DEVELOPMENT AND OPTIMIZATION OF BLACK MULBERRY RTS DRINK

Saima Parveen 1, Sharoon Masih 2, Bushra Ishfaq 3, Humaira Kausar 4, Shazia Saeed 5, Zafar Iqbal 6 and Muhammad Abrar 7

ABSTRACT

Present study was conducted during the year 2020 at the Food Technology Section of Postharvest Research Centre, Ayub Agricultural Research Institute, Faisalabad, Pakistan on the value addition of short seasoned indigenous fruit for the development of black mulberry RTS drink. The nutritional quality and effect of storage on the keeping quality of drink was evaluated. Drinks were developed by using different percentage of pulp in final ceipe, filtered, pasteurized and filled in glass bottles. The physicochemical characteristics of the drinks during shelf life were studied. Four drinks with different mulberry fruit juice percentage were prepared and stored them at ambient temperature. The total soluble solids were found to be 13.03, 13.60, 13.86 and 14.20 Brix in T1, T2, T3 and T4 respectively. During the three month storage periods TSS was reached up to 14.06, 14.13, 14.16 and 14.63 for T1, T2, T3 and T4, respectively. Reducing and total sugars % were increased and observed in the range of (7.20-7.47%), (7.65-7.89%), (7.75-7.89), (8.26-9.25%) and (12.52-12.68%), (13.20-13.69%), (13.68-14.98%), (14.52-15.4%) for T1, T2, T3 and T4 respectively. Non-reducing sugar percent was observed to be reduced from 5.32 to 5.21%, 5.89 to 5.63%, 6.23-6.11% and 6.26 to 6.20% for T1, T2, T3 and T4 respectively. During the three month storage period. Sensory evaluation showed all the treatments acceptable but highest scores for color, flavour, taste and overall acceptability were perceived in case of T4 with 25% fruit pulp at the end of storage period.

KEYWORDS: Mulberry fruit; nutritional drink; value addition; storage stability; sensory; evaluation; Pakistan

INTRODUCTION

Among various cultivars with white, purple and red colored fruits of herbaceous tree, i.e., mulberry (Morus nigra L.) is one of the most delicious fruits belonging genus Morus and family Moraceae. Black mulberry is well known for its nutritional qualities and flavor. Traditionally it is used in natural remedies due to high content of active compounds present (Imran et al., 2010). Mulberry fruits are well recognized for high antioxidant activity contributed by phytochemicals especially phenolics which functions as lipid peroxidation, atherosclerosis, anti-tumor, anti-inflammatory, antimicrobial, anti-diabetic glucosidase inhibitor in addition to its use as a conventional remedy to treat sore throat, cough, diabetes, hypertension, anemia and arthritis (Kutlu et al., 2011; Sass-Kiss et al., 2005). Its juice is used as health promoting tonic to boost immunity and metabolism and immunity. Vitamins and minerals present in mulberry fruits act as nutraceuticals to counter various chronic diseases. Its ingestion significantly contributes to assuage healthy life. Mulberry fruits contain high amount of bioactive compounds including mineral elements, vitamins, poly saccharides, fats and amino acids. Higher anthocyanins (9%) content from black mulberry showed greater bioavailability than raspberry (5.3%), sour berry (2.8%) and ruddy mulberry fruits (0.34%) (Liang et al., 2012). Anthocyanins are readily postprandially absorbed through plasma membrane in 15 to 50 min however it need 6 to 8 hours from ingestion to excretion (Hassimotto et al., 2008). Globally mulberry is exclusively used for silkworm feed; however, based on its planting locality, it may find multifarious uses including medicinal usage as infusions e.g. herbal tea from mulberry leaves, young stem and leaves consumed as delicious vegetable, appreciated as table fruit or used in preserve end juice preparation. It is also planted as landscape tree and popularly consumed in traditional animal feed comprising mixed forage ration for ruminants in
most growing localities. The sole processed form of mulberry fruit is syrup preparation which may further be utilized to contribute color and flavor to any medicine. The raw juice squeezed out from fresh mulberry fruits impart unmatched taste and fragrance. Juice also has numerous health promoting attributes like excellent nourishment, soothing effects, enriching blood, immunity booster, balance digestive secretions, boost alcohol metabolism and pacify nervous system (Kumar and Chauhan, 2008).

