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Evaluation of quality characteristics of three different colour tomato varieties in three ripening stages

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ABSTRACT

Fruit ripening and softening indicated by firmness determines the texture transportability, and shelf life of tomato products. However, the regulatory mechanism underlying firmness formation in tomato is different in different varieties and overall softening mechanism of tomato fruit is poorly understood. Therefore, in this study, physical, biochemical, and molecular properties of three different tomato varieties; 'Sarikiz' (yellow skin colour), 'Moda' (orange skin colour) and 'Red Type Cherry' (red skin colour) at three developmental stages, mature green (MG), breaker (Br) and full ripe (R) were evaluated. For this aim, colour, texture, cell wall fractionation and pectate lyase (*PL*) gene expressions were analysed at three different ripening stages. As expected, there was a dramatic difference in colour index due to different skin colours of the varieties. For textural properties, 'Sarikiz' showed the softest while 'Moda' variety had the firmest pericarp structure. The composition of the cell wall structure at three ripening stages were also resulted with significantly different fractions. The expression of pectate lyase (PL), one of the most important cell wall modification related enzyme was also studied by semi quantitative RT-PCR. Based on biochemical and molecular studies, 'Sarikiz' showed higher pectin fraction in water and *PL* gene expression at Br and R ripening stages. Based on these results, although the tomato fruits used in this study generally show the same softening trend, they show different physiological, biochemical, and molecular changes in different softening periods.

1. Introduction

The cultivated tomato *Solanum lycopersicum*, is the second most widely most produced, consumed, traded and important products in the world both as raw or processed forms (Yildizhan and Taki 2018). Botanically, the tomato is described as a berry that belongs to the nightshade family Solanaceae. This family consists of 96 genera and over 3000 species in three sub-families (Waheed et al. 2020). Worldwide production of fresh market and processed tomatoes has steadily increased during the last ten years and reached annual production of about 180 million tons with a net value of over $190.4 Billion (FAO 2019). The leading countries for this production in the World are People's Republic of China (PRC) (62.8 million tons), India (19 million tons), Turkey (12.8 million tons) and the USA (10.8 million tons) and), respectively (FAO 2019).

In addition to tomato's economic importance and worldwide cultivation, it has several features that make it a convenient plant for experimental purposes, such as efficient sexual hybridization, ease of culture under a wide range of environment and high-quality sequenced genome (Van Eck et al. 2018). Like all fleshy fruits, the tomato goes through a developmental stage known as ripening, a complex process followed by softening. The softening process is the final stage that contributes to perishability of fruit, facilitating pathogen infection, postharvest decay, and reducing shelf-life and fruit quality (Wei et al. 2010). Ripening of fleshy fruits represents a highly programmed and tightly controlled developmental process leading to textural modifications, enhanced colour, as well as increased accumulation of sugars, acids and volatile compounds culminating in a diverse array of tastes and smells that vary among species (Klee and Giovannoni 2011).

There have been many studies on the physiological and biochemical changes of fruits at different stages of development (Khan et al. 2017; Trong et al. 2019). Researches on physiological and biochemical changes of tomato fruit at different stages of development have been also carried out in many different ecological regions (Raffo et al. 2002; Guil-Guerrero and Rebolloso-Fuentes 2009; Oluk et al. 2012; Gölükçü et al. 2018). However, there are currently no full reports combining physiological, and molecular changes of different tomatoes at different developmental stages in Turkey. 'Sarikiz' tomato variety is not widely consumed by the public which makes they are not always available in markets and greengrocers. This variety is not suitable for commercial production due to their relatively short storage time; however, it is consumed for its high aromatic components. 'Moda' variety is another cherry type having relatively longer shelf life and slower ripening process compared to other two varieties used in this study. 'Red type' cherry is a common variety which is largely and freshly consumed. This variety has a shelf-life longer than 'Sarikiz', but shorter than 'Moda' variety.

Consequently, it would be interesting to study ripening process of different tomato varieties to correlate various physical characteristics like colour, texture and shelf life with pectin solubilisation and a pectin degrading enzyme pectate lyase (PL) characterized by (Uluisik et al. 2016). With the help of this research, we may introduce new data and ideas how to use these different varieties for the establishment of commercial plantations for tomato consumption.

