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Improved shelf stability of mulberry juice by combination of preservatives

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Mulberry (*Morus alba* L.) fruit is rich in sugar and has high nutritional value. Commercial mulberry juice is available in China but not readily available in India. In India the mulberry fruit trees are generally grown for rearing silkworms and to a small extent for its fruits sold in local markets. This study was carried out to standardize the preservation protocol for shelf stable mulberry fruit juice using low cost technology so that small scale entrepreneurship may be developed in this area. Effect of chemical preservatives, viz. potassium meta bisulphite (KMS) and sodium benzoate (SB) treatment (alone or in combination) followed by pasteurization, on physico-chemical, microbial and sensory qualities of stored juice (9 months) was studied. The results revealed that among all the treatments, combination of KMS and SB (0.05% each) followed by pasteurization of bottles at 100°C for 20 min. resulted in shelf stable juice with least physicochemical changes in terms of TSS, acidity, vitamin C, antioxidants, anthocyanin content, reducing and total sugars. No microbial contamination could be detected after 9 months of storage with maximum sensory acceptability.

Keywords: Mulberry, *Morus alba*, Fruit juice, Preservatives, Potassium meta bisulphite, Sodium benzoate.

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Introduction

Mulberry (*Morus alba* L.) is grown worldwide for rearing silkworms. Besides using the leaves for rearing silk worms, mulberry plants yields edible, black sweet fruits which are rich in red pigment, anthocyanins. Traditionally, mulberry fruit has been used as a medicinal agent to nourish the yin and blood, benefit the kidneys and treat weakness, fatigue, anaemia and premature greying of hair. It is also utilized to treat urinary incontinence, tinnitus, dizziness and constipation in the elderly and the anaemic[1]. Mulberry juice is full of anti-ageing properties. It enhances health, enriches the blood, tonifies the liver and kidney, calms the nerves, balances internal secretions and enhances immunity[1]. Mulberry fruit extract is reported to have protective action against peroxidative damage to bio membranes and biomolecules[2]. Kim *et al*[3] reported that black mulberry juice inhibited human cytochrome CYP3A activity in a pooled human liver microsome system. It has anti-stress, anti-HIV activities and scavenging properties against superoxide, hydroxyl and nitric acid[4]. Black mulberry has antimicrobial and anti-inflammatory properties as well[5, 6].

Unfortunately, the fresh mulberry juice has limited shelf life. The practical approaches for extending storage are rigorous attention to good sanitation from production through juice preparation and low temperature holding. Chemical preservatives often supplement other types of preservation methods, to ensure an economical, safe and flavourful product for months or even years after preparation[7]. However, while selecting preservatives, a number of factors have to be considered. Adequate preservation which ensures food safety and quality are of utmost importance. Reports are available that some preservatives act synergistically and bring about preservation even at low levels[8]. The present paper reports a low cost chemical preservative formulation for organoleptically acceptable and shelf stable mulberry juice.

Materials and Methods

In the month of March-April, fresh, mature and sound mulberry fruits (accession no. MI-497, CISH collection) having attractive red pigment were harvested in clean polyethylene bag from the experimental farm of Central Institute for Subtropical Horticulture, Lucknow and brought to processing and microbiology lab. The berries were washed followed by sorting, the juice was extracted using juice extracting hydraulic press machine. Before filling in

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glass bottles the juice was given following treatments: fresh juice (C), juice heated to 90°C (T_0), juice with 0.1 % Sodium benzoate (T_1), juice with 0.075 % Sodium benzoate (T_2), juice with 0.05 % Sodium benzoate (T_3), juice with 0.1 % Potassium meta bisulphite (T_4), juice with 0.075 % Potassium meta bisulphite (T_5), juice with 0.05 % Potassium meta bisulphite (T_6), juice with 0.075 % Potassium meta bisulphite and 0.025 % Sodium benzoate (T_7), juice with 0.05 % Potassium meta bisulphite and 0.05 % Sodium benzoate (T_8), juice with 0.025 % Potassium meta bisulphite and 0.075 % Sodium benzoate (T_9). The juice was filled in glass bottles; the bottles were pasteurized at 100°C for 20 minutes, cooled and stored at room temperature (25-30°C) under ambient condition of humidity.