Marmalade, liquors, natural dyes, juices and fruits frozen used for ice cream are processed products made from black mulberry fruit. Fruit is available for short time maximum up to 2 months in fresh form; however, it can be stored up to six weeks maximally under refer storage. Black mulberry fruit processing is therefore of great importance to increase shelf stability (Fazaeli et al., 2013). Different researchers reported variability in sugar level among ripe fruits of black mulberry i.e., 9% (Ahmad and Sadozai, 2003) however (Ercisli and Orhan (2007b) found sugar content ranged from 12% in some cultivars and may reach to peak level (20%) in other mulberry varieties. Malic and citric acids are the main organic acids present in mulberry fruit. Juice is rich in sugar (10%) and iron (2.3mg/100mL) which makes it well suited for curing sore throat, fever, dyspepsia and melancholia. Value added products like wine, cooling beverage and jam are made from it (Singh, 1992).

Despite of its health benefits, a handsome quantity of mulberry fruits is lost owing to lack of proper harvesting techniques. Limited shelf life and small quantity of mulberry being processed for medicine purpose only. No value added food product of mulberry fruits is commercially available to date in Pakistan. Keeping in view the short shelf life and potential health benefits of black mulberry fruit this study was conducted to explore its nutritional value as well as suitability for the development of ready to serve beverage and to study the storage stability to overcome the post-harvest losses during the peak season. It will also provide an insight to the industry about the suitability and shelf stability of processed mulberry fruit products and will be helpful to decrease the post-harvest losses by promoting value addition.

**MATERIALS AND METHODS**

Present study was conducted during the year 2020 at the Food Technology Section of Postharvest Research Centre, Ayub Agricultural Research Institute, Faisalabad, Pakistan. Fruit of black mulberry was obtained from local supplier and dirt, dust or other extraneous materials from fruit surface were rinsed with sterile water. Fruits were manually de-stemmed and passed through food grade stainless steel fine pulper to obtain the fruit pulp. For RTS preparation, sugar, carboxy methyl cellulose, hot water and sodium citrate were used according to treatment plan. All the ingredients were thoroughly dissolved through homogenizer to prevent phase separation drink during storage. Drinks were prepared by incorporating mulberry pulp at different ratio as given:-

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<th>Treatments</th>
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<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
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Sodium benzoate was added before filling in drink samples pasteurized at 85-90 °C. The drinks were poured at 85°C in sterilized glass bottles, capped and kept at 25±5°C under ambient conditions. During storage, prepared drinks were evaluated after 15 days for soluble solid content (TSS) by using Refractometer (Abbe Refractometer Model 2WAJ) up to 90 days. A thoroughly homogenized sample was placed on refractometer and solids were noted as described in AOAC (2006). Digital pH meter (InoLab 720, Germany) was used for determination of pH of each sample by taking 50mL of drink in 100mL beaker and pH was noted by dipping the electrode in the sample as described in AOAC (2006). The acidity in drink samples was measured following standard method by taking 10mL of mulberry drink and mixed with 100mL water and then titrated against standard NaOH (0.1 N) until light pink end point using phenolphthalein as an indicator. Non reducing and reducing sugars were determined using Lane and Eynon method according to AOAC (2006). The acidity in drink samples was measured following standard method by taking 10mL of mulberry drink and mixed with 100mL water and then titrated against standard NaOH (0.1 N) until light pink end point using phenolphthalein as an indicator. Non reducing and reducing sugars were determined using Lane and Eynon method according to AOAC (2006).

Ten trained people comprised sensory panel to record the responses by each individual for organoleptic quality on the basis of hedonic scale (9-point). The panel judged RTS samples to decide their acceptance based on flavour, taste, colour and overall acceptability. The data thus obtained were analysed using analysis of variance (ANOVA) technique and means compared by LSD test (Steel et al., 1997).

**RESULTS AND DISCUSSION**

**Analysis of fresh black mulberry juice**

The physico-chemical parameters of fresh mulberry fruit juice are illustrated in Table 1. The average total soluble solids TSS found in the juice was up to 16.40% that is in accordance with Khalid et al. (2011) who
reported 19.40 total soluble solids in mulberry juice. Similar results were reported by Koyuncu et al. (2004) i.e., 13.11 to 16.23% TSS, while studying the different genotypes of black mulberry. Likewise, in another study of black mulberry fruits quality analysis of Khyber Pakhtunkhwa area reported 8.88% TSS (Iqbal et al., 2010).

