Plant Physiology and Biochemistry 119 (2017) 328–337

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Changing of biochemical parameters and cell wall polysaccharides distribution during physiological development of tomato fruit

Monika Chylińska [a, *], Monika Szymańska-Chargot [a], Kamil Deryło [b], Dorota Tchórzewska [c], Artur Zdunek [a]

[a] Institute of Agrophysics, Polish Academy of Sciences, Doswiadczalna 4, 20-290 Lublin, Poland
[b] Department of Molecular Biology, Maria Curie-Sklodowska University, Akademicka 19, 20-033 Lublin, Poland
[c] Department of Plant Anatomy and Cytology, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland

ARTICLE INFO

Article history:
Received 9 May 2017
Received in revised form
11 September 2017
Accepted 12 September 2017
Available online 14 September 2017

Keywords:
Cell wall
Polysaccharides
Fruit ripening
Raman imaging
Immunolabeling

ABSTRACT

This study was aimed at discovering an impact of biochemical parameters (like content of cell wall polysaccharides, phenolic compounds, ascorbic acid or activity of pectinolytic enzymes) on cell wall microstructure during physiological fruit development. Cell wall microstructure as well as changes in the polysaccharides distribution were examined by confocal Raman microscopy. Also there was a need to simultaneous usage of reference method which is immunolabeling. A tomato fruit (*Solanum lycopersicum* cv Cerise) has been selected to observe the changes taking place in the fruit cell wall as it recently has been recognized as a model species for exploring fruit development processes such as fruit formation and ripening. Our studies showed that chemical images allows to depict changes in spatial distribution of polysaccharides in plant cell wall (including the middle lamella area), thus this technique allows to observation of cell wall degradation during tomato ripening (mainly pectic polysaccharides degradation). It seems that high level of pectinolytic enzymes activity and increasing content of ascorbate and hence decrease of pectins content have a significant impact on spatial distribution of biopolymers in fruit cell wall.

1. Introduction

The cell wall provides shape and structural integrity to the cell functioning as a protective barrier against pathogen invasion and environmental stress (Kalamaki et al., 2012).

Cellulose, hemicelluloses and pectins are the basic building blocks of plant cell walls. It also consists proteins and phenolic compounds. According to the model of plant cell wall cellulose macrofibrils are interlinked with hemicelluloses fibrils via hydrogen bonds, whereas pectins form an amorphous matrix (Zdunek et al., 2014). Pectins are defined as polysaccharides rich in galacturonic acid (GalA) and they appear to be generally restricted to land plants (Verhertbruggen and Knox, 2007). It is thought that there are three major classes of pectic polysaccharides: homogalacturonan (HG) and rhamnogalacturonans I and II (RG I and II).

Cell wall matrix polysaccharides, especially pectins, undergo disruption during fruit ripening. These modifications are assumed to be responsible for the decrease in tissue firmness that occurs during ripening (Dumville and Fry, 2003). An integral phenomenon accompanying the fruit ripening is softening in macro-scale. It is caused by a controlled degradation of cell wall polysaccharides and a loosening of the polymer networks of the wall, resulting in a swelling of the primary cell wall to become a softer and more hydrated (Brummell, 2006). Moreover, due to depolymerization and solubilisation of pectic and hemicellulosic cell wall polysaccharides, dissolution of the primary cell wall and middle lamella resulting in a reduction of intercellular adhesion and the decrease in fruit firmness is occurred (Brummell and Harpster, 2001).

In literature, the action of pectinolitic enzymes is considered as a main factor causing pectins degradation during ripening (Blanshard and Mitchell, 1979). One of them is pectin methylesterase (from the family of pectin esterases enzymes) which catalyzes the release of methanol from methyl-esterified of homogalacturonan (Bonnin et al., 2014). The de-esterified HG backbone is then susceptible to the activity of polygalacturonase (PG) (Steele et al. 1997). Polygalacturonase (endo-PG and exo-PG) depolymerizes HG, it catalyzes hydrolytic cleavage of the α-(1,4)

* Corresponding author.
E-mail address: m.chylinska@ipan.lublin.pl (M. Chylińska).

http://dx.doi.org/10.1016/j.plaphy.2017.09.010
0981-9428/

Abbreviations

GalA	galacturonic acid
HG	homogalacturonan
CWM	cell wall material
CDTA	trans-1,2-diaminocyclohexane-N,N,N′,N′-tetraacetate
WSP	water soluble pectins
CSP	chelator soluble pectins
DASP	dilute alkali soluble pectins
PG	polygalacturonase
PME	pectin methylesterase

glycosidic linkages between GalA in pectins.

Although lots of evidence has been presented supporting the fact that PG action is considered as the main factor of pectins degradation, the key role of this reaction in tomato fruit softening is a subject of continuous debate. For example Dumville and Fry (2003) suggested that endogenous ascorbate, released early in fruit ripening into the apoplast by membrane permeabilisation, could promote the solubilisation and depolymerization of polysaccharides, and thus contribute to fruit softening.

