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Development of Syrup from Mulberry (*Morus alba* L.) and its Quality Evaluation under Ambient and Refrigerated Storage Conditions

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Abstract

Mulberry (*Morus alba* L.) is one of the underutilized fruit which is a rich source of antioxidants like phenols and anthocyanins besides its delicious taste and strong aroma. In India the mulberry fruit trees are generally grown for rearing silkworms and to a small extent for its fruits. Therefore, investigations were conducted to develop syrup in the Department of Food Science and Technology, Dr YSP UHF Nauni, Solan (HP) during the year 2015 and its quality evaluation during storage. Different combinations of juice (20, 25, 30 and 35%) and TSS (65 and 70 °B) were tried to standardize proper combination for syrup. The syrup prepared by following the best selected recipe was packed in glass and PET bottles and stored for six months under ambient (20–25 °C) and refrigerated temperature conditions (4–7 °C). Out of 8 different treatment combinations of juice and TSS tried, syrup prepared with 25% juice, 70 °B TSS and 1.60% acid was found to be best on the basis of sensory and some physico-chemical characteristics of the product. Syrup could be safely stored for a period of six months under both the ambient and refrigerated conditions without much change in various quality characteristics. However the changes in the quality characteristics of the syrup were slower in refrigerated storage conditions as compared to ambient conditions. Both the packaging materials viz., PET and glass bottles were found suitable, with comparatively less changes occurring in glass bottle under refrigerated conditions.

Keywords: Morus alba, syrup, storage, polyethylene terephthalate, phenolic antioxidants

1. Introduction

Mulberry (Morus alba L.) a wild fruit is known as shahtut, chinni and tut in Himachal Pradesh (HP). It belongs to genera Morus in family moraceae. Morus genus has about 68 species of trees and shrubs throughout the world. This genus is widely distributed in Asia, Africa, Europe, South and North America. It is also widely found in hilly areas of Himalayas up to 3300 m elevation (Za far et al., 2013). Among various Morus species Morus alba L. has been cultivated widely in Asia especially in China, Japan and India for rearing silkworms besides fruit purpose (Ercisli and Orhan, 2007). In India, Morus alba is widely distributed in Jammu and Kashmir, UP, Karnataka, Tamilnadu, West Bengal, Kerala and to a lesser extent in HP and found at an elevation between 400 to 2000 m above mean sea level. Fruits of this species are long, ovoid or cylindrical, which are variable in colour like white, pink or purple to black. The fruits of Morus alba are rich source of anti-oxidants, besides its fruits contain essential fatty acids, vitamins, polyphenols including flavonols, carbohydrates, fibre, minerals, riboflavin, ascorbic acid, carotene and nicotinic acid (Ercisli and Orhan, 2007; Chon et al., 2009; Jiang et al., 2013). Phenols posses a wide spectrum of biochemical activities such as antioxidant,

antimutagenic and anticarcinogenic, as well as ability to modify gene expression (Gungor and Sengul, 2008). Anthocyanins from mulberry fruits can scavenge free radicals, acts as antioxidants, inhibits low-density lipoprotein oxidation and have beneficial effects on blood lipid and atherosclerosis (Du et al., 2008; Duyen et al., 2013). Mulberry known as "folk sacred fruit" is very popular (Jiang et al., 2013). Mulberry fruit is a traditional Chinese edible fruit that is used effectively in folk medicines to treat fever, protect liver from damage, strengthen the joints, facilitate discharge of urine and lower blood pressure (Bae and Suh, 2007). Mulberry fruits can be used as a worming agent, as a remedy for dysentery and as a laxative, anthelmintic, expectorant, hypoglycaemic and emetic (Ercisli and Orhan, 2007). The small edible fruits with attractive colour are sweet which can be consumed fresh and can also be processed into various fruit products. Short shelf life of fresh fruits after harvest is one of the major factors that give the necessity of developing a cheap and efficient preservation process or value-addition for growers of this fruit. Due to Short shelf life very less work on processing of this fruit has been reported so far in India. But Health beverages are also produced on commercial scale in China, Japan and

Korea from this fruit (Singhal et al., 2010). Mulberry fruit will discolor and mildew after 1-2 days at room temperature due to the high water content of more than 80% (Yang et al., 2010). Besides, mulberry fruit is not easy to store and preserve. Therefore, fresh mulberry is not directly consumed, even if it has significant functions. Generally, it needs to be further processed as an attempt to develop functional foods or health products. In HP more emphasis has been given for its use in silkworm rearing, not on the fruits, as a result the fruits goes waste. So, being a rich source of antioxidants especially phenols, this fruit can be exploited for the development of some beverages specially syrup. Thus, the present studies were under taken with the objective to develop syrup from this underutilized fruit and to study its storage life.