The total soluble solids contents of juice were recorded by using hand refractometer (Erma, Japan). Titratable acidity was determined by dissolving a known weight of sample in distilled water and titration against 0.1 N NaOH using phenolphthalein as indicator while ascorbic acid was determined by the direct colorimetric method using 2, 6-dichloro phenol indophenol as decolorizing agent by ascorbic acid in sample extract and in standard ascorbic acid solution[9]. The amount of reducing sugars was determined by spectrophotometric method as per Folin and Wu[10]. The antioxidant property of juice in terms of FRAP values was determined as per Benzie and Strain[11]. Anthocyanin content was observed following method of AOAC[12].

A panel of ten judges selected from staff and students of Postharvest Management evaluated the product fortnightly for color, flavour and overall acceptability by the method of Larmond[13] using a scale from 1 to 10, where 1 represented extremely disliked and 10 represent extremely liked.

Total yeast, mould and bacterial counts were determined by standard dilution plate method as per method of Speck[14].

The experiment was laid in two factor CRD design using values in triplicate and data was subjected to statistical analysis applying statistical package for agricultural workers developed by O.P. Sheoran of CCSHAU, Hisar[15].

Results and Discussion

Recently fresh fruit juices have become the beverages of first choice because of nutritional awareness among the people. The juice of ripe coloured mulberry fruits has attractive red colour. It is rich in sugar (TSS 13°B), extremely pleasant on the palate and contains most of the minerals and vitamins found in the original fruit.

Use of antimicrobials for extending the shelf life of juices is being practised since long. Sulphur dioxide is quite effective in inhibiting both microbial growth and enzymatic and non-enzymatic browning and its use is standard practice in juice making. However, it has bleaching property and is not suitable for coloured products[16]. Sodium benzoate is bacteriostatic and fungistatic under acidic conditions. It is widely used as preservatives in acidic foods such as salad dressings (vinegar), carbonated drinks (carbonic acid), jams and fruit juices (citric acid), pickles (vinegar), and condiments[17].

The shelf life of mulberry juice without any preservative treatment (C) was less than two days because of high sugar content and favourable acidity conditions for the growth of yeast and moulds. Heating the fresh juice to 90°C (T_0) increased shelf life for seven days but resulted in loss of colour and nutritional quality of the juice. Hence, antimicrobial additives were used in rest of the treatments.

The samples were analysed for biochemical parameters and microbial safety during storage.

The microbiological analysis of mulberry juice showed that different treatments and storage intervals had a significant effect on microbial load of mulberry juice. No microbial growth could be detected in treatments T_1-T_9 during first three months of storage. After three months, T_3 and T_6 were spoiled while T_1, T_2, T_4, and T_5, spoiled after six months of storage.

The results indicated that the initial TSS was7.4^0 B which increased to 7.8^0 B in T_7 to T_9 during 9 months of storage (Table 1). Shah *et al*[18] mentioned that increase in soluble content of the product may be due to the solubilization of fruit constituents during storage.

Sugars are the most important constituent of fruit product and are essential factor of the food product and also act as a natural food preservative. Results showed that reducing sugar increased in all samples from 1.64 to 1.85 % during nine months of storage (Table 1). The treatments and storage intervals had a significant effect on reducing sugar of the juice. Rhiz-Nielo *et al*[19] reported increase in glucose and fructose contents in strawberry fruits.

Titratable acidity of samples (T_0 to T_9) was 0.26 % at zero time, which increased up to 0.33 % in T_8 (Table 1). Results indicated that storage intervals had a significant effect on acid content of juice during storage. Nunes *et al*[20] also reported an increase in acidity of strawberry juice during storage. This increase might be due to the breakdown of pectin in to pectinic acid[21].

Ascorbic acid is the most difficult vitamin to be preserved during pasteurization as it is the least heat stable vitamin and decreases in the product during storage. Our results indicated that storage period had significant effect on ascorbic acid content of different samples. There was a gradual decrease in ascorbic acid of mulberry juice from 4.12 to 4.04 mg/100 mL (Table 1). Results show that minimum ascorbic acid content was recorded in T_7 (4.04 mg/100 mL) and maximum in T_8 (4.09 mg/100 mL) after nine months of storage. Viberg *et al*[22] reported a decrease in ascorbic acid in strawberry pulp after treatment involving freezing, heating and accelerated storage.

Anthocyanin and antioxidants decreased in all treatments with increasing storage temperature. The lowest anthocyanin 66.58 mg/100mL was observed in T_7 treatment (Table 2), whereas highest 68.02 mg/100mL in T_8 after nine month of storage. Vitamin C promoted anthocyanins degradation. A similar pattern of vitamin C degradation was also reported in strawberry juice containing ascorbic acid[23] and also in black currant nectar[24]. The Linear regression analysis provided evidence that ascorbic acid degradation fitted first order reaction kinetics.

