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Research article

Appropriate harvest maturity for exploitation of wild black raspberry (*Rubus* sp.) fruits during shelf life period from Rawalakot, Azad Jammu and Kashmir

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Abstract: Black raspberries (*Rubus* sp.) are abundantly grown and distributed widely in temperate regions of Pakistan which shows rich plant diversity of fruit species. In this study wildly grown black raspberry fruits were harvested from Rawalakot at three different maturity stages and investigated for physical, biochemical and antioxidant properties. Random samples of wild black raspberry fruits from each maturity stage [turning (75% red, 25% green), full red (100% red), black (100% black)] were stored at room temperature (22 ± 2 °C, 46% RH) for maximum 7 days and analysed on alternate days. Results showed that fruit weight, total soluble solids, pH, vitamin C, total flavonoids, total phenols, total antioxidants and total anthocyanins increased whereas fruit firmness and titratable acidity levels decreased as maturity progressed from turning to black stage. During shelf life period, total soluble solids and pH increased, while fruit weight, firmness, total anthocyanins, total flavonoids and total phenols significantly decreased with shelf life period. Moreover, titratable acidity and vitamin C decreased by advancement in shelf life period. It is advisable that wild black raspberry fruits should be harvested at black stage if used for immediate consumption. However, raspberries can be harvested at full red stage to extend storage period without compromising on fruit quality.

Keywords: antioxidants; ascorbic acid; ripening; shelf life; wild berries; skin colour

1. Introduction

Fruit maturity at harvest time is one of the critical factors that determine fruit quality and postharvest behaviour [1,2]. Moreover, for sustainable production of new fruits, harvest at proper maturity could be helpful to avoid fruit losses and wastes. Further, it is also closely related to retailers and consumers preferences [3]. Harvesting immature or overripe fruit leads to quick physiological deterioration during storage and results in lower quality and poor market value [4]. Therefore, fruits must be harvested at proper stage that ensures their optimal organoleptic quality together with the maximum shelf-life [5,6].

Quality characteristics of berry fruits include moisture content, flavour, intactness, colour and nutrients profile [7]. During berry development, fruit passes through internal and external modifications in chemical composition, texture, colour and taste [8], which depends on development stages and agronomic practices used to grow fruit plants [9]. Due to structural instability and incomplete development, pre-mature fruits are susceptible to physical injuries, moisture losses and poor development of flavour at ripe stage. If harvested at overripe stage, fruit immediately turns soft and develops bad flavour [4]. Similar situation happens with black raspberry fruits as well, which not only affects the fruit quality but also reduces the profit margins of producer.

Postharvest behaviour of Cape gooseberries was found to be strongly linked with maturity stage. Berries harvested at third maturity stage (100% orange fruit with 100% yellow calyx) showed better postharvest behaviour as compared to earlier harvests [10]. However, for immediate consumption, consumers were advised to harvest fruits at fourth maturity stage (100% orange fruit with dry and brown calyx).

Meeting nutritional demands from native fruits, vegetables and crop plants is becoming critical, especially for countries with rapidly increasing populations like Pakistan [11,12]. The increase in prices of conventional fruit crops in the most of developing countries has led to exploration of new fruit species, locally available under wild conditions [13].

Black raspberries (*Rubus* sp.) belong to the Rosaceae family and contain valuable amounts of amino acids, proteins, carbohydrates, dietary fibre, minerals and antioxidants [14]. In particular, raspberries are rich in vary nutritional compounds including vitamins and polyphenolics which contribute to the antioxidant properties of these fruit [15,16]. Black raspberries are consumed both, fresh and processed in many cooked dishes and baked products like desserts, snacks, jams and jellies [17].

Sustainable fruit production is gaining much importance in recent years. However, for successful and sustainable fruit production, the most important key principles include the utilization of natural resources and exploring the biological diversity [18,19]. Moreover, it has also been noticed during past few years that consumers demand has increased for locally grown fruits and vegetables [20,21]. In this context, locally grown and traditional varieties have the potential to be exploited for their nutritional values and sustainable production under natural climatic conditions. In fact, they possess many qualities such as taste, colour and size due to which they are gaining importance for developing new varieties in future [21–23].

Black raspberry plants grow naturally and are frequently found under wild conditions in

Rawalakot, Azad Jammu and Kashmir, Pakistan. Even though there is a huge problem of fruit harvesting as black raspberry fruits are grown as a forest fruit and often found in inaccessible areas. Fresh fruits are commonly consumed by local people in this area. The solution to this problem is the domestication and establishment of fruit orchards of this species. Despite the interest of this fruit due to its nutritional quality, research regarding the most appropriate maturity stage for harvesting is necessary for sustainable production of black raspberries in Pakistan. Therefore, the aim of this study was to identify the most suitable maturity stage for harvesting wild black raspberries in order to obtain the best physico-chemical and nutritional quality together with an extended shelf-life.