2. Materials and Methods

Seeds of each genotype were cultivated in Antalya, Turkey, and grown in controlled glasshouse conditions in Faculty of Agriculture, Akdeniz University. Three cherry type tomato fruits (Sarikiz, Moda and Red Cherry) were harvested from the greenhouse at three maturity stages: mature green (MG) breaker (Br) and ripe (R). The harvested fruits were immediately transported at Burdur Mehmet Akif Ersoy University to carry out all analysis.

2.1. Quality assessment of the fruits

The skin colour of the tomato fruits was measured by using a Colour-Meter (PCE-CSM 1) and recorded as Hunter's L*, a*, and b* values (10 fruits from each developmental stage) and colour index (CI) was calculated according to (Nangare et al. 2016). Fruit mechanical properties were investigated by pushing a probe (6 mm) into the pericarp (PCE-PTR 200 Penetrometer) and results were expressed in Newtons (N). The assessment was performed at two opposite locations along the fruit equator after peeling around a 2-centimetre square. The average of maximum forces was recorded to represent fruit firmness at the ripening stages. Finally, total soluble solids (°Brix TSS) were determined using a digital refractometer.

2.2. Isolation and extraction of cell wall components

Alcohol insoluble solids (AIS) were prepared following the procedure previously described (Lunn et al. 2013). Fractions of different cell wall pectic components were obtained by sequential chemical extraction of the cell wall material (AIS) by stirring in water (to extract water soluble pectins, WSP) for four hours at room temperature (RT). The liquid fraction was removed, and the residue treated with 50 mM of CDTA (pH 6.5) (to extract ionically bound or chelate-soluble pectins) for four hours at RT, and finally again the remaining residue were stirred with 50 mM Na_2CO_3+20mM $NaBH_4$ (to get covalently bound pectin fractions) overnight at 4°C. Quantification of uronic acids in fruit serum was carried out spectrophotometrically according to the method of Filisetti-Cozzi and Carpita (1991). 300 µL of each test sample and each standard was added to a test tube in triplicate along with 300 µL of boric acid solution and 5 mL of 12M sulphuric acid (H_2SO_4) (Fisher Scientific). The samples were then incubated at 70°C in a water bath for 40 minutes before adding 0.2 mL of the dimethyl-phenol solution. Samples were left at room temperature for 5 minutes and the colour change read at the wavelength 405 nm and 450 nm with the difference recorded. The absorbance reading at 405 nm was subtracted from that at 450 nm to correct for interference from hexoses. Averages of at three biological replicates were used for statistical analysis. Results were expressed as milligram of GA per 1 gram of AIS.

2.3. RNA extraction and gene expression analysis

Total RNA was extracted from fine powdered pericarp of tomato fruits from three developmental stages of three varieties using PureLink™ Plant RNA Reagent (Thermo Fisher Scientific) in accordance with the manufacturer's instructions. Total RNA was dissolved in 30 µL of elution buffer, and the concentration of RNA was quantified using the nanodrop (BioTek, Epoch Microplate Spectrophotometer). Total RNA was reverse transcribed into cDNA by the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). The *PL* gene primers were designed using primer 3 (http://primer3.ut.ee/). For semi quantitative RT-PCR analysis, the *ELONGATION FACTOR 1-α* gene, (*LeEF-1*, GenBank accession X14449) (Pokalsky et al. 1989) was used as an internal constitutively expressed gene (house-keeping gene). Semi RT-PCR was performed in a total volume of 20 µL Thermo Scientific PCR Master Mix. Primers sequences for *PL* and *LeEF-1* for semi-RT-PCR validation were listed in Table 1.

2.4. Statistical Analysis

The statistical analyses were conducted according to completely randomised design with at least ten different tomato fruits for fruit firmness and CI measurements. Three different fruits were used for cell wall analysis. All statistical analyses were performed through XLSTAT (version 2016.02.28451, Addinsoft, France). Duncan test was utilised for the comparison of means ($P \leq 0.05$).