In this work, the tomato fruit (*Solanum lycopersicum*) has been selected to observe the changes taking place in the fruit cell wall as it recently has been recognized as a model species for exploring fruit development processes such as fruit formation and ripening (Terao et al., 2013). This study was aimed at discovering an impact of biochemical parameters (like content of cell wall polysaccharides, phenolic compounds, ascorbic acid or activity of pectinolytic enzymes) on cell wall microstructure occurred in the cell wall during fruit ripening. Cell wall microstructure as well as changes in the polysaccharides distribution were examined by confocal Raman microscopy. This approach has allowed to obtain and analyze spectroscopic data (PCA) concerning the biopolymers and their spatial distribution within the cell wall in one experiment.

Briefly, Raman imaging of plant cell walls represents a nondestructive technique that can provide insights into chemical composition in context with structure at the micrometer level (Gierlinger et al., 2012). Taking into account the simplicity of Raman imaging and the relative easiness in sample preparation (which does not require extensive chemical treatment prior to imaging), this method would be effective to characterize the composition of cell wall with particular emphasis on dynamic changes which occur during development and maturation of fruits and vegetables (Chylińska et al., 2014).

Raman microscopy is quite new imaging method for biomaterials originating from fruits. Therefore, generally accepted reference method which is immunolabeling was used simultaneously to confirm the results obtained from Ramam imaging.

2. Materials and methods

Tomato plants (*Solanum lycopersicum* cv Cerise) were grown under greenhouse conditions (5plants). Fruit were harvested at the *mature green* and *red ripe* stages. Ripening stage was defined (named) according to Batu (2004) and identified by external colour, lycopene and starch contents. At each stage 30 fruits were sampled randomly. 15 pieces of tissue were removed from the fruit for microscopic methods. Next a laboratory sample was created by homogenizing mesocarp and ezgocarp of the rest fruit. From the laboratory sample, the material was taken for biochemical examination.

2.1. Starch content

The protocol of starch quantification in tomato fruits was based on hydrolytic conversion of starch to glucose which was performed in two phases (Megazyme Total Starch Assay Kit). In the first phase, starch, water and thermostable α-amylase were mixed and heated to boiling. The starch was totally solubilized and became partially hydrolysed to dextrins. In the second phase, the resulting starch dextrins were quantitatively hydrolysed to glucose by amyloglucosidase. Finally, the glucose oxidase/peroxidase reagent was added to samples and absorbance against a reagent blank was read at 510 nm (Szymańska-Chargot et al., 2012). Results are expressed as the mass of starch in dry matter (mg/g).

2.2. Lycopene content

Total lycopene was measured according Fish et al. (2002) on duplicate 1 g samples of puree of pericarp of tomato fruit. This assay requires 10 mL of 0.05% (w/v) butylated hydroxytoluene (BHT) in acetone, 10 mL of 95% ethanol, and 10 mL hexane per sample assayed. Absorbance against a reagent blank was read at 503 nm. The amount of lycopene in samples was estimated by the following formula:

$$\text{lycopene}(\text{mg/kg}) = (x/y) \times A_{503} \times 3.12, \quad (1)$$

where x – amount of hexane (mL), y – the weight of fruit tissue (g), A_{503} – the absorbance at 503 nm and 3.12 is the extinction coefficient.

2.3. Cell wall material extraction

Cell wall material (CWM) for study was obtained using hot alcohol insoluble solids method (Renard, 2005) with a few modifications as it was described before (Szymańska-Chargot and Zdunek, 2013).

Briefly, the material was ground by a kitchen processor (Zauberstab, ESGE, Hockenheim, Switzerland), then dropped in hot alcohol (ethanol 70%, temperature approx 82–85 °C) and left for 20 min of boiling. Then material was filtered through nylon filter and then washed successively in 70% and 96% ethanol until the test for sugar presence using the phenol-sulphuric method (Dubois et al., 1956) was negative, and finally placed in acetone. The sample was dried and ground. CWM was isolated from the pericarp and exocarp of the mature green and red ripe stages of tomato fruit.

2.4. Extraction of cell wall fractions

Sequential extraction of cell wall component method according to Redgwell (1986) with some modification (Szymańska-Chargot and Zdunek, 2013; Chylińska et al., 2016) was used to obtain different fractions of pectins from the cell wall. In each step of extraction samples were stirred using a laboratory rotor in twisted tubes. The CWM (0.1 g) was stirred in deionized water (9 mL) overnight. Then it was filtrated and the supernatant containing water soluble pectins (WSP) was collected. Residue dissolved in water was stirred in 0.1 M CDTA (5 mL, pH 6.5) for 6 h, filtrated and again stirred in 0.1 M CDTA (5 mL, pH 6.5) for 2 h. The supernatants containing calcium chelator soluble pectins (CSP) of these two steps were collected together. Then, the CDTA residue was stirred in 0.05 M Na_2CO_3 with the addition of 20 mM $NaBH_4$ (5 mL) overnight, filtrated and again stirred in 0.05 M Na_2CO_3 with the addition of 20 mM $NaBH_4$ (5 mL) for 2 h. The supernatants containing dilute alkali soluble pectins (DASP) of these two steps were collected together. All of the above extraction steps were carried out at room