PHYTOCHEMICAL PROFILE, ANTIOXIDANT POTENTIAL AND EXPRESSION OF *FLS*, *GGP* GENES IN KIWIFRUIT AT DIFFERENT HARVESTING STAGES AND DURATIONS OF STORAGE

MARIYAM, B.[4] – KHAN, N.[1] – ABBASI, A. M.[2] – AZEEM, M.[3] – KHAN, S. A.[4*] – ISMAIL, A. M.[5,6*] – ELMENOFY, W.[5] – EL-BELTAGI, H. S.[7] – EL-MOGY, M. M.[5] – ALMAGHASLA, M. I.[5]

[1]*PARC-National Tea & High Value Crops Research Institute Shinkiari, Mansehra, Khyber Pakhtunkhwa, Pakistan*

[2]*Department of Environmental Sciences, COMSATS University Islamabad - Abbottabad Campus, 22060 Abbottabad, Pakistan*

[3]*Department of Chemistry, COMSATS University Islamabad - Abbottabad Campus, 22060 Abbottabad, Pakistan*

[4]*Department of Biotechnology, COMSATS University Islamabad - Abbottabad Campus, 22060 Abbottabad, Pakistan*

[5]*Department of Arid Land Agriculture, College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa 31982, Saudi Arabia*

[6]*Pests and Plant Diseases Unit, College of Agricultural and Food Sciences, King Faisal University, P.O. Box 420, Al-Ahsa 31982, Saudi Arabia*

[7]*Agricultural Biotechnology Department, College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa 31982, Saudi Arabia*

**Corresponding authors*
e-mail: amismail@kfu.edu.sa, sabaz@cuiatd.edu.pk

(Received 28th Jul 2024; accepted 9th Dec 2024)

Abstract. Kiwifruit is recognized as a valuable source of antioxidants, flavonoids, and phenolic compounds, all of which contribute to its health-promoting properties. This study aimed to identify the optimal harvest stage for maximizing beneficial metabolites by quantifying these compounds and analyzing their gene expression profiles. Kiwifruit samples were collected at seven distinct harvesting stages. Total phenolic content (TPC) and total flavonoid content (TFC) were measured, and antioxidant capacity was assessed using HPLC-UV. Expression analysis of *flavanol synthase* (*FLS*) and *GDP-galactose phosphorylase* (*GGP*) genes was performed via RT-PCR. The results showed that TPC ranged from 0.4 to 1.3 mg of gallic acid equivalents/100 g FW, while TFC ranged from 1.1 to 2.4 mg of quercetin equivalents/100 g FW. Antioxidant activity followed a pattern similar to TPC, peaking at the early harvest stages and declining as maturity progressed. Predominant phenolic compounds included ferulic acid, chlorogenic acid, vanillic acid, vanillin, and p-coumaric acid, while catechin and epicatechin were the primary flavonoids. Quercetin and rutin were detected in smaller quantities in some samples. The *FLS* gene exhibited increased expression during later harvest stages, whereas expression of *GGP* gene remained consistently high throughout the different harvesting stages. Immature kiwifruit displayed higher TPC and stronger antioxidant activity, while mature fruit showed increased TFC. These findings provide valuable insights into the metabolic changes and gene expression patterns associated with kiwifruit quality at different harvest stages, offering a foundation for future research and applications in agriculture and food sciences.
Keywords: *flavonoids, gene expression, harvesting stages, kiwifruit, maturity, phenolic compounds*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_14011418

Introduction

Fruits and vegetables are rich sources of various biologically active compounds beneficial to human health. They contain high concentration of minerals, dietary fibers, vitamins, microelements and other metabolites with antioxidant properties (Slavin and Lloyd, 2012). Among these, kiwifruit (*Actinidia deliciosa*), belonging to the family Actinidiaceae and genus *Actinidia*, has gained attention due to its high nutrient content and potential health benefits. It is native to China, where it is known as "Yang-tao." Kiwifruit was initially called as "Chinese gooseberry" when it was first introduced into New Zealand gardens in 1900s; later, it was renamed as "kiwifruit" by Frieda Caplan (Ward and Courtney, 2013). Kiwifruit developed into a new fruit crop in New Zealand and is now commercially produced in many countries, including the United States of America, Italy, Chile, France, Greece, and Japan (Ward and Courtney, 2013). In the past 30 years, kiwifruit has grown from being a small crop in one country to becoming an important commercial crop cultivated in various regions of the world (Ward and Courtney, 2013).

There are various cultivars of kiwifruit that are commercially grown such as, *A. chinensis* var. chinesis (golden kiwifruit), *A. chinesis* var. deliciosa or *A. deliciosa* (fuzzy kiwifruit or Hayward cultivar), and *A. eriantha* (velvet vine) which are found only in China. In addition, *A. arguta* (baby kiwifruit) and *A. kolomikta* (Artic kiwifruit) are found in China, Japan, Korea and Russia (Guroo et al., 2017). Among the different species, *A. deliciosa* and *A. chinensis* hold high commercial value (Zhang et al., 2010) due to their high vitamin C (ascorbic acid) content, large fruit size, unique aroma, prolonged shelf life and flavor (Chen et al., 2013; Nishiyama, 2007).

The regular consumption of kiwifruit has been linked to reduced risks of chronic diseases, including cardiovascular disorders and digestive issues (Richardson et al., 2018). Additionally, some studies suggest that kiwifruit may be effective in lowering the cancer development and controlling its progression (Lippi and Mattiuzzi, 2019; Nishiyama, 2007). Kiwifruit is often referred to as "the king of fruits" due to its high ascorbic acid content (Huang et al., 2013). It is a nutrient rich fruit, well-balanced with various minerals, dietary fiber, elements like sodium, potassium, calcium, phosphorous, magnesium, nitrogen, and other health-promoting/beneficial secondary metabolites (Park et al., 2013; Pilkington et al., 2012). Kiwifruit contains a high amount of vitamin C content- 50% more than an orange, five times more than a banana, and ten times as much as in apple (Huang and Ferguson, 2007). It has higher vitamin C content than strawberries, lemons and grapefruits (Du et al., 2009; Landi et al., 2014).

Polyphenols are secondary metabolites in plants that, while not directly involved in basic biological functions like respiration, growth, and cell division, play an important role in plant protection and other traits (Khalifa et al., 2018). To date, thousands of polyphenols have been identified, including tannins, phenolic acids, coumarins, flavonoids, and stilbenes, with flavonoids representing the largest group, comprising of over 8150 compounds (Belščak-Cvitanović et al., 2018). Flavonoids, the most diversified phenolic group contain a C_6-C_3-C_6 skeleton. In *Actinidia* species, flavonols, flavan-3-ols, and anthocyanins have been detected. kiwifruit juice contains flavonols (quercetin and kaempferol glycosides) and flavan-3-ols (catechin and epicatechin) (Drummond, 2013).

In the flavonoids biosynthetic pathway, the phenylalanine is converted to naringenin which acts as a precursor molecule for the dihydroflavonols biosynthesis. Dihydroflavonols can follow two pathways; in one, Flavonol Synthase (*FLS*) acts on it

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_14011418

and synthesize flavonols. *FLS* is a key gene involved in the synthesis of quercetin and kaempferol (Crozier et al., 2009).

For the ascorbic acid, (AsA or vitamin C) biosynthesis, four pathways have been proposed; L-gulcose, D-mannose/L-galactose, D-glucoronic, and D-galacturonate pathways (Bulley and Liang, 2016). The L-galactose pathway is particularly notable and well documented in higher plants, including *Actinidia* species (Bulley and Liang, 2016; Zhang et al., 2018). Various studies have shown that *GGP* (GDP-L-galactose phosphorylase) is a prime modulator in the AsA biosynthesis in different plants (Liu et al., 2015; Suekawa et al., 2019; Shiri et al., 2018; Ishikawa et al., 2018). Overexpression of *GGP* was found to be directly correlated with increased AsA biosynthesis (Zhang et al., 2018).

The physicochemical properties of kiwifruit are influenced by various factors such as genotype, harvesting time, and environmental conditions, including climate, soil composition, and temperature. Post-harvest storage also significantly affects the fruit's metabolic profile, impacting nutrient retention and antioxidant capacity (Du et al., 2009; Landi et al., 2014). Previous studies have shown the benefits of kiwifruit consumption due to its high vitamin C and polyphenol levels, but research on how different maturity stages and storage durations alter these metabolites remains limited. Specifically, there is a need to understand the expression of key genes like *FLS* and *GGP*, which are essential in the biosynthesis of flavonoids and ascorbic acid, respectively, to optimize both nutritional quality and shelf life (Bulley and Liang, 2016).