3. Results

The quality analysis focused on assessment of fruit colour, firmness and TSS of the fruits. The visual appearance of three different tomato varieties at three developmental stages were shown in Figure 1. As it can be clearly seen in Figure 1, the colour changing of 'Sarikiz' has slowed down after Br stage which shows that probably it reaches maximum colour changes just after Br stage. However, when it is also looked at 'Moda' variety, there is a dramatic colour change from MG to Br and Br to R stages. This dramatic change was also supported by CI (colour index) values in which forcefully increased in three ripening stages for 'Moda' variety. 'Red type' cherry, the only red tomato in our analysis, displayed a clear change in colour from MG to Br and from Br to fully ripe tomato (Figure 1). This clear escalate was also seed in CI values which go up from 1.2 (MG) to 36.8 (R).

Colorimeters express colours in numerical terms along the L*, a* and b* axes (from white to black, green to red and blue to yellow, respectively) within the CIELAB colour sphere which are usually mathematically combined to calculate the colour indexes. The L* value was nearly similar trend for three varieties

Table 1. Primers used for semi quantitative RT-PCR validation

Gene	Forward Sequence	Reverse Sequence
PL	GCGATCAGGAGTTAGAACTGG	AATCCCCTTTTGCTTTGGTT
LeEF-1	ACCTTTGCTGAATACCCTCCATTG	CACAGTTCACTTCCCCTTCTTCG

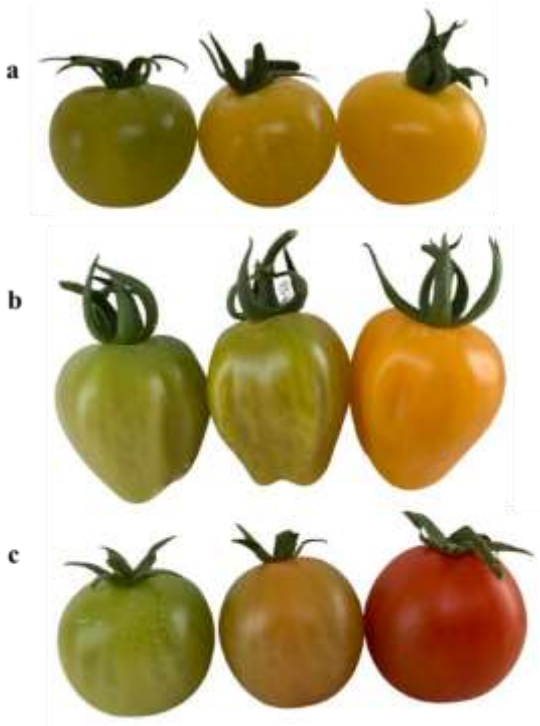

Figure 1. The visual differences of a) Sarikiz, b) Moda and c) Cherry at three ripening stages.

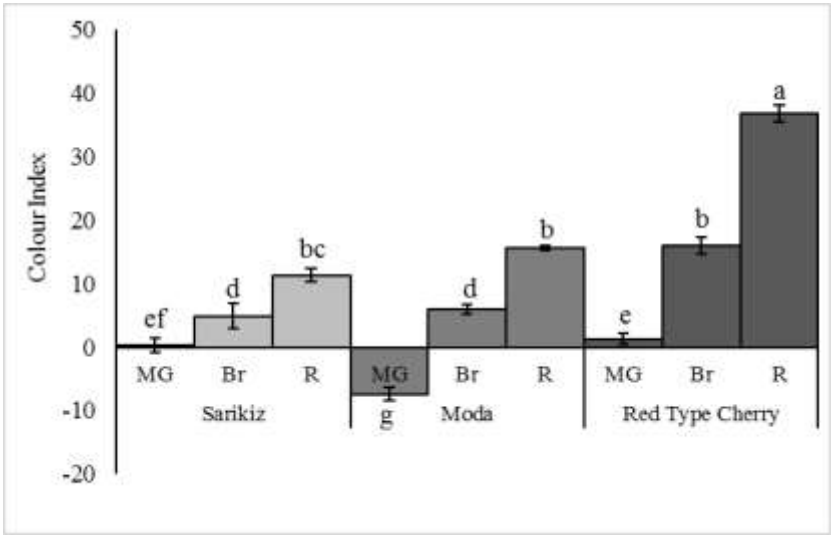

Figure 2. Colour index (CI) values for tomato fruits harvested at different ripening stages. Values represent means ± SE (n= 10). Different letters indicate significant differences, $P<0.05$.

