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Effect of mechanical impact-bruising on polygalacturonase and pectinmethylesterase activity and pectic cell wall components in tomato fruit

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Abstract

Tomato bruise damage is a common postharvest disorder that substantially reduces fruit quality. Due to lack of detailed knowledge about bruising mechanisms, effective bruise prevention is difficult. Bruises show local tissue softening in parallel with normal textural changes. Accordingly, the underlying processes at the molecular level were studied. Alterations of pectic cell wall components (degree of methylesterification, pectin solubility properties (fractionation), size exclusion of pectin extracts) and the related enzymes (pectinmethylesterase and polygalacturonase activities) were examined in mature green to red ripe tomatoes impacted at high energy. Results showed no substantial changes in PME and PG activity with bruising, although PG activity increased significantly with ripening. The degree of demethoxylation was slightly reduced in wounded tissue 3 h after impact-bruising. Bruising did not lead to significant changes in pectin solubility or degree of polymerisation within 3 h of impact. The idea of an accelerated tissue breakdown paralleling normal ripening-associated tissue softening and initiated by mechanical injury of the fruit, is suggested and might become more pronounced with longer incubation times post-impact. Changes to the xyloglucan network are also likely to be involved.

Keywords: Tomato; *Lycopersicon esculentum* Mill.; Impact bruise damage; Pectin; Polygalacturonase; Pectinmethylesterase; Size exclusion chromatography

1. Introduction

Food quality is an important issue in the agro-industry. Growing consumer awareness is reflected in the many labels that have recently been established, all of which refer to one or more aspects of quality. One quality criterion for fresh fruit such as tomatoes (*Lycopersicon esculentum* Mill.) is the absence of blemishes. Not only because external damage renders the fruit less attractive for consumption, but also because defects are preferred entry sites for pathogens and fungi. Hence, external damage creates a threat for food safety.

As consumers expect top quality tomatoes, graders discard fruit with visible defects. However, some defects may remain disguised. A bruise is a type of subcutaneous tissue failure without rupture of the skin (Mohsenin, 1986) and is listed as one of the most common postharvest disorders. Because bruises generally do not instantly develop on the fruit, defective fruit may slip through the inspection area. In many fruit species, discolouration of the injured tissue indicates the damaged spot. This is not the case with tomatoes, where instead, the affected tissue steadily softens 2–3 days after injury, and when the defect becomes apparent, the tomato has often reached the consumer. Even at a later stage, the bruises remain hardly visible to the naked eye. As a consequence, the grower has limited control on the end-quality of the product and the consumer might lose trust in the quality label.

Bruise prevention is necessary to control the quality of fresh market tomatoes. Effective prevention is only possible when the factors responsible for bruise development are known. Unfortunately, studies on internal and external bruise damage in tomato fruit are mainly phenomenological. The literature shows that the extent to which tomatoes bruise differs among cultivars (Van linden et al., 2006a) and that the damage is generally more severe in riper fruit (Halsey, 1955; Fluck and Halsey, 1973; Sargent et

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al., 1992; Kader, 1996; Ferreira et al., 2004; Van linden et al., 2006b), with almost complete absence of bruises in early ripening stages (Van linden et al., 2006b). A tomato bruise model showed that several fruit factors play a role, in particular fruit ripeness and texture, but the model was incomplete (Van linden et al., 2006b). Variation during bruise development remains unexplained by the classic impact and fruit properties. The need to characterise the processes that underlie the macroscopically visible changes in texture has been emphasised.

Limited information exists on the effect of damage on cell wall metabolism. However, three types of damage have been identified: chilling injury, mealiness and wounding (fresh cutting) of tomatoes. Basically, they all reveal an important role of cell wall modifying enzymes in the development of an unappealing texture as perceived by the consumer, and the observed textural changes relate mainly to the pectic component of the primary wall.