Knowledge gap and aim of the study

While kiwifruit is well recognized for its health-promoting compounds, there is limited knowledge on the metabolic and genetic changes that occur during different harvesting stages and extended storage periods. Existing literature lacks how these factors impact key bioactive compounds and gene expression, leaving gaps in our understanding of how to better manage kiwifruit harvest and storage for enhanced health benefits and commercial viability. Thus, the present study aims to (i) assess changes in phenolic, flavonoid, and antioxidant profiles across different maturity stages, (ii) quantify these changes over various storage durations, and (iii) analyze the expression of *FLS* and *GGP* genes at different harvesting times. This research will provide valuable insights for optimizing kiwifruit harvesting and storage practices to maintain nutritional and functional qualities.

Materials and methods

Harvesting of kiwifruits

Fruits of *A. deliciosa* variety, grown at the National Tea and High Value Crops Research Institute (NTHRI) Shinkiari, Mansehra Khyber Pakhtunkhwa, Pakistan, were harvested during the months of October-November. The plant went through various developmental stages, budding occurred in the month of March, flowering in the month of May and fruit ripening in the months of October and November. For the research purposes seven harvests (H1, H2, H3, H4, H5, H6 and H7) were done with the gap of 10 days. The fruits were brought to the laboratory using storage boxes, where after each harvest the fruits were further divided into 5 samples and each was kept at room

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_14011418

temperature for 10, 20 30 and 40 days (D0, D10, D20, D30, D40) respectively and further studies were conducted on these samples.

Extraction of phenolics and flavonoids from kiwifruits

Phenolics and flavonoids were measured at each harvest and after respective storage days 10, 20, 30 and 40. Extracts were prepared by following the procedure of Ma et al. (2017) with some modifications. For this purpose, 2 g of fresh fruit pulp was ground using pestle and mortar in 10 ml of absolute methanol. The samples were centrifuged at 5,000 rpm for 15 min and supernatant was transferred to plastic bottle. The residues were re-extracted in 10 ml of methanol and supernatants were collected and pooled together. The procedure was repeated until all the residues appeared colorless and the extracts were stored at -20°C until further use.

Determination of total phenolic content (TPC)

For quantification and measurement of the total phenolic content present in the kiwifruit extracts, Du et al. (2009) method with slight modifications was adopted. For this purpose, different concentrations of gallic acid solution i.e., 5, 10, 20, 40, 60, 80, and 100 µg/mL were used in order to make standard curve. 1 ml of each extract or gallic acid standard solution was mixed with 15% Folin -Ciocalteu reagent and the mixture was incubated for about 5 min at room temperature. After that 2 ml of 6% Na_2CO_3 solution was added and the mixtures were incubated for 90 min in the dark. The absorbance was measured at 760 nm on double beam UV/VIS spectrophotometer (PG T80, PG Instruments Limited, United Kingdom). For each extract, three replicates were employed, and the results were calculated and expressed as mg GAE/100 g FW i.e., mg gallic acid equivalent per 100 gram of sample fresh weight.

Estimation of total flavonoids content (TFC)

For the total flavonoids content estimation, the procedure of Abbasi et al. (2015) was followed with slight modifications. Different concentrations of quercetin solution i.e., 5, 10, 20, 40, 60, 80, and 100 µg/mL were used in order to make standard curve. 1 ml of each extract was mixed with the 0.5 ml of 10% solution of hydrated aluminium trichloride ($AlCl_3.6H_2O$) and was incubated for 5 min at room temperature. In the mixture, 0.5 ml of 5% $NaNO_2$ and 2 ml of NaOH solution (1 M) were added and mixed thoroughly. The absorbance of the mixtures was measured at 510 nm on double beam UV-Visible spectrophotometer (PG T80 UV/VIS spectrophotometer). For each sample, three replicates were taken, and the results were expressed as mg QE/100 g FW i.e., milligram quercetin equivalent per 100 g of sample fresh weight.

Free radical scavenging activity (DPPH)

The ability of kiwifruit to scavenge DPPH free radicals was determined using Abbasi et al. (2015) protocol with slight modifications. Briefly, 2 ml of previously prepared extract was added to 5 ml of 0.1 mM solution of DPPH in methanol. A negative control (blank sample) was also used that had the same volume of methanol (solvent) instead of extract in order to see the effect of dilution on the absorbance of DPPH solution. The mixture was incubated after placing it in dark at room temperature and the absorbance was measured at 510 nm in order to determine the remaining concentration of DPPH.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_14011418

Phytochemical profiling using HPLC analysis

The phytochemical profiling of kiwifruit was carried out through HPLC-UV by adopting method of Saeed et al. (2021). Briefly, fresh kiwifruit pulp was ground and phenolics were extracted from 1.7 g sample in 500 µl of HPLC graded methanol by an ultrasonic bath at 25°C for 30-45 min. The extract was centrifuged at 12,000 × *g* for 15 min and the supernatant was recovered followed by filtration through 0.2 µm polyamide membrane syringe filter and stored at -20°C till used for HPLC analysis. The internal standard, gallic acid solution was mixed with the extracted samples prior to HPLC analysis. HPLC analysis was performed on a Perkin Elmer Series 200 system that included a vacuum 200 degasser, a 200 series pump, an auto-sampler and a sensitive series 200 UV/Vis detector. The separation of different secondary metabolites was achieved by reversed-phase column (C_{18}, 5 µm particle size, 250 mm length × 4.6 mm ID, Supelco USA). The data were acquired and analyzed using PerkinElmer®'s TotalChrom® Chromatography Data System (CDS) software.

The mobile phase comprised of 0.1% aqueous formic acid solution used as solvent A and methanol with 0.1% formic acid used as solvent B. For the concurrent detection of phenolics and flavonoids in different kiwifruit samples, a gradient system of mobile phase was used according to the following program: initially solvent B (10%) was run isocratically for 6 min, then the proportion of B in the mobile phase was increased from 10% to 60% in next 30 min in the form of 10% increment lasted for 6 min. Finally, in order to clean the column, the 90% B solution was run for 3 min followed by 10% B for 6 min to bring the column to initial conditions. The injection volume was 20 µl and the flow rate was 1 ml/min and data were acquired at 280 nm throughout the analysis. The identification of separated compounds was made by comparing relative retention time to that of standard compounds analyzed on the same parameters used for the separation of samples. The quantification of each compound was done with respect to the peak area measurements relative to internal standard gallic acid.

Gene expression analyses

Total RNA extraction was extracted through CTAB method (Suzuki et al., 2008) and it was quantified through spectrophotometer. 2 µg of total RNA was used to synthesize cDNA using RevertAid First Strand cDNA Synthesis Kit. Specific primers were designed for two genes i.e., Flavonol Synthase (*FLS*) and GDP-L-galactosephosphorylase (*GGP*). The sequences were obtained from Kiwifruit Genome Database (http://bioinfo.bti.cornell.edu/cgi-bin/kiwi/home.cgi). The FASTA format or accession number of respective genes were used to design primers using Primer3 software http://bioinfo.ut.ee/primer3/. In present study the *FLS* and *GGP* genes were optimized and their expressions were checked in the seven harvesting stages (H1, H2, H3, H4, H5, H6 and H7) through RT-PCR. Actin was used as a control or housekeeping gene. The sequences of genes primers are listed in *Table 1*.

Table 1. *Forward and reverse primer sequences of genes GDP-L-galactosephosphorylase (GGP) and flavonol synthase (FLS)*

Gene name	Forward sequence	Reverse sequence	Product size (bp)
GGP	5' CTCTTCACATGGCAGCAGAA 3'	5' TTTCCACCCTCGAAAACAAG 3'	226
FLS	5' CTTTGAGCTCCCACAAGAGG 3'	5' TGCAGCCACTTTGCATACTC 3'	225

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_14011418

Gel electrophoresis was performed for the quantification of PCR products. For this purpose, the PCR products of each gene were analyzed by making 2% agarose gel. A total of 6 µl of PCR product was loaded onto gel. Along with the samples a 100 bp marker was also run-in order to measure the band size of each gene. Gene expression was only studied on freshly freeze-dried samples and not on samples stored at room temperatures which were used for other analysis.

Statistical analysis

All the experimental results were means ± SD of three parallel measurements. The SPSS software 20.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. One-way ANOVA followed by Tukey test was applied to find significant difference among various treatments at $p \leq 0.05$.

Results

Total phenolics (TPC) in kiwifruits

TPC was significantly higher ($p < 0.05$) at the start of fruit development, decreasing significantly in the later stages as the fruit matured (*Fig. 1*). During initial stages the effect of storage was low compared to later stages. The range of TPC across all samples was 0.34-1.30 mg GAE/100 g FW, with the highest value in early-stage and storage (H1, D0) samples and the lowest in late-stage, long-stored (H7, D40) samples (*Fig. 1*).

