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Araştırma
Research

CHANGES IN SOME ENZYMATIC PARAMETERS OF SIX APRICOT CULTIVARS DURING RIPENING

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ABSTRACT: In this study, some enzymatic and physical changes were investigated during different ripening periods in fruits of some apricot cultivars grown in Malatya province, Turkey. Firmness, polygalacturonase and pectin methylesterase enzyme activities were monitored in fruits of six apricot cultivars ('Hasanbey', 'Canino', 'Turfanda Eskimalatya', 'Hacihaliloglu', 'Ozal' and 'Levent') harvested at unripe (hard green), half ripe (mature green) and ripe stages. The polygalacturonase and pectin methylesterase enzyme activities increased during fruit development while fruit flesh firmness decreased. The highest enzyme activities were found in 'Levent' cultivar.
Keywords: *Prunus armeniaca* L., flesh firmness, pectin methylesterase, polygalacturonase

OLGUNLAŞMA SÜRECİNDE ALTI KAYISI ÇEŞİDİNİN ENZİM PARAMETRELERİNDEKİ DEĞİŞİMLER

ÖZET: Bu çalışmada, Malatya bölgesinde yetiştirilen farklı kayısı çeşitlerinin olgunlaşma periyodu boyunca bazı enzimatik ve fiziksel değişimleri incelenmiştir. Ham, yarı olgun ve olgun dönemde hasat edilmiş olan altı kayısı çeşidinin ('Hasanbey', 'Canino', 'Turfanda Eskimalatya', 'Hacihaliloglu', 'Ozal' ve 'Levent') meyvelerinde meyve eti sertliği, poligalakturonaz ve pektin metilesteraz enzim aktiviteleri belirlenmiştir. Poligalakturonaz ve pektin metilesteraz enzim aktiviteleri meyve gelişimi boyunca artarken, meyve eti sertliği azalmıştır. En yüksek enzim aktiviteleri 'Levent' kayısı çeşidinde saptanmıştır.
Anahtar Sözcükler: *Prunus armeniaca* L., pektin metilesteraz, poligalakturonaz, olgunlaşma basamakları

1. INTRODUCTION

It has been estimated that apricot is being cultivated from more than 5,000 years in a wide area that covers Iran, Turkistan, Afghanistan, Middle Asia and Western China. It was brought to Armenia from a more eastern center of origin, much earlier as evidenced by archeological excavations of the pre-Christian sites. It was brought to Anatolia in the 4th century BC from Persia during the voyages of Alexander the Great. Thus Anatolia became the second homeland of apricot. During the Roman and Persian wars in 1st century BC, it spread first to Italy, and then to Greece. Eventually it spread to Spain and England in the 13th century and to France and America in the 17th century (Buttner, 2001; Ercisli, 2004; Faust et al., 1998).

Turkey as a globally leading apricot producer annually produces about 600-800 thousand tons of apricot. The most famous apricots in the world in terms of color, taste, odor, flavor, and dry matter are grown in Malatya province located in Eastern Anatolia (Asma, 2000).

Apricot is very popular among consumers because of its distinct taste and aroma. It is also rich in bioactive substances such as carotenoids, polyphenols, vitamin C, etc. (Hegedus et al., 2010; Caliskan et al. 2012). Apricot cultivars differ from each other in terms of maturation time, fruit size, biochemical content, fruit firmness, shape, etc. (Asma, 2000).

Genetically programmed fruit ripening is defined as physiological and biochemical changes occurring in the characteristics of fruits during the fruit maturation (Giovanni, 2001; Yentur, 1995). Fruit flesh firmness decreases, and the activities of some destructive enzymes in cell wall increase as a result of the maturation (Deng et al., 2005; Sturm et al., 2003).

Fruit flesh firmness provides resistance against external mechanical damages. Mechanical resistance is accepted as one of the important criteria of maturity for fruits such as apricot (Jiang and Li, 2000; Pelayo et al., 2002). Fruit softening, which occurs at the end of the fruit maturation period, is associated with the destruction of cell wall components such as pectic materials in the middle lamella and increase in the activity of the hydrolytic enzymes responsible for this destruction (Figueroa et al., 2010). Studies conducted on climacteric fruits show that the modification of cell wall polymers is carried out by means of cell wall-modifying enzymes such as polygalacturonase, pectate lyase, pectin methylesterase, galactosidase, α-L-arabinofuranosidase, endo-1,4-β-D glucanase,

xyloxidase, expansin, xyloglucan endotrasglycosylase and endo-mannanase (Brummell and Harpster, 2001).

