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STORAGE STABILITY OF BIOACTIVE COMPOUNDS IN PROCESSED MULBERRY PRODUCTS FROM TWO SPECIES

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ABSTRACT

This study was conducted to investigate the sensory characteristics, bioactive compounds and free radical scavenging activity of value added products namely crush, syrup and leather prepared from two mulberry species and the changes were determined over a period of 8 months of storage. Among the processed products, leather from black mulberry whereas crush and syrup from red mulberry showed the highest overall acceptability based on the sensory scores. Leather from black mulberry had 65-67% higher total phenolics, 18-33% higher flavonoids than crush and syrup whereas crush from black mulberry had 25-31% higher anthocyanins than syrup and leather. Ascorbic acid content was higher in crush prepared from black mulberry than red mulberry. The study revealed that leather retained 19-23% more antioxidant activity than syrup and crush. The results suggest that processing mulberry into products enhances their utilization and offer a potential health promoting alternative that can be stored upto four months without substantial quality loss.

Keywords: Anthocyanins, Free radical scavenging activity, Flavonoids, Sensory characteristics, Total phenols

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Mulberry (*Morus* spp.) is a fast growing, perennial and deciduous tree belonging to family Moraceae. Mulberry species having three different colours are known to have their origin in eastern and central China (*Morus alba*), North America (*Morus rubra*) and western Asia (*Morus nigra*) (Watson and Dallwitz, 1999). In recent years, food industry has shown its interest in underutilized fruits rich in bioactive compounds resulting in their use as substitution for nutraceuticals and synthetic chemicals (Kaur *et al*., 2023). Mulberry is one such fruit which is rich in compounds like anthocyanins, flavonoids, polyphenols, essential amino acids and vitamins. These components render mulberry its ani-inflammatory, antioxidant and health promoting properties which make it favourable for functional food applications (Jiang and Nie, 2015). In spite of its nutritional and therapeutic potential, mulberry prevail neglected in conventional food processing and product development resulting in seasonal wastage and underutilization (Tewari *et al.,* 2023).

Processing mulberry fruits into value added products such as jam, jellies, syrup and juices can not only prolong their shelf life but also improve consumer acceptability and market value (Ercisli and Orhan, 2007; Gundogdu *et al*., 2011; Acharya *et al*., 2022). Globally, the utilization of mulberry fruits through different products is a common practice in developed nations (Meena *et al*., 2022). However, in India this versatile fruit is still missing its identity at commercial and industrial scale (Jan *et al*., 2021). The processing of fruits into final products leads to certain changes in their characteristics and nutritional components. Thus, ensuring the retention of nutritional and antioxidant properties during storage is important.

Given this, the present study was conducted with the aim of formulating and assessing different value added products from mulberry namely syrup, crush and leather with special emphasis on their nutritional and storage stability. This practical approach will not only promote the application of an underutilized fruit but also provide potential health oriented products with improved shelf life, supporting both nutritional security and economic sustainability.

MATERIALS AND METHODS

Sample collection

Fruits of red mulberry (*Morus rubra* L.) and black mulberry (*Morus nigra* L.) were collected from 10 randomly selected mulberry trees planted under natural agro-climatic conditions at Punjab Agricultural University, Ludhiana campus which lies at 30º 56' N latitude and 75º 52' E longitude. During two consecutive years (2022-24), mulberry fruits were manually harvested in April when they were ripe and moved right away to the Department of Food and Nutrition laboratory at Punjab Agricultural University, Ludhiana. The procured fruits were sorted based on ripening stage, physical quality, size uniformity and divided into two separate lots with the first one washed and dried immediately to store in the refrigerator at 4°C for the organoleptic evaluation of

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the fresh fruit. The second lot was immediately washed with 0.01% sodium hypochlorite and rinsed with distilled water to eliminate any microbial load and debris for the development of processed mulberry products with the determination of their bioactive compounds and antioxidant potential. All the processed products of mulberry were prepared on the same day and stored at refrigerated conditions at 4ºC for shelf life and antioxidant potential studies.

Chemicals and glassware

Analytical grade chemicals of purity ≥ 98-99% procured from Fischer Scientific, India and Hi-Media, India were used for the study. Laboratory glassware were cleaned thoroughly using 0.5% Labolene detergent solution rinsed with distilled water then dried in an oven at 130°C for 60 minutes.

Preparation and packing of mulberry products

Three products namely syrup, crush and leather were prepared from red (*M. rubra*) and black (*M. nigra*) mulberry species using the standardized protocols as per the FPO specifications.

Mulberry crush was prepared by using 1 kg of coarsely blended mulberry pulp, 2 kg sugar, 3 g citric acid. Sugar syrup was prepared by cooking at low flame with continuous stirring taking double amount of sugar as that of pulp, while water was taken in equal amount as that of pulp. Citric acid was added to the syrup and the scum formed was removed with a ladle. Finally, the blended mulberries were added to the sugar syrup and mixed well with further cooking at low flame for 2-3 minutes. The crush was then filled in 250 ml pre sterilized glass bottles leaving 2.5 cm headspace and stored at 4-8º temperature.

Mulberry syrup was prepared as per FPO specification using 25% fruit pulp, 65% TSS and 3.5 % citric acid. Mulberries (500 g) were finely blended into puree to strain out the juice and mixed with sugar syrup followed by cooking at low flame for 2-3 minutes. The syrup was then filled in 250 ml pre sterilized glass bottles leaving 2.5 cm headspace and stored at refrigerated conditions at 4°C for shelf life and antioxidant potential studies.

