Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2014; 2(3): 177-180 **ISSN 2347-9515 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com **DOI:** 10.36347/sajb.2014.v02i03.002

Research Article

Cellulase, Polygalacturonase and β-galactosidase Activity in Ripening Raspberry (*Rubus caesius* L.) fruit

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Abstract: Activities of the cell wall degrading enzymes cellulase, polygalacturonase, and β-galactosidase were determined on unripe, semi-ripe, and ripe raspberry (*Rubus caesius L.*) fruit. The enzyme activity, measured as µmoles of released product.g^{-1} of fruit h^{-1} indicated the presence of polygalacturonase, cellulose, and β-galactosidase in raspberry fruit. Enhanced fruit ripening was reflected by increased values for cellulase, polygalacturonase abd β- galactosidase activity. In raspberry cellulose, polygalacturonase, and βgalactosidase appear to be involved in fruit softening during unripe to the ripe stages.
Keywords: Raspberry, Fruit ripening, Cellulase, Polygalacturonase, β-galactosidase

INTRODUCTION

The delicate nature of raspberry fruits is a major difficulty for growers and processors. The ripe fruit are easily ruptured during harvesting, transport and commercial operations [1]. Continued softening after harvesting exacerbates this problem and is a contributory factor to their extremely short shelf life [2, 3]. The recent transfer of genes into raspberry plants [4] raises the prospect of being able to manipulate raspberry softening. Such methods have been successfully employed in tomatoes although this was only possible because the role of ethylene [5, 6], and wall degrading enzymes [7, 8, 9] was well established. Very little research has been done into the nature of the corresponding changes in raspberries.

There is an increase in ethylene production as raspberries ripen until physiologically active concentrations are found in red fruit [10, 11]. The softening of fruit appears to be a multicomponent process. Underneath the epidermis and hypodermis the thin walled mesocarp cells become distended during fruit expansion and the delicate nature of these cells contributes to the textural changes [12, 13]. It seems very likely that there is also extensive cell wall breakdown since Duclos and Latrassse [14] report a halving in the total pectin content of Malling Exploit fruit during maturation. Wall degradation is usually accompanied by increases in cell wall hydrolases such as polygalacturonase, cellulase, β-galactosidase, and pectin methyl esterase (PME) hydrolase cell walls [15]. These cell wall softening enzymes degrade the pectin fraction in cell walls, through intermediary steps, to glucose and galactose [15]. Cell wall softening enzymes differ among fruit. PG, cellulase, and β-galactosidase are found in tomatoes, apples, and avocadoes [15].

Our objective in this study was to quantify cell wall degrading enzyme activity in ripening raspberry fruit and to define their presence and possible involvement in fruit softening during ripening.

MATERIAL AND METHODS

Raspberries that were evaluated in this study (*Rubus caesius L.*) were collected from the northwest (Kivi - Ardebil province) of Iran. Fruit representing the maturity stages of unripe, semi-ripe, and ripe were harvested on 18 May, 7 June, 22 June 2013. Unripe fruit were of small size, green color, with no signs of pink color, semi-ripe fruit had attained almost maximum size, and had a mixture of pink and red colors. Ripe fruits were firm, fully black, and easily detached from the receptacle. The receptacle was not retained. Fruit were frozen at -20 ˚C within 2 h of harvest. Each experimental unit consisted of 40- 50 berries. For analysis, fruit were thawed at room temperature and homogenized with di water. After homogenization, the mixture was centrifuged at 900 x g for ten minutes.

