*Pak. J. Bot.*, 55(6): 2131-2141, 2023. DOI: http://dx.doi.org/10.30848/PJB2023-6(20)

# FRUIT THINNING IMPROVES POMEGRANATE FRUIT QUALITY BY ACTIVATING PHYSICAL, PHYSIOLOGICAL, BIOCHEMICAL AND BIOACTIVE ATTRIBUTES

**GHULAM MUSTAFA[1]*, ISHTIAQ A. RAJWANA[1], HAFIZ NAZAR FARIED[1], TANVEER UL HAQ[2] AND MUHAMMAD AZHAR BASHIR[3]**

*[1]Department of Horticulture, Muhammad Nawaz Shareef University of Agriculture, 66000 Multan, Pakistan*
*[2]Department of Soil and Environmental Science, Muhammad Nawaz Shareef University of Agriculture, 66000 Multan, Pakistan*
*[3]Horticultural Research Station, 63100 Bahawalpur, Pakistan*
**Corresponding author's email: gmustafabzu@gmail.com*

**Abstract**

The excessive fruit load on various fruit trees is an obstacle in achieving sustainable production. Fruit thinning helps to improve fruit quality attributes by reducing crop load. Therefore, a field study was carried out to evaluate the impact of fruit thinning on pomegranate. The four levels of fruit thinning were applied that including without thinning of a cluster (control), keeping three fruits ($T_1$), two fruits ($T_2$) and one fruit per cluster ($T_3$). The effect of fruit thinning on physical and biochemical quality attributes of three pomegranate cultivars (Sindhuri, Kalehar and Sava) were evaluated during two consecutive growing seasons (2018 and 2019). The results revealed that Sindhuri followed by Kalehar and Sava showed significant improvement in fruit weight, fruit length, fruit width, A-grade fruit percentage, arils weight percentage, hundred arils weight, juice contents and peel redness (a*) when applied $T_3$ (one fruit per cluster) while plant yield, peel weight percentage, peel thickness, lightness (L), yellowness (b*), fruit firmness, percentage of B grade and C grade fruits were significantellyreduced. The value of total soluble solid (TSS), pH, ascorbic acid, anthocyanin, antioxidant activity (AA), superoxide dismutase (SOD) and catalase (CAT) was also improved in $T_3$ while titratable acidity (TA), total phenolic contents (TPC) and polyphenol oxidase (PPO) were reduced significantly. These observations indicate that reducing crop load by thinning (one fruit per cluster) could be a viable strategy to improve fruit quality attributes in pomegranate fruit. Conclusively, one fruit per cluster ($T_3$) significantly improved fruit's physical and biochemical attributes in pomegranate cultivars (Sindhuri, Kalehar and Sava).

**Key words:** Antioxidative enzymes, Crop load, Cultivar, Fruit thinning, Phytochemicals and Quality.

## Introduction

Pomegranate (*Punica granatum* L.) is a deciduous fruit crop that belong to the Lythraceae family. It has been grown successfully in tropical, subtropical and Mediterranean regions (Ozcan *et al*., 2019; Fattahi *et al*., 2020). The crop is being cultivated in India, Iran, China, USA, Turkey, Egypt, South Africa, Spain, France, Italy, Chile, Pakistan and Portugal (Mansouri *et al*., 2010). More than 500 cultivars were reported worldwide while 50 cultivars are grown commercially (Anon., 2001). It is also known as a superfruit due to its high medicinal value, nutritional impact, antioxidant and anti-carcinogenic activities (Varasteh *et al*., 2012). In Pakistan, pomegranate is ranked at position number 12$^{th}$ with a cultivated area of 7293 hectares and annual production of 37613 metric tons (Anon., 2020). The excessive fruit load on trees is becoming an obstacle in achieving sustainable production and maintaining tree health potency and fruit quality due to depletion of plant assimilates (Turk *et al*., 2018). The plant yield, fruit size and quality affected by heavy crop load, profuse fruit setting and different diseases (Kahramanoglu *et al*., 2018; Fattahi *et al*., 2020). Therefore, crop load management has become an obligatory tool to reduce competition among fruits for early maturation, quality of fruit and consumers acceptability by improving leaf to fruit ratio on the plant canopy (Hehnen *et al*., 2012). The fruits produced in clusters are uneven in size, having blemishes on skin, provide a favorable environment for pests anddeteriorate fruit quality attributes (Costa *et al*., 2013). The pomegranate plant produced abundant flowers (hermaphrodite, intermediate and functionally male) in three waves, from early April to mid May (Jafari *et al*., 2014). Only hermaphrodite flowers can produce quality fruits that appear on the terminal and axillary buds (Kahramanoglu & Usanmaz, 2018). The fruits developed from the early emergence of flowers are superior in quality with the highest commercial value (Mohsen & Osman, 2015; Fattahi *et al*., 2020).

Fruit thinning is preferred over flower thinning due to extreme weather conditions and lack of technical skill (Webster & Spencer, 2000). Thinning is the judicious removal of fruit from the plant using various thinning methods (hand, chemical and mechanical thinning). Hand thinning is an utmost reliable method to achieve good quality and optimum crop load on plant canopy by removing de-shaped, undersized, diseased and injured fruits while retaining healthy ones (Fallahi *et al*., 2006; Jafari *et al*., 2014). The cultivars may require a different level of fruit thinning due to variations in genetic and physiological characteristics (Mohsen & Osman, 2015). Fruit size is influenced by thinning severity, crop load, wood age, flower bud quality, number of fruits per cluster and fruit position on canopy (Link, 2000). If the number of sinks increased, it would reduce the size of fruits and various quality attributes due to increased competition for photosynthates, minerals, nutrients and water (Rodrigues *et al*., 2019). Thinning just after fruit setting help to improve fruit weight, size, juice contents, titratable acidity (TA), pH, total soluble solids (TSS) and maturity index (Seehuber *et al*., 2012). Fruit thinning increases the accumulation of extra photosynthates (carbohydrates) among remaining

fruits that enhance various physical (percentage of A-grade fruit, total peel weight, total arils weight, hundred arils weight (HAW), fruit firmness, shelf life, juice contents) and biochemical attributes (TSS, TA, pH, ascorbic acid and antioxidants) of fruits by reducing competition among remaining fruits on the plant (Kahramanoglu *et al*., 2018). Fruit thinning improves fruit firmness, fruit colour, distribution of photoassimilates and sunlight (Seehuber *et al*., 2012). Fruit matures earlier in light-loaded plants with improved TSS and other organic compounds than heavy loaded (Wünsche *et al*., 2000). Improvements in fruit quality and colour through thinning are usually accompanied by increased fruit length, width, volume, weight of 100 arils, TSS, pH, ascorbic acid, anthocyanin and antioxidant activity with a slight reduction in yield and total acidity as compared to untinned plants (Link, 2000; Mohsen & Osman, 2015). The fruit taste also increased, which is a blend of TSS, acidity and the percentage between TSS and acidity, improved by reducing the crop load (Opara *et al*., 2009). Despite its vast health benefits, consumer demand and economic importance, there is little information regarding productional issues like crop load management or fruit thinning (Fattahi *et al*., 2020). Pome fruitpractices for fruit thinning are adopted regularly to improve fruit size and various quality attributes (Vasantha *et al*., 2006). Moreover, no guidelines for fruit thinning of Pakistani pomegranate cultivars are documented; therefore, a gap existsin identifying how thinning affects various fruit quality parameters. Thus, the current study was planned to evaluate the impact of hand thinning on plant yield, fruit size and quality attributes of three commercially grown pomegranate cultivars (Sindhuri, Kalehar and Sava) under conditions in South Punjab, Pakistan.