For the preparation of leather 800g mulberry was pureed in a food processor (Philips, Food processor HL1661700 Watts) until a smooth mixture was obtained followed by the addition of sugar (80 g) and citric acid (2 g) to it. This pureed mixer was then processed at low flame for 1-2 minutes and was spread into a thin layer in trays measuring 30 cm × 15 cm and 2 cm deep and put in a dehydrator set at 55°C for 6-7 hours until the moisture content reached the level of 15%. The fruit leather was then cooled and cut into pieces and the layers were scrapped off into rolls and packed in biaxially-oriented polypropylene (BOPP) films having moisture vapor transmission rate (MVTR) of 4 x 10^{-3} kg/m^2/d at 38°C, 90% RH with an oxygen transmission rate (OTR) of 2.5 L/m^2/d atmosphere at 25°C for shelf life and antioxidant potential studies. A set of three replicates of each fruit product sample was taken for studying the antioxidant and storage potential of the processed products.

The prepared products were evaluated for bioactive compound and antioxidant activity after 2, 4, 6 and 8 months of storage.

Sensory evaluation tests

A semi-trained sensory panel consisting of 30 members including faculty, postgraduate and research scholars from the Department of Food and Nutrition, Department of Food Science and Technology and Department of Fruit Science conducted sensory evaluation tests on standardized products. These tests were based on Hedonic test method (Meilgaard *et al*., 2007) and the panelists were oriented prior to the sensory tests. The products from different varieties were coded and presented to the panelists along with proforma randomly to avoid each others' influence on the rating. The products prepared from red and black mulberry were scored by the panelists for appearance, aroma, taste, texture and overall acceptability using nine point hedonic scale. The scoring for liking of the product was given as 9, 8, 7, 6, 5, 4, 3, 2 and 1 points, to express: like extremely, like very much, like moderately, like slightly, neither like nor dislike, dislike slightly, dislike moderately, dislike very much and dislike extremely, respectively.

Extract preparation

For each product, three independent extractions were carried out according to the method described by El-Hadary and Taha (2020) with slight modification. One gram of each sample was extracted with 5 ml of 80% methanol containing 0.1% (v/v) formic acid for 15 min. The treated samples were centrifuged for 5 min at 10,000 rpm and the supernatant was collected. The volume was made to 20 ml with 80% methanol and the prepared extracts were stored at -20ºC until analysis.

Total monomeric anthocyanins (TMA)

UV-vis spectrophotometer (Model T60U, PG Instruments) was used to determine total monomeric anthocyanins by a pH differential method (Giusti and Wrolstad, 2001). Ten millilitres of 1% hydrochloric acid was used to extract 2 gm of product sample for two hours and the mixture was centrifuged for twenty minutes. Total monomeric anthocyanin was calculated

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using the given equation 1. TMA was expressed as mg cyanidin-3-glucoside equivalent/100g fresh weight.

$$A = [(AA - AB) \text{ at pH } 1.0 - (AA - AB) \text{ at pH } 4.5] \quad (1)$$

where,

A = absorbance of extract

AA = absorbance at 510 nm

AB = absorbance at 700 nm

Total flavonoids content (TFC)

The aluminium chloride colorimetric method as described by Josipović *et al.* (2016) with slight modification was used to quantify the total flavonoid content. Two millilitres of product extract in a test tube was mixed with 2.8 ml of distilled water, 0.1 ml of potassium acetate (1M) and 0.1 ml of 10% aluminium chloride. For half an hour, the solution mixture was incubated at room temperature. At 415 nm, the solution's absorbance was measured and a standard curve of quercetin was made. TFC was expressed as mg of quercetin equivalent per 100 gm of sample.

Total phenolic content (TPC)

Total phenolic content in mulberry product samples extract was determined according to Han *et al.* (2018). Methanolic sample extract of 0.5 ml was mixed with 5 ml of 0.2 mol/L Folin-Ciocalteu reagent. After 5 min, 4 ml of 7.5% saturated sodium carbonate solution and 5 ml of deionized water were added. For two hours, the mixture was incubated in the dark at room temperature. At 765 nm, the reaction mixture's absorbance was measured and a standard curve of gallic acid solution was made. TPC was expressed as mg of gallic acid equivalent (GAE) per 100 g of sample.

Ascorbic acid content (AAC)

Ascorbic acid content was estimated using 6 per cent metaphosphoric acid solution (20 ml) added slowly to 5 grams of product sample and diluted to 100ml in a volumetric flask using metaphosphoric acid solution. 10ml of this extract was taken in a conical flask and titrated against 2, 6-dichlorophoenol indophenol dye till the pink colour appeared which persisted at least for 15 seconds. A standard ascorbic acid solution was prepared by dissolving 100 mg of pure ascorbic acid in 100 ml of 6% metaphosphoric acid. The amount of dye used in the titration was determined volumetrically and used in the calculation of the ascorbic acid content mg/ 100ml of the product sample (AOAC, 2007).