The cell wall degrading enzymes, cellulose, polygalacturonase, and β-galactosidase, were assayed for each sample. For cellulose, samples consisted of 100 µL of supernatant plus 900 µL of substate (2% low

viscosity carboxymethyl-cellulose (CMC) sodium salt in 0.05 M acetate buffer, pH 4.5). A 100 µL (5.1 units) 1% cellulose solution (Sigma, Saint Louis, MO. Cellulase [EC 3.2.1.4 from *Aspergillus niger*]) plus 900 µL of 2% CMC substrate was used as a standard. The enzyme-substrate treatments were incubated for 24 h at 37 ˚C. Cellulase activity was not detectable when enzyme-substrate solution was measured at an earlier time. The reaction was stopped with 1 mL dinitrosalicylc acid (DNS) reagent (2.5 g DNS, and 15 g Na-K tartrate in 2 N NaOH) (Victor).The mixtures were then heated for 5 min at 100 ˚C. Deionized water (4 mL) was added to the reaction tubes before measuring the absorbance of the samples with a spectrophotometer (Sequoia-Turner, Model 690, Chicago, III) set at 490 nm.

For polygalactrunase, 100 µL of supernatant was added to 900 µL of substrate (1% polygalacturonic acid in 0.05 M acetate buffer, pH 4.5). A standard solution with 25 µL (3.0 units) of pectinase (Sigma, {EC 3.2.1.15., fom *Aspergillus niger*}), plus 75 µL H_2O and 900 µL of 1% polygalacturonic acid substrate was utilized to compare units of activity. Reducing sugars were measured as previously described.

β-galactosidase activity in fruit homogenate samples was measured by mixing 100 µl of supernatant with 900 µl of subatrate (0.05 M acetate buffer, pH 4.5, 0.015 M NaCl, 0.06% bovine serum albumin (BSA), and 1% p-nitrophenyl-β-D-galactopyranoside). The controls were 100 mL water plus 900 mL of substrate, and 25 mL (3.0 units) of pectinase, combined with 75 µL H_2O and 900 mL of substrate. These treatments were incubated for 1 h at 37 ˚C, and the reaction was stopped with 1 mL of 0.2 M Na_2CO_3. Four mL of di water were added before measuring absorbance at 400 nm to determine the p-nitrophenol groups released and activity expressed as µmol.g fresh weight^{-1}.h^{-1}.

Statistical analysis

Statistical analyses were performed using the SPSS for Windows version 16.0 (SPSS Inc.,USA). Differences between means were first analyzed by ANOVA test and then least significant difference (LSD) test ($P < 0.05$)

RESULTS AND DISCUSSION

Cellulose activity depended on ripeness stage (Fig 1). Ripe fruits had more cellulose activity than unripe or semi-ripe fruits. Ethylene increases in raspberry, paralleling cellulose activity. Increasing cellulose activity in ripe fruits relative to unripe or semi-ripe fruits may be due to changes associated with cessation of growth and the onset of maturation. Different cellulose isozyme forms may be appearing with ripening raspberries [16]. The type of isozyme present at the time of assay influences cellulose activity [17].

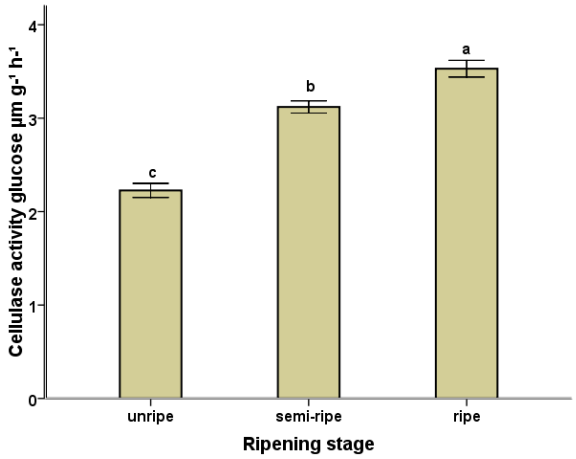

Fig. 1: Variation of cellulase activity in ripening raspberry fruit

(The bars represent the mean of 3 replicates with standard deviation. Means followed by the same letters are not significantly different for p=0.05)

PG activity was low at the unripe and semi-ripe stages, but increased sharply between the semi-ripe and ripe stage (Fig 2). The highest level of PG activity, detected at the ripe stage, correlated with maximum fruit softening. PG activity also increased during raspberry ripening [18]. A few numbers of studies have analyzed cell wall modifications during development and ripening of raspberry fruits.