2. Materials and methods

2.1. Plant material and treatments

Black raspberry fruit were harvested in August, 2022 from plants naturally grown in Rawalakot, Azad Jammu and Kashmir (Latitude: 33–36°N, Longitude: 73–75°E, Altitude: 1,638 m above sea level) and transported to the University of Poonch Rawalakot for further analysis. Fruit were harvested (1000 g for each maturity stage) at the turning (75% red, 25% green), full red (100% red) and black (100% black) stages using naked eyes (Figure S1). An initial sorting was performed to select non-damaged and free from decay symptoms fruit. The raspberries were analysed immediately after harvest (D1), and at 3 (D3), 5 (D5) and 7 (D7) days after shelf life period. All the fruits were packed in Styrofoam boxes and stored at room temperature (22 ± 2 °C, 46% RH).

2.2. Physical properties

Fruit diameter (cm) for each maturity group was measured on 10 fruits per replication using a Vernier calliper (Mitutoyo, Japan) and fruit weight (g) was determined on 30 fruits per replication by using a digital scale (GF-6100, A&D Co. Ltd., Japan).

Firmness was measured on 10 fruits per replication using a hedonic scale from 0 to 9 (0 = very soft; 9 = very hard). Chroma meter (CR-400, Konica Minolta, Japan) was used for $L*$ [white 100-black 0], $a*$ [green (-60) – red (+60)] and $b*$ [blue (-60) – yellow (+60)] colour coordinates determination (30 fruits per replication).

2.3. Biochemical properties

Total soluble solids (TSS) content and titratable acidity (TA) were determined on fruit extract, following the AOAC [24] methods. TSS was measured with a hand refractometer (Kyoto Company, Japan) and expressed in percentage. Titratable acidity was determined through titration method. Briefly, the filtrate (5 mL) with 2–3 drops of 0.1% phenolphthalein solution as an indicator was titrated using 0.1 N NaOH to an end point pink (pH 8.1). The results were expressed as the percentage of malic acid per 100 mL fruit juice. pH was determined on fruit juice using a pH meter (Model: WTW 82362 Inolab, Germany).

Dye used to determine vitamin C was 2, 6-dichlorophenol indophenols. Fruit juice (5 mL) plus 4% Meta phosphoric acid (5 mL) was mixed and the solution was titrated by using dye until the persistent of light pink colour. The results were expressed as mg per 100 mL fruit juice [24].

2.4. Antioxidant properties

Total flavonoids were measured according to the method described by Maqbool et al. [25] and the results were expressed as mmol quercetin 100 g^{-1} FW. Total phenols were measured using spectrophotometer (Model: UV 4000, O.R.I., Reinbeker, Hamburg, Germany). Mixture was prepared using 10% Folin-Ciocalteau's Reagent (2.5 mL), 7.5% sodium carbonate solution (2.0 mL) and aqueous extract (0.5 mL). This mixture was incubated at 45 ℃ for 40 minutes and the absorbance was measured at 765 nm in the spectrophotometer. Obtained results were presented as µg of gallic acid 100 g^{-1} FW. Total antioxidants were determined by using the procedure of Benzie and Strain [26]. Spectrophotometer was used to check absorbance at 593 nm, while results obtained were presented as antioxidants concentration having ferric reducing activity which was equivalent to 1 mg $FeSO_4$ g^{-1} FW.

Total anthocyanins were measured using spectrophotometer method of pH dilution [27]. Spectrophotometer at 510 nm and 700 nm was used for measuring absorbance of each dilution, while distilled water was used to fill blank cell. Anthocyanin pigments as mg of cyaniding-3-glucoside 100 g^{-1} FW were measured using 29,600 as an extinction coefficient and 449.2 as a molecular weight.

2.5. Sensory properties

A sensory evaluation was carried out by a 10-member panel that was trained to become familiar with the characteristics of the fruits. The panellists evaluated the fruit samples using a hedonic scale [28]. According to their preferences, the panellists were asked to score the fruit samples on a 0–10 points hedonic scale taking into account taste, texture, flavour and acceptability. Each member of the panel evaluated three samples harvested at the three different maturity stages (turning, full red and black stage) (50 fruits per replication).

2.6. Statistical analysis

The factorial experiment was arranged as in a completely randomized design with three replications. The two factors were: maturity stages on the basis of fruit colour (3 levels: turning, red and black) and shelf life period (4 levels: day 1, 3, 5 and 7). Analysis of variance was carried out using Statistix 8.1 software. To compare the differences of means, least significance difference (LSD) test was used at 0.05 level of significance [29].