Antioxidant activity

DPPH (1, 1-diphenyl-2-pierylhydrazyl) method as outlined by Brand-Williams *et al*. (1995) was employed to estimate each product sample's capacity to scavenge free radicals. In a test tube, 0.1 ml of methanolic extract was mixed with 2.9 ml of DPPH solution. After giving the mixture a good shake, it was left in the dark for half an hour. At 517 nm, the absorbance of the resultant solution was measured in comparison to a blank. Equation 2 was used to calculate the percentage inhibition.

$$\% \text{ Inhibition} = AB - AA/AB \times 100 \quad (2)$$

where, AA = absorbance of product samples

AB = absorbance of the blank sample

The DPPH radical scavenging activity was calculated as a percentage of DPPH discolouration.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using SAS (Statistical Analysis System) 9.3 (2011) and the statistical significance of mulberry genotypes was compared by considering least significant difference (LSD) values at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Sensory characteristics of mulberry products during storage

Crush

Crush from berry fruits is becoming popular as it has been used to make various kinds of shakes, smoothies and diluted drinks. Among the fresh products, crush of *M. rubra* scored more for appearance (8.3) and aroma (8.0) in comparison to *M. nigra* (7.9 and 7.5, respectively). Both varieties scored similar for taste indicating non-significant difference. In regard of overall acceptability, *M. rubra* (8.2) was preferable to *M. nigra* (7.7).

During storage, both varieties showed a decline in sensory scores with increasing storage period (Fig 1A & 1B). Flavour and taste score showed the most significant decrease suggesting the influence of oxidative reaction, volatile losses, and potential acid-sugar changes during storage. Better stability was shown in *M. rubra* crush with a final score for flavour and overall acceptability remaining above 7.2. The decline was sharper in *M. nigra* with the score of flavour, taste, colour and overall acceptability dropping to 6.4, 6.6, 6.8 and 7.0, respectively by the end of storage. Similarly, Bishnoi *et al.* (2016) highlighted that the organoleptic scores of crush and jam decreased during storage but still remained in the acceptable range even after three months of storage at room temperature. Crush prepared from *M. rubra* outperformed *M. nigra* in sensory attributes and storage stability due to its high sugar content and balanced acidity. The product

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retained its sensory attributes and was acceptable upto 4 months making it fit for medium term storage under refrigeration.

Syrup

Fruit syrups are used in various cuisines as well in flavoring of ice creams, smoothies, puddings and cakes. The sensory screening of syrup showed that syrup prepared from both the varieties was acceptable during storage however the extent of stability varied (Figure 1C and D). The colour score for syrup prepared from *M. nigra* initially was 7.8 and had a gradual decline during storage showing pigment degradation over time. Slight decline was also observed in taste and flavour falling to around 7.0-7.2 with an overall acceptability of 7 by the end of fourth month. In contrast, *M. rubra* syrup displayed superior sensory retention during storage. Colour, taste and flavor score remained stronger by the end of fourth month (8.6, 7.5 and 7.8, respectively) with an overall acceptability of 8 which suggests better stability of sugars, acids and aromatic compounds. The results indicated that while both varieties of mulberry are suitable for syrup, *M. rubra* is more preferable due to its longer storage stability and better retention of sensory attributes.

Leather

Fruit leather is a nutritionally dense product prepared from fruit pulp which can be consumed as a snack or dessert (Alam *et al*., 2024). Different drying methods have been verified by Suna and Ozkan-Karabacak (2019) for pestil (leather) with microwave drying to be the most feasible method due to its reduced drying time and enhanced bioactive content.

Leather prepared from *M. nigra* had a higher colour score of 8.8 and remained stable during the storage (Fig. 1E and F). A good stability for texture and taste was observed with a consistent score above 8, while slight decline was observed in flavour. Throughout the storage overall acceptability remained above 8, reflecting its stability. In contrast, leather from *M. rubra* showed slightly lower sensory scores with colour score ranging from 8.4-8.6. Texture and taste had a gradual decline and flavour was the most affected parameter (7) by the end of storage. Mulberry leather known as mulberry pestil in Turkey with different variations was evaluated by Yildiz (2013), where leather with mulberry alone as well as with addition of hazelnuts or walnuts was prepared showing that the leather produced from mulberry alone had higher total antioxidant activities than other products prepared with the addition of walnut and hazelnuts which exhibited higher protein content. The comparative study reveals that *M. nigra* leather showed higher stability in terms of colour, texture and overall acceptability.

Bioactive components and antioxidant potential of fresh mulberry products and changes during storage

Ascorbic acid content (AAC)

The ascorbic acid in freshly prepared mulberry products namely crush, syrup and leather from red (*Morus rubra*) and black (*Morus nigra*) mulberry varied from 21.67 to 27.82 mg/100g (Table 1). Among the products prepared from black mulberry, crush had the highest ascorbic acid (27.82 mg/100g) content followed by leather (27.39 mg/100g) and syrup (26.10 mg/100g). On the other hand, crush prepared from red (*Morus rubra*) species had the highest ascorbic acid (26.43 mg/100g) followed by syrup (22.82 mg/100g) and leather (21.67 mg/100g). Lucia *et al.* (2016) analyzed the ascorbic acid content of products derived from black mulberry which ranged from 0.27 mg/100g in wine prepared from mulberry to 1.10mg/100g in fruits dipped in honey without the sterilization process. It was further established that increased cooking time or thermal exposure to product preparation decreased the amount of ascorbic acid than in unprocessed foods. The variability in ascorbic acid in these products and those analyzed in the present study may be due to the genetic variability between the fruits grown in different geographical areas. Gundogdu *et al.* (2017) in their study showed that the biochemical content and antioxidant activity of the genotype and cultivars were significantly different from one another.