## Materials and Methods

**Plant selection and application of treatments:** Two years (2018 and 2019), a field study was conducted at two commercial pomegranate orchards; Maral fruit farm (30° 01’ 74” N; 71° 03’ 98” E and 311.7 feet elevation from sea level) at Multan and Raees fruit farm (28° 94’ 15 N; 70° 85’ 66” E and 398.6 feet elevation from sea level) at Liaqat pur, South Punjab of Pakistan. The commercially grown three cultivars of pomegranate (Kalehar, Sindhuri and Sava) were selected for this study. Two cultivars (Sindhuri and Sava) were chosen from Raees fruit farm, Rahim Yar Khan, and third cultivar (Kalehar) was selected from Marral fruit farm, Multan. The healthy plants of eight years old plants with homogenous vigour and size were chosen for this experiment. The recommended cultural practices were adopted in commercial pomegranate orchards. Four thinning treatments were used based for this study, i.e., without cluster thinning or control ($T_0$), three fruits per cluster ($T_1$), two fruits per cluster ($T_2$), and one fruit per cluster ($T_3$) as shown in (Fig. 1).

The thinning treatments were applied after twenty days of fruit setting (Jafari *et al*., 2014; Mohsen & Osman, 2015) during both growing seasons. A total of sixteen healthy plants having homogenous vigor were selected by adjustingfifteen fruit clusters per tree. There were four treatments with four replications, and each plant was considered as an experimental unit. The fruits from the experiments plants were harvested according to experimental design at commercial maturity 3rd week of August) and shifted to Postharvest Science and Technology Lab, MNS-University of Agriculture, Multan, Pakistan, for different physiochemical analyses under ambient conditions (25 ± 2°C; 55–65% relative humidity).

**Determination of physical and physiological characteristics of fruits:** The plant yield (kg), weight of individual fruit, total arils, total peel, 100 arils and fruit weight loss (%) were measured using an electronic weighing balance (PA4102, OHASU Corporation, USA). The average length and width (mm) of fruit were calculated by using a digital vernier caliper (Mitutoyo, 938882, Seiko Corporation, Japan). The percentage of A-grade (200 gram or above), B-grade (150 to 199 gram) and C-grade (100 to 149 gram) fruits were also classified based on fruit weight described by Kahramanoglu *et al*., (2018). The aril juice contents were estimated by using Juicer/Blender (MJ-M176P Panasonic, Malaysia). The fruit colour was determined using the chromameter (CR-400 Konica MinoltiaSencing, Inc., Japan) and values of L, a* and b* were recorded. The fruit firmness was determined from both sides of the fruits by using a penetrometer or digital fruit hardness tester (FR-5120, Lutron Electronics Enterprises Co., Ltd. Taiwan) with a probe of 5mm.


Fig. 1. One fruit per cluster (A), two fruits per cluster (B), three fruits per cluster (C) and four fruits per cluster (D).

**Determination of biochemical andbioactiveattributesof fruits:** Total soluble solids of arils juice were estimated by using a digital refractometer (PAL-1, ATAGO, Japan ATAGO, Japan) and its value was expressed in °Brix. At the same time, titratable acidity (TA) was recorded with automatic titrator (HI84532, Hanna Instruments, U.K.) and expressed in percentage. The pH of arils juice was determined by using a pH meter (Milwaukee MW804, Romania). The maturity index (TSS/TA) was determined by using method adopted by Fattahi *et al*., (2020). The vitamin-C (mg/100 mL) contents were measured by indophenol's titration-based method described by Hussain *et al*., (2017). After filtering 5 mL ofaliquot was titrated against dyes of sodium bicarbonate ($NaHCO_3$) and 2-6-dichloroecdophenol and its value was expressed as, mg/100mL. The 5g frozen samples of pomegranate arils (stored at -80°C) were each homogenized in 10 ml reaction mixtures (methanol, acetone and HCl with a ratio of 90:8:2) and centrifuged at 27586×gn at 4°C for 4 min in a refrigerated centrifuge (Centrifuge, Z326 K, Hermle, Germany) and the supernatant was collected. For estimation of total phenolic contents (TPC), Folin-Ciocalteu reagent method was used as described by Razzaq *et al*., (2013). Absorbance was recorded at 760 nm with a spectrophotometer (Cecil Aquarius, CE 7400S, Cecil Instruments, U.K.) and expressed as µg ml $FW^{-1}$. Antioxidant scavenging activity (% inhibition) was determined by using the 1,1 diphenyl-2-picrylhydrazyl (DPPH) method as described by Razzaq *et al*., (2013) with some modification, and absorbance was noted at 520 nm. Total anthocyanin from pomegranate juice was determined by calculating $\Delta Ag^{-1}FW$= (A530-A620)-0.1(A650-A620) method described by Zheng *et al*., (2006) and absorbance was observed at three different wavelengths (530nm, 620nm and 650nm).

**Determination of antioxidativeenzyme:** One gram of frozen sample was homogenized with 2 mL of phosphate buffer (7.2 pH) in pestle mortar to estimate antioxidative enzymes. The homogenized mixture was centrifuged at 9000 rpm at 04°C for 4 minutes, and the supernatant was collected. The activity of antioxidative enzymes, catalase (CAT), superoxide dismutase (SOD), and peroxidase (POX) was carried out by following the method of Razzaq *et al*., (2013) and readings recorded using Epoch, Eliza Reader (Bio-Tek). The activity of SOD was recorded by measuring 50% inhibition of photochemical reduction of nitro blue tetrazolium (NBT). The 500 µL phosphate buffer (pH 05), 100 µL NBT, 200 µL Triton-X, 200 µL of methionine, 800 µL distilled water were dissolved in test tube and finally added 100 µL of enzymes extract supernatant was obtained. The tubes were placed in a laminar airflow cabinet under ultraviolet light for 15 minutes and then added 100 µL Riboflavin as a substrate. Finally, the absorbance was recorded at 560 nm. The activity of CAT (U/mg of protein) was calculated by adding 5.9 mM of $H_20_2$ (100 µL) in enzymes extract (100 µL), and absorbance was recorded at 240 nm. The POX activity was determined by using a reaction mixture containing phosphate buffer (pH 5), 40 mM $H_2O_2$ and 20 mM guaiacol with the ratio of 8:1:1, respectively. To determine the POX value (U/mg of protein), enzymes extract (100 µL) was added to the reaction mixture (100 µL) and absorbance was measured at 470nm. PPO activity was determined by taking 1.4 mL of 100 mM citrate buffer (pH 6.8), and 0.5 mL of 100 mM of 4-methyl catechol was added in enzyme extract, and absorbance was recorded at 412 nm. The activity of enzym was expressed as U/mg of protein as described by Mustafa *et al*., (2023).

**Statistical analysis**

The experimental data were subjected to analysis of variance (Steel *et al*., 1996) by using Statistix 8.1 software (Tallahassee Florida, USA), based on four replications under 2-factors factorial (Thinning levels and Genotype). As the year effect was non-significant, the data of this study were pooled before statistical analysis. The means were compared using the LSD Fisher's test (least significance differences) at 5% probability level ($p \leq 0.05$).

**Results**

**Physical quality attributes:** The result indicated that interactive effect of fruit thinning and cultivar related to plant yield, fruit weight (g), percentage of A-grade fruit, fruit width, peel weight, peel thickness, lightness (L), redness (a*), yellowness (b*) and fruit firmness (FF) were found significant while plant yield and fruit length showed non-significant interaction ($p \leq 0.05$). The maximum plant yield (54.34kg) produced by Sindhuri cultivar when applied $T_0$ while maximum fruit weight (299.82g), fruit length (124.85mm) and fruit width (110.83mm) produced by Sava cultivar when applied $T_3$ (Table 1). The maximum arils weight (65.57%) produced by Sindhuri cultivar whereas maximum peel weight percentage (53.80%) and peel thickness produced by Kalehar under control condition (Table 1). Maximum hundread aril weight (39.72) and A-grade fruit per cent (58.44%) produced by Sava cultivar when applied $T_3$ (one fruit per cluster) while Sava cultivar produced maximum value of fruit firmness under control condition. The maximum value of lightness (68.85) and yellowness (43.74) produced by Sava cultivar when applied $T_1$ where as the highest value of redness (44.17) obtained from Sindhuri cultivar when applied $T_3$ (Table 2). Irrespective of cultivar response, fruit width was 60.64% higher in $T_3$ than $T_0$. In contrast, a reduction in peel thickness has been observed with increasing fruit thinning levels. The maximum decrease in peel thickness (13.16%) was observed when applied $T_3$, irrespective of the cultivar effect (Table 3). The fruit width of Sava cultivar was 1.19 and 1.07 folds higher than Sindhuri and Kalehar, respectively. While among cultivars response, Kalehar gave 1.07- and 1.41-fold higher peel weight and peel thickness than Sava and Sindhuri, which was 1.09 and 1.17-fold higher than Sava and Sindhuri cultivar, respectively, regardless of treatment.The cultivar Sava presented a higher percentage of A-grade fruits (1.10 and 1.25-fold), total arils weight (1.05 and 1.00 folds), 100 aril weights (1.11 and 1.04-fold), and juice contents (1.04 and 1.05-fold) as compared to Sindhuri and Kalehar, respectively (Table 2). Irrespective of treatments effect, the maximum FF (68.95N) value was recorded from Kalehar, 1.34 and 1.42-fold higher than Sava and Sindhuri, respectively (Table 4).