3. Results and discussion

3.1. Physical properties

Fruits harvested at black stage recorded maximum fruit diameter (1.07 cm), followed by fruits at red stage (0.81 cm), while minimum fruit diameter was observed in fruits at turning stage (0.66 cm) (Table 1). Increase in diameter of fruit was observed with advancement of maturity ($P \leq 0.05$). Šnajder and Duralija [30] studied various pomological characteristics of blackberry fruits harvested at different maturity stages. They reported that harvest time had great impact on those characteristics. Higher values of length, width and weight were recorded in blackberry fruits after first harvest time while the value of total soluble solids was higher in fruits picked in the third harvest time. Similarly, increase in fruit size with advancement in maturity stage was also reported in Andean blackberries by Horvitz et al. [31].

Maximum value of fruit colour $L*$ was recorded in fruits harvested at turning stage (39.47) which was followed by fruits at full red stage (37.31), while minimum value of fruit colour $L*$ was observed in fruits at black stage (28.56). Results regarding $a*$ value of black raspberry fruits showed maximum $a*$ value at turning stage (16.94) which was followed by fruits at red stage (15.58) whereas minimum value was recorded in fruits at black stage (1.08). Similarly, $b*$ value of black raspberry fruits showed maximum value of fruits harvested at turning stage (12.82) which was followed by fruits harvested at red stage (8.15) whereas minimum $b*$ value was observed in fruits at black stage (1.22) (Table 1).

Table 1. Fruit diameter and peel colour of wild black raspberry (*Rubus* sp.) fruits harvested at different maturity stages. Means with different letters are significantly different according to LSD test at 0.05 level of significance, ±SEM (n = 3).

Maturity stages	Fruit diameter (cm)	Fruit peel colour		
		*L** value	*a** value	*b** value
Turning stage	0.66±0.01 c	39.47±0.56 a	16.94±0.05 a	12.82±0.45 a
Full red stage	0.81±0.01 b	37.31±0.32 a	15.58±0.08 a	8.15±0.13 b
Black stage	1.07±0.01 a	28.56±0.30 b	1.08±0.00 b	1.22±0.04 c

Black raspberry fruits showed highest fruit weight at black stage (11.47 g) followed by fruits harvested at red stage (5.31 g), while the lowest fruit weight value was recorded in fruits at turning stage (5.30 g) (Figure 1a). During shelf life, black raspberries harvested in all maturity stages exhibited loss of fresh weight significantly ($P \leq 0.05$) but the highest loss in fresh weight was noted in raspberries harvested at black maturity stage. Highest fresh weight was measured at D1 (11.66 g) which decreased as the shelf life period prolonged, while the lowest fresh weight was measured at D7 (3.94 g) during shelf life period. Weight of black raspberry was lower at early mature stage and increased significantly towards late maturity. Loss in fruit weight appeared to be attributed to difference in maturity [32], while during shelf life period, fruit weight decreased gradually. Samaniego et al. [33] also reported increase in weight of black raspberries as fruits matured and decreased during shelf life period.

Black raspberry fruit firmness was the highest in fruits harvested at turning stage (7.91) followed by fruits at red stage (5.66) whereas lowest firmness was recorded in fruits harvested at black stage (3.25) (Figure 1b). Fruit firmness gradually decreased during shelf life period in fruits at any maturity stage ($P \leq 0.05$) and the rate of decrease was almost similar among all maturity stages. Results regarding shelf life period showed maximum fruit firmness at D1 (7.11 score) followed by firmness at D3 (6.0 score), while minimum firmness was observed at D7 (4.0 score). Decrease in firmness may be due to loss in weight, higher metabolic activity and loss in membrane permeability. Fruit firmness decreased as maturity progressed which could be due to the higher concentration of protopectin which are responsible for consistency and strength of cell wall tissues [34]. This phenomenon can also be linked with increase in weight loss, as this process also leads to higher cell and tissue breakdown and consequently accelerating the process of aging which includes the loss of firmness [1]. In this regard, many researchers have previously reported a similar phenomenon in which weight loss and loss in firmness were correlated [1,5,35,36].