β-Gal activity was relatively low at the unripe and semi-ripe stages but increased between the semi-ripe and ripe stages (Fig 3). These results are similar to reports where higher activity during the semi-ripe and ripe stages in other fruits. For instance, β-Gal activity increased with maturity in apples [19], in peppers [20], and boysenberry [21]. Pressey [17] found total β-Gal activity was high in tomatoes while only one β-Gal

isozyme increased during ripening. Xylose is the main non-cellulosic neutral sugar in blackberry and raspberry, and the loss of galactose during ripening is more noticeable in other fruit species [22]. All the same, a reduction in cell wall galactose, often indicative of rhamnogalacturonan degradation, was observed in two different raspberry cultivars [23].

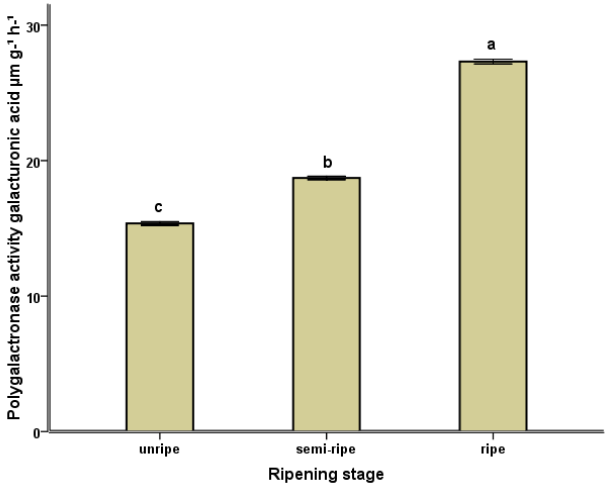

Fig. 2: Variation of polygalacturonase (PG) activity in ripening raspberry fruit
(The bars represent the mean of 3 replicates with standard deviation. Means followed by the same letters are not significantly different for p=0.05.)

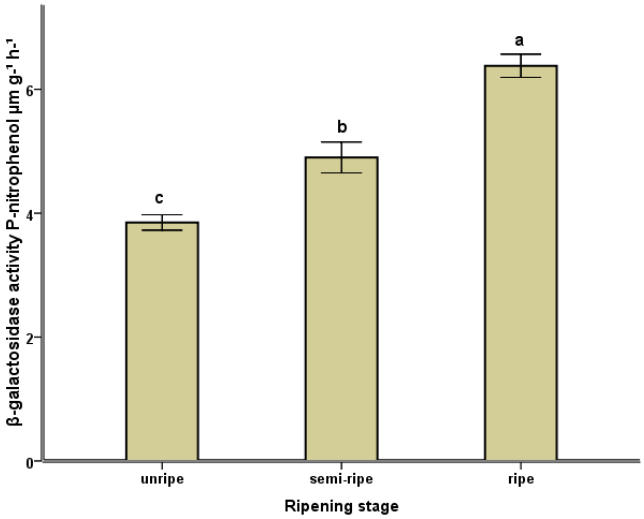

Fig. 3: Variation of β-galactosidase (BG) activity in ripening raspberry fruit
(The bars represent the mean of 3 replicates with standard deviation. Means followed by the same letters are not significantly different for p=0.05.)

Activity of cell wall degrading enzymes in maturing raspberry fruit differed from that of ripening tomatoes [24] apples [15], and peppers [20]. PG activity is thought to influence fruit softening more than cellulose [22]. Interpretation of our data indicates that PG, β-Gal and cellulose have similar activity levels in raspberries. These enzymes appear important in raspberry fruit softening, with activity peaking in ripe fruit. The increased PG, β-Gal and cellulose activities in raspberry fruit coincide with color changes, indicating a possible role for these enzymes in raspberry softening.

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