Early stages (H1–H3)

The initial stages (H1 and H2) demonstrated relatively higher TPC values, with H1, D0 and H2, D0 showing 1.30 and 1.25 mg GAE/100 g FW, respectively. These values decrease moderately with each subsequent storage day (D10, D20, etc.) within the same maturity stage. For example, H1 shows a drop from 1.302 mg GAE/100 g FW on D0 to 1.167 mg GAE/100 g FW on D40.

Mid stages (H4–H5)

There is a noticeable decline in TPC in the mid-maturity stages. H4, D0 starts with a TPC of 1.174 mg GAE/100 g FW, but by D40, it has decreased significantly to 0.793 mg GAE/100 g FW. Similarly, H5, D0 begins at 0.842 mg GAE/100 g FW and drops to 0.558 mg GAE/100 g FW by D40.

Late stages (H6–H7)

The later stages, H6 and H7, showed even lower initial TPC values. H6, D0 starts with 0.618 mg GAE/100 g FW, decreasing to 0.391 mg GAE/100 g FW by D40. H7 samples, representing the most mature stage, had the lowest TPC across all storage periods, with H7, D0 at 0.467 mg GAE/100 g FW and declining to 0.339 mg GAE/100 g FW by D40.

When the kiwifruits were stored for 10, 20, 30 and 40 days at room temperature (15-20°C), a significant impact on their TPC was observed. TPC did not decrease during the first 20 days of storage, however, the content decreased greatly afterwards.

TPC was significantly higher ($p < 0.05$) at the start of fruit development, decreasing significantly in the later stages as the fruit matured (*Fig. 1*). During initial stages the

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_14011418

effect of storage was low compared to later stages. The range of TPC across all samples was 0.34-1.30 mg GAE/100 g FW, with the highest value in early-stage and storage (H1, D0) samples and the lowest in late-stage, long-stored (H7, D40) samples (*Fig. 1*).

The analysis of TPC across all harvesting stages (H1 to H7) and storage days (D0 to D40) revealed significant patterns. At early stages H1, H2, and H3, TPC showed no significant differences during storage ($p > 0.05$), with values remaining relatively stable up to 40 days. For H4 and H5, a noticeable decline in TPC was observed over the storage period. For example, TPC in H4 decreased from 1.17 mg GAE/100 g FW (D0) to 0.79 mg GAE/100 g FW (D40), while H5 values dropped from 0.84 mg GAE/100 g FW (D0) to 0.56 mg GAE/100 g FW (D40). Similarly, at later stages H6 and H7, TPC was inherently lower at harvest and exhibited a more pronounced decline with prolonged storage. H6 showed a reduction from 0.62 mg GAE/100 g FW (D0) to 0.39 mg GAE/100 g FW (D40), while H7 values fell from 0.47 mg GAE/100 g FW (D0) to 0.33 mg GAE/100 g FW (D40). TPC values significantly decreased with prolonged storage ($p < 0.05$), suggesting greater sensitivity to storage duration in later harvests.

This observed stability in TPC during the initial storage period, followed by a marked decrease, could be due to the phenolic compounds initially resisting degradation due to the fruit's natural protective mechanisms. As storage time progresses beyond 20 days, these compounds may be increasingly susceptible to oxidative degradation, enzymatic reactions, or other biochemical processes that reduce their levels, especially at room temperature (15-20°C) as observed in other studies on phenolic stability in fruits (Slavin and Lloyd, 2012; Bulley and Liang, 2016).

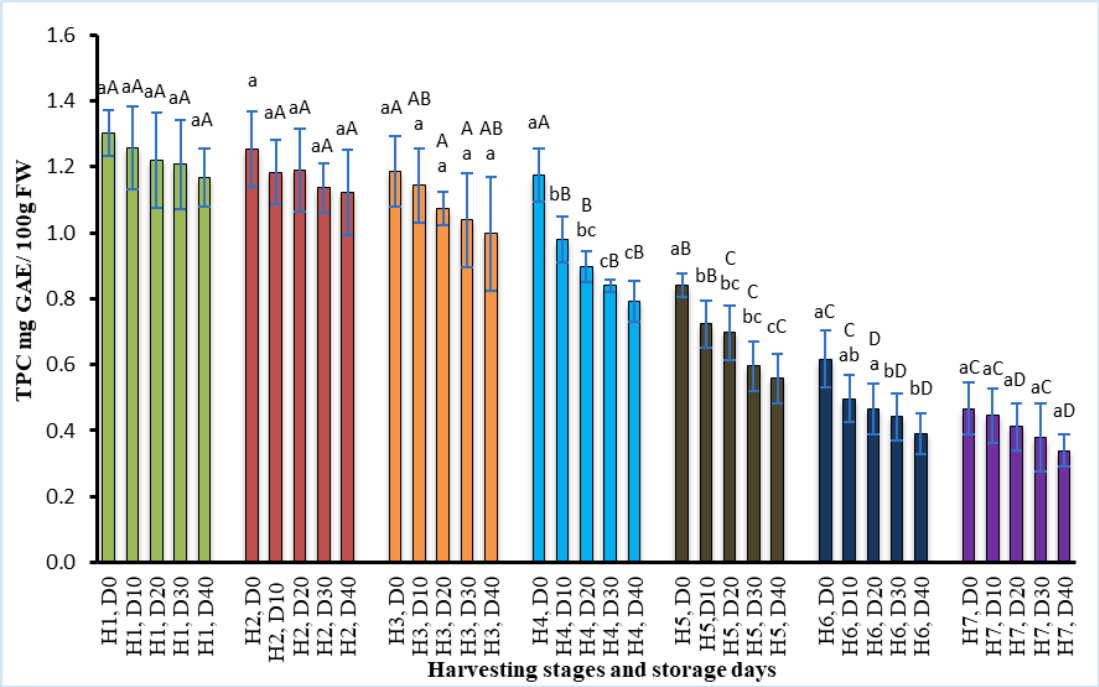

Figure 1. *The total phenolic content (TPC) levels in methanol extracts of kiwifruit at different harvesting stages and storage periods. H = Harvest stages, D = Days of storage. Different lower-case letters on columns indicate statistically significant difference among different storage times of same harvesting independently (ANOVA post-hoc Tukey test, $p < 0.05$). Different higher case letters on columns indicate significant difference among different harvestings at same days of storage independently (ANOVA post-hoc Tukey test, $p < 0.05$). Error bars indicate standard deviation replicates n = 3*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_14011418

Total flavonoids (TFC) in kiwifruits

The TFC trend was opposite to that of TPC, increasing as the fruit matured. TFC values ranged from 1.14-2.52 mg QE/100 g FW, with the highest level found in late-stage, 40-day-stored samples (H7, D40) (*Fig. 2*).

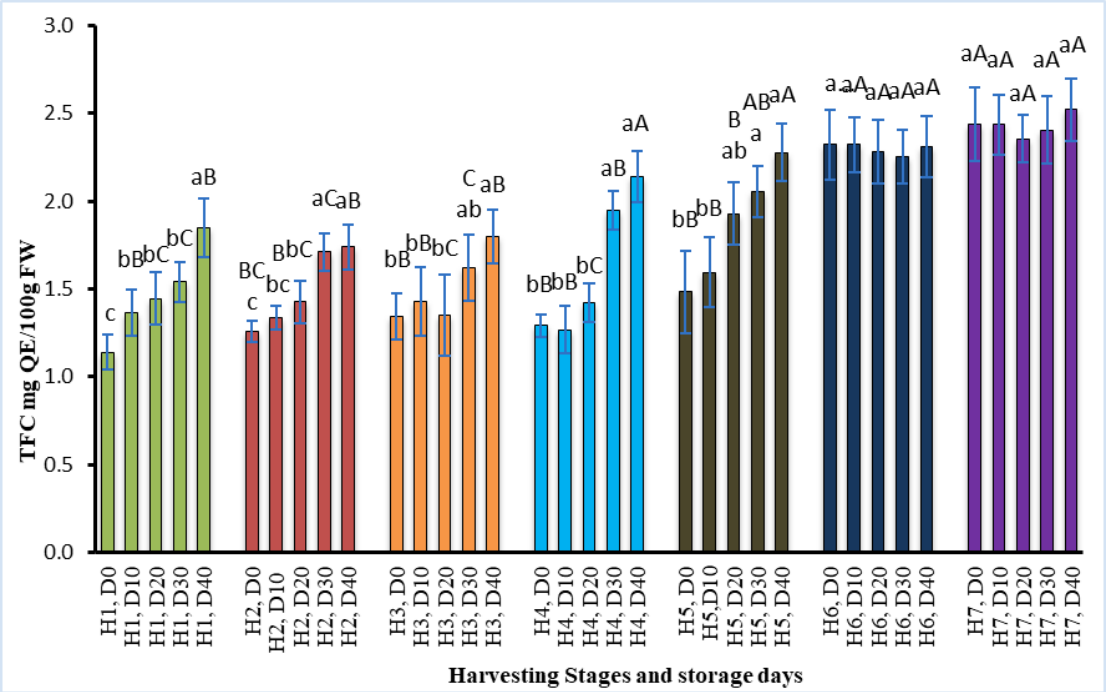

Figure 2. *The total flavonoids content (TFC) levels in methanol extracts of kiwifruit at different harvesting stages and storage periods. H = Harvest stages, D = Days of storage. Different lower-case letters on columns indicate statistically significant difference among different storage times of same harvesting independently (ANOVA post-hoc Tukey test, $p < 0.05$). Different higher case letters on columns indicate significant difference among different harvestings but same storage time independently (ANOVA post-hoc Tukey test, $p < 0.05$). Error bars indicate standard deviation of replicates n = 3*

