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A transcriptomics-based kinetic model for enzyme-induced pectin degradation in apple (*Malus* × *domestica*) fruit

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ABSTRACT

Modifications of cell wall pectin during apple softening involve the concerted action of several enzymes. A mathematical model was developed to describe the action of cell wall hydrolases resulting in specific modifications in cell wall pectin polysaccharides during softening of 'Jonagold' apple. A conceptual model of enzyme-induced pectin breakdown was formulated based on current knowledge. Activities of different cell wall enzymes were considered to be directly correlated to the expression levels of the respective genes. The first pectin modification to occur was assumed to be cleavage of side chain neutral sugars from the polymer backbone, by the action of side chain debranching enzymes, beta-galactosidase and alpha-L-arabinofuranosidase. It was considered that this increases cell wall porosity, facilitating the action of pectin methyl esterase, which de-methylates pectin polysaccharides, providing a substrate for polygalacturonase. Polygalacturonase degrades the de-methylated pectin chain, producing smaller pectin fragments which are easily solubilised in water. Coupled differential equations were written based on these reactions. The model was calibrated using data on expression of cell wall-related genes, activities of enzymes encoded by these genes, and the associated modifications in pectin polysaccharides during softening in 'Jonagold' apples stored at 1 °C under regular atmosphere or controlled atmosphere. The model could explain 79% of the variation in the data. Using the model, *in silico* investigations on the impact of downregulation of different key cell wall-related genes on softening were carried out, and the results were compared successfully with currently available literature data.

1. Introduction

Loss in flesh firmness resulting from enzyme-induced modifications in cell wall pectin is a major cause of postharvest quality losses in apples. Consequently, several studies aimed at understanding the modifications that occur within cell wall pectin polysaccharides and how these modifications are regulated during softening (Wu et al., 1993; Atkinson et al., 2012; Ng et al., 2013; Gwanpua et al., 2016b). A number of cell wall hydrolases have been implicated in pectin modifications during fruit softening, such as polygalacturonase (PG), pectin methyl esterase (PME), beta-galactosidase (β-GAL), alpha-arabinofuranosidase (α-AF), and pectate lyase (Goulao et al., 2007; Gwanpua et al., 2014). Also, non-pectolytic proteins, in particular expansins, have been shown to be involved in reorganizing the cellulose–xyloglucan network (Rose et al., 1997).

Characterization of cell wall pectin changes provides information on its degradation (Hadfield, 1998). Pectin characterization is particularly important when studying softening because *in vitro* activities measured for most enzymes may significantly deviate from *in vivo* activities (Christiaens et al., 2012). The relationship between expression of cell wall-related genes, activities of cell wall hydrolases, and pectin modifications is not straightforward. The current hypothesis assumes that PG action results in pectin depolymerisation and solubilisation, while β-GAL, α-AF, and softening-related expansins increase cell wall porosity (Brummell, 2006). A useful way to ascertain this hypothesis is to quantify the relationships using mathematical models (Hertog et al., 2011). Several models have been developed to understand and predict apple softening (Tijskens et al., 1999; Hertog et al., 2001; Johnston et al., 2001; Gwanpua et al., 2012, 2013; van der Sman and Sanders, 2012). However, these models so far were limited to describing the effect of temperature and composition of atmospheric gases on firmness, as a physical property, rather than explaining the underlying

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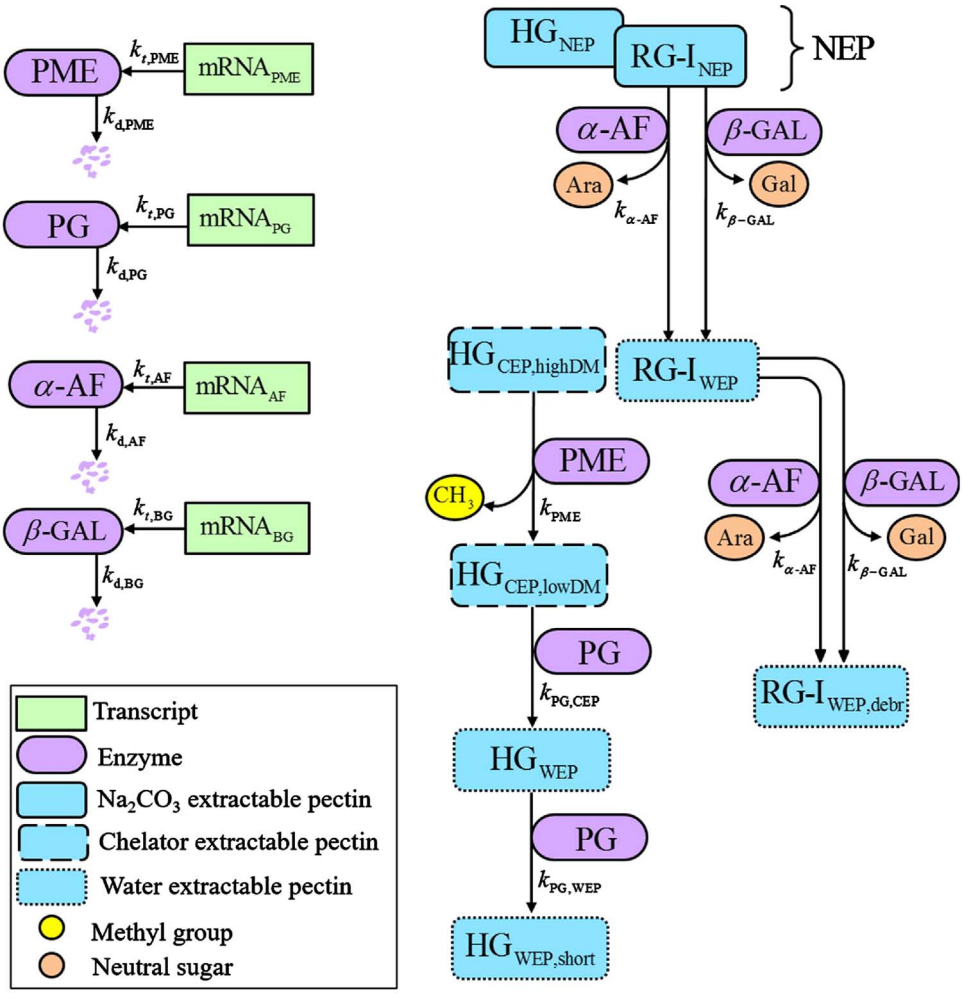