Pectin methylesterase (PME) is one of the key enzymes involved in plant carbohydrate metabolism (Hubbard and Pharr, 1992). This enzyme causes the formation of pectic acid and methanol by hydrolyzing the components of pectin (Jayani et al., 2005). Polygalacturonase (PG) is the other enzyme which plays an important role in the degradation of pectin components in plants (Prasanna et al., 2005). It is responsible for the destruction of polygalacturonic acid that holds together the matrix of the cell wall and loosens cell adhesion (Brummel Harpster, 2001).

There are very few studies addressing biochemical compounds in apricot. Therefore, in this study, the activity of the enzymes, polygalacturonase (PG) and pectin methylesterase (PME), and fruit firmness have been determined in the unripe, half ripe and ripe stages of fruits belonging to different apricot cultivars grown in Malatya province.

2. MATERIALS AND METHODS

2.1. Plant Material

Apricot fruit samples were obtained from the apricot collection orchard established in the Apricot Research and Application Center of İnönü University (Altitude: 938 m, Latitude: 38:20:20.50 N, Longitude: 38:26:28,79 E), Malatya-Turkey.

Apricot trees were 8 years old at the time when fruit samples were collected. The apricot seedlings were reproduced by grafting rootstock 'Zerdali'. The trees were planted at the intervals of 7×7 meters. Organic fertilizer (15 kg) was applied once in every three years in autumn season. In addition, 2 kg of 33% ammonium nitrate and 1 kg of triple super phosphate were applied in every February. The trees were watered at regular intervals from the first week of June (8 times). Every tree was given about 50-70 liter of water at each time of irrigation.

In early maturated cultivars, such as 'Canino', 'Turfanda Eskimalatya', 'Hasanbey', and 'Hacihaliloglu', fruits harvested 30 days after the full blooming time were considered as unripe, 60 days after as half ripe, and pre-harvested fruits as ripe. In late maturated cultivars, such as Levent and Ozal, fruits harvested 30 days after the full blooming time were considered as unripe, 90 days after as half ripe, and pre-harvested fruits as ripe. Fruit samples were collected randomly from different regions of a single tree at each of the three maturation periods.

2.2. Fruit Flesh Firmness

The fruit flesh firmness of ten fruits was measured by using a digital penetrometer (Bareiss HPE II-Fff model) (diameter: 8 mm).

2.3. Enzyme Extraction and Assay

Pulp homogenate (10 % w/v) was prepared by homogenizing 4 g fruit tissue in the homogenization buffer: Tris-HCl (20 mM, pH 7), cysteine-HCl (20 mM), EDTA (20 mM), and Triton X-100 (0.05 %). Homogenate was centrifuged for 30 min, 15000 ×*g*, at 4 ºC and the supernatant was used for enzyme analysis (Manoj et al., 2000).

2.4. Determination of PG Activity

PG activity was determined by using the method of Nelson (1944) and Somogyi (1952) by measuring the decreased band formation. The reaction mixture (1 mL total volume) contained 0.2 mL sodium acetate buffer (200 mM, pH 4.5), 0.1 mL NaCl (200 mM), 0.3 mL polygalacturonic acid (PGA) (1%, pH 4.5) and 0.1 mL supernatant. Reaction was initiated by adding substrate PGA, and the reaction mixture was incubated for 1 hour at 37 ºC. The substrate was added to control tubes after starting the incubation. Then, 1 mL DNS (3,5-dinitrosalicylic acid) was added into the mixture, and the reaction was stopped by keeping the tubes in boiling water for 5 min. Absorbance at 540 nm was taken, and the enzyme activity was calculated by using standard spinning.

2.5. Determination of PME Activity

PME activity was determined using the method described by Hangermann and Austin (1986). The reaction mixture including 1 mL pectin solution (0.01 %), 0.2 mL NaCl (0.15 M), 0.1 mL bromothymol blue solution (0.01 %) and 0.2 mL distilled water was prepared in 3 mL glass cuvettes. After adding 0.1 mL supernatant into the mixture, absorbance was measured at 0 and after 3 min at 620 nm. The activity of PME was calculated according to the difference between absorbance at 0 and after 3 min by using a standard curve.

2.6. Determination of Total Soluble Protein

The amount of total soluble protein was determined by Bradford's method (1976) using a microplate reader system (Molecular Devices Corp., Versamax). The prepared homogenate (5 µL) and Bradford reagent (250 µL) were added to each well on the microplate and kept in dark at room temperature (~25 ºC) for 15 min. The color-dependent changes in absorbance were measured at 595 nm wavelength. The obtained optical density (OD) of the samples was compared to standard graph prepared using bovine serum albumin (BSA), and the amount of total protein in the samples was calculated by using a software package (Slide). The measurements were repeated three times and average value was considered.