2. Materials and Methods

The mature fruits of Morus alba procured from Bela area of Hamirpur district of Himachal Pradesh in the month of April 2015 and used for various physico-chemical analysis and juice extraction. The juice from the mulberry fruits was easily extracted by using hydraulic press machine. Mulberry syrup was prepared by mixing its juice with sugar syrup in different concentrations as given in Table 1. To get the desirable concentration of acid (1.60%) in syrup, citric acid was added in different treatment combinations. Sodium benzoate (600 ppm) was added at the end of product preparation of syrup in all the treatment combinations as preservative. The synthetic colour carmosine at the rate of 0.0025% was added in all treatment combinations.

Table 1: Treatment detail of syrup								
Treatments (T)	T ₁	T ₂	T_3	T ₄	T_{5}	T_6	T ₇	T ₈
Juice (%)	20	25	30	35	20	25	30	35
TSS (°B)	65	65	65	65	70	70	70	70

2.1. Packaging and storage

The syrup prepared by following the best selected recipe was packed in pre-sterilised glass and PET bottles (700 ml capacity). All the packed products were properly labelled and stored in ambient (20–25 °C) and low temperature (4–7 °C) conditions for six months. The physico-chemical and sensory characteristics of all the products were estimated at zero, three and six months of storage.

2.2. Physico-chemical analysis and sensory evaluation

The colour of syrup in terms of different units (Red and Yellow) was observed with Tintometer (Lovibond Tintometer Model-E). TSS, reducing sugars, total sugars, titratable acidity, ascorbic acid and anthocyanins of prepared products were determined according to method described by Ranganna (1997) and AOAC (1990). The pH of the samples was determined by using a digital pH meter (CRISON Instrument, Ltd, Spain). Total phenols content was determined by Folin-Ciocalteu procedure given by Singleton and Rossi (1965). Nine points hedonic rating test was followed for conducting

the sensory evaluation of mulberry syrup (Joshi, 2006). The panel of ten judges were selected to evaluate the product for sensory parameters such as colour, body, taste, aroma and overall acceptability.

2.3. Statistical analysis

Data on physico-chemical characteristics of syrup was analysed by Completely Randomized Design (CRD) before and during storage, whereas, data pertaining to the sensory evaluation were analyzed by using Randomized Block Design (RBD). The experiments on recipe standardization and for storage studies were replicated three times.

3. Results and Discussion

3.1. Standardization of recipe for the preparation of mulberry

Data pertaining to physico-chemical characteristics of mulberry syrup was given in Table 2. TSS of first four recipes was maintained 65 °B and for rest they were kept at 70 °B, during the preparation of the product. With the increase in juice content in different recipes a significant effect on physicochemical characteristics of syrup has been observed. Data presented in Table 2 show that recipe T₁ (20% juice and 65°B TSS) and T_s (20% juice and 70 °B TSS) contain less content of anthocyanins, total phenols and ascorbic acid, it might be due to less percentage of juice used as compared to other recipes like T₄ (35% juice and 65 °B TSS) and T₆ (35% juice and 70°B TSS), which contains higher content of anthocyanins, total phenols and ascorbic acid, because of higher percentage of juice used in these recipes. The change in juice content has also

Table 2: Physico-chemical characteristics of different recipes of mulberry syrup

Treat-	Physico-chemical characteristics							
ments	Colour (TCU)		Antho- cyanins	Total phe- nols	Total phenols			
	R	Υ	(mg 100 g ⁻¹)	(mg 100 g ⁻¹)	(mg 100 g ⁻¹)			
T ₁	20.25	3.12	4.00	28.95	2.52			
T ₂	20.52	2.90	5.37	36.72	3.10			
T ₃	20.80	2.78	6.12	44.58	3.69			
$T_{_{4}}$	20.94	2.64	7.52	50.15	4.34			
T ₅	20.15	3.20	4.02	28.92	2.53			
T_6	20.30	3.02	5.40	36.68	3.11			
T ₇	20.60	2.90	6.12	44.52	3.74			
T ₈	20.85	2.76	7.54	50.20	4.36			
CD	0.07	0.04	0.06	0.08	0.09			
(p=0.05)								

