Structural changes of pectin methylesterase from orange peel subjected to thermal and high pressure processing

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ABSTRACT

Pectin methylesterase (PME) from orange peel (Valencia cv.) was extracted and purified using cation exchange and gel filtration chromatography. The purified orange peel PME contained a single band of 32000 Da with an isoelectric point higher than 9. The effect of High Pressure (HP) processing on the activity of PME in Tris-HCl buffer solution (pH 7.5) was studied, irreversible HP-induced conformational changes were investigated by far and near – UV Circular dichroism (CD). The relative residual activity of PME decreased with the increase of pressure and treatment duration. HP inactivation of purified orange peel PME can be described by a first-order kinetic model. The inactivation rate constant k values were calculated as 0.018, 0.037, 0.085 and 0.169 min⁻¹ at 200, 400, 500 and 700 MPa and 40°C respectively. As far as the CD analysis of PME samples is concerned, a characteristic for β-structure (β-sheets and turns) minimum at ~218 nm was observed. The secondary structure of PME was changed following HP treatment. Spectra of the thermal and high pressure treated samples showed different profiles in comparison to the native PME as the intensity of the minimum at the CD spectra decreased with the increase of pressure and treatment duration. It was observed that the conformational change of the secondary structure was accompanied by a corresponding loss of enzyme activity. The obtained results explain the inactivation of this enzyme after processing at certain conditions where irreversible conformational change was induced.

Keywords: pectinmethylesterase ; high pressure ; circular dichroism ; secondary structure ; tertiary structure

INTRODUCTION

Pectinmethylesterase (PME), an endogenous enzyme found in fruits and vegetables, affects the quality characteristics of final products (such as juices). In the literature there is a significant number of papers describing the effect of high pressure (HP) and temperature on PME activity from different fruits and vegetables, such as orange, tomato, peach, strawberry, green beans and papaya. The results found in the literature and the obtained results from experiments conducted from our laboratory show that the degree of inactivation depends on the origin of the enzyme[1,6]. Despite the extensively studied PME inactivation using thermal and HP processing, there is limited work done on the description of structural changes of this enzyme processed by HP. The pressure-induced changes in the rate of enzyme-catalysed reactions may be attributed to (a) changes in the structure of PME, (b) changes in the reaction mechanism and (c) the effect of a rate-determining step on the overall rate. Mozhaev et al., reports that high pressure accelerates reactions that are accompanied by a decrease in volume [5]. Hence, the relationship between the activity and conformation of PME as well as the mechanism of HP-induced inactivation of this enzyme is worth investigating.

The objective of this research was to study the structural changes of PME before and after treatment of the enzyme at temperatures 40-80°C, combined with pressures 0.1-800 MPa.
MATERIALS & METHODS

Materials
The PME was produced from orange peel (Valencia cv.) using the procedure for purification as described by Cameron [4]. After ammonium sulphate fractionation (30-75%), the PME was purified by cation exchange (UNOsphereS, Biorad) and gel filtration (Biogel P30, Biorad) chromatography. All fractions were analyzed for PME activity and protein concentration. The protein concentration was determined spectrophotometrically according to Bradford [2] and the PME activity was measured using a continuous spectrophotometric assay to a kinetic microplate reader [3]. Apple pectin with 70-75% degree of esterification was used as substrate for PME activity measurement and it was obtained from Sigma Chemical Co.(USA). All other reagents in the investigation were of analytical grade.

High pressure treatment
High pressure treatments were achieved using a pilot scale HHP equipment with a maximum operating pressure and temperature of 1000 MPa and 100°C respectively (Food Pressure Unit FPU 1.01, Resato International BV, Roden, Holland) consisting of an operation high pressure unit with a pressure intensifier and a multivessel system consisting of six vessels of 45 mL capacity each. Vessels were surrounded by liquid circulating jackets connected to a heating-cooling system. Experiments were performed for pressures 100-750 MPa and temperature 30-80°C. Pressure was released after preset time intervals independently for each vessel by opening the pressure valve. Pressure and temperature were constantly monitored and recorded (in 1s intervals) during the process.

Circular dichroism spectroscopy (CD)
The secondary and tertiary structure of PME was determined by CD spectroscopy. CD measurements were conducted using a JASCO-715 spectropolarimeter with a Peltier type cell holder, which allows for temperature control. Wavelength scans in the far (190 to 260 nm) and the near (260 to 340 nm) UV regions were performed in Quartz SUPRASIL (HELLMA) precision cells of 0.1 cm and 1 cm path length, respectively. Each CD spectra was obtained by averaging four successive accumulations with a wavelength step of 0.5 nm at a rate of 50 nm min\(^{-1}\), response time 8 s and band width 2 nm. Buffer spectra were accumulated and subtracted from the sample scans. The far and near UV spectra were analyzed by curve-fitting software ORIGIN 8.0 (OriginLab Corporation, USA) and for the calculation of secondary structural contents of the protein the CDNN program (Institut für Biotechnologie, Martin-Luther Universität Halle-Wittenberg) was used.
RESULTS & DISCUSSION