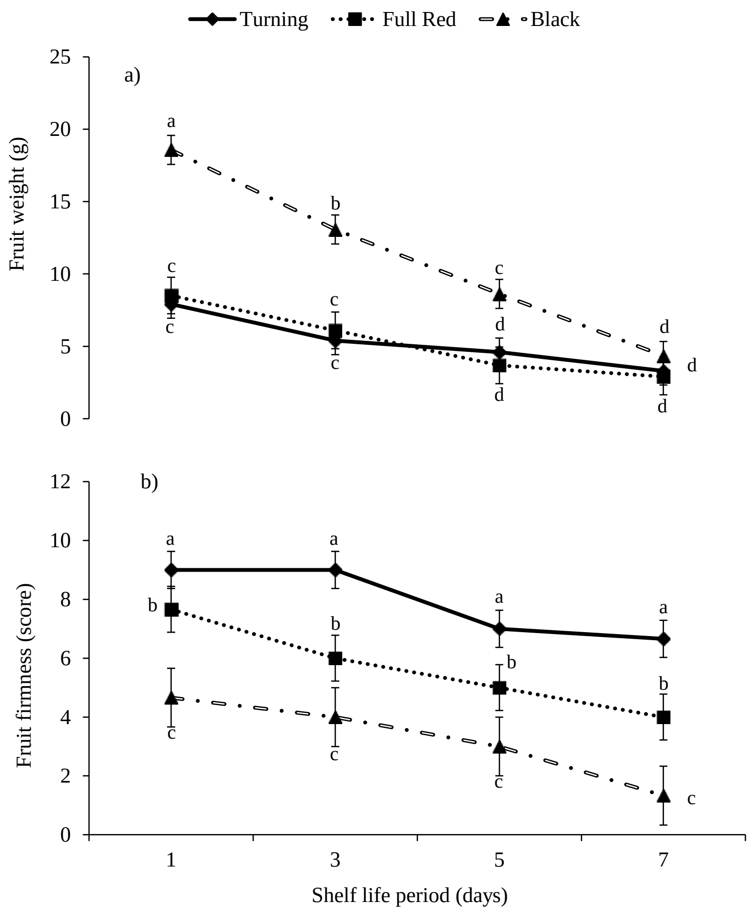

Figure 1. Effect of fruit maturity and shelf life period on a) fruit weight and b) fruit firmness of wild black raspberry (*Rubus* sp.) fruits. Vertical bars indicate average ± standard error (n = 3).

3.2. Biochemical properties

Total soluble solids content in raspberry harvested at black stage was the highest (12.94%), followed by fruits harvested at red stage (3.38%) whereas the lowest total soluble solids were recorded in fruits harvested at turning stage (2.69%) (Figure 2a). Raspberries at either maturity stages exhibited slight but statistically significant increase in total soluble solids ($P \leq 0.05$). Rate of increase in total soluble solids in raspberries harvested at black stage was higher than those harvested at turning and full red maturity stages. During shelf life period, maximum value was recorded at D7 (7.80%), while minimum value was observed at D1 (4.56%). This increase in total soluble solids during maturity stages and shelf life period is a marked characteristic in black raspberry. It is a fact that mature fruits have accumulated high amount of total soluble solids which results in increase in sensory characteristics and nutritional value. Sequentially, early stages of maturity of fruits showed lower enzymatic and metabolic activities and low amount of soluble sugars accumulation which is due to low production of ethylene [37]. In support of this argument, a study conducted by Lopez et al. [10] found a similar trend in Cape gooseberries, where higher increase in total soluble solids in ripe fruits was observed as compared to fruits harvested at earlier stage of maturity. Horvitz et al. [1] also found similar results when they harvested Andean blackberries (*Rubus glaucus* Benth) at two maturity stages and stored them at room temperature (18 ± 2 °C) and cold storage (8 ± 1 °C) conditions. It was obvious in that study that more mature fruits showed lower values of acidity and higher values of total soluble solids, total anthocyanins and sensory scores as compared to the fruits harvested earlier.

