PLANT PECTIN METHYLESTERASE TREATMENTS DRAMATICALLY REDUCE WATER-BINDING IN SUGAR BEET PULP VIA CALCIUM CROSSLINKING

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Abstract:

We are investigating a novel application of a thermally-tolerant plant pectin methylesterase (TT-PME) to improve energy efficiency in sugar beet processing. Sucrose diffusion from cossettes occurs in water heated to about 70°C, the optimal temperature for TT-PME activity. Drying pulp is necessary to stabilize it for storage and commercialization as animal feed, but this consumes up to 30% of total energy in a processing plant. Beet pulp's high water binding is associated to its high content of pectin and associated polysaccharides. We hypothesize TT-PME action on pectin in the presence of calcium, can reduce water-binding in beet pulp by promoting calcium-mediated pectin crosslinking, resulting in a denser more compressible tissue. This may lower the energy cost of drying pulp through more efficient mechanical pressing. To test this hypothesis, we treated beet pulp with TT-PME and its identically acting homolog PME2, in the presence of calcium, under optimal enzymatic conditions. Calcium addition without enzyme treatment could improve water separation by 10%, while PME treatments without calcium addition showed no significant difference over untreated controls. PME treatments of beet pulp in combination with calcium showed dramatically reduced water binding, which was greater than 20% than by calcium alone. This establishes the potential utility for developing biotech beets that express TT-PME to provide an improved processing trait.

Introduction:

Sucrose is extracted from sliced beet roots (cossettes) by diffusion with hot water (70°C). This process generates millions of tons of wet pulp residue, which is subsequently mechanically pressed to reduce water contents, then heat-dried to allow storage and commercialization as an animal feed ingredient. Calcium, in the form of gypsum, is used as a pressing aid to improve water separation (E.D. Bosse, 1997). Drying beet pulp is highly energy intensive, consuming up to 30% of the energy of a beet processing plant (Asadi, 2007). Reducing energy consumption in the factory is desired to reduce energy costs and associated green-house gas emissions, thus improving sustainability.

We are investigating a novel application for the enzyme thermally-tolerant pectin methylesterase (TT-PME; Savary et al., 2003; Savary et al., 2013). Pectin is an abundant cell wall polysaccharide in beet pulp (25% dry weight; McCready, 1966). Pectin functions in root tissue structure (pulp) to entrap and bind water, hence it contributes to the high energy needs for drying beet pulp. PME action on pectin introduces charged uronic acid residues in pectin's homogalacturonan regions, and these sites can interact with multivalent cations such as calcium to promote cooperative interchain cross-linking (Figure 1). Native beet pectin contains about 45% unesterified galacturonic acid contents (Savary and Nunez, 2003), which is sufficient to support calcium's benefit as a pulp pressing aid.

We hypothesize that plant PMEs' processive action (Cameron et al., 2011) on pectin in beet pulp can improve calcium's ability to reduce water binding by providing more extensive cooperative binding. TT-PME is optimally active at the thermal conditions used for sucrose diffusion (Figure 2; Savary et al., 2010). At this high temperature, most plant PMEs are denatured, presumably including native sugar beet PMEs. Our objective was to assess TT-PME's effect on water binding in beet pulp following exogenous treatment under process conditions. This will establish a proof of principle for our long term goal to develop biotech beets that express TT-PME in tap roots for an improved processing trait. This preliminary report provides evidence for PME's beneficial action to significantly improve mechanical separation of water from beet pulp.

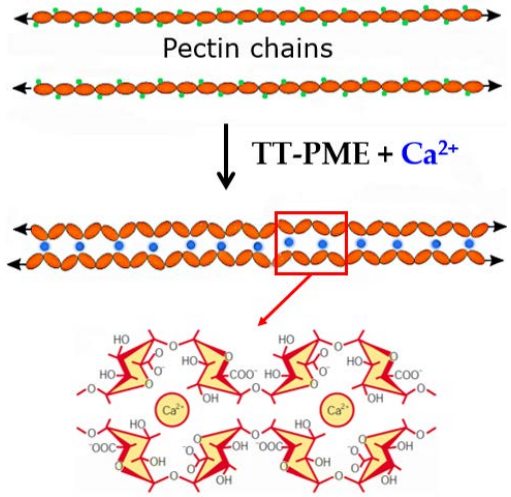

Figure 1. TT-PME action promotes cooperative calcium cross-linking in pectin. This may improve tissue compressibility.

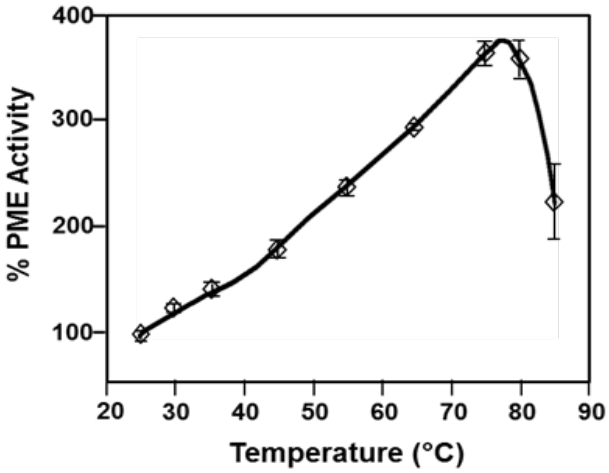

Figure 2. TT-PME is highly active at the water temperature conditions used during cossette diffusion (60 to 70 °C), conditions that denatures other PMEs.