There was a significant ($p \leq 0.05$) and gradual reduction in ascorbic acid content during the storage period in all the three products (Fig. 2). However, among the different products prepared from red and black mulberries, significantly higher ascorbic acid content was retained in crush and syrup even after 4 months of storage as compared to leather. Among the two varieties black (*Morus nigra*) mulberry maintained higher ascorbic acid content as compared to red (*Morus rubra*) mulberry throughout the storage period of the processed products. It was observed that sufficient amount of ascorbic acid (>16 mg/100g) can be retained up till 6 months of storage in crush and syrup prepared from black (*Morus nigra*) mulberries which can still satisfy sufficient recommended dietary allowance (RDA) for vitamin C which is 75-90 mg/day.

Degradation of 11 to 12% in total ascorbic acid content during the storage of sea buckthorn berry juice for 7 days at cold temperature (6ºC) was observed by Gutzeit *et al.* (2008). Ascorbic acid being an antioxidant gets readily oxidized into dehydroascorbic acid and further to diketogluconic acid thus reducing its content in stored products (Steskova *et al.,* 2006).

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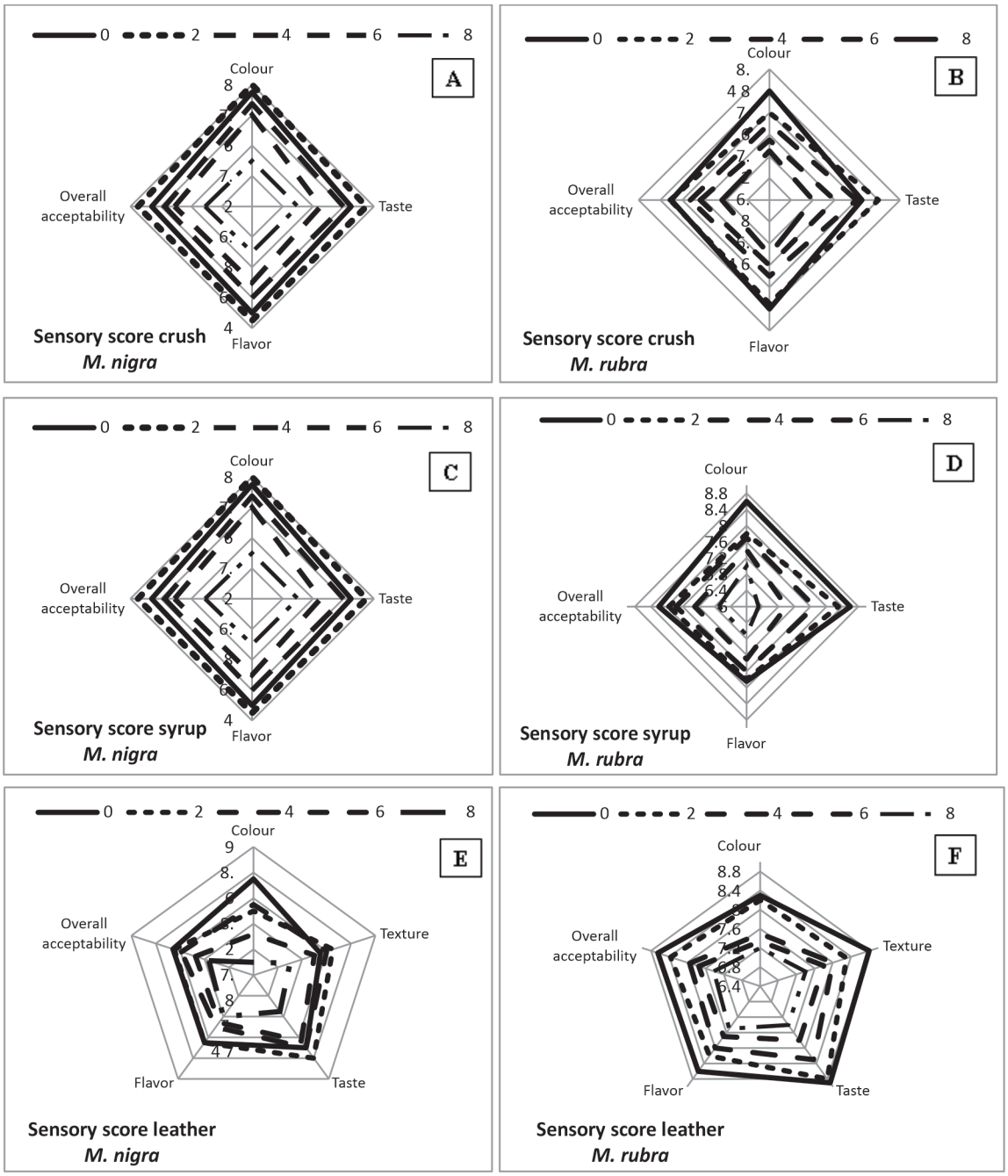


Fig. 1. Changes in sensory properties of mulberry products prepared from different species during 0, 2, 4, 6 and 8 months of storage

Total phenolic content (TPC)

Among the products prepared from fresh mulberries of two different varieties, significantly higher phenolic content was recorded in leather (2350.84 mg GAE/100g) prepared from black (*Morus nigra*) mulberry followed by red (*Morus rubra*) mulberry leather (1293.30 mg/100g).