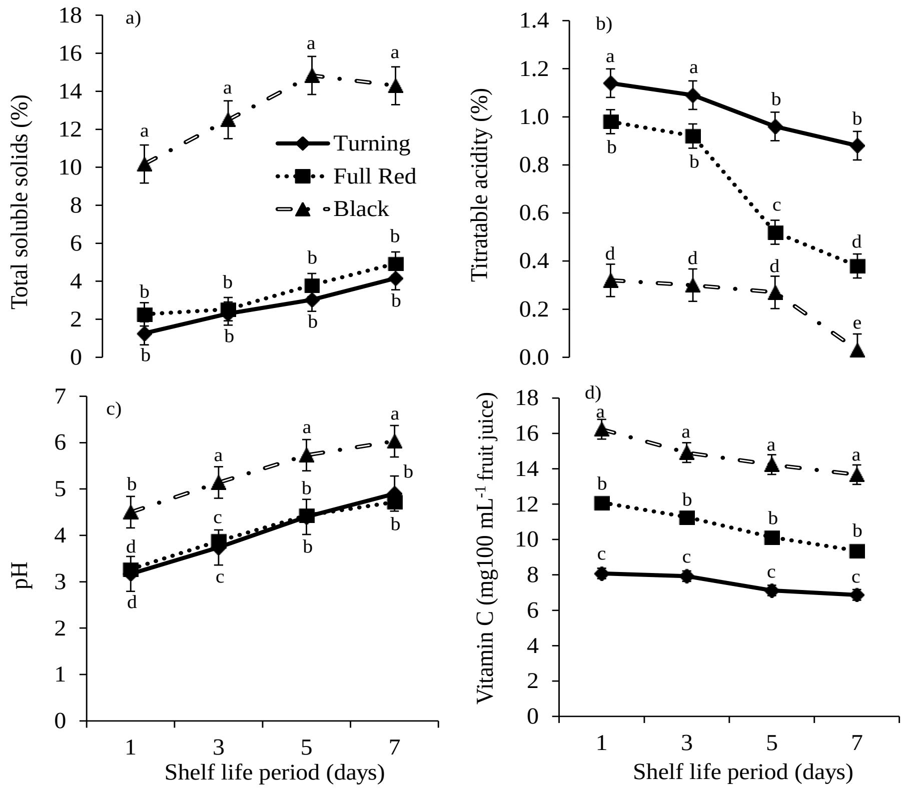

Figure 2. Effect of fruit maturity and shelf life period on a) total soluble solids, b) titratable acidity, c) pH and d) vitamin C in wild black raspberry (*Rubus* sp.) fruits. Vertical bars indicate average ± standard error (n = 3).

Titratable acidity in black raspberry fruits was the highest in fruits harvested at turning stage (1.01%) followed by fruits harvested at red stage (0.70%) whereas the lowest titratable acidity was determined in fruits harvested at black stage (0.23%) (Figure 2b). Titratable acidity gradually declined in raspberries harvested at any maturity stage during shelf life period. Rate of decrease was slowest in raspberries at turning stage whereas raspberries harvested at full red stage exhibited fastest decline. In contrast, raspberries harvested at black stage exhibited decline in titratable acidity during later stage of shelf life period. During storage, maximum value was observed at D1 (0.81%), which was followed by D3 (0.77%), while minimum value was observed at D7 (0.42%). Titratable acidity levels decreased with advancement in fruit maturity as the organic acids are converted into sugars [38]. Fruit, during early stage of development, have higher amount of organic acids which are available for respiration during long-term storage [38].

Black raspberry fruits harvested at black stage had highest pH (5.35) whereas minimum pH value was observed in black raspberry fruits harvested at turning stage (4.05) (Figure 2c). During storage, fruit pH increased with advancement in storage period. Maximum pH was recorded at D7 (5.21), while minimum pH value was observed at D1 (3.64). Observed increase in fruit pH potentially can be attributed to increase in total soluble solids and decrease in titratable acidity levels. The decrease in acidity can be attributed to the use of organic acids as respiratory substrates [38]. Tosun et al. [39] also reported increase in blackberry pH with progression in maturity. Similarly, Françoso et al. [40] reported that pH and acidity of strawberries were significantly affected only by storage time, when treated with different doses of Co-60 gamma irradiation and stored at 4 °C for 29 days. They observed a significant increase in pH and a decrease in acidity.

Raspberries harvested at black stage exhibited the highest level of vitamin C (14.77 mg 100 mL^{-1} fruit juice) whereas raspberries harvested at turning stage had the least concentration of vitamin C (7.50 mg 100 mL^{-1} fruit juice) (Figure 2d). Ascorbic acid in raspberries harvested at any maturity stages showed gradual decline during shelf life period. Overall, maximum concentration of vitamin C was recorded at D1 (12.14 mg 100 mL^{-1} fruit juice) which decreased with increase in shelf life period and minimum vitamin C concentration in black raspberry fruits was observed at D7 (9.97 mg 100 mL^{-1} fruit juice). Besides many other essential nutrients which human body require but cannot synthesize, includes vitamins and the most important among them is vitamin C which not only strengthens the immune system but also acts as antioxidant and protects against cardiovascular diseases [41]. Vitamin C is considered the most prone to loss and changes to a less active form during storage at any temperature [42]. Similar decreasing trend in vitamin C concentration in different fruits was reported during storage by Abeysuriya et al. [43].

3.3. Antioxidant properties

Maximum concentration of total flavonoids was observed in fruits harvested at black stage (15.35 mmol quercetin 100 g^{-1} FW), while total flavonoids concentration was minimum in fruits harvested at turning stage (7.81 mmol quercetin 100 g^{-1} FW) (Figure 3a). Moreover, total flavonoids in black raspberry gradually decreased during shelf life period, from D1 (12.41 mmol quercetin 100 g^{-1} FW) to D7 (10.81 mmol quercetin 100 g^{-1} FW). Similar trends were reported in Ivorian Gnagnan berries by Dri et al. [44] who found increase in flavonoids as ripening progressed but also reported gradual decrease in flavonoids during storage. This decrease in flavonoids could be ascribed to high respiration rate and tissue degradation [45].