at the three developmental stages (Table 2). However, the luminosity L* value decreases while other two parameters increased during ripening in tomato. This trend was seen more obvious in proceeded ripening stages of 'Red Type Cherry'. The increasing of a* value indicates the loss of green colour in which no major changes were observed in fruits in still predominantly green (MG to Br). For example, the biggest change from MG to Br was in 'Moda' variety (from -3.1 to 2.2) for a* value. However, there was more dramatic increase in a* for all varieties during ripening transition from Br to R, especially in 'Red Type Cherry' with final a* value 12.89. In overall, the total CI increased in all different colour tomatoes during three ripening stages. This is increasing values was seen much clear in 'red type' variety which are probably based on lower L* and higher a* and b* values. The degree of °Brix was higher in 'Sarikiz' in all three different ripening stags compared to other two varieties. Fully ripe 'Sarikiz' tomatoes had the highest °Brix of all tested fruits (Table 2).

Measurement of the maximum load (Newton, N) on pericarp samples for the three different ripening stages of fruits are shown in Figure 3. The fruit firmness continuously decreased during the tested period in all varieties. The firmness differences between the stages for all varieties was statistically significant ($P<0.05$). Although the 'cherry' was the firmest at MG stage, the 'Moda' was slightly the firmest at the end of the ripening process but was not significantly different than the second firmest variety red type cherry'. 'Sarikiz' has a pericarp which is very juicy and

Table 2. L*, a* and b* mean values at three different ripening stages of tomato fruits, (n= 10).

Samples	Ripening Stages	Colour Values			°Brix TSS
		*L**	*a**	*b**	
Sarikiz	MG	41.19	0.32	10.69	5.9
	Br	40.04	2.43	11.32	6.1
	R	43.02	3.99	15.89	6.15
Moda	MG	43.50	-3.14	12.78	4.9
	Br	45.71	2.28	16.93	5.0
	R	43.66	8.20	22.56	5.02
Red Type	MG	41.13	0.29	10.13	5.05
	Br	39.65	4.22	12.36	5.12
	R	35.94	12.89	14.49	5.12

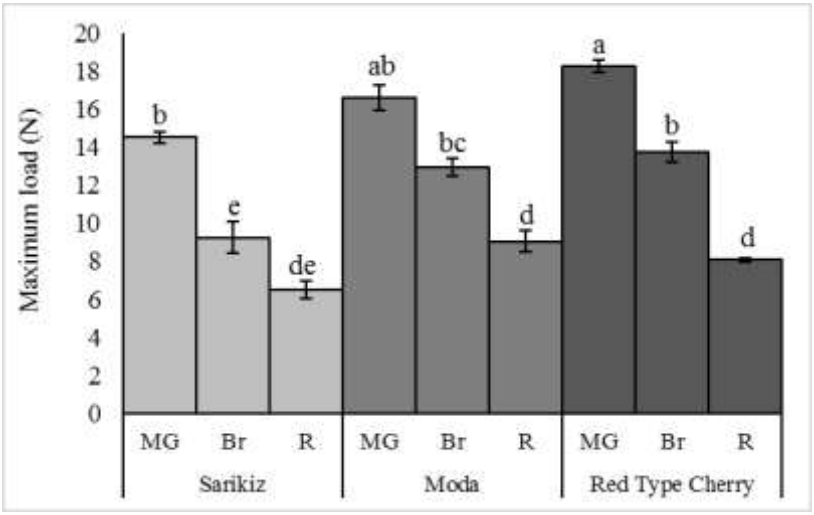

Figure 3. Maximum load (N) for pericarp of the tomato fruits at three different ripening stages. Values represent means ± SE (n= 10). Different letters indicate significant differences, $P<0.05$.

soft pericarp compared to other two varieties, which resulted significantly softer fruits at three developmental stages compared other two varieties. However, although the 'Sarikiz' had the softest fruits in R stage, the 'Cherry' had a total firmness loss of 55.73% from MG to R in where the 'Moda' had of 45.42%. These results suggest us that, 'Moda' variety was the firmest and probably has a longer shelf life among three varieties.