**Table 1. Effect of fruit thinning on plant yield (PY), fruit weight (FW), fruit length (FL), fruit width (FW), total aril weight percentage (TAW), total peel weight percentage (TPW), peel thickness (PT) and 100eight.**

| Cultivars | Treatment | Plant yield (Kg) | Fruit weight (g) | Fruit length (mm) | Fruit width (mm) | Total arils weight (per cent) | Total peel weight (per cent) | Peel thickness (mm) |
|---|---|---|---|---|---|---|---|---|
| Sindhuri | Control | 54.34 ± 1.03a | 105.71 ± 0.39i | 66.54 ± 0.83h | 57.31 ± 1.11h | 53.92 ± 0.05g | 46.08 ± 0.06d | 0.35 ± 0.005de |
| | 3 Fruit/cluster | 53.13 ± 0.47a | 155.96 ± 1.43g | 88.45 ± 2.66f | 67.90 ± 0.64f | 59.39 ± 0.08c | 40.61 ± 0.08h | 0.34 ± 0.003ef |
| | 2 Fruit/cluster | 50.06 ± 0.24b | 199.06 ± 3.37ef | 98.852 ± 0.80d | 74.03 ± 0.47e | 62.02 ± 0.16b | 37.98 ± 0.16i | 0.33 ± 0.004g |
| | 1 Fruit/cluster | 48.29 ± 0.67bc | 249.65 ± 6.10c | 111.98 ± 3.05c | 87.50 ± 1.79c | 65.57 ± 0.10a | 34.43 ± 0.10j | 0.31 ± 0.003h |
| Kalehar | Control | 50.22 ± 0.72b | 138.50 ± 3.12h | 74.02 ± 1.86g | 64.57 ± 0.17g | 46.20 ± 0.12j | 53.80 ± 0.12a | 0.42 ± 0.003a |
| | 3 Fruit/cluster | 47.41±0.61cd | 191.87 ± 1.71f | 92.98 ± 1.5f | 73.37 ± 0.89d | 51.21 ± 0.14h | 48.79 ± 0.14c | 0.40 ± 0.001b |
| | 2 Fruit/cluster | 46.63 ± 0.59de | 229.93 ±3.57d | 109.13 ± 1.09c | 88.06 ± 0.90c | 54.81 ± 0.19f | 45.19 ± 0.19e | 0.37 ± 0.002c |
| | 1 Fruit/cluster | 44.83 ± 0.50e | 282.05 ± 1.48b | 119.57 ± 1.23b | 99.82 ± 0.71b | 57.62 ± 0.31d | 42.41 ± 0.32g | 0.36 ± 0.004de |
| Sava | Control | 53.90 ± 0.86a | 149.92 ± 1.52g | 76.63 ± 0.99g | 68.94 ± 0.96f | 50.72 ± 0.14i | 49.28 ± 0.14b | 0.38 ± 0.003c |
| | 3 Fruit/cluster | 53.81 ± 0.60a | 199.74 ± 2.87e | 95.94 ± 0.82de | 87.06 ± 1.13c | 56.83 ± 0.09e | 43.17 ± 0.09f | 0.37 ± 0.009c |
| | 2 Fruit/cluster | 53.04 ± 0.92a | 251.28 ± 3.68c | 113.09 ± 0.92c | 101.82 ± 0.84b | 59.57 ± 0.16c | 40.43 ± 0.16h | 0.35 ± 0.001de |
| | 1 Fruit/cluster | 49.63 ± 027b | 299.82 ± 1.62a | 124.85 ± 1.29a | 110.83 ± 1.21a | 62.22 ± 0.13b | 37.78 ± 0.13i | 0.33 ± 0.002fg |

The ± indicates a standard error (SE) of the mean. The values with different letters show a significant difference at $p \leq 0.05$

**Table 2. Effect of fruit thinning on fruit firmness, percentage of A-grade fruit, percentage of B-grade fruit, percentage of C-grade fruit, lightness (L), redness (a*) and yellowness (b*).**

| Cultivars | Treatments | Hundred aril weight (g) | Fruit firmness (N) | A- grade fruit (Per cent) | B-grade fruit (Per cent) | C-grade fruit (Per cent) | Lightness (L) | Redness (a*) | Yellowness (b*) |
|---|---|---|---|---|---|---|---|---|---|
| Sindhuri | Control | 22.88 ± 0.55i | 54.34 ± 1.03d | 26.87±1.34e | 33.74±1.45a-c | 39.39±2.57a | 46.08 ± 0.06d | 30.35 ± 0.05d | 22.88 ± 0.55i |
| | 3 Fruit/cluster | 24.55 ± 0.66gh | 33.98 ± 1.17k | 38.47±2.09d | 38.26±1.49a | 23.27±0.84b-d | 48.56 ± 0.64cd | 33.38 ± 1.35c | 25.53 ± 0.49f |
| | 2 Fruit/cluster | 30.42 ± 0.46e | 39.68 ± 1.15ij | 51.20±.60b | 32.02±2.23c | 16.79±2.43e | 43.42 ± 0.53fg | 37.97 ± 0.39b | 22.37 ± 0.38g |
| | 1 Fruit/cluster | 34.30 ± 0.32c | 42.06 ± 1.41hi | 60.51±2.58a | 21.16±1.33d | 18.33±1.42de | 36.42 ± 0.75h | 44.17 ± 1.1a | 18.49 ± 0.40h |
| Kalehar | Control | 24.33 ± 0.27h | 45.90 ± 0.43fg | 25.23±1.20e | 35.88±0.69a-c | 39.89±1.63a | 30.37 ± 0.59i | 11.40 ± 0.74h | 16.61 ± 0.34i |
| | 3 Fruit/cluster | 25.49 ± 0.45gh | 47.55 ± 0.82ef | 36.44±1.80d | 37.23±1.59a | 28.33±0.89bc | 56.65 ± 1.26b | 13.79 ± 0.41g | 34.99 ± 0.77c |
| | 2 Fruit/cluster | 31.87 ± 0.42d | 57.26 ±1.37c | 49.27±1.96bc | 32.07±1.89c | 18.66±3.29de | 50.58 ± 0.57c | 14.82 ± 0.39g | 29.78 ± 0.37e |
| | 1 Fruit/cluster | 38.01 ± 0.25b | 61.01 ± 1.49b | 60.14±.73a | 19.67±1.91d | 21±1.74de | 44.46 ± 0.46ef | 22.43 ± 0.74f | 26.84 ± 0.52f |
| Sava | Control | 27.13 ± 0.21f | 68.95 ± 0.64a | 26.51±1.43e | 33.50±0.61bc | 39.99±1.87a | 40.82 ± 0.49g | 27.76 ± 0.84e | 23.18 ± 0.47g |
| | 3 Fruit/cluster | 28.05 ± 0.38f | 37.76 ± 1.07j | 35.44±1.60d | 37.40±1.35ab | 27.16±2.26b | 68.85 ± 1.39a | 3.71 ± 0.23j | 43.74 ± 0.31a |
| | 2 Fruit/cluster | 34.40 ± 0.20c | 43.83 ± 0.70gh | 46.52±1.13c | 31.12±2.57c | 22.37±3.45b-e | 58.06 ± 1.86b | 6.305 ± 0.33i | 40.23 ± 0.54b |
| | 1 Fruit/cluster | 39.72 ± 0.24a | 50.33 ± 0.85de | 58.44±1.64a | 22.4±1.50d | 20.48±1.34c-e | 47.03 ± 0.81de | 8.18 ± 0.18i | 33.60 ± 0.32d |