2.7. Statistical Analysis

Statistical analysis was performed using SPSS 10.0 software. Duncan's test was used to estimate the significance ($p<0.05$) following variance analysis (Duncan, 1955).

3. RESULT AND DISCUSSION

3.1. Fruit Flesh Firmness

Statistically significant loss in fruit flesh firmness with increasing maturity period was observed in all the cultivars used in the study (Fig 1) ($p<0.05$). In the unripe stage, the highest fruit firmness was determined in 'Hasanbey' cultivar (78.3 N) and the lowest fruit flesh firmness was observed in 'Hacihaliloglu' cultivar (71.7 N). At the half ripe stage, the partial fruit flesh firmness decreased ranging between 70.5 and 75.5 N among the cultivars. However, in the period of ripening, compared to the other periods, fruit flesh firmness decreased significantly in the all cultivars. In this period, 'Hasanbey' cultivar (61.8 N) had the highest fruit flesh firmness and 'Turfanda Eskimalatya' cultivar (31.0 N) had the lowest fruit flesh firmness value. Previous studies conducted on different fruit species indicated decrease in fruit flesh firmness, in particular, at the ripening stage (Lohani et al., 2004; Usenik et al., 2008). Pinzon et al. (2007) reported that fruit flesh firmness was 12% higher in green stage compared to ripening stage for gulupa fruits. Prinsi et al. (2011) measured the fruit flesh firmness of the peach cultivars 'Oro A' and 'Bolero' at two different fruit development periods and reported that the firmness was 31.7 N in ripe fruits, 46.8 N at unripe stage for 'Oro A' cultivar, while it was 13.7 N at ripe stage and 55.9 N at unripe stage in 'Bolero' cultivar. Villarreael et al. (2008) determined that the values of fruit flesh firmness for strawberry varieties, 'Selva', 'Camarosa', and 'Toyonaka', were 20.2, 20.3 and 13.4 N at unripe stage and 1.8, 1.4 and 0.74 N in the ripe stage, respectively.

3.2. Enzyme Activity

PG activity was analyzed and a significant increase in enzyme activities was determined during all maturity periods investigated ($p<0.05$) (Fig. 2).

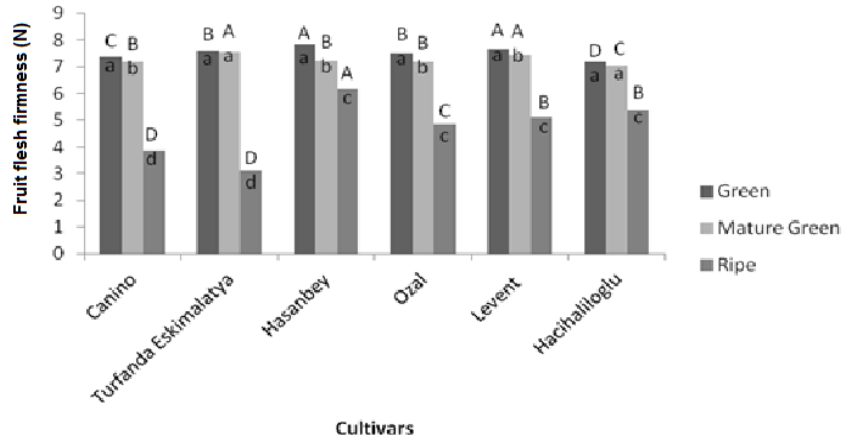

Figure 1. Fruit firmness of different cultivars at the same ripening stages (capital letters) and at different ripening stages (small letters). Data depicted by different letters are significantly different from each other ($p<0.05$) according to Duncan's test

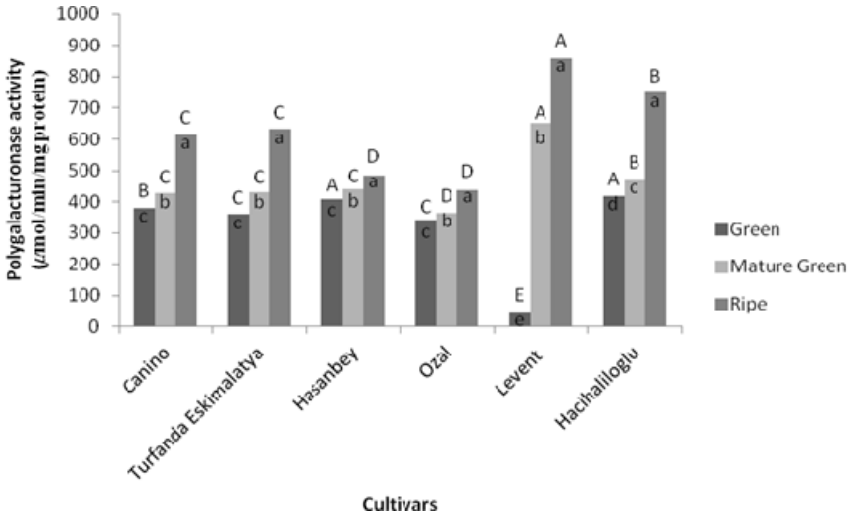

Figure 2. PG enzyme activities of different cultivars at the same ripening stages (capital letters) and at different ripening stages (small letters). Data depicted by different letters are significantly different from each other ($p<0.05$) according to Duncan's test