The antioxidant property measured as FRAP values revealed no appreciable change in antioxidant values of juice. The lowest antioxidant (0.925 mM) content was observed in T_7 while highest (1.321 mM) in T_8 after nine month of storage (Table 3). Analysis of

Table 1—Biochemical changes of preserved mulberry juice during storage

Parameters	Storage period (Months)	Treatments											C.D. at p=0.05		
		C	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	Due to treatments	Due to periods	Due to interaction
TSS (^{0}B)	0	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	0.02	0.02	0.05
	3	-*	-**	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6			
	6	-	-	7.6	7.6	-	7.8	7.8	-	7.8	7.8	7.8			
	9	-	-	-	-	-	-	-	-	7.8	7.8	7.8			
Acidity (%)	0	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.01	0.01	0.02
	3	-*	-**	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.28	0.27			
	6	-	-	0.29	0.29	-	0.29	0.29	-	0.29	0.30	0.30			
	9	-	-	-	-	-	-	-	-	0.31	0.33	0.32			
Ascorbic acid (mg/100ml)	0	4.12	4.12	4.12	4.12	4.12	4.12	4.12	4.12	4.12	4.12	4.12	0.01	0.01	0.02
	3	-*	-**	4.11	4.11	4.10	4.10	4.11	4.11	4.09	4.11	4.10			
	6	-	-	4.09	4.09	-	4.09	4.09	-	4.07	4.09	4.08			
	9	-	-	-	-	-	-	-	-	4.04	4.09	4.05			
Reducing sugar (g %)	0	1.64	1.64	1.64	1.64	1.64	1.64	1.64	1.64	1.64	1.64	1.64	0.01	0.01	0.02
	3	-*	-**	1.68	1.66	1.66	1.66	1.67	1.63	1.68	1.69	1.66			
	6	-	-	1.71	1.70	-	1.68	1.70	-	1.71	1.76	1.68			
	9	-	-	-	-	-	-	-	-	1.77	1.85	1.72			
Total sugar (g %)	0	2.78	2.78	2.78	2.78	2.78	2.78	2.78	2.78	2.78	2.78	2.78	0.01	0.01	0.02
	3	-*	-**	2.82	2.83	2.83	2.81	2.85	2.80	2.85	2.85	2.83			
	6	-	-	2.86	2.85	-	2.85	2.92	-	2.89	2.95	2.89			
	9	-	-	-	-	-	-	-	-	2.92	3.04	2.95			

Treatments: C- Fresh juice; T_0 - juice heated to 90^{0}C; T_1 - juice with 0.1 % Sodium benzoate; T_2 - juice with 0.075 % Sodium benzoate; T_3 - juice with 0.05 % Sodium benzoate; T_4 - juice with 0.1 % Potassium meta bisulphite; T_5 - juice with 0.075 % Potassium meta bisulphite; T_6 - juice with 0.05 % Potassium meta bisulphite; T_7 - juice with 0.075 % Potassium meta bisulphite and 0.025 % Sodium benzoate; T_8 - juice with 0.05 % Potassium meta bisulphite and 0.05 % Sodium benzoate; T_9 - juice with 0.025 % Potassium meta bisulphite and 0.075 % Sodium benzoate.

-→ Spoiled; -* → Spoiled after 2 days; -**→ Spoiled after one week

Table 2—Changes in anthocyanin content (mg/100 mL) during storage of preserved mulberry juice

Treatments	Storage periods (Months)			
	0	3	6	9
C	68.52	-*	-	-
T_0	68.52	-**	-	-
T_1	68.52	67.78	66.28	-
T_2	68.52	67.65	66.21	-
T_3	68.52	67.1	-	-
T_4	68.52	67.23	67.06	-
T_5	68.52	67.25	67.09	-
T_6	68.52	67.32	-	-
T_7	68.52	67.23	66.98	66.58
T_8	68.52	68.2	68.1	68.02
T_9	68.52	67.2	67.1	66.82

CD at p=0.05; Due to treatments: 0.008; Due to periods: 0.006; Due to interaction: 0.02

C- Fresh juice; T_0 - juice heated to 90^0C; T_1 - juice with 0.1 % Sodium benzoate; T_2 - juice with 0.075 % Sodium benzoate; T_3 - juice with 0.05 % Sodium benzoate; T_4 - juice with 0.1 % Potassium meta bisulphite; T_5 - juice with 0.075 % Potassium meta bisulphite; T_6 - juice with 0.05 % Potassium meta bisulphite; T_7 - juice with 0.075 % Potassium meta bisulphite and 0.025 % Sodium benzoate; T_8 - juice with 0.05 % Potassium meta bisulphite and 0.05 % Sodium benzoate; T_9 - juice with 0.025 % Potassium meta bisulphite and 0.075 % Sodium benzoate.