Free radical scavenging activity (DPPH)

In order to find the free radical scavenging activity, the DPPH assay was performed that showed a similar trend to that of the quantification of TPC in all the fruit harvests and the sample storage periods. The antioxidant capacity was higher during the initial harvesting stages, however, the tendency decreased as the fruit matured. The highest scavenging activity was observed at H1 with value 80.011 ± 2.01% (*Fig. 3*). The antioxidant activity showed significant differences across harvesting stages and storage durations ($p < 0.05$). In the early harvesting stages, H1, D0 had the highest scavenging activity (80.01 ± 2.01%), which gradually declined to 67.30 ± 1.78% at H1, D40 ($p < 0.05$). Similarly, H2, D0 started at 78.14 ± 1.90% and decreased significantly to 63.15 ± 1.68% by D40 ($p < 0.05$). These results highlight the strong antioxidant potential of fruits in early stages, likely due to higher phenolic content and stability of antioxidant compounds. In mid-harvesting stages, reductions in scavenging activity during storage were also significant ($p < 0.05$). For example, H4, D0 (62.50 ± 1.88%) decreased to 45.25 ± 1.55% by H4, D40. Similarly, H5, D0 (50.11 ± 1.67%) declined to

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_14011418

37.09 ± 1.44% by D40 ($p < 0.05$), indicating diminished antioxidant capacity with storage. For the later harvesting stages, variations in scavenging activity between initial and intermediate storage periods were not statistically significant ($p > 0.05$). For instance, H6, D0 (30.33 ± 1.55%) and H6, D10 (28.80 ± 1.42%) showed no significant difference ($p > 0.05$), but activity significantly reduced to 20.44 ± 1.20% by H6, D40 ($p < 0.05$). Similarly, H7, D0 (24.09 ± 1.30%) and H7, D10 (22.80 ± 1.25%) had no significant difference ($p > 0.05$), but by H7, D40, activity declined significantly to 15.10 ± 1.12% ($p < 0.05$). These findings indicate a clear reduction in antioxidant activity with fruit maturation and extended storage, especially for early and mid-harvesting stages, whereas late-stage fruits exhibited consistently lower antioxidant levels.

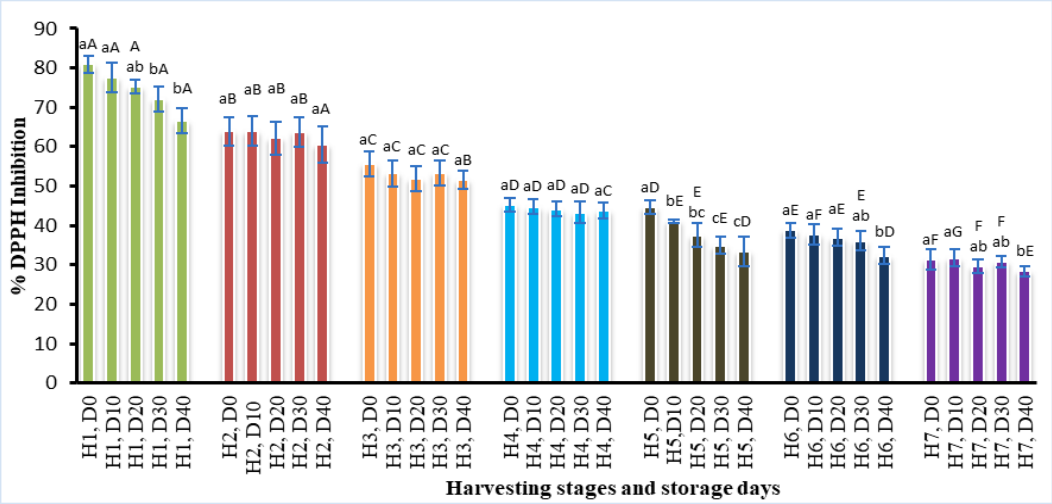

Figure 3. *Free radical scavenging activity of methanolic extracts of kiwifruit at different harvesting stages and storage periods. Different lower-case letters on columns indicate statistically significant difference among different storage times of same harvesting independently (ANOVA post-hoc Tukey test, $p < 0.05$). Different higher case letters on columns indicate significant difference among different harvestings after same days of storage independently (ANOVA post-hoc Tukey test, $p < 0.05$). Error bars indicate standard deviation of replicates n = 3*

Phytochemical profiling using HPLC analysis

The concentration of 12 different types of phenolic compounds in kiwifruit at different harvesting stages and storage durations are summarized in *Table 2*. The chromatograms of a standards mixture (*Fig. 4a*) and a sample extract (*Fig. 4b*) illustrate the presence and distribution of these compounds. As shown in *Figure 4b*, not all phenolic compounds were consistently detected across all samples, with some compounds only appearing at specific harvesting or storage stages. For example, CA was detected only in few samples with the highest value of 2.371 µg/g FW in H5 on day 20 (D20) (*Table 2*). Chlorogenic acid (CHA) was similarly limited in occurrence, reaching its peak concentration of 0.334 µg/g FW in H6 sample at D40. Ferulic acid (FA) though present at all harvesting stages, was undetectable or found in trace amounts after 30 or 40 days of storage, with its highest concentration of 0.179 µg/g FW in the H3 sample at D0. p-Coumaric acid was primarily detected in initial harvesting samples,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_14011418

peaking at 0.293 µg/g FW in the H4 sample at D0. Vanillic acid reached its highest level of 4.720 µg/g FW in the H7 sample at D40, while vanillin, found only in some extracts, reached a maximum of 1.162 µg/g FW in the H6 sample at D0. These data underscore the variation in phenolic compound levels across different stages of harvest and storage.

Table 2. *Levels of phenolic compounds present in methanolic extract of kiwifruit at different harvesting stages and periods*