The levels of WSP (water-soluble pectin), CDTA (chelator soluble pectin), and Na_2CO_3 (carbonate soluble pectin) that could be extracted from the cell wall materials (CWM) of three tomato cultivars at three different ripening stages are presented (Figure 4). The fractionation of the CWM revealed a variety of differences in the levels of WSP at different ripening stages. As expected, CWM of WSP was the least at the MG stage, and there was a significant increase during shelf-life ripening in three cultivars. The cultivars 'Sarikiz' had the highest WSP with 40.76 mg UA g^{-1} at the R stage, while cultivar 'Red Cherry' and 'Moda' had around 28 mg UA g^{-1} of WSP at their ripe stages. The amount of CDTA pectin was found to be a higher fractionation in all cultivars at all ripening stages. The highest CDTA soluble pectin was found in R stage of all cultivars. The Na_2CO_3 soluble pectin of the three cultivars collected at different ripening stages decreased significantly during the storage period. Opposite to WSP, the cultivar 'Sarikiz' had the least Na_2CO_3 soluble pectin at R stage compared to other two cultivars. Based on cell wall fractionation analysis, the results showed that the cultivar 'Sarikiz' had the most WSP and the least Na_2CO_3 fraction at R stage compared to other two cultivar, which could be the reason of softer texture of 'Sarikiz'.

The *PL* gene expression was determined at a range of stages of fruit development developmental and ripening (MG, Br and R) stages of three tomato varieties. As shown in Figure 5, there was relatively strong *PL* gene expression in all varieties during all stages compared to housekeeping gene *LeEF-1*. However, it is clearly visible that, the expression of the *PL* is higher at Br stage of 'Sarikiz' compared to its MG and R stage. However, in 'Moda' e level of transcripts of PL is the highest at MG stage. The gene accumulation in 'cherry' cultivar looks similar to 'Sarikiz' in which the expression was higher in Br stage compared to other two ripening stages.

4. Discussion

Tomato fruits are usually harvested at MG stage either for transportation purposes or longer storage. The fruits harvested at MG stage exhibit maximum shelf life and they can ripe on their own due to its climacteric condition to the best of the ripening attributes (Sharma et al. 2020). As with all fleshy fruits, different tomato varieties show different ripening processes and mechanisms. In other words, distinct variability in postharvest ripening behavior was observed among tomato varieties when assessed in terms of colour, texture and shelf-life change based parameters. Therefore, in this study, we evaluated three different tomatoes that differ in colour, firmness and shelf-life characteristics at three different stages of ripening.

Tomato fruit changes its colour from green to red, yellow, or orange during ripening, because of chlorophyll degradation simultaneously to carotenoid biosynthesis like β-carotene (Carrillo-López and Yahia 2014). The changes in a* value was