When the two varieties were compared for total phenols in the selected products, a significant ($p \leq 0.05$) difference in phenols was observed. The highest phenols were found in black (*Morus nigra*) mulberry products (766.29 to 2350.84 mg GAE/100g) followed by red (*Morus rubra*) mulberry products (405.53 to 1293.30 mg GAE/100g). Okatan (2020) also observed significant variation in phenolic contents of the different mulberry genotypes. Kamiloglu *et al.* (2013) studied the biochemical composition of black mulberry products and found the total phenols to be 976.40, 266.80 and 108.70, 25 mg GAE/100 g for dried mulberry, mulberry juice, jam and syrup, respectively. While for products

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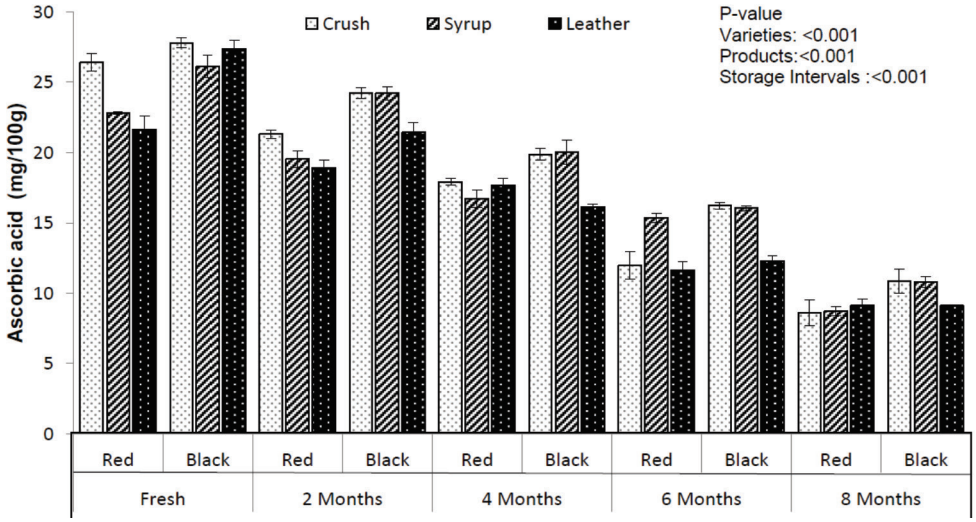


Fig. 2. Changes in ascorbic acid content (mg/100g) of mulberry fruit products prepared from two different species during storage

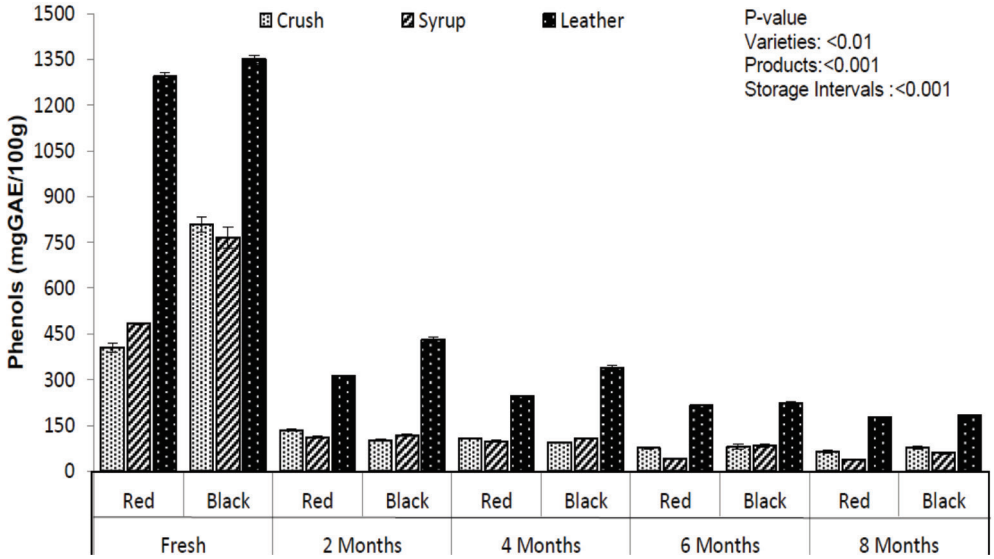


Fig. 3. Changes in total phenolic content (mg GAE/100g) of mulberry fruit products during storage prepared from two different species

like mulberry wine, mulberry molasses and ice cream, the value for total phenols varied from 377.90 to 737.30 mg GAE/100g.

The total phenols in freshly prepared crush, syrup and leather from black (*Morus nigra*) mulberry was 808.73, 766.29 and 2350.84 mg GAE/100g, respectively. The corresponding value for red (*Morus rubra*) mulberry was 405.53, 483.34 and 1293.30 mg GAE/100g. There was a significant ($p \leq 0.05$) and gradual reduction in total phenols during eight months of storage period in all the three products (Fig. 3). Among the different mulberry products kept for the storage studies, significant amount of phenols were retained in crush and syrup till 4 months of storage while the leather maintained higher phenolic content till 6 months of storage. Among red (*Morus rubra*) and black (*Morus nigra*) mulberries, black (*Morus nigra*) mulberries retained higher phenolic content throughout the storage period. The fluctuation in the phenolic content of six dark fruit juices of currant, cranberry, blueberry, pomegranate, strawberry and cherry over the course of 29 days in storage at 4ºC was studied by Piljac-Zegarac *et al.* (2009). A significant increase in total phenols content in all juices was observed after 48 hours and thereafter a gradual decrease in total phenols was observed with each passing day. The drying treatment involved in the

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Table 1. Bioactive compounds and antioxidant potential of different processed products prepared from two species of mulberry