Pectinmethylesterase was purified from orange peels. The steps of purification included precipitation with ammonium sulphate, cation exchange chromatography and gel filtration. The PME binds strongly to a cation-exchange column (UNOsphere S) media at pH=7.5 whereas most of the proteins were eluted. Increasing the NaCl gradient, four peaks of PME activity were eluted. The major activity peak eluted at NaCl concentration of 0.12 M (figure 1A). This sample was rechromatographed on cation-exchange column at pH=7.5 for further purification. The activity peak was finally resolved by a Biogel P30 gel filtration column as a single activity peak corresponding to a molecular mass of 32 kDa. The final purification factor for the PME (with major activity peak) was 14.7.

Figure 1. (A) UNO Sphere S cation exchange chromatography of PME in 20mM Tris-HCl at pH 7.5. Bound proteins were eluted with a 0 to 500 mM NaCl gradient. (B) Biogel P30 gel filtration chromatography of UNO S binds PME at pH 7.5.

High-Pressure Inactivation

The effect of high pressure and temperature on the inactivation of PME was studied. In Fig. 2 the effect of pressure on the inactivation of PME at 40°C in a pressure range between 200 MPa and 700 MPa is shown. The isothermal and HP inactivation of purified orange peel PME can be described by a first-order kinetic model. The inactivation rate constants, k (min⁻¹) were calculated as 0.018, 0.037, 0.085 and 0.169 min⁻¹ for processing at 200, 400, 500 and 700 MPa, respectively. As it was expected, application of higher pressure resulted in a higher loss of activity. When the inactivation rate constants for enzyme at 40°C were plotted as a function of pressure, the activation volume was estimated as -11.9 mL/mol.

In Fig. 3 the effect of temperature during HP treatment is shown. As it can be seen, for the same pressure (400MPa) temperature had a profound effect. At a temperature of 55°C PME activity appeared to have a significant decrease. Comparing these data with those found in the literature for pressure inactivation of orange PME in different matrices, it can be seen that the obtained k values are significantly lower. This could be attributed to the degree of purification of the enzyme, since it is evidenced that a purified enzyme is more pressure resistant compared to the unpurified same enzyme [7].
Figure 2. High Pressure inactivation of purified PME (orange peel, Valencia cv.) at 200, 400, 500 and 700 MPa and 40°C

Figure 3. Effect of temperature (40-55°C) on the inactivation rate constants of HP treated (400MPa) PME

Analysis of PME structure by Circular Dichroism

The far and near-UV CD characterization of HP treated and native PME are depicted in Fig.4 and 5. The UV CD spectra of all samples were analyzed immediately after HP treatment. A characteristic for β-structure (β-sheets and turns) proteins minimum at ~218 nm was observed for PME. Spectra of the thermal and high-pressure treated samples showed different profiles compared to the native PME. Irreversible conformational changes of the secondary and the tertiary structure were accompanied by a corresponding loss of enzyme activity. As it can be seen, the structural changes of pressure-treated PME are not similar to their thermally induced process. For similar loss of enzymatic activity (residual activity ~ 0.74), the short thermal treatment at 60°C for 5 min had higher effect on the PME structure than the high pressure treatment. Furthermore, as shown in figure 4, the spectra of HP-treated PME at 200 MPa exhibited a slight modification, whereas the spectra of HP-treated at 600 MPa exhibited significant conformational change from native PME.

The near-UV CD spectra of PME samples showed significant tertiary structure changes for pressures higher than 200MPa. The spectra Δθ (difference in ellipticity between the untreated and HP-treated PME (200 and 600 MPa at 30°C)) vs. λ, depicting changes in the enzyme’s tertiary structure are shown in Fig. 5 A and B. In addition, this figure is providing evidence that the conformational changes of the tertiary structure is correlated to a significant change in the activity of PME.
Figure 4. Far-UV CD spectra of untreated and HP treated PME.

Figure 5. A. The difference $\Delta \theta$ vs. $\lambda$ (native PME at 25 °C– treated PME at 600 MPa and 30 °C for 10 min (residual activity = 0.8)) near-UV CD spectra B. The difference $\Delta \theta$ vs. $\lambda$ (native PME at 25 °C– treated PME at 200 MPa and 30 °C for 10 min (residual activity = 0.96)) near-UV CD spectra
CONCLUSION

The secondary and tertiary structure of PME treated by HP changed as evidenced by CD analysis. The intensity of negative peak in the CD spectra as well as the relative residual activity of PME decreased with an increase of pressure and treatment duration. The obtained results explain the reduced enzyme activity (inactivation) of this enzyme after processing at certain conditions where irreversible conformational changes were induced.

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REFERENCES