The ± indicates a standard error (SE) of the mean. The values with different letters show a significant difference at $p \leq 0.05$

**Table 3. Main effect of fruit thinning on plant yield, fruit physical, physiological, biochemical, bioactive and enzymatic attributes.**

| Parameters | $T_0$ | $T_1$ | $T_2$ | $T_3$ | LSD |
|---|---|---|---|---|---|
| Plant yield | 52.83a | 51.45b | 49.91c | 48.03d | 1.15 |
| Fruit weight | 130.00d | 182.52c | 226.74b | 277.17a | 4.15 |
| Fruit length | 72.04d | 92.11c | 106.49b | 118.21a | 2.43 |
| Fruit width | 63.61d | 77.44c | 87.97b | 99.38a | 1.63 |
| Total aril % | 50.28d | 55.81c | 58.80b | 61.80a | 0.26 |
| Total peel % | 49.72a | 44.19b | 41.20c | 38.21d | 0.25 |
| Peel thickness | 0.38a | 0.37b | 0.35c | 0.33d | 0.01 |
| 100 arils weight | 24.78d | 26.03c | 32.23b | 37.34a | 0.65 |
| Palatability rate | 6.49d | 7.12c | 7.86b | 8.84a | 0.13 |
| Fruit firmness | 39.77d | 46.93c | 51.14b | 55.50a | 1.71 |
| A-grade fruit % | 26.20d | 36.78c | 49.00b | 59.70a | 2.65 |
| B-grade fruit % | 34.37b | 37.96a | 31.74b | 21.43c | 2.71 |
| C-grade fruit % | 39.43a | 25.25b | 19.27c | 18.87c | 3.54 |
| Lightness (L) | 58.02a | 50.69b | 42.64c | 38.08d | 1.88 |
| Redness (a*) | 16.97d | 20.27c | 25.50b | 30.82a | 1.41 |
| Yellowness (b*) | 34.75a | 30.79b | 26.31c | 23.32d | 0.91 |
| Total soluble solids | 13.77d | 14.40c | 15.30b | 16.23a | 0.2 |
| Titratable acidity | 0.66a | 0.61b | 0.57c | 0.53d | 0.01 |
| Maturity index | 21.18d | 23.92c | 27.00b | 31.07a | 0.60 |
| pH | 3.81d | 3.86bc | 3.90b | 3.99a | 0.01 |
| Anthocyanin | 0.33d | 0.37c | 0.41b | 0.43a | 0.02 |
| Vitamin-C | 33.31c | 36.456b | 36.839b | 37.954a | 0.66 |
| Total phenolics | 409.6a | 406.9b | 400.8c | 396.6d | 18.35 |
| Antioxidants | 69.50d | 73.82c | 77.86b | 82.07a | 0.84 |
| Superoxide dismutase | 42.98d | 48.89c | 53.85b | 56.31a | 1.81 |
| Catalase | 74.42d | 82.71c | 88.04b | 93.66a | 2.47 |
| Peroxidase | 10.04d | 12.09c | 13.34b | 14.45a | 0.49 |
| Polyphenol oxidase | 0.75a | 0.75a | 0.71b | 0.70b | 0.03 |

The different letters show a significant difference at $p \leq 0.05$

**Table 4. Main effect of cultivars on plant yield, fruit physical, physiological, biochemical, bioactive and enzymatic attributes.**

| Parameters | Sindhuri | Kalehar | Sava | LSD |
|---|---|---|---|---|
| Plant yield | 52.83a | 51.45b | 49.91c | 1.01 |
| Fruit weight | 130.00c | 182.52b | 226.74a | 3.45 |
| Fruit length | 72.04c | 92.11b | 106.49a | 2.11 |
| Fruit width | 63.61c | 77.44b | 87.97a | 1.41 |
| Total aril % | 50.28c | 55.81b | 58.80a | 0.21 |
| Total peel % | 49.72a | 44.19b | 41.20c | 0.22 |
| Peel thickness | 0.38a | 0.37b | 0.35c | 0.01 |
| 100 arils weight | 24.78c | 26.03b | 32.23a | 0.56 |
| Palatability rate | 39.77c | 46.93b | 51.14a | 1.48 |
| Fruit firmness | 26.20c | 36.78b | 49.00a | 2.30 |
| A-grade fruit % | 34.37b | 37.96a | 31.74b | 2.35 |
| B-grade fruit % | 39.43a | 25.25b | 19.27c | 3.07 |
| C-grade fruit % | 6.49b | 7.12b | 7.86a | 0.11 |
| Lightness (L) | 58.02a | 50.69b | 42.64c | 1.63 |
| Redness (a*) | 16.97c | 20.27b | 25.50a | 1.25 |
| Yellowness (b*) | 34.75a | 30.79b | 26.31c | 0.79 |
| Total soluble solids | 12.77c | 14.40b | 15.30a | 0.17 |
| Titratable acidity | 0.66a | 0.61b | 0.57c | 0.01 |
| Maturity index | 21.18c | 23.92b | 27.00a | 0.52 |
| pH | 3.81b | 3.86ab | 3.90a | 0.01 |
| Anthocyanin | 0.33c | 0.37b | 0.41a | 0.01 |
| Vitamin-C | 33.31c | 36.456b | 36.839a | 0.57 |
| Total phenolics | 409.6a | 406.9b | 400.8c | 5.91 |
| Antioxidants | 69.50c | 73.82b | 77.86a | 0.73 |
| Superoxide dismutase | 42.98c | 48.89b | 53.85a | 1.56 |
| Catalase | 74.42c | 82.71b | 88.04a | 2.14 |
| Peroxidase | 10.04c | 12.09b | 13.34a | 0.42 |