Ercisli and Orhan (2007) depicted similar findings while working on the chemical composition of mulberry cultivars. Their results illustrated TSS in the range of 15.9 to 20.4%, titratable acidity from 0.25 to 1.40% and ascorbic acid were found to be in the range of 19.4 to 22.4 mg/100g in different juice samples. It showed that this specific crop had high sugar contents and a modest amount of ascorbic acid. The average ascorbic acid contents in fresh black mulberry juice was around 22.45 mg per 100 mL. The said results are in accordance to that of Lale and Ozcagiran (1996) and Ercisli et al. (2010). Iqbal et al. (2010) also testified percent ascorbic acid up to 32.25mg/100g in black mulberry juice.

Analysis of drink

Total soluble solids (%)
The observed values of total soluble solids (TSS) in Fig. 1 revealed substantial difference (13.03-14.63%) among treatments. A consistent increase in total soluble solids was observed in all treatments (14.06-14.63%) during storage period. This gradual increasing trend in TSS can be attributed to conversion of complex carbohydrates (polysaccharides) into primary ones i.e., monosaccharide and oligosaccharides by hydrolysis. At zero day storage TSS of drinks were as (13.03-14.06%), (13.60-14.13%), (13.86-14.16%) and (14.20-14.63%) for T1, T2, T3 and T4, respectively. A significant change was observed in the RTS drink samples as the storage period preceded. Maximum percentage of TSS was recorded in T4 (14.63%) followed by T3 (14.16%) against minimum in T1 (14.06%) and T2 (14.13%) at the end of storage period. Similar trend of increased total soluble solids was observed by Partibha et al. (2020) and Hussain et al. (2010) from 12.00% to 13.2% and from 8.90% to 9.46% percent during storage of green mango mint RTS drink and apple and apricot blended juice respectively. Comparable results were portrayed by Shanta et al. (2021) for TSS i.e., 11.32-11.50% during three months storage of date palm RTS drink.

Titratable acidity (%)
The statistical results (Fig. 2) depicted that acidity varied significantly due to addition of fruit pulp percentage as well as in storage. There was significant decrease in acidity among treatments with the increased percentage of pulp. The storage behavior showed significant rise with the passage of time. It was depicted that percent acidity was in the range of 0.44 to 0.37 % at zero day. The storage study showed no significant variation in RTS drink. After 15 days of storage a gradual increase in percent acidity was observed in all the samples of RTS drink.

pH
Mean values (Fig. 3) revealed that pH varied significantly among the treatments and significant decrease during storage period of 90 days. Maximum pH was observed from 4.04 to 4.42 at zero day for all treatments i.e. gradually decreased upto 3.84 to 4.16
at the end of 90 days storage period. Effect of storage period revealed gradual decrease in pH. Earlier study of Rehman et al. (2021) also showed decrease in pH from 3.97 to 3.81 during storage. Similarly a pH decline (4.0-3.93) in ash gourd-mint leaves juice during storage was observed by Majumdar et al. (2008). Analogous results were illustrated by Kumar et al. (2016) during storage study of carbonated pomegranate fruit juice. Increasing trend in acidity and decrease of pH might be attributed to acetic acid and lactic acid production.

Reducing sugars (%)
The results for change in reducing sugars in drink samples indicated significant effect during storage (Fig. 4). At the initial stage of storage reducing sugars of drinks were as (7.20%), (7.65%), (7.75%) and (8.26%) for T_1, T_2, T_3 and T_4 respectively and gradual increase was observed during storage. Maximum value for reducing sugar was documented in T_4 (9.25%) followed by T_3 (7.89%) against minimum in T_1 (7.47%) at the end of storage period. This increase in reducing sugars may be credited to sucrose inversion under acidic environment. Similar results were depicted by Majumdar et al. (2011) from 6.38 to 7.23 percent during storage of antioxidant rich Apple and autumn olive drink.

Non-reducing sugars (%)
Non-reducing sugars contents in mulberry RTS drink increased among the treatments while gradual decrease was observed during storage. The mean values ranged from 5.32 to 6.26 percent for treatments (Fig. 5). The percent decrease was observed as (5.32-5.21%), (5.89-5.63%), (6.23-6.11%) and (6.26-6.20%) for T_1, T_2, T_3 and T_4 respectively during storage. The highest value was observed in T_4 (6.2%) and lowest in T_1 (5.21%) followed by T_2 (5.63%) and T_3 (6.11%) at the end of storage. Similar trend was observed by Hussain et al. (2010) depicting percent decline in non reducing sugars from 2.56 to 1.88 percent in apple-apricot blended juice. Dhaliwal and Hira (2004) also recounted decrease in non-reducing sugars from 2.47-2.13 percent in apple and autumn olive antioxidant rich RTS drink during storage.

