PECTIN ACETYLESTERASE – ANALYSIS AND APPLICATION FOR SUGAR BEET PECTIN UTILIZATION

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ABSTRACT

Sustainable technologies are being sought to provide new and higher-value coproducts from sugar beet pulp. Pectin is a complex plant cell wall polysaccharide that represents a major fraction of sugar beet pulp. One distinguishing feature of sugar beet pectin is a high content of C2 and C3 acetyl esters. Such esters impart unique physical, chemical, and functional properties. Acetyl esters probably function biologically to restrict pectin depolymerizing enzyme activities. The enzyme pectin acetylesterase (EC 3.1.1.6) can specifically hydolyze such acetyl esters in homogalacturonan regions of pectin, thus it may play a critical role in cell wall modification during root development and during pest/pathogen interactions. Pectin acetylesterase may be used technically in an efficient, environmentally-friendly bioprocess for modifying sugar beet pectin structure to improve performance and increase functionality. Such pectins can be used to fabricate new biobased materials for application in drug delivery systems. This presentation will summarize our recent developments in four areas supporting enzymatic modification of sugar beet pectin: 1) A new GC-MS method, with headspace solid-phase microextraction, for efficient determination of ester content in pectin and of esterase enzyme activity. 2) A specific polyacrylamide gel electrophoresis method to distinguish pectin esterase types. 3) The purification and biochemical characterization of pectin acetylesterase. 4) The preparation of porous "microsponge" matrices using enzyme-modified sugar beet pectin.

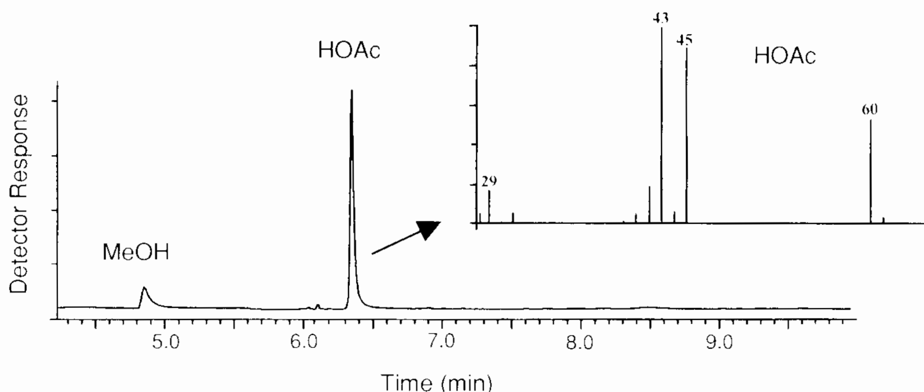
INTRODUCTION

We are conducting new research to develop a better understanding of enzyme systems involved in modifying sugar beet cell wall structure. No pectinase or related enzymes that act on cell-wall polysaccharide have been isolated or described from sugar beet. These enzymes are implicated functionally not only in quality of cell wall residues (pulp), but also in the protective function of the cell wall. We seek to determine which of these enzymes may be useful in bioprocesses to prepare unique polysaccharides with improved performance and greater functionality. This report presents some of our recent results targeting development of pectin acetyl esterase and its use in preparation of new biomaterials from pectin derived from sugar beet pulp.

RESULTS

A new GC-MS method was developed to provide a simple, fast, and direct means for measuring the acetic acid and methanol contents of pectin. The contents from as a little as 1 mg pectin sample are readily detected and quantified (Figure 1). This method can also be used to specifically determine esterase enzyme activities (Savary and Nuñez, 2003), and it will be generally useful in pectin characterization (Fishman et al., 2001).

Figure 1. Representative chromatogram from GC-MS for the separation and determination of methanol and acetic acid contents in sugar beet pectin. Products from hydrolyzed pectin esters were recovered by headspace solid-phase microextraction (Carboxen-PDMS fiber), separated by GC on a PoraPlot Q column, and then analyzed by electron impact mass spectrometry with selected ion monitoring. Stable deuterated isotopomers of methanol and acetic acid were used as internal standards and construction of calibration curves for absolute quantification of analytes. Insert provides mass spectrum confirming identify of acetic acid.



Pectin acetylesterase and pectin methylesterase activities can be differentially detected by analytical SDS and IEF polyacrylamide gel electrophoresis (Figure 2). Pectin methylesterase and acetylasterase activities isolated from orange peel were initially separated from each other by cation-exchange and affinity chromatography (Savary et al., 2002).

Pectin acetylesterase and pectin methylesterase have been used to selectively modify sugar beet and citrus pectins (Hotchkiss et al., 2002; Yoo et al., 2003), and pectin-based bioproducts have been fabricated (Figure 3). The biomatrix formed is manifested as a sponge-like structure, and it can be further modified by infiltration with a secondary polymer. Such materials with their unique physical and chemical properties are now being investigated for new biomedical and industrial applications (Liu et al., 2003).