Processed product	Mulberry species	Ascorbic acid (mg/ 100g dw)	Total phenols (mg GAE/ 100g dw)	Flavonoids (mg QE/ 100g dw)	Anthocyanins (mg/100g dw)	Antioxidant activity (%)
Crush	*M. nigra*	27.82^{a}±0.34*	808.74^{bc}±23.19	175.35^{b}±0.86	28.92^{a}±0.12	49.46^{c}±0.54
	M. rubra	26.43^{a}±0.61	405.53^{c}±14.15	171.20^{b}±1.04	13.07^{c}±0.40	46.12^{d}±2.42
Syrup	*M. nigra*	26.10^{a}±0.80	766.29^{c}±33.74	143.81^{bc}±0.20	21.48^{b}±0.06	51.87^{b}±0.27
	M. rubra	22.82^{b}±0.11	483.34^{c}±3.54	124.30^{c}±1.05	14.48^{c}±2.44	48.69^{c}±2.70
Leather	*M. nigra*	27.39^{a}±0.65	2350.84^{a}±12.75	214.92^{a}±8.81	19.82^{b}±0.36	64.77^{a}±0.22
	M. rubra	21.67^{b}±0.96	1293.30^{b}±12.25	196.87^{a}±1.48	16.61^{bc}±0.26	49.87^{c}±0.27

*Mean±SE
Within a column, means followed by different alphabets differ significantly at p<0.05

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preparation of leather (pestil) caused an increment in total phenols and antioxidant capacity with the highest results of 209.14 mg GAE/100 g dw for total phenols and 181.37 µ mol TE/100 g dw for antioxidant capacity by 180 W microwave heating treatment (Suna and Ozkan-Karabacak, 2019).

Flavonoids

Among the processed products from the black (*Morus nigra*) and red (*Morus rubra*) mulberry, a significant (p≤0.05) difference in flavonoid content was observed (Table 1), the highest being in products prepared from black mulberry. Leather from black (*Morus nigra*) mulberry had a significantly higher flavonoids content than other products (p≤0.05) whereas, syrup and crush had the lower values of 143.81 and 175.35 mg QE/100g, respectively. A similar trend was observed in products prepared from red (*Morus rubra*) mulberry, with a significantly (p≤0.05) higher flavonoids in leather followed by crush and syrup. Kamiloglu *et al*. (2013) reported that the total flavonoid contents of dried mulberry, mulberry juice, mulberry jam and mulberry syrup produced from black mulberry species were found to be 380.0, 205.7, 91.8 and 4.7 mg CE/100g, respectively. In other products namely wine, molasses and ice cream the range for flavonoids was 84.8-751.1 mg CE/100g.

There was a significant (p≤0.05) and gradual reduction in flavonoids during eight months of storage period for the processed products (Fig. 4). The reduction in flavonoids in crush, syrup and leather prepared from black (*Morus nigra*) mulberry was 175.35, 143.81 and 214.92 mg QE/100g in fresh state to 7.45, 6.33 and 55.65 mg QE/100g after eight months of storage, respectively. Among the red (*Morus rubra*) and black (*Morus nigra*) mulberries used for processing, black mulberries retained higher flavonoids throughout the storage period. Among the different mulberry products kept for the storage studies, significant amount of flavonoids were retained in leather till 6 months of storage whereas crush and syrup maintained sufficient flavonoids content till 4 months of storage. Morin (3, 5, 7, 2, 4-pentahydroxy flavone) is a flavonoid that has been identified in mulberry fruit which is reported to be high in antioxidant and anti-inflammatory potential. Quantitative differences in the concentration of flavonoids among the different mulberry varieties observed were in accordance with previous studies reporting higher total phenolics and flavonoids in black mulberries (Ercisli and Orhan, 2007). Moreover, the genotypic characteristics as well as the processing conditions or protocols are usually responsible for the variation in phenolics and flavonoids among the varieties as well as the products prepared from them as their metabolic pathways are highly responsive to these factors (Pehluvan *et al.,* 2015). The effect of domestic processing and storage on flavonols in lingoberry, black currant, bilberry, strawberry and raspberry showed a considerable loss of 40% quercetin in traditional preservation of crushed lingo berries in their own juice as well as a significant reduction in quercetin over 40% was seen in bilberries and lingoberries during 9 months of storage at -20ºC (Hakkinen *et al.,* 2000). Processing of mulberry jam reduced the total phenolics (88%), total flavonoids (89%), anthocyanins (97%) and antioxidant capacity (88-93%) but the fruit phenolic components may be preserved by food processing optimization which could assist the food sector reduce antioxidant loss and enhance the quality of its finished products (Tomas *et al.,* 2017).

Anthocyanins

The appealing colour of black and red mulberry is bestowed to the presence of anthocyanins. Hence, as expected, a significantly higher anthocyanins were found in black (*Morus nigra*) (54.79 mg/100g) as compared to red (*Morus rubra*) (28.97 mg/100g) mulberries (p≤0.05). The anthocyanins content of the products from black