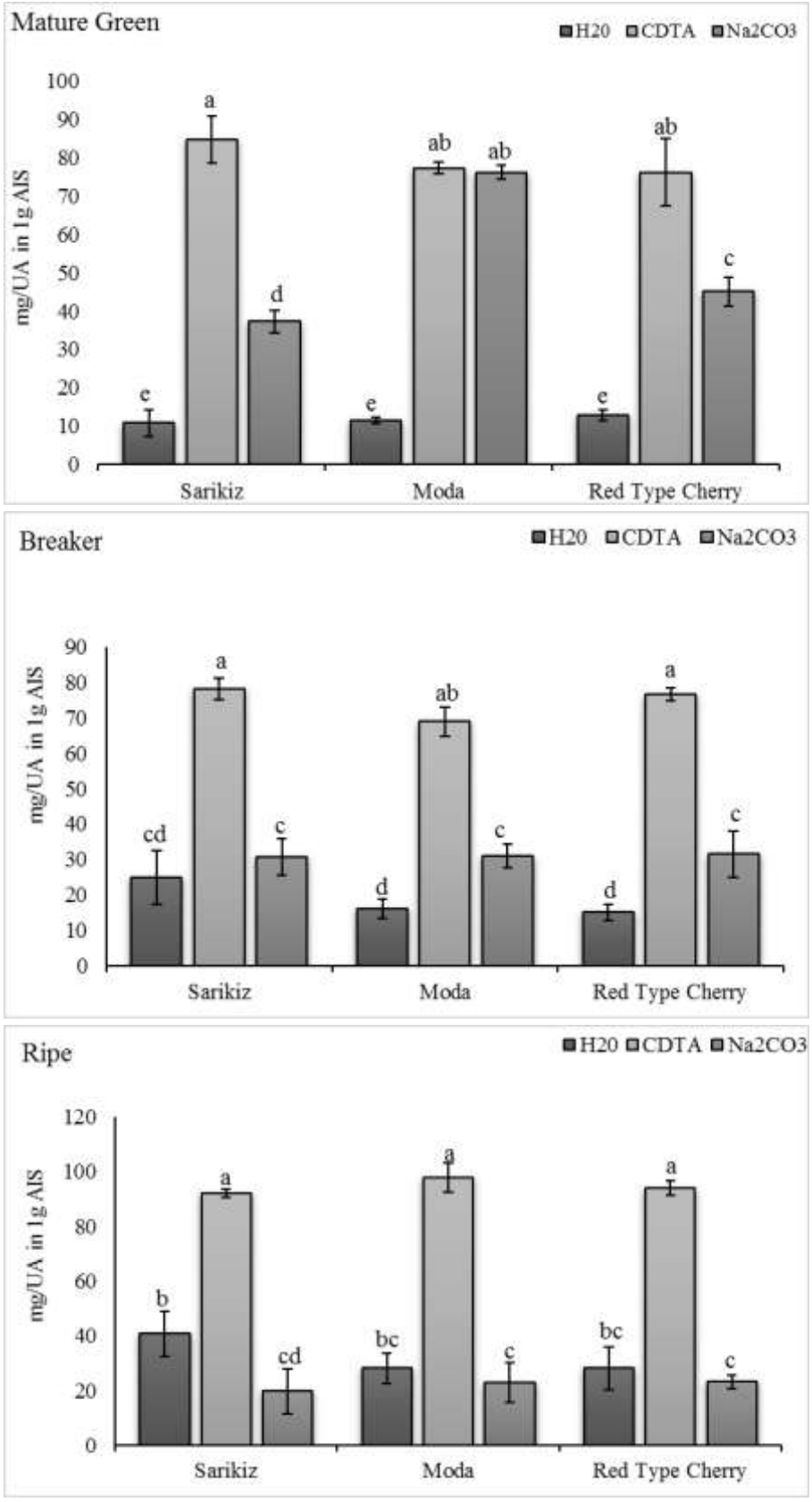

Figure 4. Changes in WSP, CDTA and Na_2CO_3 soluble pectin of Sarikiz, Moda and Red Type Cherry at MG, Br and Ripe stages. Error bars indicate the standard error for three replicates. There is a statistical difference between bars containing different letters, $P<0.05$.

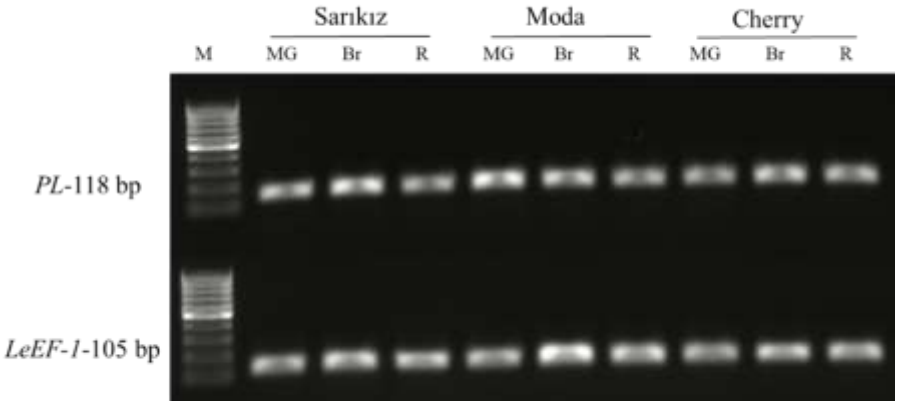

Figure 5. Semi quantitation RT-PCR gel electrophoresis image for the *PL* gene expression at three ripening stages of three tomato cultivars.

expected to be a good indicator for colour changes, because of a* perpendicular axes represent the green to red axis at the CIELAB colour system in which some researchers have only used a* values (Cantwell 1998). In this respect, a specific tomato ripeness stage can be determined by a* value of pericarp. However, in our case, it was impossible to evaluate the ripening stage only by a* value. Therefore, when it is also looked at b* values (blue to yellow), the gradual increase can easily be seen during ripening stages, especially in 'Sarikiz' and 'Moda' varieties. Overall, these results showed us that, the ripening process have normally gone throughout ripening process in three different colour tomatoes.