Sample/ compounds	CA	EPi-CAT	QE	RU	GA	CA	CHA	FA	p-CA	SA	VA	VN
H1, D0	0.432	0.026	0.000	0.000	14.706	0.000	0.153	0.144	0.186	0.023	0.174	0.000
H1, D10	1.966	0.000	0.000	0.000	14.706	0.000	0.302	0.080	0.046	0.042	0.580	0.000
H1, D20	1.274	0.000	0.000	0.000	14.706	0.000	0.944	0.027	0.000	0.000	0.000	0.000
H1, D30	3.735	0.000	0.000	0.000	14.706	0.000	1.270	0.000	0.000	0.000	0.000	0.287
H1, D40	4.035	0.086	0.000	0.000	14.706	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H2, D0	0.110	0.000	0.000	0.000	14.706	0.000	0.000	0.083	0.172	0.000	0.000	0.224
H2, D10	0.296	0.000	0.000	0.000	14.706	0.000	1.818	0.058	0.110	1.341	0.119	0.000
H2, D20	0.077	0.000	0.000	0.000	14.706	0.000	1.564	0.014	0.000	0.000	1.471	0.000
H2, D30	0.865	0.000	0.000	0.000	14.706	0.000	0.935	0.055	0.063	0.000	0.000	0.000
H2, D40	0.072	0.044	0.000	0.000	14.706	0.000	0.000	0.061	0.038	1.230	0.988	0.000
H3, D0	0.178	0.047	0.096	0.085	14.706	0.000	0.107	0.179	0.263	0.000	0.508	0.039
H3, D10	0.191	0.000	0.000	0.043	14.706	1.172	0.000	0.081	0.000	0.000	0.000	0.170
H3, D20	2.514	0.000	0.082	0.000	14.706	0.937	0.000	0.019	0.000	0.000	0.000	0.000
H3, D30	2.075	0.015	0.000	0.061	14.706	0.000	0.000	0.015	0.000	0.000	0.000	0.000
H3, D40	2.428	0.068	0.208	0.032	14.706	0.000	0.000	0.017	0.090	0.000	0.000	0.102
H4, D0	0.196	0.000	0.000	0.172	14.706	0.125	0.381	0.041	0.293	0.000	0.321	0.000
H4, D10	0.181	0.000	0.000	0.000	14.706	0.000	0.000	0.025	0.069	0.000	0.142	0.000
H4, D20	0.184	0.000	0.000	0.000	14.706	0.000	0.000	0.000	0.057	0.000	0.000	0.000
H4, D30	1.953	0.017	0.000	0.000	14.706	0.000	0.000	0.028	0.038	0.000	0.000	0.000
H4, D40	3.666	0.088	0.000	0.000	14.706	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H5, D0	3.361	0.366	0.000	0.000	14.706	0.000	0.000	0.037	0.000	0.126	0.000	0.000
H5, D10	0.178	0.111	0.000	0.000	14.706	0.465	0.000	0.000	0.133	0.000	0.000	0.103
H5, D20	2.025	0.134	0.000	0.000	14.706	2.371	0.000	0.071	0.000	0.000	0.000	0.000
H5, D30	0.000	0.000	0.052	0.000	14.706	0.000	0.000	0.049	0.000	0.000	0.000	0.000
H5, D40	0.307	0.027	0.086	0.000	14.706	0.000	0.000	0.065	0.071	0.000	0.000	0.030
H6, D0	0.343	0.028	0.000	0.022	14.706	0.000	1.337	0.000	0.000	0.000	0.173	1.162
H6, D10	1.673	0.028	0.000	0.000	14.706	0.075	0.000	0.090	0.067	0.042	1.154	0.000
H6, D20	0.000	0.046	0.000	0.000	14.706	0.000	0.000	0.020	0.010	0.000	2.886	0.000
H6, D30	0.000	0.409	0.092	0.000	14.706	0.000	0.000	0.027	0.000	0.000	2.472	0.000
H6, D40	0.000	0.045	0.000	0.050	14.706	0.000	3.071	0.000	0.047	0.000	3.718	0.230
H7, D0	0.057	0.000	0.000	0.000	14.706	0.000	0.066	0.086	0.000	0.000	0.000	0.206
H7, D10	0.000	0.020	0.000	0.000	14.706	0.183	1.418	0.060	0.000	0.000	4.311	0.000
H7, D20	0.000	0.000	0.019	0.000	14.706	0.000	0.000	0.025	0.000	0.424	1.612	0.000
H7, D30	0.068	0.028	0.000	0.000	14.706	0.000	0.739	0.000	0.000	0.000	3.027	0.000
H7, D40	0.291	1.354	0.399	0.028	14.706	1.148	0.000	0.000	0.048	0.000	4.720	0.000

CAT: Catechin, EPI-CAT: Epi-catechin, QE: Quercetin, RU: Rutin. GA: Gallic acid, CA: Caffeic acid, CHA: Chlorogenic acid, FA: Ferullic acid, pCA: p- Coumaric acid, SA: Syringic acid, VA: Vanillic acid, VN: Vanillin

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_14011418

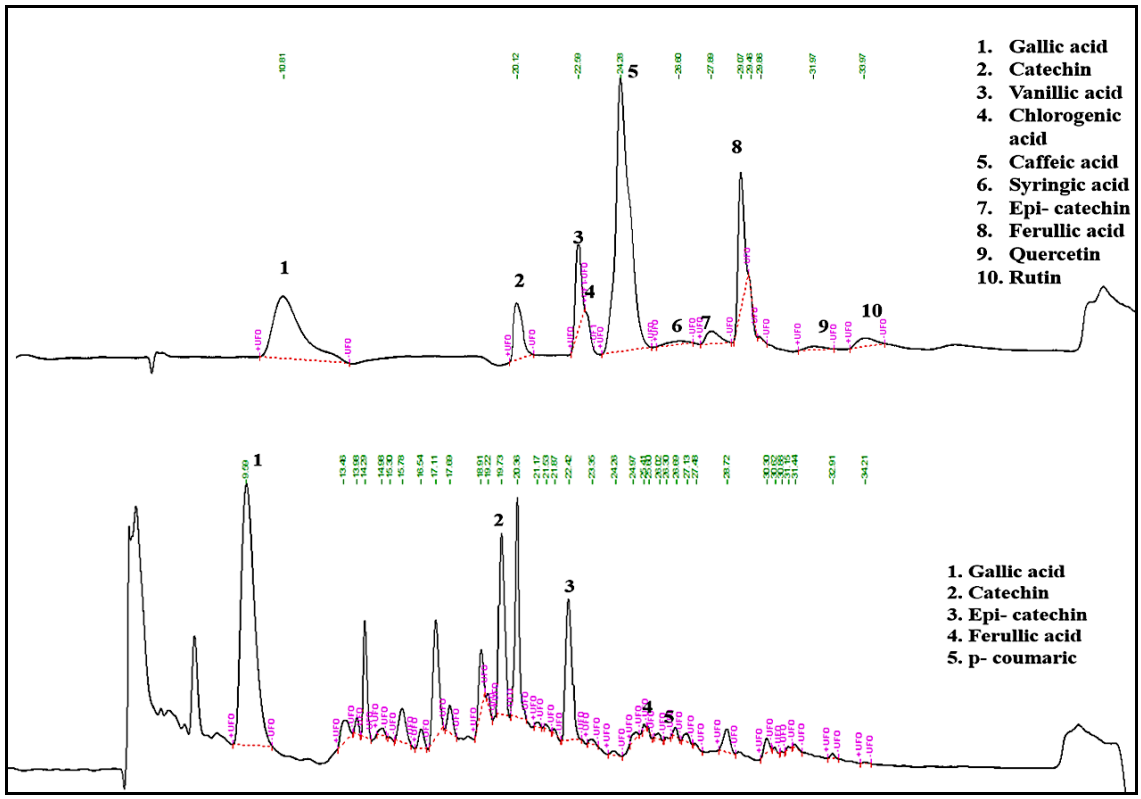

Figure 4. *(a) The HPLC chromatogram obtained of the mixture of standards used for the identification and quantification purposes. (b) Compounds present in an extract of harvest 1, day 40, the gallic acid was used as an internal standard*

Gene expression analysis

GDP-L-galactose phosphorylase (GGP) gene

The expression pattern of the *GGP* gene, which plays a crucial role in ascorbic acid synthesis, was analyzed across seven harvesting stages. Actin served as the housekeeping gene in RT-PCR (*Fig. 5A*). The intensity of *GGP* gene bands was highest during the initial harvesting stages but declined in the later stages, suggesting a decrease in *GGP* expression as the fruit matured (*Fig. 5B*). This decrease may correspond to a reduction in vitamin C biosynthesis, as *GGP* is a pivotal gene in ascorbic acid production.

Flavonol synthase (FLS) gene

The *FLS* gene, involved in flavonoid biosynthesis, showed a different pattern: its expression increased notably from the fourth harvest stage onwards (*Fig. 5C*). This trend indicates a rise in flavonoid production during later stages of fruit maturation, which aligns with the observed increase in total flavonoid content.

Hierarchical clustering analysis (HCA)

The relationships among seven harvesting stages (H1, H2, H3, H4, H5, H6, and H7) and storage periods (D0, D10, D20, D30, and D40) of kiwifruit are illustrated in *Figure 6* based on the measured parameters.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_14011418