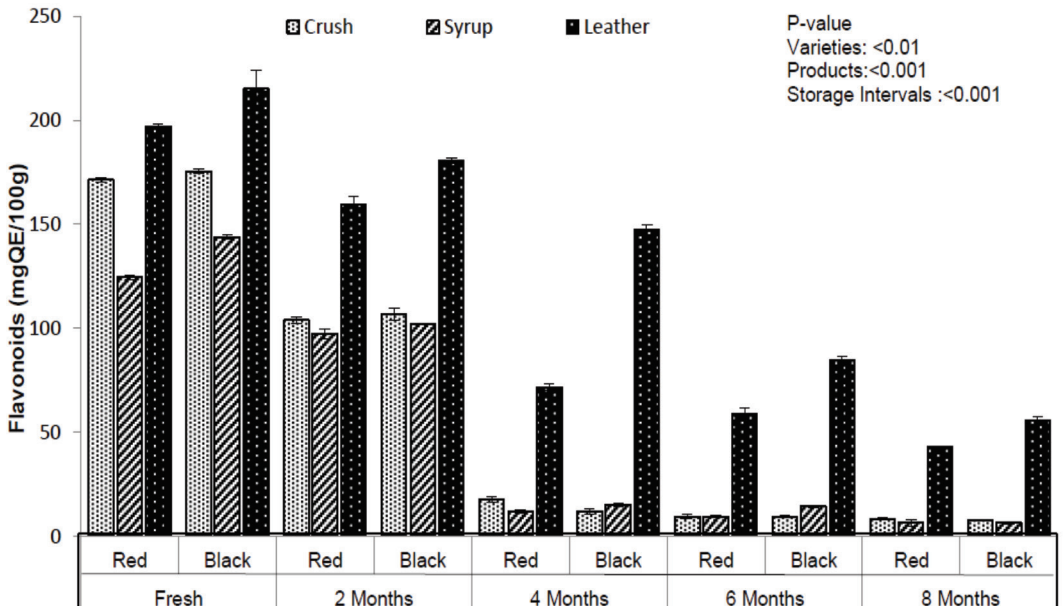


Fig. 4. Changes in flavonoids (mg QE/100g) of mulberry fruit products prepared from two different species during storage

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(*Morus nigra*) mulberry was highest in crush (28.92 mg/100g) followed by syrup (21.48 mg/100g) and leather (19.82 mg/100g). On the other hand, among the products prepared from red (*Morus rubra*) mulberry, a significantly higher anthocyanins were found in leather (16.61 mg/100g) ($p \leq 0.05$). The products namely, crush (13.07 mg/100g) and syrup (14.48 mg/100g) had a significantly ($p \leq 0.05$) lower anthocyanins. The total anthocyanins as reported by Kamiloglu *et al.* (2013) for different products made from black mulberry varied from 0.1-1221.0 mg C3G/100g. The values for dried mulberry, mulberry juice, mulberry jam and syrup were 61.3, 19.2, 4.0 and 0.1 mg C3G/100g, respectively. The values for mulberry wine, molasses and ice cream varied from 2.4-173.1 mg C3G/100g. Similarly, Lucia *et al.* (2016) observed variation in anthocyanins content in black mulberry products ranging from 21.4 to 106.4 mg/dm^3. The literature reports highlighted the importance of mulberry processing to obtain appreciable levels of anthocyanins which are considered as an effective antioxidant compound found in fruits. There was a significant and gradual reduction in anthocyanins during eight months of storage period of all the three products ($p \leq 0.05$) (Fig. 5). The anthocyanins in freshly prepared crush, syrup and leather from black (*Morus nigra*) mulberry were 28.91, 21.48 and 19.82 mg/100g, respectively. The corresponding value for red (*Morus rubra*) mulberry was 13.07, 14.48 and 16.61 mg/100g. The reduction in anthocyanins of crush, syrup and leather prepared from black (*Morus nigra*) mulberry ranged between 28.91 to 7.95, 21.48 to 7.08 and 19.82 to 7.63 mg/100g, respectively. The corresponding reduction for the products prepared from red (*Morus rubra*) mulberry ranged from 13.07 to 2.65, 14.48 to 2.72 and 16.61 to 5.94 mg/100g. Thus, it is clear from the studies that the maximum anthocyanins were retained in crush prepared from the black mulberries followed by syrup from black mulberries throughout the storage period. Sufficient amount of anthocyanins (>12 mg/100g) can be retained up till 6 months of storage in crush and syrup prepared from black (*Morus nigra*) mulberries.

Brownmiller *et al*. (2008) observed 28 to 59 per cent reduction in total anthocyanins content in blueberry products during the storage of 6 months. Similarly, Zou *et al*. (2017) reported 99 per cent decrease in total anthocyanins content of mulberry juice after 8 weeks of storage at 30°C. However, mulberry syrup maintains stable component levels during storage for 30 days which declined thereafter (Trung *et al.,* 2018).

Antioxidant activity

The presence of high amounts of bioactive compounds namely phenols, flavonoids and anthocyanins imparted a very high antioxidant activity to black mulberry species as compared to red mulberries and the processed products obtained from them. Among the products prepared from black (*Morus nigra*) mulberry, the highest antioxidant activity was in leather (64.77%). The least antioxidant activity was in crush (49.46%). The products prepared from red (*Morus rubra*) mulberries also maintained similar trend with a significantly higher antioxidant activity in leather (49.87%) in comparison to crush and syrup (46.12 and