Total phenols concentration in black raspberry fruits significantly differed at different harvesting stages ($P \leq 0.05$), and was maximum in fruits harvested at black stage (4.96 µg of gallic acid 100 g^{-1} FW), while minimum in fruits at turning stage (1.45 µg of gallic acid 100 g^{-1} FW) (Figure 3b). During storage, a decreasing trend was observed in total phenols as the maximum value were recorded at D1 (3.63 µg of gallic acid 100 g^{-1} FW), and minimum value was observed at D7 (2.73 µg of gallic acid 100 g^{-1} FW).

Raspberries are considered rich fruits in terms of their chemical composition as they possess a variety of vitamins, minerals and polyphenols [16]. Polyphenols are responsible for sensory and nutritional attributes in plants. As they are present in significant quantities in plant based food products, therefore, it has been reported in many studies that their continuous consumption has reduced the risk of many serious human diseases [46,47]. These results were supported by previous findings of Dri et al. [44] who also observed decreasing trend in total phenols during storage. This decreasing trend in total phenols could be the result of breakdown of acids, decreasing level of ascorbic acid and formation of glucose and fructose [48]. Another study [49] found that concentrations of total phenols, flavonoids, anthocyanins and antioxidants of five strawberry cultivars varied significantly depending on cultivar and harvest time. In case of phenolics, the highest quantity was recorded in strawberry cultivars at early harvest time while the lowest quantity of total anthocyanins was reported at the third harvest time.

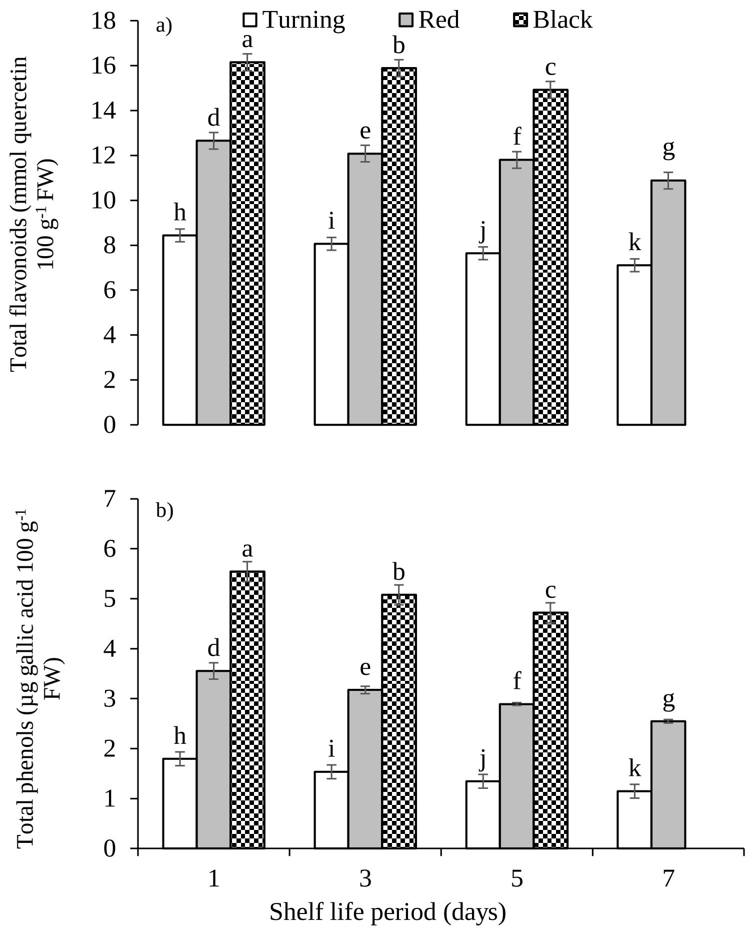

Figure 3. Effect of fruit maturity and shelf life period on a) total flavonoids (mmol quercetin 100 g^{-1} FW) and b) total phenols (μg of gallic acid 100 g^{-1} FW) in wild black raspberry (*Rubus* sp.) fruits. Vertical bars indicate average ± standard error (n = 3).

Results regarding antioxidant activity of black raspberry showed that maximum value was observed in fruits which were harvested at black stage (14.27 mg $FeSO_4$ 100 g^{-1} FW), while minimum value was recorded in fruits at turning stage (7.74 mg $FeSO_4$ 100 g^{-1} FW) (Figure 4a). Similar findings were shared by Wang and Lim [50] who observed increase in antioxidants with maturity. Results regarding storage showed that maximum antioxidant activity was recorded at D1 (11.63 mg $FeSO_4$ 100 g^{-1} FW), while minimum antioxidant activity was observed at D7 (9.89 mg $FeSO_4$ 100 g^{-1} FW). Fawole and Opara [51] also reported decreasing trend in antioxidant activity of pomegranates during storage.