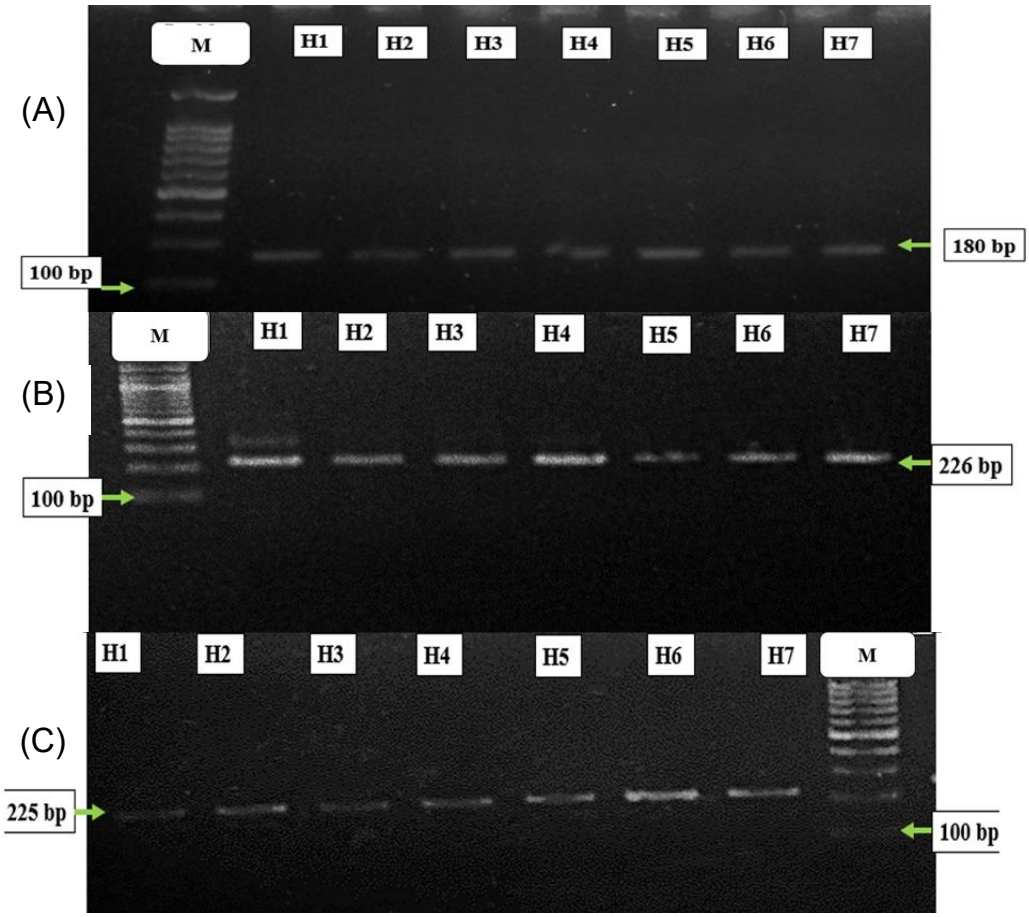

Figure 5. *The RT-PCR expression of (A) Actin (B), GDP-L-galactosephosphorylase (GGP) (C), and Flavonol Synthase (FLS) genes in the seven harvesting stages H1, H2, H3, H4, H5, H6 and H7. M: DNA size Marker (1 Kb), PCR amplification of these gene is shown in lane 1 to lane 7*

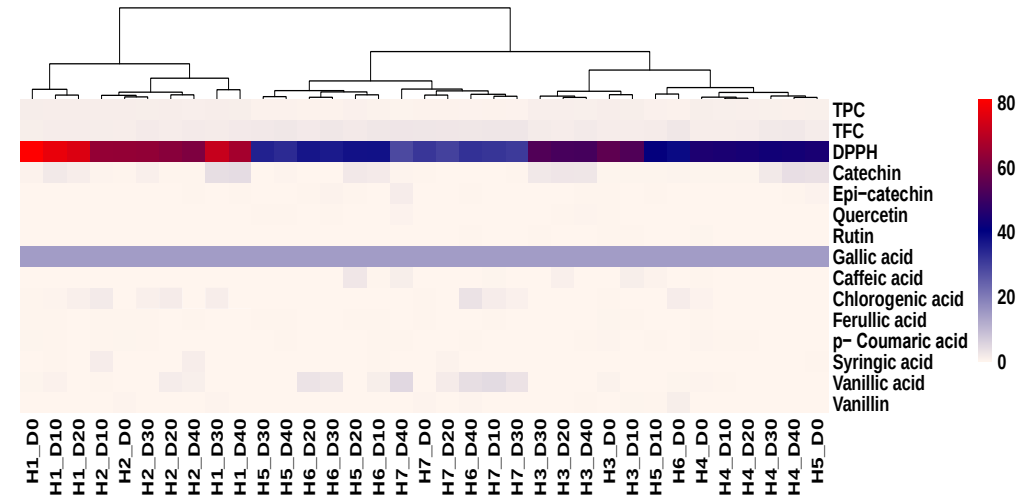

Figure 6. *The heatmap diagram presents the variations in the assessed parameters of kiwifruit (columns) across seven distinct harvesting stages (H1, H2, H3, H4, H5, H6, and H7) and storage periods (D0, D10, D20, D30, and D40) (rows). The heatmap employs a gradation of red for higher numerical values and blue for lower numerical values (refer to the scale located in the upper right quadrant of the image)*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_14011418

The heatmap analysis effectively delineated the collective fluctuations across all parameters. It is clear that TPC exhibited a substantial decline during the later phases of fruit maturation, which corresponded to harvest time. On the contrary, the TFC exhibited a minimal value during the early phases of harvesting; nevertheless, it increased substantially with the fruit's maturation. The DPPH assay demonstrated that the antioxidant capacity was greatest at the time of fruit harvest; nevertheless, this trend declined as the fruit matured. The heatmap clustering demonstrated that the phenolic compounds differed among samples according to the stage of harvesting and the duration of storage (*Fig. 6*).

Discussion

When kiwifruits were stored for 10, 20, 30 and 40 days at room temperature (15-20°C), storage time had a significant impact on the TPC (Total Phenolic Content) of fruits. TPC remained relatively stable during the first 20 days of storage but showed a substantial decrease afterwards. Overall, this trend is consistent with the findings of Hui-Na et al. (2008), who reported higher TPC at the initial stages of fruit development in the kiwifruit (*A. setosa*). Similar results were observed in other fruits like oranges, where TPC was affected by extended storage duration (Klimczak et al., 2007). While previous literature often addresses the influence of different storage conditions on phenolic content (Kalt, 2005), in this study, the storage temperature was kept constant, and only the storage time varied. Thus, it was the storage time, not the conditions, that influenced the TPC observed here. Additionally, studies indicate that the temperature significantly affects TPC in kiwifruits with faster decrease observed at lower temperatures like 5-10°C compared to temperatures 0, 15 and 20°C (Asiche et al., 2017). However, in commercial practice, kiwifruits after harvest are stored at lower temperatures (0-4°C). At this temperature range all the metabolic processes are suppressed, thus leading to the prolonged fruit storage (Marsh et al., 2004). The decrease in TPC over time may be due to the natural metabolic breakdown of phenolic compounds as fruits continue to ripen at post-harvest. Research also suggests that phenolic compounds are sensitive to oxidative stress, which could contribute to their decline over prolonged storage periods at room temperature (Kalt, 2005; Pal et al., 2013).

In contrast, TFC showed stability and even slight increases over the storage period, the results are similar to observations in other fruits such as mangoes and apples, where flavonoid content rises due to stress-induced metabolic changes during storage (Huang et al., 2019; Zhong et al., 2018). Similarly, it is indicated that TFC remained stable in various fruits like plum, apricot, yellow pepper, grape etc. (Benlloch-Tinoco et al., 2015; Kevers et al., 2007: Kao, 2006). These results reinforce the observation that flavonoid content is less sensitive to storage time, potentially due to different biosynthetic pathways or stability compared to phenolics.

The results of free radical scavenging activity were similar to previous study (Kao, 2006). The decrease in the activity was due to decrease of vitamin C and polyphenolic content. Both are responsible for providing free radicals scavenging in different fruits and vegetables (Pal et al., 2013). As kiwifruits and other citrus fruits are rich source of ascorbic acid (vitamin C) and are thus a great source of antioxidants (Nishiyama et al., 2004), so, as the fruit matures, the vitamin C and phenolic content decrease that leads to a decline in the antioxidant activity (Tavarini et al., 2008; Kalt, 2005).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_14011418

Studies showed that the antioxidant activity is more affected by the storage time than the storage temperature (Tavarini et al., 2008). As the storage periods increased, the phenolic content decreased that in turn lead to a decrease in antioxidant activity. There was a reduction of antioxidant activity after 20 days period mainly due to decrease in phenolic content. The results are supported by the previous findings (Tavarini et al., 2008; Shivashankara et al., 2004).

The values of different phenolic compounds varied in different samples. In previous studies these compounds have been quantified in the A. deliciosa. However, no syringic acid and gallic acid were detected. Gallic acid presence has been reported in the A. deliciosa variety from China. The extracts contained chlorogenic acid, similar to those present in pear, apple and quince (Kim et al., 2009; Fattouch et al., 2008). From the flavonoids group catechin and epi-catechin were predominantly found in different kiwifruit extracts. Quercetin and rutin were present in few samples only.