Materials and Methods

Sugar beet pulp preparation. Sugar beets were collected from a Betaseed energy beet field site in Newport, Arkansas, then washed, peeled, chopped and the pulp separated from the juice by grinding with a commercial juicer. The ground pulp was heat-dried at 80°C in a vacuum oven, and blended to a powder to facilitate treatments.

PME extraction and activity quantification. TT-PME, and its 9-fold more abundant thermolabile isoform PME2 (both with identical action on pectin: Savary et al., 2010; Cameron et al., 2011) were isolated from orange pulp, and PME enzymatic activity quantified as described by Savary et al. (2013).

PME treatment of beet pulp. Two grams of dry powdered pulp were hydrated in dilute phosphate buffer (pH 7.5) containing combinations of 0, 0.5, or 5 PME units per gram of dry powdered pulp and 0, 10, or 30 mM $CaCl_2$, then incubated overnight at 30°C). Replicate trials with TT-PME were performed at 60°C). Calcium concentrations approximate those used for beet processing (Asadi, 2007). The treated powdered pulp was centrifuged at 16,000 x g for 1 hour, to separate excess water.

Determination of water binding. Water bound to powdered pulp after PME treatment was determined as follows, then converted to a percentage of the untreated control:

$$\frac{\text{Weight of wet treated pulp (g) – weight of dry pulp (g)}}{\text{Weight of dry pulp (g)}}$$

Statistical analysis. The assay was replicated 3 times for each treatment. Significant effects of PME treatments (p-value < 0.05) were established by 2-factor (PME dosage, and calcium concentration) analysis of variance (ANOVA), followed by a least significant difference test.

Results and Discussion

To establish if PME treatment of beet pulp can promote calcium crosslinking to improve water separation, a benchtop assay was developed for small-scale screening trials. Ground beet pulp was treated in 50 ml centrifuge tubes with three dosage levels of enzyme and calcium chloride (including zero additions for controls) followed by centrifugation to measure supernatant volumes. With no PME treatment, 10-30 mM calcium alone reduced water binding about 10%, which is consistent with the benefit of pressing aids containing calcium. Enzyme treatments with no supplemented calcium showed no difference with untreated controls, which indicates the necessary function of calcium in pectin crosslinking. Remarkably, low enzyme dosage (0.5 PME U/g) with 10 mM calcium reduced water binding in the pulp pellet by an additional 20%. Ten-fold higher enzyme dosage provided an additional 5% reduction. Thus there was an approximate maximum 35% total reduction when 5 PME U/g was applied with supplemented calcium, as compared to the untreated control. Statistical analysis confirmed these effects as highly significant (p-value <0.0001, Figure 3).

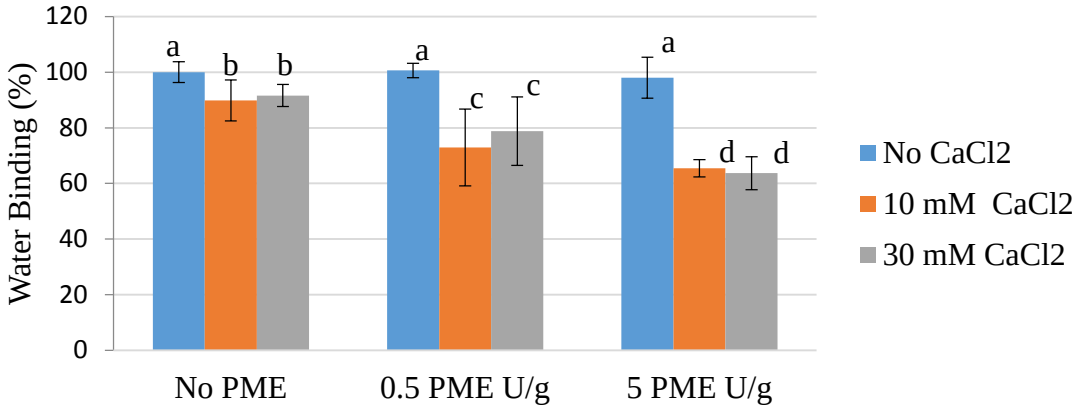

Figure 3. Water binding in beet pulp following treatments with two PME dosages and 10-30 mM calcium chloride. Water binding is shown as a percentage of the untreated control. Letters above bars indicate statistical LSD test's grouping.

Conclusion:

PME action combined with supplemented calcium demonstrated dramatically improved reduction in the water binding capacity of ground beet pulp. This supports the proposed mechanism that improved cooperative ionic crosslinking drives a denser, more compressible tissue. This establishes proof of principle for localized expression of TT-PME in beet roots, which will be

active in the diffuser with calcium that is already added for cossette processing. Transgenic sugar beets expressing TT-PME may thus deliver the benefit observed in our studies to beet processors.

Acknowledgments:

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