The lowest enzyme activity was determined at unripe stage and the PG activity increased slightly in the half ripe stage, and the highest level was found in ripe fruits. The fastest PG activity increase was observed in 'Levent' cultivar. PG activity increased from 42.8 µmol min^{-1} mg^{-1} to 857.2 µmol min^{-1} mg^{-1} in this cultivar. In other five apricot cultivars, a parallel increase was observed in three maturity stages. There are several studies reporting PG activity increase at the ripening period (Li et al., 2009; Playas et al., 2004; Zhou et al., 2000). The PG activity in strawberry fruits at different maturation periods showed that enzyme activity was the highest in ripe fruits and the lowest in the unripe fruits in all the three cultivars studied (Villarreal et al., 2008).

Similar to PG activity, PME activity was also increased rapidly with increasing maturity (Fig. 3) ($P<0.05$).

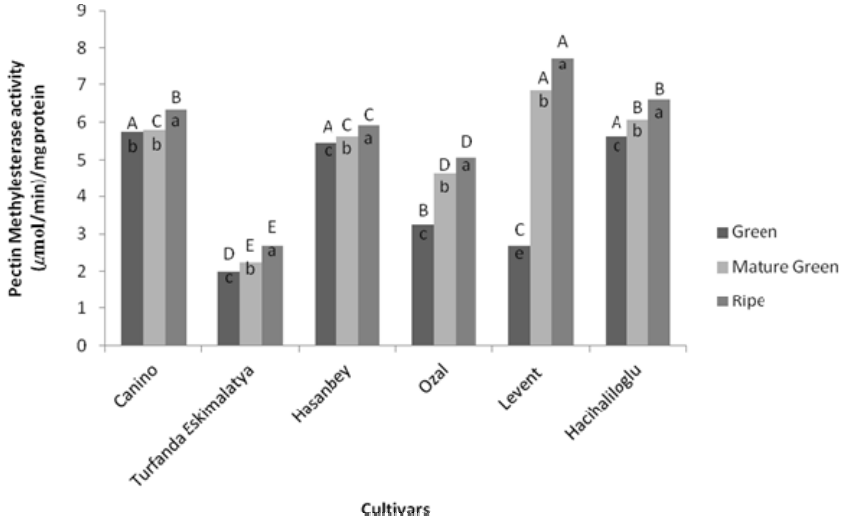

Figure 3. PME enzyme activities of different cultivars at the same ripening stages (capital letters) and at different ripening stages (small letters). Data depicted by different letters are significantly different from each other ($p<0.05$) according to Duncan's test

The highest PME activity was found in the ripe stage. Compared to other cultivars, a significant difference was observed with regard to enzyme activity in the unripe (2.7 µmol min^{-1} mg^{-1}) and ripe fruits (7.7 µmol min^{-1} mg^{-1}) in 'Levent' cultivar. Similar results were observed for the other five apricot cultivars in the three maturity periods (Fig. 3).

Previous studies conducted on different apricot cultivars reported that PME activity increased from unripe- to ripe stage (Arancibia and Motsenbocker, 2004; Deng et al., 2005; Lohani et al., 2004). Belleau et al. (2008) showed that fruit flesh firmness decreased during the maturation period, and these variations resulted from the destructive enzymes of the cell wall. Similarly, PG, and PME enzyme activities in plum fruit increased with ripening (Nunes et al., 2009). Jain et al. (2003) searched PG and PME activities in four fruit development stages, such as mature green, color turning, ripe and overripe in guava fruits. PG activity was 85 units g fwt^{-1} (fruit weight) and PME activity was 36.6 units g fwt^{-1} at the unripe stage and the enzyme activities increased in the overripe stage.

4. CONCLUSION

In the study, some variations in both tissue structure and the enzymatic activity were determined based on fruit maturity periods. As a result, during the maturing, loss in fruit firmness was observed, whereas increase in the activity of cell-wall destructive enzymes such as PG and PME was found. In addition, PG and PME enzyme activities were highest in Levent cultivar at each harvest stage.

5. ACKNOWLEDGEMENTS

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