The different letters show a significant difference at $p \leq 0.05$

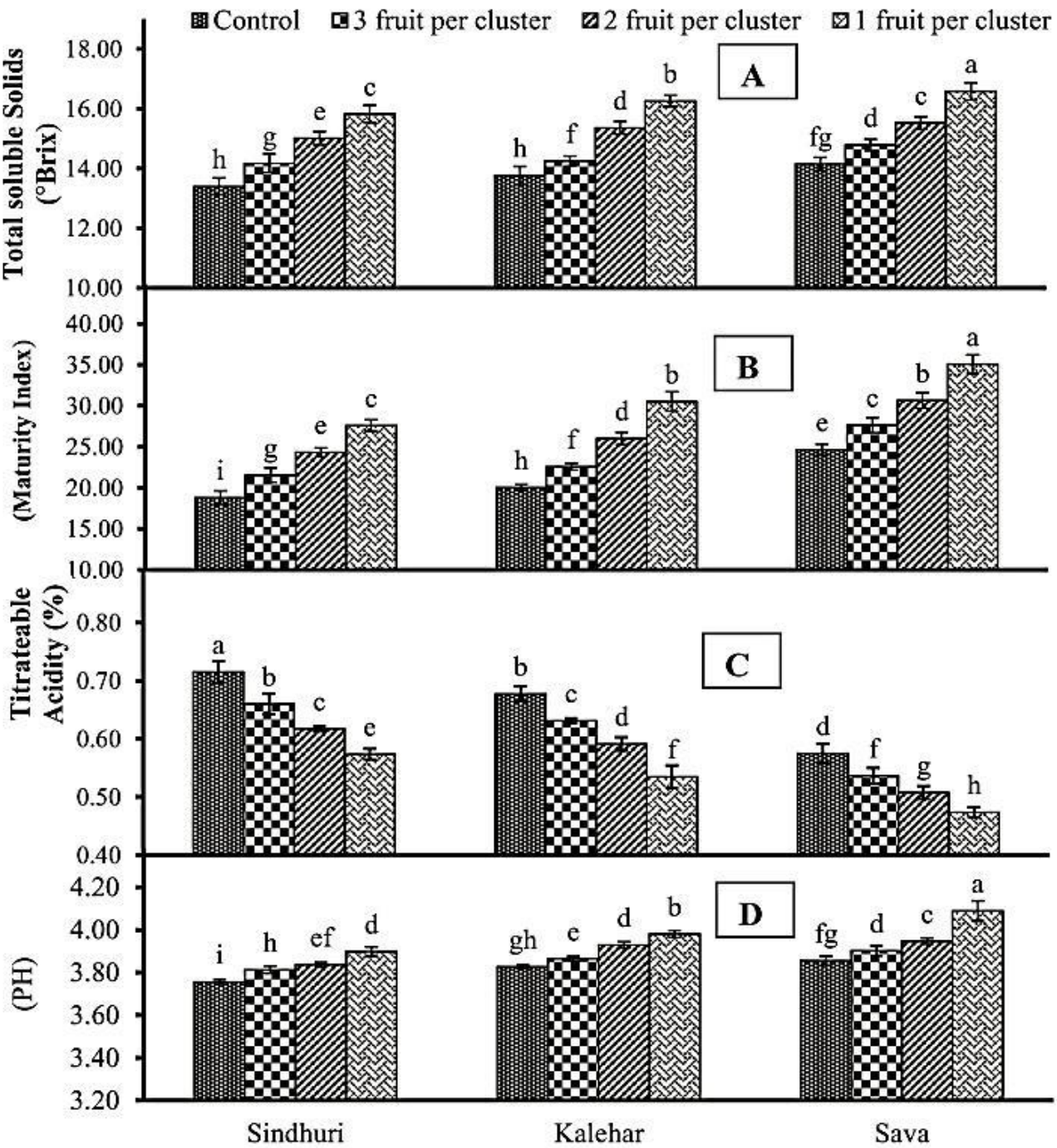


Fig. 2. Effect of fruit thinning on Total soluble solid (A), Titratable acidity (B), Maturity index (C) and pH (D) on three cultivars of pomegranate.Every value in the above figures is the mean of 4 replicates. The vertical bars indicate ± standard error (SE) of the mean. Bars showing different letters are significantly difference at $p \leq 0.05$. $T_0$= Control, $T_1$= Three fruits/cluster, $T_2$= Two fruits/cluster and $T_3$= One fruit/cluster. LSD values for total soluble solids = 0.34, Titrateable acidity= 0.02, Maturity index= 1.03 and pH of juice= 0.01.

**Biochemical attributes:** The interactive effect of fruit thinning and cultivar was found significant ($p \leq 0.05$) related to TSS, TA, maturity index (TSS/TA) and pH of fruit juice. The highest value of TSS (16.59 °Brix), maturity index (35.1) and pH (4.09) while the lowest value of TA (0.48) obtained from Sava cultivar when applied $T_3$ as shown in Figure 2 (A, B, C and D respectively). Regardless of the varietal response TSS and MI were found 19.41% and 53.3% higher, respectively, while 20.02 per cent reduction has been observed in $T_3$ (Table 3). Among cultivar response, TSS and maturity index in Sava cultivar was higher while TA was 1.02 and 1.23 lower than Sindhuri and Kalehar. At the same time, the minimum value of TA (0.53%) got from Sava that was 1.06 and 1.23-fold lesser than Kalehar and Sindhuri regardless of treatments. The pH of fruit juice in the Sava cultivar was 1.02 to 1.04 folder higher than Kalehar and Sindhuri, respectively (Table 4).

**Bioactive compounds (vitamin-C, total antioxidants, anthocyanin and total phenolic contents):** The interactive effect of fruit thinning and cultivar was

significant ($p \leq 0.05$) related to vitamin-C and total antioxidant where as a non-significant interaction has been observed related to TPC and anthocyanin. The maximum values of vitamin-C (40.55 mg/100 mL) and total antioxidant (78.47 % inhibition) were found from Sava cultivar when applied $T_3$ (one fruit per cluster (Fig. 3-A and B). The maximum value of TPC (472.6 mg GAE/100g) and anthocyanin ($0.69\Delta Ag^{-1}FW$) was observed from $T_3$(one fruit per cluster) regardless of varietal response (Fig. 3-C and D). Among treatments effects maximum values of Vitamin-C and antioxidants were obtained from highest level of thinning (one fruit per cluster) regardless of cultivar effect. TPC was 1.47 and 1.27-fold higher in Sava compared to Sindhuri and Kalehar, respectively, regardless of the treatment effect (Table 3). Where as among cultivar response, the Sava cultivar produced maximum Vitamin-C contents that were 1.29 and 1.44-fold higher, while total antioxidant contents were 1.03 and 1.08-fold higher than Sindhuri and Kalehar, respectively regardless of treatment effect. Among cultivar responses, the maximum anthocyanin contents obtained from Sindhuri was 1.29 and 9.24-fold higher than Kalehar and Sava irrespective of treatment effects (Table 4).

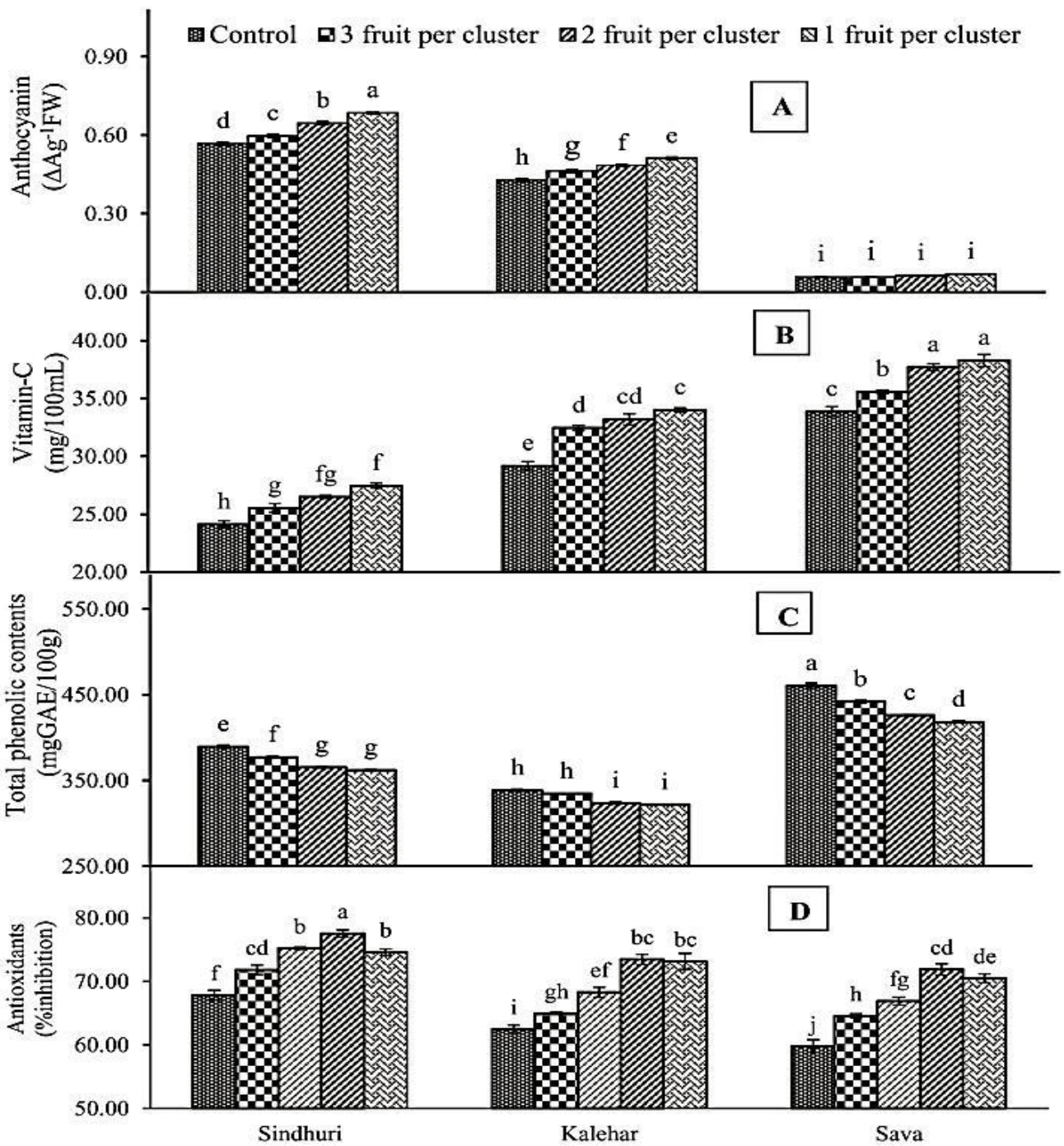


Fig. 3. Effect of fruit thinning on Anthocyanin (A), Vitamin-C (B), Total Phenolics (C) and Total Antioxidants (D) on three cultivars of pomegranate. Every value in above figures is the mean of 4 replicates. The vertical bars indicate ± standard error (SE) of mean. Bars showing different letters are significantly difference at $p \leq 0.05$. $T_0$= Control, $T_1$= Three fruits/cluster, $T_2$= Two fruits/cluster and $T_3$= One fruit/cluster. LSD values for anthocyanin = 0.02, Vitamin-C= 0.67 total phenolic contents = 2.81 and antioxidants = 1.32.