- →Spoiled; -* →Spoiled after 2 days; -** → Spoiled after one week

Table 3—Changes in antioxidant content (mM/ mL) during storage of preserved mulberry juice

Treatments	Storage periods (Months)			
	0	3	6	9
C	1.166	-*	-	-
T_0	1.166	-**	-	-
T_1	1.166	1.162	0.930	-
T_2	1.166	1.397	1.343	-
T_3	1.166	1.02	-	-
T_4	1.166	1.004	0.942	-
T_5	1.166	1.095	0.965	-
T_6	1.166	1.058	-	-
T_7	1.166	1.123	1.054	0.925
T_8	1.166	1.332	1.321	1.321
T_9	1.166	1.311	1.304	1.293

CD at p=0.05; Due to treatments: 0.001;Due to periods: 0.001; Due to interaction: 0.001

Treatments: C- fresh juice; T_0 - juice heated to 90^0C; T_1 - juice with 0.1 % Sodium benzoate; T_2 - juice with 0.075 % Sodium benzoate; T_3 - juice with 0.05 % Sodium benzoate; T_4 - juice with 0.1 % Potassium meta bisulphite; T_5 - juice with 0.075 % Potassium meta bisulphite; T_6 - juice with 0.05 % Potassium meta bisulphite; T_7 - juice with 0.075 % Potassium meta bisulphite and 0.025 % Sodium benzoate; T_8 - juice with 0.05 % Potassium meta bisulphite and 0.05 % Sodium benzoate; T_9 - juice with 0.025 % Potassium meta bisulphite and 0.075 % Sodium benzoate

- → Spoiled; -* → Spoiled after 2 days -**→ Spoiled after one week

variance of principal effects showed a significant effect of all analyzed factors on changes in antioxidant activity[25]. Numerous publications confirm changes in antioxidant activity during heating and storage of plant products.

Organoleptic analysis indicated treatment T_8 as best during storage (Fig. 1). The HPLC analysis of T_8 juice indicated the presence of phenolics, viz. Gallic acid (13.4 µg/100 mL), Epi-catechin (21.0 µg/100 mL), Caffeic acid (1.18 µg/100 mL).

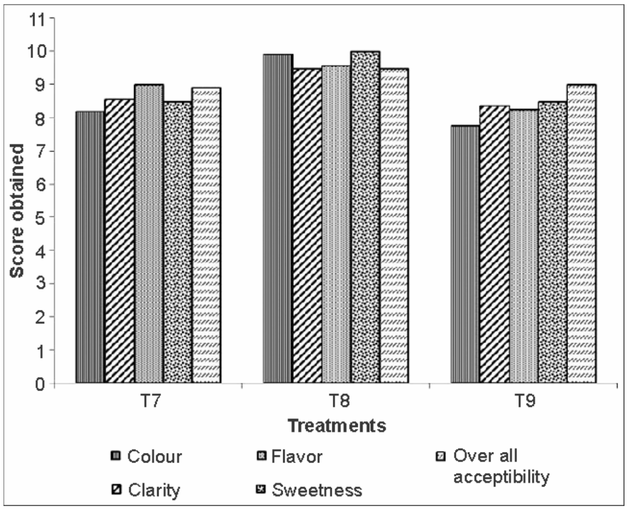

Fig.1—Sensory evaluation of mulberry juice after nine months of storage

Conclusion

Mulberry juice preserved with a combination of preservatives, viz. Potassium meta bisulphite (0.05 %) and Sodium benzoate (0.05 %) resulted in shelf stable mulberry juice with high organoleptic acceptability and stable nutritional quality. The protocol may be followed by industry for shelf stable mulberry juice.

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References

1 http://www.itmonline.org/arts/morus.htm.

2 Naderi G A, Asgary S, Sarraf-Zadegan N, Oroojy H and Afshin-Nia F, Antioxidant activity of three extracts of *Morus nigra*, *Phytother Res*, 2004, **18**, 365-369.