Texture is a sensory property and is recognised as a combination of different features, most likely dependent on the anatomical properties of the primary cell wall (Chylińska et al. 2017). Instrumental measurements are generally used to determine firmness related to mechanical properties of tissue. It is commonly known that tomato fruit softens quickly during development and ripening. One of the most obvious changes in the cell walls of ripening tomato fruits is the degradation of the pectic polysaccharides. This involves an increase in their solubility and a reduction in their molecular weight (Seymour et al. 1987).

Pectin content was estimated by the uronic acid concentration as galacturonic acid is the main component of pectins. Among cell-wall polysaccharides, water soluble pectin (WSP) chelator soluble pectin (CDTA) and carbonate soluble pectin (Na_2CO_3) are important part of cell walls and closely related to the ripening and softening of fruit (Brummell 2006).

Pectin which is loosely bound at cell wall characterized as the polysaccharides water soluble fraction (WSF). As fruit undergoes from mature to ripening transition, an increase is expected in WSF due to increase in loosely bound polyuronides, such as hydrolysis of homogalacturonan and neutral sugars present in the lateral chains of rhamnogalacturonan I (Gross and Sams 1984). In our analysis, although there was much less WSF in 'Sarikiz' compared to other cultivars, it has significantly higher amount of WSF in Br and R ripening stages. This result supports the firmness measurement results in which 'Sarikiz' was determined as the softest tomato. Moreover, it is a good hint to say that more pectin already lost its stronger bounds at Br and R ripening stages in 'Sarikiz'. Reduced amounts of WSF in 'Moda' and 'Red Cherry' at Br and R stages, suggesting that more of the pectins in fruit cell walls were covalently associated with the wall matrix. To summarize, our cell wall fractionation indicated a higher amount of WSP in 'Sarikiz' especially at Br and R ripening stages might corresponded to softer texture of this variety.

Although it would obviously be more reliable to evaluate the expression levels of genes by qPCR, due to for unforeseen reasons, we had to evaluate the expression levels of *PL* gene by semi q-RT PCR. In the present work, we evaluated the expression of *PL*, one of the main tomatoes softening related gene by semi q-RT-PCR. Silencing of a gene coding for PL enzyme improved tomato shelf-life, which is caused by alternating the levels of soluble pectin and total pectin (Uluisik et al. 2016). In our study, the expression of *PL* gene was slightly higher in 'Sarikiz' compared to other two cultivars at Br stage. The expression of the gene looks like lesser in 'Moda' especially at R stage, which could be one the reasons in lesser CWF and firmer texture of the fruit. The overall data from semi q-RT-PCR and cell wall composition led us to think that total activity of PL and other cell wall related enzymes were likely to be responsible for the wall changes that converted interconnected wall components into the WSF, mainly in 'Sarikiz'.

5. Conclusion

Although a great deal has been made in understanding the softening mechanism of the tomato, this complex mechanistic structure is open to new discoveries with integrating different varieties and cultivars into the research area. Therefore, in this study we evaluated quality parameters of three different colour tomatoes grown in Turkey, from physical, biochemical and molecular perspective. The softer tomato variety 'Sarikiz' at Br stage correlated with an increase in water-soluble pectin and expression level of cell wall degrading enzyme PL. Evaluation of these cultivars by different omic technologies (like transcriptomic and metabolomic) would be chance to identify different genes or compounds could be used in different breeding studies to create better quality of tomatoes.

Conflict of Interest

The author declares that he has no conflict of interest.

Authors Contribution

SU designed the study and carried out the experiments. He evaluated the data and wrote the manuscript.

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