Previous literature showed that GGP, act as a key gene in vitamin C biosynthesis (Zhang et al., 2017; Liu et al., 2015; Suekawa et al., 2019; Shiri et al., 2018; Ishikawa et al., 2018). It was found in the present study that expression of is decreased in the late harvesting stages which could suggest that there is a decrease in vitamin C level, as GDP-L-galactose phosphorylase (GGP) is the key ascorbic acid biosynthesis gene (Mellidou and Kanellis, 2017) and serves as the control point in various plant species including Arabidopsis (Bulley et al., 2009; Yoshimura et al., 2014), tobacco (Bulley et al., 2009; Wang et al., 2014), tomato (Mellidou et al., 2012; Bulley et al., 2012; Wang et al., 2013), citrus (Alós et al., 2013), blueberry (Liu et al., 2015), kiwifruit (Li et al., 2010) and strawberry (Bulley et al., 2012). The gene involved in the flavonoids biosynthesis is over-expressed in the later harvesting stages and that may have increased total flavonoids content as observed in this study. It has been observed that the vitamin C quantity decreased with the fruit maturation, especially in case of citrus fruits (Lee and Kader, 2000) therefore, the expression of the genes involved in its biosynthesis decreased as well in this study. While this study provides comprehensive data on TPC, TFC, and gene expression, it is essential to highlight that the impact of storage time, rather than variable storage conditions, was the primary focus. Future research could extend these findings by examining different temperature conditions or controlled light exposure to assess their combined effects on kiwifruit's metabolic profile.

Conclusion

This study provides novel insights into the metabolic composition and antioxidant potential of kiwifruit across various harvest stages and storage durations. Kiwifruit was shown to contain several bioactive metabolites including chlorogenic acid (CHA), ferulic acid (FA), p-coumaric acid (p-CA), vanillic acid (VA), and catechin (CAT), which varied in concentration at different harvest times. Early stages of harvest showed higher total phenolic content (TPC), contributing to increased antioxidant activity, which diminished as the fruit matured. TFC exhibited stability, slightly increasing due to stress-induced metabolic adjustments over prolonged storage, which is consistent with patterns observed in other fruits like mangoes and apples. Gene expression analysis revealed a clear correlation between *GGP* and *FLS* genes with their corresponding metabolites, vitamin C, and flavonoids, underscoring genetic regulation as a determinant of kiwifruit nutritional quality.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_14011418

Contrary to initial assumptions, the storage time, rather than the storage conditions, played a critical role in influencing the metabolic profile at post-harvest. These findings indicate that kiwifruit's nutritional value can be optimized through strategic harvesting, particularly if the goal is to maximize antioxidant activity, phenolic content, and vitamin C levels by harvesting at earlier stages.

As kiwifruit cultivation has only recently been introduced in Pakistan, these results are invaluable for guiding local and global researchers and growers in improving yield quality and maximizing health benefits. Aligning with prior studies yet extending beyond them, this work highlights practical applications for enhancing kiwifruit's commercial and nutritional potential, contributing to a deeper understanding of kiwifruit as a functional crop.

Author contributions. Sabaz Ali Khan conceived the original idea. Muhammad Azeem, Arshad M. Abbasi designed the experiments. Bibi Mariyam and Noorullah Khan performed the experiments. Ahmed Mahmoud Ismail, Wael Elmenofy and Mustafa I. Almaghasla analyzed the data. Ahmed Mahmoud Ismail, Hossam S. El-Beltagi, Mohamed M. El-Mogy wrote the manuscript. Funding Acquisition, Ahmed Mahmoud Ismail. All authors have read and approved the manuscript.

Conflict of interests. The authors declare that they have no conflict of interest.

Acknowledgements. Authors extend their gratefulness to the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia, for supporting this research for work through grant number KFU241376. Authors greatly acknowledge the field staff of NTHRI for helping during the samples collections.

Data availability. Data will be made available on request.

REFERENCES

[1] Abbasi, A. M., Guom, X., Nazir, A. (2015): Preliminary assessment of phytochemical contents and antioxidant properties of *Pistacia integerrima* fruit. – Pakistan Journal of Pharmaceutical Sciences 28(4): 1187-94.

[2] Alós, E., Rodrigo, M. J., Zacarías, L. (2013): Transcriptomic analysis of genes involved in the biosynthesis, recycling and degradation of L-ascorbic acid in pepper fruits (*Capsicum annuum* L.). – Plant Science 207: 2-11.

[3] Asiche W. O., Mitalo, O. W., Kasahara, Y., Tosa, Y., Mworia, E. G., Ushijima, K., Kubo, Y. (2017): Effect of storage temperature on fruit ripening in three kiwifruit cultivars. – The Horticulture Journal 86(3): 403-410.

[4] Belščak-Cvitanović, A., Durgo, K., Huđek, A., Bačun-Družina, V., Komes, D. (2018): Overview of Polyphenols and Their Properties. – In: Galanakis, C. M. (ed.) Polyphenols: Properties, Recovery, and Applications. Woodhead Publishing, Sawston, pp. 3-44.

[5] Benlloch-Tinoco, M., Igual, M., Rodrigo, D., Martínez-Navarrete and N. (2015): Superiority of microwaves over conventional heating to preserve shelf-life and quality of kiwifruit puree. – Food Control 50: 620-629.

[6] Bulley, S. M., Laing, W. (2016): Ascorbic Acid-Related Genes. – In: Testolin, R. et al. (eds.) The Kiwifruit Genome. Springer, Cham. p 163-177.

[7] Bulley, S. M., Rassam, M., Hoser, D., Otto, W., Schünemann, N., Wright, M., Laing, W. (2009): Gene expression studies in kiwifruit and gene over-expression in Arabidopsis indicates that GDP-L-galactose guanyltransferase is a major control point of vitamin C biosynthesis. – Journal of Experimental Botany 60(3);765-778.

[8] Bulley, S., Wright, M., Rommens, C., Yan, H., Rassam, M., Lin-Wang, K., Laing, W. A. (2012): Enhancing ascorbate in fruits and tubers through over-expression of the

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_14011418

l-galactose pathway gene GDP-l-galactose phosphorylase. – Plant Biotechnology Journal 10(4): 390-397.
[9] Chen, Y. A., Yang, H., Liu, Y. F. (2013): GAP Production Technology in Kiwifruit. – Northwest Agriculture and Forestry University of Science and Technology Press, Xianyang.
[10] Crozier, A., Jaganath, I. B., Clifford, M. N. (2009): Dietary phenolics: chemistry, bioavailability and effects on health. – Natural Product Reports 26(8): 1001-1043.
[11] Drummond, L. (2013): The composition and nutritional value of kiwifruit. – Adv Food Nutr Res 68: 33-57.
[12] Du, G., Li, M., Ma, F., Liang, D. (2009): Antioxidant capacity and the relationship with polyphenol and vitamin C in Actinidia fruits. – Food Chemistry 113(2): 557-562.
[13] Fattouch, S., Caboni, P., Coroneo, V., Tuberoso, C., Angioni, A., Dessi, S., Cabras, P. (2008): Comparative analysis of polyphenolic profiles and antioxidant and antimicrobial activities of Tunisian pome fruit pulp and peel aqueous acetone extracts. – Journal of Agricultural and Food Chemistry 56(3): 1084-1090.
[14] Guroo, I., Wani, S. A., Wani, S. M., Ahmad, M., Mir, S. A., Masoodi, F. A. (2017): A review of production and processing of kiwifruit. – Journal of Food Processing and Technology 8: 699. DOI: 10.4172/2157-7110.1000699.
[15] Huang, H., Ferguson, A. R. (2007): Genetic resources of kiwifruit: domestication and breeding. – Horticultural Reviews 33: 1-121.
[16] Huang, H., Jiang, X., Xiao, Z., Yu, L., Pham, Q. (2019): Influence of storage temperature on bioactive compounds and antioxidant capacity of mango pulp. – Food Chemistry 276: 252-260.
[17] Huang, S., Ding, J., Deng, D., Tang, W., Sun, H., Liu, D., Yu, J. (2013): Draft genome of the kiwifruit *Actinidia chinensis*. – Nature Communications 4: 2640. DOI: 10.1038/ncomms3640.
[18] Hui-Na, C., Cheng-Chu, N., Andi Shau-Mei, O., Tse-Heng, C., Chia-Chen, C. (2008): Characterization of the physico-chemical and antioxidant properties of Taiwanese kiwifruit (*Actinidia setosa*). – Botanical Studies 49: 215-224.
[19] Ishikawa, T., Maruta, T., Yoshimura, K., Smirnoff, N. (2018): Biosynthesis and Regulation of Ascorbic Acid in Plants. – In: Gupta, D. K. et al. (eds.) Antioxidants and Antioxidant Enzymes in Higher Plants. Springer, Cham, pp. 163-179.
[20] Kalt, W. (2005): Effects of production and processing factors on major fruit and vegetable antioxidants. – Journal of Food Science 70(1): 11-19.
[21] Kao, M. W. (2006): A comparative study of antioxidant and physicochemical properties of blackberry and kiwifruit. – A thesis submitted to the Graduate Faculty of Auburn University in partial fulfillment of the requirements for the degree of master of science, Auburn, Alabama.
[22] Kevers, C., Falkowski, M., Tabart, J., Defraigne, J. O., Dommes, J., Pincemail, J. (2007): Evolution of antioxidant capacity during storage of selected fruits and vegetables. – Journal of Agricultural and Food Chemistry 55(21): 8596-8603.
[23] Khalifa, I., Zhu, W., Li, K. K., Li, C. M. (2018): Polyphenols of mulberry fruits as multifaceted compounds: compositions, metabolism, health benefits, and stability—A structural review. – Journal of Functional Foods 40: 28-43.
[24] Kim, J. G., Beppu, K., Kataoka, I. (2009): Varietal differences in phenolic content and astringency in skin and flesh of hardy kiwifruit resources in Japan. – Scientia Horticulturae 120(4): 551-554.
[25] Klimczak, I., Małecka, M., Szlachta, M., Gliszczyńska-Świgło, A. (2007): Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. – Journal of Food Composition and Analysis 20(3-4): 313-322.
[26] Landi, M., Tardelli, F., Remorini, D., Massai, R., Guidi, L. (2014): Do sun-versus shade-grown kiwifruits perform differently upon storage? An overview of fruit maturity and

