (A) Molecular mass standards. (B) Strawberry PME after affinity chromatography.

Figure 3. Activity of strawberry PME as a function of substrate concentration. Assay conditions: apple pectin (DE 70–75%), pH 7.0, 35 °C, 0.117 M NaCl.
between 6.9 and 7.0 and an optimum temperature between 59 and 60 °C.

Autohydrolysis of Pectin at Different pH and Temperature. At elevated temperatures, apple pectin solution showed a pH- and temperature-dependent autohydrolysis, which leads to the formation of hydrogen ions. In our experiments, the autohydrolysis of pectin was investigated in a temperature range from 50 to 65 °C and a pH range from 6.5 to 9.0. Assays were performed using an apple pectin solution of 3.5 mg/mL containing 0.117 M NaCl, following the same procedure for determining PME activity without the addition of PME samples. The autohydrolysis of a pectin solution is expressed in mL/min (volume of 0.01 N NaOH solution required per min to keep the pH of pectin solution constant at different combinations of pH and temperature). The pectin autohydrolysis response surface was plotted (Figure 5) to show the effects of pH and temperature on pectin autohydrolysis. Within the pH and temperature range studied, the autohydrolysis of the apple pectin solution increases with elevated pH and temperature. This reaction should be taken into account when PME activity is to be measured at elevated temperatures.

Thermal Inactivation Kinetics of Purified Strawberry PME. Isothermal inactivation at atmospheric pressure of purified strawberry PME could be accurately described by a fractional-conversion model in a temperature range from 54 to 63 °C (Figure 6). The thermostable strawberry PME fraction contributed about 12% of the total activity. Further separation of strawberry PME isozymes and thermal stability studies of individual isozymes might be useful to gain insight in the observed fractional-conversion behavior during thermal processing.

Inactivation rate constants, estimated using nonlinear regression analysis of fractional-conversion equation, are reported (Table 2). As expected, the inactivation rate constants increase with increasing temperatures. These data show that the thermal stability of purified strawberry PME was comparable to that of orange PME (11). However, purified strawberry PME is less thermostable than purified banana PME thermally treated in the same experimental conditions by Ly-Nguyen et al. (9), who found an inactivation rate constant at 65 °C of 0.009 min\(^{-1}\) for purified banana PME. The temperature dependence of inactivation rate constants of purified strawberry PME in the temperature range studied could be adequately described by the Arrhenius equation yielding an activation energy of 206.7 kJ/mol.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Rate Constant (min(^{-1}))</th>
<th>% Remaining Activity</th>
<th>(E_a) (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>54</td>
<td>0.0376 ± 0.0049(^a)</td>
<td>12.7 ± 1.7</td>
<td>206.7</td>
</tr>
<tr>
<td>57</td>
<td>0.0555 ± 0.0055</td>
<td>10.1 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0.1271 ± 0.0130</td>
<td>11.2 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>0.4940 ± 0.0747</td>
<td>12.6 ± 2.4</td>
<td></td>
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</table>

\(^a\) Standard error of regression.

High-Pressure Inactivation Kinetics of Purified Strawberry PME. The high-pressure inactivation at 10 °C of purified strawberry PME could be adequately described by a fractional-conversion model in the pressure range of 850–1000 MPa (Figure 7), indicating the presence of a first-order inactivating pressure-sensitive strawberry PME fraction and the occurrence of a pressure-stable PME fraction. The inactivation rate constants of the pressure-sensitive strawberry PME fraction increase with increasing pressure levels. Kinetic data in
Tor isolated from kiwi fruits. Isothermal and isobaric single affinity chromatography step using a PME-inhibitor-PM.

Improvement of processed strawberries by enhanced pressure treatments, which can be beneficial for structure stability. However, strawberry PME is very stable under pressure treatments, which can be beneficial for structure improvement of processed strawberries by enhanced PME activity during high-pressure processing.

**Figure 7.** High-pressure inactivation at 10 °C of purified strawberry PME dissolved in 20 mM Tris–HCl buffer (pH 7.0), modeled using a fractional-conversion model, at 850 MPa (○), 925 MPa (▲), and 1000 MPa (▲).

**Table 3.** Kinetic Parameter Estimates of a Fractional-Conversion Model for Isothermal–Isobaric Inactivation of Purified Strawberry PME at 10 °C

<table>
<thead>
<tr>
<th>P (MPa)</th>
<th>k (min⁻¹)</th>
<th>A∞ (%)</th>
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<tbody>
<tr>
<td>850</td>
<td>0.0131 ± 0.0009</td>
<td>9.4 ± 1.7</td>
</tr>
<tr>
<td>925</td>
<td>0.0177 ± 0.0036</td>
<td>11.6 ± 5.1</td>
</tr>
<tr>
<td>1000</td>
<td>0.0260 ± 0.0045</td>
<td>9.2 ± 2.9</td>
</tr>
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</table>

\[ V_a = -10.77 \pm 0.67 \text{ cm}^3/\text{mol} \]

\[ R^2_a = 0.996 \]

* Standard error of regression.

Table 3 show that purified strawberry PME is much more stable toward high-pressure treatments as compared to banana PME (9) and orange PME (11). The pressure-stable PME fraction contributed about 10% of the total activity. The pressure dependence of the inactivation rate constants of the pressure-labile PME fraction within the pressure range investigated could be adequately modeled by the Eyring relationship yielding an activation volume of \(-10.77 \text{ cm}^3/\text{mol}\).

**Conclusions**

Strawberry PME can be successfully purified by a single affinity chromatography step using a PME-inhibitor isolated from kiwi fruits. Isothermal and isobaric–isothermal inactivation of purified strawberry PME follows fractional-conversion models. Strawberry PME is comparable to orange PME with regard to its thermostability. However, strawberry PME is very stable under pressure treatments, which can be beneficial for structure improvement of processed strawberries by enhanced PME activity during high-pressure processing.

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**References and Notes**


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