T₄: (20% Juice+65 °B TSS); T₅: (25% Juice+65 °B TSS); T₅: (30% Juice+65 °B TSS); T₄: (35% Juice+65 °B TSS); T₅: (20% Juice+70 °B TSS); T₆: (25% Juice+70 °B TSS); T₇: (30% Juice+70 °B TSS); T_s: (35% Juice+70 °B TSS);

affected the colour units of different recipes of syrup. Data on sensory characteristics of different recipes of mulberry syrup as mentioned in Table 3 indicate that the recipe with 25% juice and 70 °B TSS (T_6) was the best on the basis of sensory and some physico-chemical characteristics of syrup. The higher overall acceptability scores for recipe T_6 might be due to better combination of juice-acid-syrup blend as compared to other recipes.

Table 3: Sensory characteristics (score) of different recipes of mulberry syrup

Treatments (T)	Colour	Body	Taste	Aroma	Overall acceptability
T ₁	8.00	7.00	6.52	6.58	7.03
$T_{_{2}}$	7.23	7.42	7.10	6.60	7.00
T ₃	6.55	5.50	7.20	6.78	6.55
$T_{_{4}}$	6.41	5.22	7.54	7.32	6.63
T ₅	8.10	7.56	6.25	6.70	7.15
$T_{_{6}}$	8.10	7.90	8.20	7.95	8.02
T ₇	6.70	6.10	7.26	7.54	6.90
T ₈	6.20	6.00	7.04	7.60	6.71
CD (p=0.05)	0.34	0.29	0.32	0.35	0.29

3.2. Storage of mulberry syrup

3.2.1. Physico-chemical characteristics

3.2.1.1. Colour

There was a significant decrease in red (R) and yellow (Y) TCU (Figure 1a and 1b) during storage of mulberry syrup. More decrease in red and yellow colour units of syrup was recorded under ambient storage conditions as compared to refrigerated conditions. Decrease in colour units during storage might be due to degradation of anthocyanin pigments. The reason for less decrease in colour units of syrup during storage might be due to stable nature of synthetic colour towards heat and light. Similar trend of decrease in red and yellow TCU has been reported by Thakur et al. (2016) in box myrtle appetizer.

3.2.1.2. Apparent viscosity

Apparent viscosity of mulberry syrup increased significantly (Figure 1c) during the storage period. Increase in apparent viscosity may be due to increase in TSS and soluble sugar which increased strain and shearing rate and decreased the flow index. As the flow index decreases it helps to develop pseudo plasticity and increased the apparent viscosity of the product. This increase in apparent viscosity was observed more in syrup stored under ambient temperature conditions as compare to refrigerated storage conditions. Similar results have been reported by Khurdiya and Lotha (1994) for kinnow mandarin juices and Jain et al. (1996) in mango RTS beverage.

3.2.1.3. TSS

The TSS content of syrup increased slightly during storage (Figure 1d) and this increase in TSS during storage might be

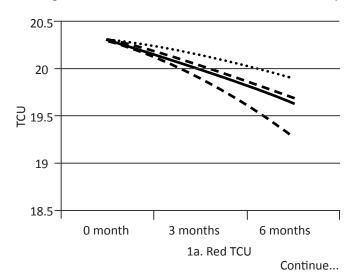
due to hydrolysis of polysaccharides into monosaccharide and soluble disaccharides. More increase in TSS was found in syrup stored under ambient conditions (Increased from 70–70.80 and 70.95 °B in glass and PET bottles) as compared to refrigerated storage conditions (Increased from 70–70.40 and 70.65 °B in both glass and PET bottles) and this might be due to the faster rate of reaction because of high temperature in ambient conditions. The TSS content of this product was found to be non-significant with respect to both the packaging material during storage. Our results are in conformity with the findings of Khurdiya and Roy (1984) in jamun syrup and Thakur et al. (2013) in wild pomegranate syrup (with arils).