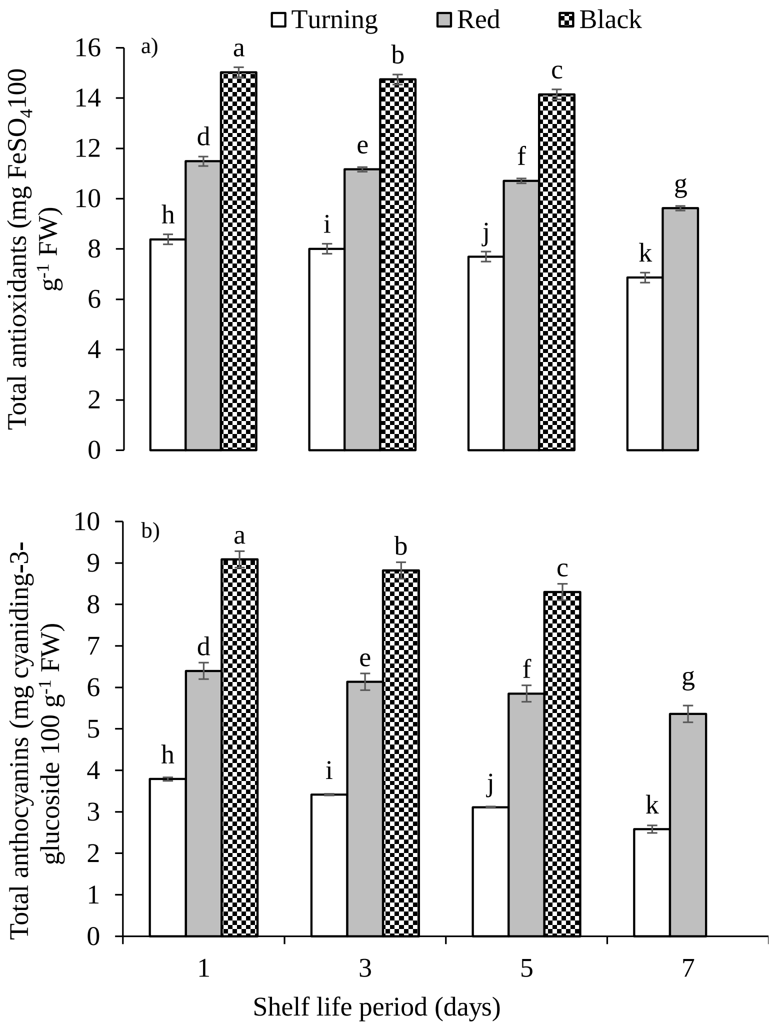

Figure 4. Effect of fruit maturity and shelf life period on a) total antioxidants (mg $FeSO_4$ 100 g^{-1} FW) and b) total anthocyanins (mg of cyaniding-3-glucoside 100 g^{-1} FW) in wild black raspberry (*Rubus* sp.) fruits. Vertical bars indicate average ± standard error (n = 3).

Anthocyanins are naturally produced bioactive compounds which are abundantly found in fruits and vegetables and are considered beneficial for human health. These days, they are considered effective against the most common human diseases which include cardiovascular and cancer [52–55]. Maximum total anthocyanins were determined in fruits harvested at black stage (8.50 mg of cyaniding-3-glucoside 100 g^{-1} FW), while least amount of anthocyanins was recorded in fruits harvested at turning stage (3.22 mg of cyaniding-3-glucoside 100 g^{-1} FW) (Figure 4b). In a previous study by Wang and Lim [50], it was found that total anthocyanins increased as the maturity progressed. Storage study revealed maximum total anthocyanins at D1 (6.42 mg of cyaniding-3-glucoside 100 g^{-1} FW), while minimum value was observed at D7 (5.25 mg of cyaniding-3-glucoside 100 g^{-1} FW). Similar trends in total anthocyanins were obtained by Fawole and Opara [51] when they stored pomegranates which were harvested at five different maturity stages.

3.4. Sensory properties

Sensory evaluation of black raspberry gave maximum taste score to fruits harvested at black stage and at D1 (9.00), which decreased with the passage of time during storage and after 7 days of shelf life it reached to a minimum level (2.33). While in case of fruits harvested at turning stage (6.67) and full red stage (8.00), fruits attained good taste scores after 7 days of storage, respectively. Similar, results were observed for texture, flavour and overall acceptability of black raspberry fruits harvested at different stages of maturity and a shelf life period of 7 days (Figure 5).