Fig. 1. A conceptual model of enzyme-induced cell wall pectin modifications during apple fruit softening. The Na_2CO_3 extractable pectin (NEP) constitutes Rhamnogalacturonan −I (RG-I) molecules to which are attached Homogalacturonan (HG) with high degree of methylation (DM). Beta-galactosidase (β-GAL) and alpha-arabinofuranosidase (α-AF) catalyse the removal of galactose (Gal) and arabinose (Ara), respectively, from RG-I, with rate constants $k_{\beta\text{-GAL}}$and $k_{\alpha\text{-AF}}$, respectively. This results to the release of HG molecules with high DM, $HG_{CEP,\ high\ DM}$ and RG-I molecules that are extractable with water, $RG\text{-}I_{WEP}$. Further debranching occurs in $RG\text{-}I_{WEP}$, while pectin methyl esterase (PME) acts on $HG_{CEP,\ high\ DM}$, removing methyl groups (CH_3) from the galacturonic acid backbone to form HG molecules with low DM, $HG_{CEP,\ low\ DM}$ with rate constant k_{PME}. Polygalacturonase (PG) catalyses the breakdown of $HG_{CEP,\ low\ DM}$ to form HG molecules that are solubilisable in water, HG_{WEP}, with a rate constant $k_{PG,CEP}$. Further breakdown of HG_{WEP} by PG to form shorter chains HG molecules, $HG_{WEP,\ short}$ is possible, with a rate constant $k_{PG,WEP}$. The activities of the different enzymes are controlled by translation and protein degradation, with rate constants $k_{t,p}$and $k_{d,p}$, respectively (p represents respective enzyme).

biochemical changes responsible for fruit softening. An exception is the model developed by Róth et al. (2008) for predicting enzymatic softening of apples, based on a simplified mechanism of sequential degradation of pectin backbone by β-GAL and PG. However, no data on pectin properties was used in that model, and the actual mechanism of modifications in pectin polysaccharides during apple softening is much more complex.

With the goal of developing a detailed kinetic model to mechanistically describe enzyme-induced cell wall breakdown during apple softening, we collected data on cell wall pectin characterization, activities of cell wall enzymes, and expression of cell wall-related genes during ripening in 'Jonagold' apples kept under different storage conditions (Gwanpua et al., 2014, 2016a). The present study uses these data to develop a kinetic model describing the events from the expression of cell wall-related genes, over synthesis of cell wall hydrolases to modifications in pectin polysaccharides leading to softening of 'Jonagold' apples, similar to an approach earlier used in modelling ethylene biosynthesis in apples (Van de Poel et al., 2014). The model was used *in silico* to investigate the impact of downregulation of different cell wall-related genes on cell wall modifications, and the findings were compared to literature data.

2. Materials and methods

Apples (*Malus* × *domestica* Borkh.) cv 'Jonagold' were harvested in 2012 from an orchard located at Opvelpsestraat, Bierbeek, Belgium. Fruit were harvested at commercial maturity and stored at 1 °C, either under controlled atmosphere (CA) storage or regular atmosphere for 6 months. Before storage and at intervals during storage, firmness, cell wall pectin properties, and activity of various cell wall hydrolases (PG, PME, β-Gal, and α-AF) were quantified. This data was earlier reported in Gwanpua et al. (2014). Firmness was measured as the maximum force needed for a self-cutting cylindrical probe, with a surface area of 1 cm^2, to penetrate the fruit to a depth of 8 mm. Cell wall material was isolated from apple cortex tissue as alcohol insoluble residue (AIR). The AIR was fractionated by extracting sequentially with water, CDTA, and Na_2CO_3, to obtain water extractable pectin (WEP), chelator extractable pectin (CEP), and Na_2CO_3 extractable pectin (NEP) fractions, respectively. The uronic acid content of the AIR and the different fractions were quantified. Also, the degree of methylation of the AIR and the WEP fraction were quantified. Furthermore, the molar mass and the neutral sugar content of the WEP fraction were quantified using size exclusion chromatography and high-performance anion exchange chromatography, respectively. Optimized protocols were used for obtaining