3.2.1.4. Reducing sugar

During storage of mulberry syrup there was a gradual increase in reducing sugars (Figure 1e). More increase in reducing sugars was found in syrup stored under ambient conditions (Increased from 48.85-53.28 and 54.08% in glass and PET bottles) as compared to refrigerated storage conditions (Increased from 48.85-51.10 and 51.74% in glass and PET bottles). Increase in sugars during storage might be attributed to the hydrolysis of starch into sugars. Similar, increase in reducing sugars during storage has been reported by Thakur et al. (2013) in wild pomegranate syrup (with arils). As far as packaging material is concerned, higher increase in reducing sugars was recorded in syrup packed in PET bottle as compared to glass bottle. This might be due to the faster rate of chemical reactions in the product packed in PET bottle because of faster heat absorption of PET packaging material than glass as a result of difference in their thermal conductance properties.

3.2.1.5. Titratable acidity

The titratable acidity of syrup showed slight decrease during storage (Figure 2a) which was higher under ambient storage conditions (decreased from 1.60–1.49 and 1.46% in glass and PET bottles) than refrigerated conditions (decreased from 1.60–1.56 and 1.52% in glass and PET bottles). Decrease in titratable acidity during storage might be due to copolymerization of organic acids with sugars and amino acids. The slower rate of reactions as a result of low temperature in refrigerated conditions. Similar results were recorded by



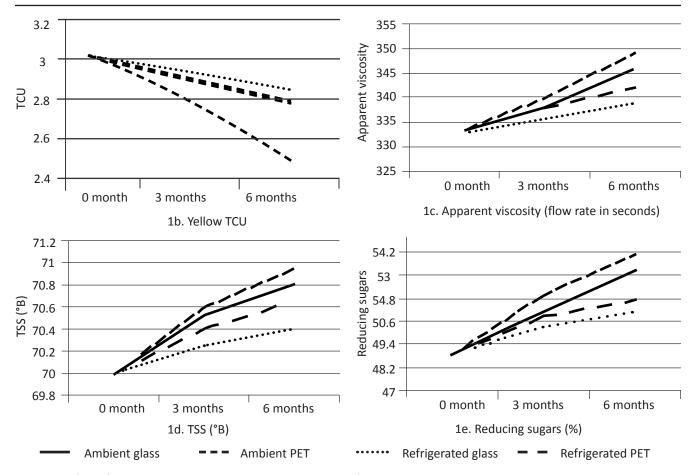


Figure 1: Effect of storage on physico-chemical characteristics of mulberry syrup

Khurdiya and Roy (1984) in jamun syrup.

3.2.1.6. Ascorbic acid

There was a continuous decrease in ascorbic acid content of syrup with advancement of storage period (Figure 2b), however, decrease was significantly lower under refrigerated conditions (decreased from 3.11-2.58 and 2.16 mg 100 g⁻¹ in glass and PET bottles) as compared to ambient conditions (decreased from 3.11-1.52 and 1.04 mg 100 g-1 in glass and PET bottles). Decrease in ascorbic acid content during storage might be due to its degradation into dehydro-ascorbic acid or furfural. Ascorbic acid is highly sensitive to heat; therefore its degradation was more in ambient conditions. Our findings are in agreement with the results reported by Thakur et al. (2013) in wild pomegranate syrup (with arils) and Khurdiya and Roy (1984) in jamun syrup. Lower decrease in ascorbic acid of syrup packed in glass bottle observed during storage might be due to the slower rate of reactions in it as glass materials absorb heat slower than PET material.

3.2.1.7. Anthocyanins

A significant decrease in anthocyanins content of mulberry syrup was recorded during storage (Figure 2c) which was more in ambient storage conditions (decreased from 5.40-4.18 and 3.70 mg 100 g⁻¹ in glass and PET bottles) than refrigerated conditions (decreased from 5.40-4.88 and 4.52 mg 100 g⁻¹ in glass and PET bottles). Loss of anthocyanins in syrup might be due to their high susceptibility to auto oxidative degradation during storage. However, less loss of this attribute in the product might be due to slower rate of its auto oxidation in refrigerated storage conditions as compared to ambient conditions. Similar findings were recorded by Khurdiya and Roy (1984) in jamun syrup and Thakur et al. (2013) in wild pomegranate syrup (with arils).

3.2.1.8. Total phenols

A gradual decrease in total phenols content of syrup was observed during storage (Figure 2d) which was slower under refrigerated storage conditions (decreased from 36.68-33.96 and 32.47 mg 100 g⁻¹ in glass and PET bottles) than ambient conditions (decreased from 36.68-35.34 and 34.71 mg 100 g⁻¹ in glass and PET bottles). Significant decrease in total phenols content during storage might be due to their involvement in the formation of polymeric compounds, complexing of phenols with protein and their subsequent precipitations as observed by Abers and Wrolstad (1979) in strawberry preserve. Similar trend of decrease in phenols content has been reported by Thakur et al. (2013) in wild pomegranate syrup (with arils) and Thakur et al. (2016) in box myrtle appetizer.