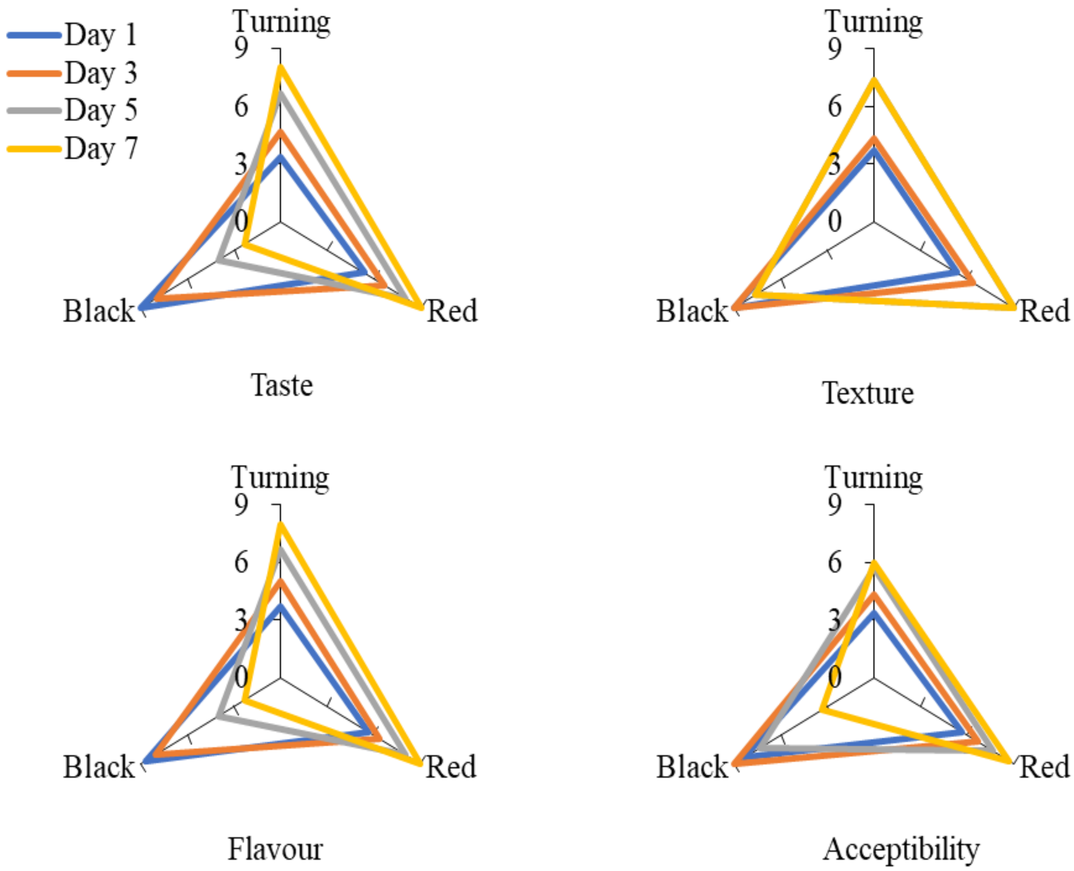

Figure 5. Effect of fruit maturity and shelf life period on sensory properties (score) of wild black raspberry (*Rubus* sp.) fruits. Vertical bars indicate average ± standard error (n = 3).

4. Conclusions

This study concludes that as the maturity progressed from turning to black stage, fruit size, fruit weight, total soluble solids, pH, vitamin C, total soluble solids, total flavonoids, total phenols, total antioxidants and total anthocyanins increased. During shelf life period, total soluble solids and pH increased, however, with increase in storage time fruit weight and firmness, total anthocyanins, flavonoids and phenols decreased. It could be recommended that wild black raspberry fruits should be harvested at black maturity stage for fresh consumption and at full red stage for longer storage and extended transportation. For sustainable production on commercial scale, further studies are needed on growth and yield of wild growing black raspberries.

Author contributions

Sana Hayyat: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft; Mehdi Maqbool: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing; Abdul Hamid: Funding acquisition, Project administration; Muhammad Shehzad: Data curation, Formal analysis, Supervision; Raheel Anwar: Formal analysis, Validation, Writing – original draft; Sandra Horvitz: Formal analysis, Validation, Writing – original draft; Noosheen Zahid: Conceptualization, Data curation, Formal analysis; Muhammad Azam Khan: Resources, Visualization, Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

Use of Generative-AI tools declaration

The authors declare that they have not used artificial intelligence tools in the creation of this article.

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Conflict of interest

The authors state that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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