Total sugars (%)
Total sugars percentage in Mulberry RTS drink was found to be increased among the treatments and during storage period. The mean value increased from 12.52% to 14.52% within the treatments with pulp percentage addition. At the end of storage the values for total sugars ranged from 12.68 to 15.37%. The storage study showed an increasing trend in total sugars (13.55 to 14.06%). Fig. 6 showed gradual increase of total sugars percentage form (12.5-12.68%), (13.20-13.69%), 13.68-14.98% and (14.52-15.37%) for T_1, T_2, T_3 and T_4 correspondingly. Similar increase in total sugars from 14.52 to 16.06% after 90 days of storage, was observed by Kumar et al. (2016) during storage study of carbonated pomegranate fruit juice.

Sensory evaluation
Organoleptic perception and its implication in traditional foods are to be evaluated to satisfy consumer demand (Cayot, 2006). Statistical analyses for the sensory characteristics data of food products are very imperative to decide its marketability. Significant differences were found in this study among the treatments and during storage.

Colour: The data (Fig. 7) showed increasing trend in colour score with an increase in pulp percentage while
the gradual decrease in scores for colour was observed during storage period. $T_4$ (25% pulp) gained maximum score (8.4) followed by $T_3$ (8.2) while minimum score was recorded for $T_1$ (7.4) followed by $T_2$ (7.4). The storage exhibited steady decline in color. The change in color parameter is attributed to maillard reaction between sugars and amino acids.

**Taste:** The results for taste (Fig. 8) depicted that taste scores were improved with gradual increase in pulp percentage from $T_1$ (7.2) to $T_4$ (8.4) followed by $T_3$ (8.2) and $T_2$ (7.4). It showed a gradual decline of scores for taste perceptive of drinks during storage but remain in acceptable limit till the end of storage. Loss of flavor and taste might be characterized to ascorbic acid degradation and production of furfural.

**Flavor:** The organoleptic evaluation for flavor depicted decreasing trend from 7.4 to 7.0 for $T_1$, 7.6 to 7.0 for $T_2$, 7.8 to 7.6 for $T_3$ and 8.2 to 7.9 for $T_4$ (Fig. 9). Maximum mean score was recorded for $T_4$ (7.8) followed by $T_3$ (7.60) while minimum score was recorded in $T_1$ and $T_2$. A drop in flavor scores may be due to the acidity increase of drinks. These changes in flavor might be attributed to heat treatment applied while processing.

**Overall acceptability:** The statistically analyzed data for sensory scores presented noteworthy variations for overall acceptability during storage. $T_4$ obtained maximum scores (8.6) for overall acceptability followed by $T_3$ (8.2) and $T_2$ (7.0) and while $T_1$ found minimum scores (6.6) (Fig. 10) at the end of storage period. The judges concluded that decrease in pulp percentage of
mulberry fruit in drink samples decreases the overall acceptability for the drinks. Martin et al. (2003), Tariq et al. (2020) represented a decrease in overall acceptability of beverages prepared from different ratios of apple and olive pulp. This decline may be owing to colour degradation along with modification in flavour and taste of stored samples. However, a better maintenance of colour, taste and flavour was observed for treatment T_4. Similar results were reported for mixed fruit juice RTS beverage (Kumar and Manimegalai, 2001) and fruit product (Vaidya et al., 1998).

![Graph showing effect of treatments and storage on overall acceptability of mulberry RTS drink](image)

**Fig. 10. Effect of treatments and storage on overall acceptability of mulberry RTS drink**

**CONCLUSION**

Short shelf-life of mulberries, as well as the difficulties in harvesting large amounts of this fruit from trees due to the lack of appropriate harvest techniques, improper handling leads us to the conclusion that its post-harvest losses occur on large scale (40-50%). It is need of time to implement processing and preservation techniques to overcome this huge loss in the form of syrup, preserves, beverages, concentrate, dried powder, fruit leathers etc. The characteristic flavour and attractive taste of the beverages obtained in this study considered to be popular with the consumer being a rich source of anthocyanins and phenols, with a high potassium and citric acid content and low sodium level that could be recommended for young children and elderly people.

**REFERENCES**


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Pak. J. Bot. 43:91-96
## CONTRIBUTION OF AUTHORS

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<th>Sr. No.</th>
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<td>1.</td>
<td>Saima Parveen</td>
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<td>Muhammad Abrar</td>
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