3.2.2. Sensory characteristics of mulberry syrup during storage The decrease in colour, body, taste, aroma and overall

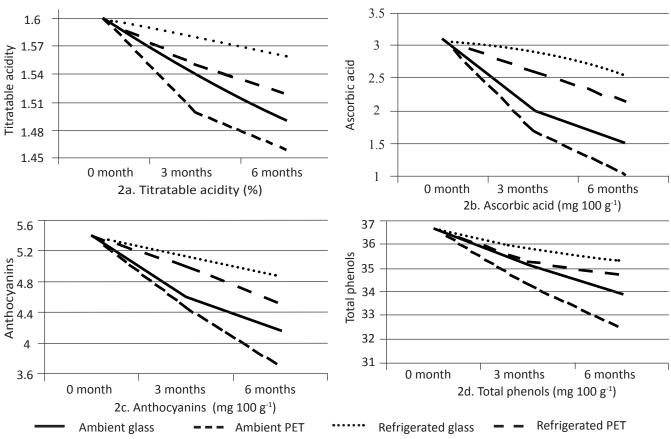


Figure 2: Effect of storage on physico-chemical characteristics of mulberry syrup

acceptability scores of syrup was observed during storage. However, decrease in score was less in refrigerated storage conditions (Figure 3) than ambient (Figure 4). Decrease in colour scores during storage might be due to degradation of colour pigment (anthocyanins) and browning caused by copolymerization of organic acids of the product. As far as packaging material is concerned, there was non-significant effect of packaging materials on the colour scores of syrup. While, the possible reason for decrease in body scores might be due to the interactions between phenols and protein as well as the formation of cation complexes with pectin and

Figure 3: Effect of storage and packaging on sensory characteristics of mulberry syrup stored under ambient conditions

phenols which led the judges to award lower scores to the product during storage. Whereas, a decrease in taste scores of syrup with advancement of storage period might be due

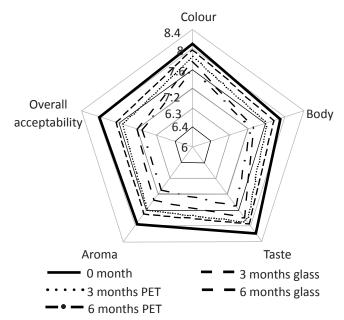


Figure 4: Effect of storage and packaging on sensory characteristics of mulberry syrup stored under refrigerated conditions

to the loss of sugar-acid-juice blend responsible for taste during storage. However, this decrease was significantly lower under refrigerated storage conditions (Figure 3 and 4) than ambient conditions. Retention of higher taste scores in refrigerated conditions might be due to the better retention of original sugar-acid-juice blend as a result of slow reaction rate contributing change in this blend. The loss of aroma scores during storage might be due to the possible loss of volatile aromatic compounds. The aroma scores of syrup packed in glass bottles were retained better than PET bottles during storage. Retention of higher scores of aroma in refrigerated conditions might be due to the lower losses of aromatic compounds at low temperature during storage as compared to ambient conditions. Decrease in overall acceptability scores might be due to the loss in appearance, flavour compounds and uniformity of the product during storage. Higher overall acceptability scores retained in refrigerated conditions might be due to the minute losses of colour, flavour compounds and uniformity of the product during storage. However, the retention of better overall acceptability scores of syrup in glass bottle might be due to the better retention of above mentioned factors as a result of slower reaction rates in glass bottle as compared to PET. The similar results have also been reported earlier by Khurdiya and Roy (1984) in jamun syrup, Thakur et al. (2013) in wild pomegranate syrup (with arils) and Thakur et al. (2016) in box myrtle appetizer.

4. Conclusion

Out of 8 different treatment combinations mulberry syrup recipe (T_c) containing 25% juice, 70 °B TSS and 1.60% acid was found to be best on the basis of its physico-chemical characteristics and sensory parameters. The syrup could be stored safely for a period of 6 months under both storage conditions and in both packaging materials. The best quality of this beverage could be maintained in glass bottle stored under refrigerated storage conditions as compared to PET bottle.

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