3 Kim H, Yoon Y J, Shon, I J C and Liu H H, Inhibitory effects of fruit juices on cytochrome P450 3A(CYP3A) activity, *Drug Metab Dispos*, 2006, **34**, 521-523.

4 Sakagami H[1], Asano K, Satoh K, Takahashi K, Kobayashi M, Koga N, Takahashi H, Tachikawa R, Tashiro T, Hasegawa A, Kurihara K, Ikarashi T, Kanamoto T, Terakubo S, Nakashima H, Watanabe S and Nakamura W, Anti-stress, anti-HIV and vitamin C-synergizedradical scavenging activity of mulberry juice fractions, *In vivo*, 2007, **21**, 499-06.

5 Butt M S, Nazir A, Sultan M T and Schroen K, *Morus alba L.* nature's functional tonic, *Trends Food Sci Technol*, 2008, **19**, 505-512.

6 Khalid N, Fawad SA and Ahmed I, Antimicrobial activity, phytochemical profile and trace minerals of black mulberry (*Morus nigra* L.) fresh juice, *Pak J Bot*, 2011, **43**, 91-96.

7 http://www.ehow.com/about_6162327_use-chemical-preservatives_.html.

8 http://microsites.schuelke.com/wetwipepreservation/documents/Aslittlepossiblenewconceptstominimizetheuseofclassicalpreservativesschlke.pdf.

9 Ranganna S, Handbook of analysis and quality control for fruit and vegetable products, IInd edn. Tata Mc Graw Hill Publication Co Ltd, New Delhi, ISBN: 0074518518, 2000, pp.1152.

10 Folin O and Wu H, Estimation of blood sugar by alkaline copper reducing method, *J Biol Chem*, 1920, **41**, 367.

11 Benzie FF and Strain JJ, Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration, *Methods in Enzymology*, 1999, **299**, 15.

12 Association of Official analytical Chemists (A.O.A.C.), 1984, Official methods of analysis. *Washington*, D. C., U. S. A.

13 Larmond Larmond E, Method of sensory evaluation of food, Canada Dept. of Agriculture, Publ 1977, 1286, 36-37.

14 Speck M, Compendium of methods for the microbiological examination of foods, Second edition, American Public Health association Inc, 1985, pp. 644-649.

15 www.hau.ernet.in/opstat.html.

16 Jurd, L, Reactions involved in sulfite bleaching of anthocyanin, *J Food Sci,* 1964, **29**, 16-19.

17 Pitt J I and Hocking A D, Fungi and food spoilage, Third edition, Springer Dordrecht Heidelberg, London, New York, 2007, 401-419.

18 Shah W H, Sufi N A and Zafar S I, Studies on the storage stability of guava fruit juice, *Pak J Sci Ind Res*, 1975, **18** (3-4), 179-183.

19 Rhiz-Nielo A, Lopez A J, Lopez M R, Lopez M J and Dijkstra D J, Analysis of sugar from strawberry cultivar of commercial interest-contents evaluation, *Acta Hortic*, 1997, **439**, 663-664.

20 Nunes MCN, Brecht J K, Morais A M M A and Sargent S A, Physical and chemical quality characteristics of strawberries after storage are reduced by a short delay to cooling, *Post Harvest Bio and Tech*, 1995, **6**, 17-28.

21 Riaz R A, Ali A and Saleem M, Studies on the preparation and storage stability of comminuted Kinnow fruit beverage bees, *Pak J Sci Ind Res*, 1988, **32**(8), 574-578.

22 Viberg U, Alklint C, Åkesson B, Önning G *and* Sjöholm I, *The effect of processing on total antioxidative capacity in strawberries,* In*: Kumpulainen J T, Salonen J T (Eds),* Natural antioxidants and anticarcinogens in nutrition, health and disease*, The Royal Society of Chemistry, Cambridge* 1999, pp. 338-340

23 Garzon G A and Wrolstad E R, Comparison of the stability of pelargonidin-based anthocyanins in strawberry juice and concentrate, *J Food Sci,* 2002, **67**(4), 1288-1299.

24 Iversen C K, Black current nectar: effect of processing and storage on anthocyanin and ascorbic acid content, *J Food Sci,* 1999, **64**(1), 37-41.

25 Arabshahi D S, Devi D V and Urooj A, Evaluation of antioxidant activity of some plant extracts and their heat, pH and storage stability, *Food Chem*, 2007, **100**, 1100-1105.