Figure 2. Representative electropherograms for Polyacrylamide Gel Electrophoresis analysis of acetylesterase and methylesterase preparations. Panel A, Non-reducing SDS-PAGE (12% monomer) with Coomassie blue staining of enriched orange acetylesterase (A) and pectin methylesterase (M). Panel B, Following SDS-PAGE, recovery of acetylesterase activity detected with β-NAA substrate. Panel C, Native IEF-PAGE (pH 8-10.5) with detection of acetylesterase activity. Panel D, IEF-PAGE with pectin methylesterase activity detected by ruthenium red staining of pectin-agarose overlay gel.

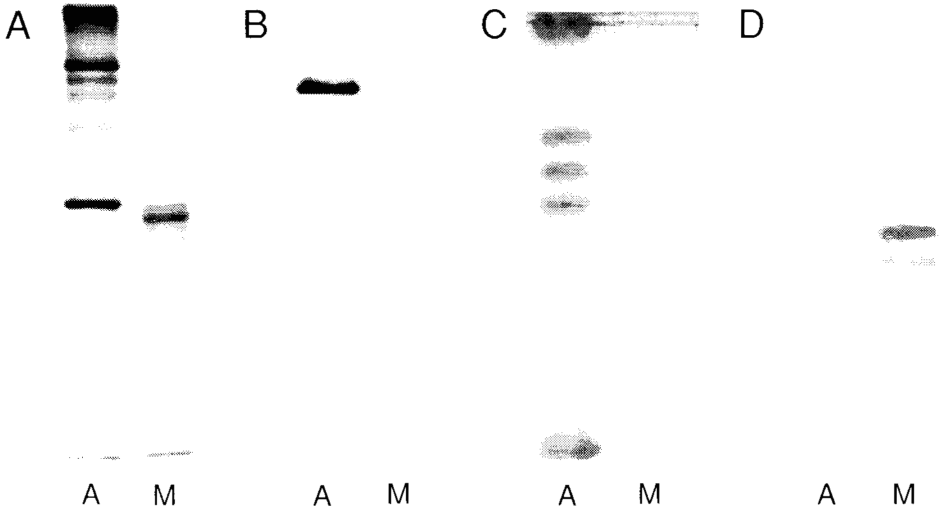
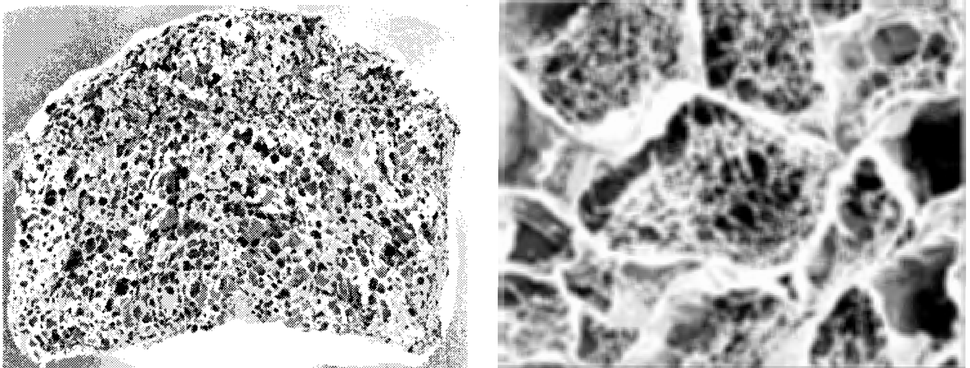


Figure 3. Scanning electron microscopy of a new biomatrix fabricated from sugar beet pectin. A pectin "microsponge" prepared from sugar beet pectin (left panel). A closer view of a pectin microsponge generated with an interpenetrating polymeric network (right panel).



CONCLUSION

We have developed new tools for analyzing pectin acetylesterase and its activity on sugar beet pectin. These tools are now being used to isolate and characterize this enzyme from sugar beet tissues. Pectin acetylesterase and related esterases are being used to prepare new bioproducts from sugar beet pectin.

REFERENCES

1. SAVARY, B.J., & NUÑEZ, A.: GC-MS quantification of the methanol and acetic acid contents of pectin using headspace microextraction with stable isotope dilution. (Submitted to *J. Chrom. A.)*
2. SAVARY, B.J., HOTCHKISS, A.T, & CAMERON, R.G.: Characterization of a salt-independent pectin methylesterase isolated from Valencia orange peel. *J. Agric. Food Chem. 50,* 3553-3558, 2002.
3. FISHMAN, M.L., CHAU, H.K., HOAGLAND, P., & AYYAD, K.: Characterization of pectin, flash-extracted from orange albedo by microwave heating, under pressure. *Carbohydr. Res., 323,* 126-138, 2001.
4. HOTCHKISS, A.T., SAVARY, B.J., CAMERON, R.G., CHAU, H.K. FISHMAN, M.L., & LUZIO, G.A.: Enzymatic modification of pectin to increase its calcium sensitivity while preserving its molecular weight. *J. Agric. Food Chem. 50,* 2931-2937, 2002.
5. YOO, S.-H., FISHMAN, M.L., SAVARY, B.J., HOTCHKISS, A.T.: Monovalent salt-induced gelling system of enzymatically deesterified pectin. (Submitted to *J. Agric. Food Chem.*)
6. LIU, L.S., FISHMAN, M.L., KOST, J., & HICKS, K.B.: Pectin-based systems for colon-specific drug delivery via oral route. (Submitted to *Biomaterials.*)