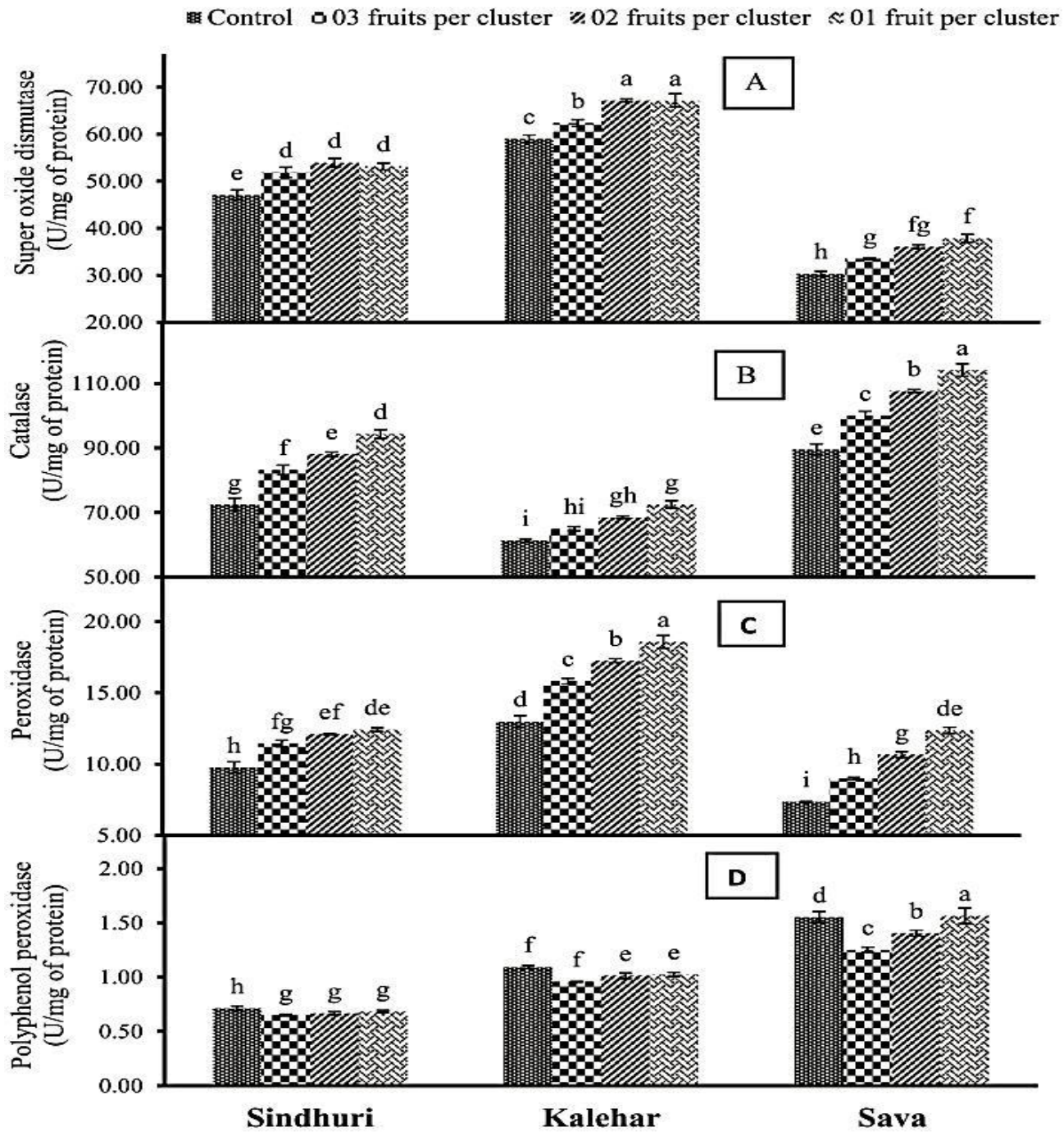


Fig. 4. Effect of fruit thinning on Super oxide dismutase (A), Catalase (B), Peroxidase (C) and Polyphenol peroxidase (D) on three cultivars of pomegranate. The vertical bars indicate ± standard error (S.E) of mean. Bars showing different letters are significantly different at $p \leq 0.05$. LSD values for SOD= 3.13 CAT= 4.28, POX= 0.84 and PPO= 0.06.

**Catalase (CAT), Superoxide dismutase (SOD), Peroxidase (POX) and Polyphenol oxidase (PPO):** The effect of fruit thinning on CAT, SOD, POX and PPO was significant ($p \leq 0.05$). The maximum value of CAT (112.14 U/mg of protein) by Sawa cultivar and SOD (65.68 U/mg of protein) from Kalehar cultivar while minimum value of PPO (1.14 U/mg of protein) by Sindhuri cultivar was found when applied $T_3$ (one fruit per cluster) compared to other treatments. Similarly, the maximum value of POX (17.57U/mg of protein) was observed from Kalehar under one fruit per cluster condition (Fig. 4). The maximum value of SOD (56.31U/mg of proteion), CAT (93.66U/mg of proteion) and POX (14.45 U/mg of proteion) while the lowest value of PPO (0.70U/mg of proteion) was obtained by $T_3$ (one fruit per cluster), regardless of cultivar effect (Table 3). Among cultivar response maximum values of SOD (53.85U/mg of proteion) CAT (88.04U/mg of proteion) and POX (13.34U/mg of proteion) attained from Sava cultivar followed by Kalehar nd Sindhuri cultiuvars. The highest value of PPO was found in the Sava, which was 3.08 and 2.48-fold higher in Sindhuri and Kalehar, respectively. The maximum value of POX was obtained from Kalehar, which was 1.77 and 3.03-fold higher than Sindhuri and Sava (Table 4).

**Discussion**

Sustainable fruit production relies on various factors such as environment, relative humidity, tree genotype, sunlight, soil texture, pruning, irrigation, nutritional management, and crop load (Ahmad *et al*., 2006). The tissues from developing fruits act like strong sinks for photosynthetic products, nutrients and water. The increasing trend in fruit weight, length, width, shape index, 100 arils weight, peel thickness, and juice percentage through thinning practices were observed during current study. The obtained results are aligned with Kahramanoglu *et al*., (2018) and Mohsen & Osman (2015). By reducing crop load, more assimilates share by remaining fruits that produce more giant cells, with improved weight and size (Mohsen & Osman, 2015). The fruits from light crop load have giant cells and have immense weight and size than heavy fruit settings (Kahramanoglu *et al*., 2018). Fruit firmness is a vital tool to improve shelf life. The improvement in fruit firmness is due to advancement in fruit maturity. Our results align with Milic *et al*., (2015), who reported that fruits from thinned trees had improved firmness than control. The increase in firmness may be due to more accumulation of carbon assimilates and advancement in maturity as reported in cherry (Milic *et al*., 2015). Our results related to fruit firmness are found contradictory (Whiting *et al*., 2004; Osborne & Robinson, 2008) who found that increased fruit thinning level decreases the fruit firmness. Improvements in TSS through thinning positively contribute to fruit quality with improved taste (Solomakhin & Blanke, 2010). The TSS, ascorbic acid, TA and anthocyanin play an essential role in fruit quality, consumer's acceptability, and fruit maturity as in various fruits like pomegranate (Mir *et al*., 2012). The maturity is determined by assessing skin colour, TSS, TA, and flavor of the fruit. The increase in TSS and other organic acids due to fruit thinning taste improved significantly (Al-Said *et al*., 2009). So, increasing the TSS and maturity index of thinned fruit substantially enhances the palatability (Opara *et al*., 2009; Bchir *et al*., 2012). The results obtained during our investigations related to TSS and TA are also in agreement with Al-Said *et al*., (2009), who found that the maximum amount of TSS were obtained from the highest level of thinning (one fruit per cluster), and the lowest value was recorded from control in pomegranate. The increased leaf to fruit ratio after fruit thinning, produced more photosynthetic materials that ultimately increased soluble solids (Fallahi *et al*., 2020). During the current study value of TA is deceased. The value of pH is improved significantly with increasing the fruit thinning level in pomegranate due to antagonistic interaction between TA and pH (Al-Said *et al*., 2009; Mohsen & Osman, 2015). The highest fruit maturity index (37.40) was recorded from $T_3$ during the current investigation that is in line with Mohsen & Osman (2015). The increase in pH juice could be due to the early maturity, and photosynthetic assimilates in the fruit and breakdown of various acids that reduced TA value and improved pH of juice (Fattahi *et al*., 2020).