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_14011418

nutraceutical properties of whole and fresh-cut produce. – Journal of Agricultural and Food Chemistry 62(19): 4377-4383.
[27] Lee, S. K., Kader, A. A. (2000): Preharvest and postharvest factors influencing vitamin C content of horticultural crops. – Postharvest Biology and Technology 20(3): 207-220.
[28] Li, M., Ma, F., Liang, D., Li, J., Wang, Y. (2010): Ascorbate biosynthesis during early fruit development is the main reason for its accumulation in kiwi. – PloS ONE 5(12): e14281.
[29] Lippi, G., Mattiuzzi, C. (2019): Kiwifruit and cancer: an overview of biological evidence. – Nutrition and Cancer 72(4): 547-553.
[30] Liu, F., Wang, L., Gu, L., Zhao, W., Su, H., Cheng, X. (2015): Higher transcription levels in ascorbic acid biosynthetic and recycling genes were associated with higher ascorbic acid accumulation in blueberry. – Food Chemistry 188: 399-405.
[31] Ma, T., Sun, X., Zhao, J., You, Y., Lei, Y., Gao, G., Zhan, J. (2017): Nutrient compositions and antioxidant capacity of kiwifruit (*Actinidia*) and their relationship with flesh color and commercial value. – Food Chemistry 218: 294-304.
[32] Marsh, K., Attanayake, S., Walker, S., Gunson, A., Boldingh, H., MacRae, E. (2004): Acidity and taste in kiwifruit. – Postharvest Biology and Technology 32(2): 159-168.
[33] Mellidou, I., Kanellis, A. K. (2017): Genetic control of ascorbic acid biosynthesis and recycling in horticultural crops. – Frontiers in Chemistry 5: 50.
[34] Mellidou, I., Keulemans, J., Kanellis, A. K., Davey, M. W. (2012): Regulation of fruit ascorbic acid concentrations during ripening in high and low vitamin C tomato cultivars. – BMC plant Biology 12(1): 239.
[35] Nishiyama, I. (2007): Fruits of the Actinidia genus. – Advances in Food and Nutrition Research 52: 293-324.
[36] Nishiyama, I., Yamashita, Y., Yamanaka, M., Shimohashi, A., Fukuda, T., Oota, T. (2004): Varietal difference in vitamin C content in the fruit of kiwifruit and other *Actinidia* species. – Journal of Agricultural and Food Chemistry 52(17): 5472-5475.
[37] Pal, R. S., Kumar, R. A., Agrawal, P. K., Bhatt, J. C. (2013): Antioxidant capacity and related phytochemicals analysis of methanolic extract of two wild edible fruits from north western Indian Himalaya. – International Journal of Pharma and Biosciences (2): 113-123.
[38] Park, Y., Im, M., Ham, K., Kang, S., Park, Y., Namiesnik, J., Gorinstein, S. (2013): Nutritional and pharmaceutical properties of bioactive compounds in organic and conventional growing kiwifruit. – Plant Foods for Human Nutrition 68(1): 57-64.
[39] Pilkington, S. M., Montefiori, M., Jameson, P. E., Allan, A. C. (2012): The control of chlorophyll levels in maturing kiwifruit. – Planta 236(5): 1615-1628.
[40] Richardson, D. P., Ansell, J., Drummondm, L. N. (2018): The nutritional and health attributes of kiwifruit: a review. – European Journal of Nutrition 57(8): 2659-2676.
[41] Shiri, S., Zebarjadi, A. (2018): Isolation and characterization of GDP-L-galactose phosphorylase gene of vitamin C biosynthesis pathway from kiwi. – Journal of Applied Biotechnology Reports 5(2): 59-63.
[42] Shivashankara, K. S., Isobe, S., Al-Haq, M. I., Takenaka, M., Shiina, T. (2004): Fruit antioxidant activity, ascorbic acid, total phenol, quercetin, and carotene of Irwin mango fruits stored at low temperature after high electric field pretreatment. – Journal of Agricultural and Food Chemistry 52(5): 1281-1286.
[43] Slavin, J. L., Lloyd, B. (2012): Health benefits of fruits and vegetables. – Advances in Nutrition 3(4): 506-516.
[44] Suekawa, M., Fujikawa, Y., Inoue, A., Kondo, T., Uchida, E., Koizumi, T., Esaka, M. (2019): High levels of expression of multiple enzymes in the Smirnoff-Wheeler pathway are important for high accumulation of ascorbic acid in acerola fruits. – Bioscience, Biotechnology and Biochemistry 83(9): 1713-1716.
[45] Suzuki, Y., Mae, T., Makino, A. (2008): RNA extraction from various recalcitrant plant tissues with a cethyltrimethylammonium bromide-containing buffer followed by an acid

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_14011418

guanidium thiocyanate-phenol-chloroform treatment. – Bioscience, Biotechnology and Biochemistry 72(7): 1951-1953.
[46] Tavarini, S., Degl'Innocenti, E., Remorini, D., Massai, R., Guidi, L. (2008): Antioxidant capacity, ascorbic acid, total phenols and carotenoids changes during harvest and after storage of Hayward kiwifruit. – Food Chemistry 107(1): 282-288.
[47] Vissers, M. C., Carr, A. C., Pullar, J. M., Bozonet, S. M. (2013): The bioavailability of vitamin C from kiwifruit. – Adv Food Nutr Res 68: 125-47.
[48] Wang, L., Meng, X., Yang, D., Ma, N., Wang, G., Meng, Q. (2014): Overexpression of tomato GDP-L-galactose phosphorylase gene in tobacco improves tolerance to chilling stress. – Plant Cell Reports 33(9): 1441-1451.
[49] Wang, L. Y., Li, D., Deng, Y. S., Lv, W., Meng, Q. W. (2013): Antisense-mediated depletion of tomato GDP-l-galactose phosphorylase increases susceptibility to chilling stress. – Journal of Plant Physiology 170(3): 303-314.
[50] Ward, C., Courtney, D. (2013): Kiwifruit: taking its place in the global fruit bowl. – Advances in Food and Nutrition Research. https://doi.org/10.1016/B978-0-12-394294-4.00001-8.
[51] Yoshimura, K., Nakane, T., Kume, S., Shiomi, Y., Maruta, T., Ishikawa, T., Shigeoka, S. (2014): Transient expression analysis revealed the importance of VTC2 expression level in light/dark regulation of ascorbate biosynthesis in Arabidopsis. – Bioscience, Biotechnology, and Biochemistry 78(1): 60-66.
[52] Zhang, J. Y., Pan, D. L., Jia, Z. H., Wang, T., Wang, G., Guo, Z. R. (2018): Chlorophyll, carotenoid and vitamin C metabolism regulation in *Actinidia chinesis'* Hongyang'outer pericarp during fruit development. – PloS ONE 13(3): e0194835.
[53] Zhang, L., Li, Z., Wang, Y., Jiang, Z., Wang, S., Huang, H. (2010): Vitamin C, flower color and ploidy variation of hybrids from a ploidy-unbalanced Actinidia interspecific cross and SSR characterization. – Euphytica 175: 133-143.
[54] Zhang, S., Sun, H., Zhang, L. (2017): Multi-regulated GDP-l-galactose phosphorylase calls the tune in ascorbate biosynthesis. – Journal of Experimental Botany 68(16): 4201-4214.
[55] Zhong, Y., Fan, X., Zhu, M. (2018): Effect of postharvest treatments on flavonoid accumulation in apples. – Journal of Agricultural and Food Chemistry 66(5): 1157-1163.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_14011418