A different mechanism of softening was found in chilled fruit compared to normal ripening, which was characterised by the development of a gel structure composed of high molar mass de-esterified pectin, retaining moisture. Water was translocated to the modified wall, which was associated with the dry mouth feel of chilling-injured tomatoes (Jackman et al., 1992; Marangoni et al., 1995). Another textural disorder is mealiness, which is known to depend on the equilibrium between the cell wall strength and the adhesion between cells. Stronger cell walls and weaker cell-to-cell adhesion favour cell separation during mastication, which results in the typical dry feel in the mouth. Studies showed, amongst other things, that cell walls of mealy fruit display distinct properties in the pectic component (Devaux et al., 2005). Increased pectin degradation was found in wounded tissue of fresh-cut tomatoes (Huber et al., 2001). Three possible causes were summarised, notably a facilitated pectin hydrolysis by alterations in the apoplastic conditions (pH, ion balance) (Huber and Lee, 1989), which presumably (Huber et al., 2001) alleviate *in planta* constraints (Brummell and Labavitch, 1997) on pectin hydrolysis, the activation of pre-existing polygalacturonase (PG) (Huber et al., 2001), or wound-induced enzyme synthesis (Huber and Lee, 1989; Moretti et al., 1998). In contrast, more recent work compared extractable pectolytic enzyme activities in intact and fresh-cut tomatoes and found limited activities in the cut fruit (Chung et al., 2006). However, they did not examine the associated changes in the cell wall pectin.

The above studies on the effect of postharvest physical damage on tissue disintegration show that a series of biochemical events are dictating the externally perceptible texture anomalies in tomato fruit. Along with reported changes in the xyloglucan network (Maclachlan and Brady, 1994; Rose et al., 1998; Rose and Bennett, 1999), changes in the pectin network will lead to cell wall swelling and ultimately tissue deterioration, and both phenomena are likely to be associated with bruising (Hadfield and Bennett, 1998). For this reason, the presence, activity and probable changes in activity of the two most common pectolytic enzymes, pectinmethylesterase (PME) and PG, are likely to be associated with bruise development. Accordingly, modifications of the cell wall pectins brought about by either one of these enzymes are of principal interest.

This paper aims to shed some light on bruising-associated textural changes that are observed in tomato fruit. The work focussed on the short-term immediate changes (max. 3 h). Two objectives were: (1) to investigate the effect of a mechanical impact on the pectolytic enzyme activity and (2) to investigate the associated modifications of the cell wall pectins. Changes in PME and PG activity were examined, along with alterations in the degree of pectin methylesterification (DM) and the uronide content and molar masses of the water-soluble, chelator-soluble and alkali-soluble pectin fractions.

2. Material and methods

2.1. Materials

Fresh market tomatoes (*Lycopersicon esculentum* Mill. cv. Admiro) were hand-picked in a greenhouse of the Vegetable Research Station (Sint-Katelijne-Waver, Belgium). Tomatoes were selected to span the complete physiological range of ripening (Grierson and Kader, 1986) from mature green to the red ripe stage. Fruit were grouped in six ripening classes based on their external colour as described by the USDA standards (USDA, 1991) and with special attention to equal colour development within each group. The following ripening stages were used: mature green (MG), breaker (BR), turning (TU), pink (PI), orange red (OR) and red ripe (RR) stage, each containing 30–36 tomatoes.

2.2. Bruise treatment and tissue extraction

Sample preparation was carried out at 25 °C and fruit were acclimatised prior to treatment. The samples were prepared in two steps. In a first, bruise damage was induced in 2/3rd of the tomatoes at all six stages of ripening, while the other 1/3rd served as control fruit. Bruise treatment involved a single dynamic impact at the fruit equator and was located over the locule. Each tomato was impacted at two spots that were 90° apart. A pendulum device was used and a sufficiently high impact energy (>0.250 J) was applied in order to cause bruising. The applied bruise method has been described in detail in previous work (Van linden et al., 2006a). Of the impacted tomatoes, half of the fruit were kept and stored at 25 °C for 20 min, the other half for 3 h. In a second step, the impacted pericarp tissue was excised and carefully peeled using a scalpel, yielding two discs per fruit. Each tissue disc was cut in two halves, one of which was used for the enzyme activity study and the other for the cell wall study. Tissue discs were immediately frozen in liquid nitrogen and stored in two separate groups at −40 °C until further analysis. The control samples were similarly brought to 25 °C and prepared.

2.3. Enzyme activity after bruising

2.3.1. Extraction and assay of tomato PME

Approximately 10 g of frozen tissue, pooled from 10 tomatoes per treatment, was ground in a mortar and centrifuged at 10,000 × *g* and 4 °C for 30 min. The pellet was recovered and