Anthocyanin is a potent antioxidant found in various fruits, responsible for developing pinkish to reddish color in fruits (Tzulker *et al*., 2007). The maximum accumulation of photosynthates, more light penetration due to thinning and improved maturity index are directly linked with the conversion of chloroplast into chromoplast (Mohsen & Osman, 2015). Thinning trigger production of anthocyanin due to light penetration on fruits (Fallahi *et al*., 2006) and results obtained during our investigations are aligned with Jafari *et al*., (2014), who reported that maximum anthocyanin contents were received at the highest fruit thinning level. Vitamin-C acts as a potent source of antioxidants due to redox reactions activity. It is present abundantly in pomegranate fruit (Kulkarni & Aradhya, 2005). During the current study, the value of vitamin-C increased significantly in all cultivars. The maximum value was obtained from the highest thinning level compared to control, which are in line with Fattahi *et al*., (2020). The maximum accumulation of vitamin-C contents in fruits from thinned clusters may be due to earliness in maturity and ultimate production of TSS and organic acids (Riaz *et al*., 2015). Total phenolic content in fruits has important sensory attributes and potential health benefits (Radunic *et al*., 2015). Reducing astringency in fruit pomegranate fruit is desirable during the ripening process due to a decrease in TPC (Fawole & Opara, 2013; Mohsen & Osman, 2015). Several studies proved that TPC is directly linked with maturity index and its value is reduced when maturity index increases in pomegranate fruits (Fawole & Opara, 2013). Another reason for reduction in TPC may be tdue to oxidation of phenolic compounds by polyphenol oxidase that acts as a substrate for the browning process at theonset of maturity (Zarei *et al*., 2011). SOD being the first line of defense shields in plants cells against oxidative stresses, catalyzes the dismutation of superoxide into $H_2O_2$ to water and oxygen (Powers *et al*., 2008; Barros, 2011). It is well known that SOD converts superoxide anion ($O^{-2}$) to $H_2O_2$ which later on disputed to $O_2$ and water by CAT, and POX (Tian *et al*., 2010). Catalase (CAT) is a $H_2O_2$ oxidoreductase enzyme present in almost every living organism. It acts as a primary source of defense by either catalyze the decomposition of $H_2O_2$ to water and $O_2$ (Chelikani *et al*., 2004). As fruit thinning reduced the competition among remaining fruits by advancing the fruit maturity and maximum value of CAT, SOD and PPO is produced at the highest level of thinning due to advances in fruit maturity as reported earlier in mango (Razzaq *et al*., 2013). Another reason for increasing SOD, CAT and PPO by fruit thinning may be the maximum availability of photosynthates for remaining fruits. In contrast, PPO is increased due to earliness in maturity (Fallahi *et al*., 2020). In plant cells, PPO enzyme travels through the thylakoid membrane and acts upon the substrate in the surrounding cytoplasm, resulting in browning (Engelbrecht, 1982). During the current study the increase of PPO activity in fruits obtained from the highest level of fruit thinning associated with a proportionate reduction in TPC due to the enzymatic oxidation of phenolic compounds by PPO to the highly reactive compounds (o-quinones), leading to fruit browning (Richard & Gauillard, 1997).

## Conclusion

The results obtained from the current study showed that crop load or number of fruits on tree significantly influencefruit weight, Hundred ari weight, % of A-grade fruits, fruit maturity, TSS, AA and various antioxidants activity. On the other hand, it was found that one fruit per cluster ($T_3$) reduced yield upto 15% but % of A-grade fruits, fruit firmness and various quality attributes were improved in three cultivars under south Punjab conditions. These observations indicate that reducing crop load by thinning (one fruit per cluster) could be a viable strategy to improve fruit quality attributes in pomegranate fruit. Conclusively, one fruit per cluster ($T_3$) significantly improved physico and phytochemical attributes of fruit in pomegranate. Furthermore, the impact of fruit thinning at different stages of fruit setting and plant age can be evaluated to improve the quality and productivity of pomegranates.

## Acknowledgments

The authors gratefully acknowledge the financial assistance of Higher Education Commission of Pakistan for these studies under 5000-PhD Indigenous Fellowship and NRPU Project No. 10561 and MNSUAM central Lab (Post harvest lab).

### References

Ahmad, S., Z.A. Chatha, M.A. Nasir, A. Aziz, N.A. Virk and A.R. Khan. 2006. Effect of pruning on the yield and quality of Kinnow fruit. J. Agric. Soc. Sci. 2:51-53.

Al-Said, F.A., L.U. Opara and R.A. Al-Yahyai. 2009. Physico-chemical and textural quality attributes of pomegranate cultivars (*Punica granatum* L.) grown in the Sultanate of Oman. *J. Food Engin.*, 90: 129-134.

Anonymous. 2001. Regional report CWANA 1999-2000. *International Plant Genetic Resources Institute*, Rome, Italy, 20-28.

Anonymous. 2020. Fruit vegetables and condiments statistics 2018-19 - Ministry of National Food Security and Research Islamabad, Pakistan.

Barros, L., L. Cabrita, M.V. Boas, A.M. Carvalho and I.C. Ferreira. 2011. Chemical, biochemical and electrochemical assays to evaluate phytochemicals and antioxidant activity of wild plants. *Food Chem.*,127: 1600-1608.

Bchir, B., S. Besbes, R. Karoui, M. Paquot, H. Attia and C. Blecker. 2012. Osmotic dehydration kinetics of pomegranate seeds using date juice as an immersion solution base. *Food Bioproc. Technol.*, 5: 999-1009.

Chelikani, P., I. Fita and P.C. Loewen. 2004. Diversity of structures and properties among catalases. *Cell. Mol. Life Sci.*,61: 192-208.

Costa, G., M.M. Blanke and A. Widmer. 2013. Principles of thinning in fruit tree crops-needs and novelties. *EUFRIN Thinning Working Group Symposia*, 998. pp. 17-26.

Engelbrecht, A.H.P. 1982. Localization of polyphenoloxidase activity in avocado fruit. In: *10th International Congress on Electron Microscopy.Congress Proceedings*.3: 323-324.

Fallahi, E., B. Fallahi, R.E. Byers, R.C. Ebel, R.T. Boozer and J. Pitts. 2006. The influence of Tergitol-TMN-6 surfactant on blossom thinning of stone fruits. *Hort Sci.*, 41: 1243-1248.

Fallahi, E., W.M. Colt and M.M. Seyedbagheri. 1997. Influence of foliar application of nitrogen on tree growth, precocity, fruit quality, and leaf mineral nutrients in young 'Fuji'apple trees on three rootstocks. *J. Tree Fruit Prod.*, 2: 1-12.

Fattahi, E., A. Jafari and E. Fallahi. 2020. Hand Thinning Influence on Fruit Quality Attributes of Pomegranate (*Punica granatum* L. cv. 'MalaseYazdi'). *Int. J. Fruit Sci.*, 20: S377-S386.

Fawole, O.A. and U.L. Opara. 2013. Developmental changes in maturity indices of pomegranate fruit: A descriptive review. *ScientiaHorticulturae*, 159: 152-161.