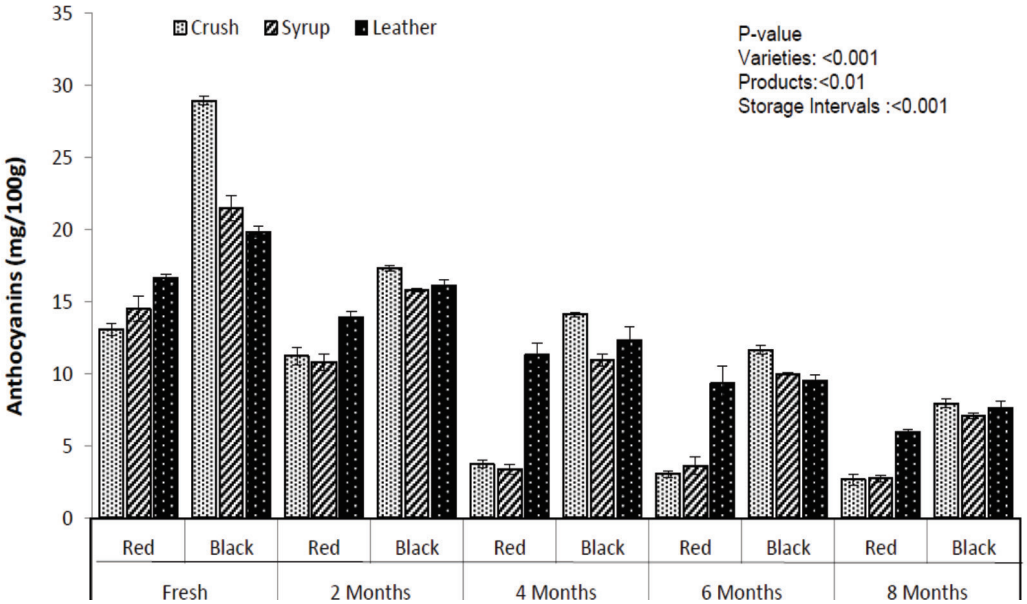


Fig. 5. Changes in anthocyanin content (mg /100g) of mulberry fruit products prepared from two different species during storage

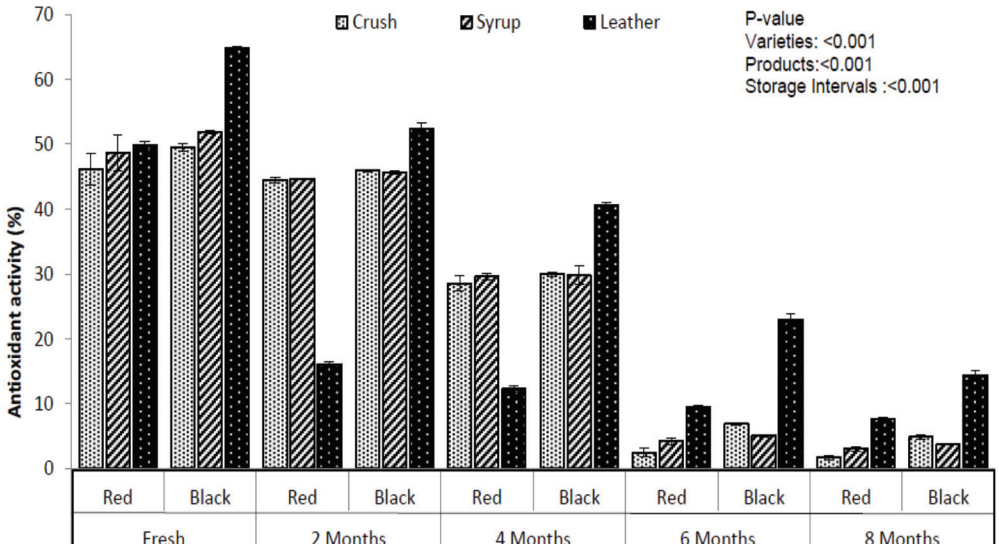


Fig. 6. Changes in antioxidant activity (%) of mulberry fruit products prepared from two different species during storage

48.69%) ($p \leq 0.05$). Kamiloglu *et al.* (2013) reported wide variation in antioxidant activity of mulberry products with DPPH values varying from 2114.70 in fresh mulberry, 580.0 mg TEAC/100g in dried mulberry, 313.40 mg TEAC/100g in juice, 94.0 mg TEAC/100g in jam and 10.8 mg TEAC/100g in syrup. Black (*Morus nigra*) mulberry was used for preparation of food products with high phytotherapeutic properties due to the presence of anthocyanins ranging from 21.4 in wine to 106.4 mg/ dm^{-3} in fresh juice with an accompanying antioxidant activity from 50.81 in wine to 74.02% in fruits sterilized in honey (Lucia *et al.,* 2016).

There was a significant and gradual reduction in antioxidant activity during eight months of storage period in all the three products ($p \leq 0.05$) (Fig. 6). The percent reduction in antioxidant activity after 6 months of storage as compared to freshly prepared black (*Morus nigra*) mulberry products varied from 65% in leather, 90% in syrup and 86% in crush whereas the corresponding values for products prepared from red mulberry were 81% in leather, 91% in syrup and 95% in crush. Thus it was clear that crush and syrup prepared from black (*Morus nigra*) and red (*Morus rubra*) mulberry can maintain sufficient antioxidant activity for 4 months

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whereas leather from these two varieties has a potential for storage up to 6 months with sufficient antioxidant potential as well as permissible organoleptic scores. Brownmiller *et al.* (2008) observed a reduction of 43 to 71% in free radical scavenging activity in blueberry products namely, juice, canned in water, canned in syrup and pureed blueberries. Singhal *et al.* (2010) produced different products of mulberry such as anti-obesity drink, mulberry fruit tea, sauce, cakes, fruit powder, fruit wine and food colorant.

All these products were found to be acceptable and development of such value-added products provides an effective way of enhancing the nutritional, functional, and sensory attributes while reducing the postharvest losses of mulberry fruit. Thus, industrial processing of mulberry to produce different range of products can result in growth of mulberry as a food crop apart from fodder crop for animals and in sericulture.

Authors' contribution

Conceptualization and design of experiment (KB, KKK); Execution of field/lab experiment (AT, KKK, HK); Analysis of data and interpretation (AT, KKK, KB); Preparation of manuscript (AT, KKK).

Conflict of interests

The authors declare that there is no conflict of interests.

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