Hehnen, D., I. Hanrahan, K. Lewis, J. McFerson and M. Blanke. 2012. Mechanical flower thinning improves fruit quality of apples and promotes consistent bearing. *Scientia Horticulturae*, 134: 241-244.

Hussain, S.B., M.A. Anjum, S. Hussain, S. Ejaz and H.M. Kamran. 2017. Agro-climatic conditions affect fruit quality of mandarin (*Citrus reticulata* Blanco) cultivars. *Fruits*, 72: 341-349.

Jafari, A., K. Arzani, E. Fallahi and M. Barzegar. 2014. Optimizing fruit yield, size, and quality attributes in "Malase Torshe Saveh" pomegranate through hand thinning. *J. Amer. Pomol. Soc.*, 68: 89-96.

Kahramanoglu, I., S. Usanmaz and T. Alas. 2018. Effects of fruit thinning on the quality and size of 'Wonderful' pomegranate. *Adv. Food Sci.*, 40: 114-119.

Kulkarni, A.P. and S.M. Aradhya. 2005. Chemical changes and antioxidant activity activity in pomegranate arils during fruit development. *Food chemistry*, 93: 319-324.

Link, H. 2000.Significance of flower and fruit thinning on fruit quality. *Plant growth Regul.*, 31: 17-26.

Mansouri, Y.S., J. Khazaei, S.R. Hassan Beygi and S.S. Mohtasebi. 2010. Statistical modeling of pomegranate (*Punica granatum* L.) fruit with some physical attributes. *J. Food Proc Technol.*, 1: 1-4.

Milic, B., Z. Keserović, M. Dorić, V. Ognjanov and N. Magazin. 2015. Fruit set and quality of self-fertile sweet cherries as affected by chemical flower thinning. *Hort. Sci.*, 42: 119-124.

Mir, M.M., I. Umar, S.A. Mir, M.U. Rehman, G.H. Rather and S.A. Banday. 2012. Quality evaluation of pomegranate crop-A review. *Int. J. Agri. Biol.*, 14(4):

Mohsen, F.S. and A. Osman. 2015. Improving physico-chemicalaspects of wonderful pomegranate fruits through fruit thinning. *Int. J. Curr. Res. Biosci. Plant Biol.*, 2: 95-103.

Mustafa, G., I.A. Rajwana, H.N. Faried, T.U. Haq and M.A. Bashir. 2023. The influence of pruning intensity on physical and biochemical fruit attributes of pomegranate. *Erwerbs-Obstbau*., 1-11.

Opara, L.U., M.R. Al-Ani and Y.S. Al-Shuaibi. 2009. Physico-chemical properties, vitamin-C content, and antimicrobial properties of pomegranate fruit (*Punica granatum* L.). *Food Bioproc. Technol.*, 2: 315-321.

Osborne, J.L. and T. Robinson. 2008. Chemical peach thinning: Understanding the relationship between crop load and crop value. *New York Fruit Quarterly* 16: 19-23.

Ozcan, M.M., F. Aljuhaimi, N. Uslu, I.A. Mohamed Ahmed, M.A. Osman, M.A. Gassem and H.A. Salih. 2019. Effect of oven drying on antioxidant activity, phenolic compounds, fatty acid composition and tocopherol contents of pomegranate aril and oils. *J. Food Proc. Preserv.*, 43(3): 13885.

Powers, T., T. Harris, R. Higgins, P. Mullin, L. Sutton and K. Powers. 2011. MOTUs, morphology, and biodiversity estimation: a case study using nematodes of the suborder Criconematina and a conserved 18S DNA barcode. *J. Nematol.*, 43: 35.

Radunić, M., M.J. Špika, S.G. Ban, J. Gadže, J.C. Díaz-Pérez and D. MacLean. 2015. Physical and chemical properties of pomegranate fruit accessions from Croatia. *Food Chem.*, 177: 53-60.

Razzaq, K., A.S. Khan, A.U. Malik and M. Shahid. 2013. Ripening period influences fruit softening and antioxidative system of 'Samar Bahisht Chaunsa' mango. *Sci. Hort.*, 160: 108-114.

Riaz, M., T. Zamir, N. Rashid, N. Jamil, S. Rizwan, Z. Masood, A. Mushtaq, H. Tareen, M. Khan and M. Ali. 2015. Comparative study of nutritional quality of orange (*Citrus sinensis*) at different maturity stages in relation to significance for human health. *Amer-Eurasian J. Toxicol. Sci.*, 7: 209-213.

Richard-Forget, F.C. and F.A. Gauillard. 1997. Oxidation of chlorogenic acid, catechins, and 4-methylcatechol in model solutions by combinations of pear (*Pyrus communis* cv. Williams) polyphenol oxidase and peroxidase: a possible involvement of peroxidase in enzymatic browning. *J. Agri. Food Chem*., 45: 2472-2476.

Rodrigues, J., D. Inzé, H. Nelissen and N.J. Saibo. 2019. Source–sink regulation in crops under water deficit. Trends in plant science 24: 652-663.

Seehuber, C., L. Damerow and M.M. Blanke. 2012. Concepts of selective mechanical thinning in fruit tree crops. EUFRIN Thinning Working Group Symposia, 998: 77-83.

Solomakhin, A.A. and M.M. Blanke. 2010. Mechanical flower thinning improves the fruit quality of apples. *J. Sci. Food Agri*., 90: 735-741.

Steel, R.G.D., J.H. Torrie and D.A. Dickey. 1996. Principles and Procedures of Statistics: A Biometrical Approach, Subsequent edition. McGraw-Hill College, New York.

Tian, L., E. Dell and W. Shi. 2010. Chemical composition of dissolved organic matter in agroecosystems: Correlations with soil enzyme activity and carbon and nitrogen mineralization. *App. Soil Ecol*., 46: 426-435.

Türk, G., M. Sönmez, M. Aydin, A. Yüce, S. Gür, M. Yüksel, E.H. Aksu and H. Aksoy. 2008. Effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats. *Clinical Nutrition*, 27: 289-296.

Tzulker, R., I. Glazer, I. Bar-Ilan, D. Holland, M. Aviram and R. Amir. 2007. Antioxidant activity, polyphenol content, and related compounds in different fruit juices and homogenates prepared from 29 different pomegranate accessions. *J. Agri. Food Chem*., 55: 9559-9570.

Varasteh, F., K. Arzani, M. Barzegar and Z. Zamani. 2012. Changes in anthocyanins in arils of chitosan-coated pomegranate (*Punica granatum* L. cv. Rabbab-e-Neyriz) fruit during cold storage. *Food Chem*., 130: 267-272.

Vasanthakumar, A., I. DelaliberaJr, J. Handelsman, K.D. Klepzig, P.D. Schloss and K.F. Raffa. 2006. Characterization of gut-associated bacteria in larvae and adults of the southern pine beetle, Dendroctonusfrontalis Zimmermann. *Environ. Entomol*., 35: 1710-1717.

Webster, A.D. and J.E. Spencer. 2000. Fruit thinning plums and apricots. *Plant Growth Regul*., 31: 101-112.

Whiting, M.D. and G.A. Lang. 2004. Bing'sweet cherry on the dwarfing rootstock Gisela 5': thinning affects fruit quality and vegetative growth but not net $CO_2$ exchange. *J. Amer. Soc. Hort. Sci*., 129: 407-415.

Wünsche, J.N., J.W. Palmer and D.H. Greer. 2000. Effects of crop load on fruiting and gas-exchange characteristics of Braeburn'/M. 26 Apple Trees at Full Canopy. *J. Amer. Soc. Hort. Sci*., 125: 93-99.

Zarei, M., M. Azizi and Z. Bashir-Sadr. 2011. Evaluation of physicochemical characteristics of pomegranate (*Punica granatum* L.) fruit during ripening. *Fruits*, 66: 121-129.

Zheng, Y., S.Y. Wang, C.Y. Wang and W. Zheng. 2007. Changes in strawberry phenolics, anthocyanins, and antioxidant capacity in response to high oxygen treatments. *LWT-Food Sci. Technol*., 40: 49-57.

(Received for publication 21 June 2022)