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RESEARCH PAPER

Screening of Mulberry Accessions for Wine Preparation

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Abstract

Mulberry (*Morus spp.*) is a fruit known for its nutritional qualities and flavor. Traditionally, it is used as a natural medicine due to high content of active therapeutic compounds. Its fruits are rich in sugar and therefore, are potential substrate for wine production. Mulberry accessions viz., MI-118, MI-497 and MI-362 were screened for wine preparation. The sensory evaluation revealed that the wine prepared from variety MI-497 was the best due to an attractive deep red colour, 7.6^{0}B TSS; 0.34 per cent acidity; 0.16% tannins; 0.23% reducing sugar; 0.41% total sugar; 2.89 mM/ml antioxidants; 10.17 mg/100ml anthocyanin and 13.2 % (v/v) alcohol. The wine from mulberry accessions MI-362 was comparatively sweeter with higher TSS and acidity but low in anthocyanins content (2.46 mg/100 ml). HPLC analysis detected gallic acid, p – coumaric acid, catechins, kaempferol and epi-catechin as major phenolic compounds present in the wines. The data obtained revealed that the mulberry accessions MI-497 and MI-362 are suitable for wine production. Wine prepared from Indian mulberry accession MI-497 had the high antioxidant (2.89 mM/ml) and anthocyanins (10.17 mg /100 ml) content and thus, could have good market potential.

Keywords: Mulberry, accessions, wine, alcohol, phenolics

Mulberry (*Morus spp.*) is a very hardy plant belonging to the family *Moraceae*. It is both wild or cultivated in India for its foliage, which is a primary source of food for silkworms. In general, there are three types of Mulberry -white, red and purple. The fruits possess medicinal properties and have delicious taste (Srivastava *et al.,* 1997). These are consumed fresh due to their short storage life. Processing of mulberry is not at commercial level due to lack of economy of scale. Mulberry fruits have been reported to exhibit a variety of biological activities, such as anti-thrombotic, antioxidant, and neuron-protective (Ramesh *et al.,* 2014). These activities are generated by anthocyanins, which are a group of naturally occurring phenolic compounds that are also responsible for the color of mulberries (Liu *et al.,* 2004). If these fruits are industrially exploited for various commercially viable products, mulberry can become an important crop throughout the world. Yadav *et al.* (2013) have worked out protocol for shelf-stable mulberry juice from Indian accessions. Red grape wines are known for therapeutic value due to the presence of red pigment (Yang *et al.,* 2009). Some of the red colour mulberry accessions have been formed as rich source of anthocyanins. There are reports of wine preparation from mulberry having therapeutic qualities for the control of inflammations of the throat, tongue and mouth (Kumar and Chauhan 2008; Soufleros *et al.,* 2004; Quer, 1995). Mulberry wines are not common. Present study aims at screening of Indian mulberry accessions for preparation of mulberry wines and their storage evaluation.

MATERIALS AND METHODS

Fruit collection and Juice extraction

In the month of March-April, fresh, mature and sound mulberry fruits (accessions MI-118, MI-497, MI-362; CISH collection) procured from the experimental farm of ICAR-Central Institute for Subtropical Horticulture, Lucknow, were filled in clean polythene bags and brought to laboratory. The fruits were washed separately under running tap water, crushed in fruit mill and the juice was extracted by a hydraulic press.

Yeast culture

The yeast culture, *Saccharomyces cerevisiae* var. *ellipsoideus* used in the present investigation, was obtained from the culture collection of Microbiology Laboratory of ICAR-CISH, Lucknow. The culture was maintained on Yeast Extract Peptone Dextrose (YEPD) Agar slants and was re-cultured every month.

Preparation of wine

The total soluble solids (T.S.S) and acidity of mulberry juice were maintained at 20 °Brix and 0.5%, by adding sucrose and citric acid, respectively. The juice was inoculated with yeast (*Saccharomyces cerevisiae*), transferred into narrow mouth glass jars with fermentation lock and kept for fermentation up to 15 days at low temperature (16 ± 2°C) till TSS became constant. The wine was siphoned, aged, bottled in 300ml capacity glass bottles, pasteurized at 65°C for 5 min and stored at 15 ± 2°C temperature.

Physico-chemical, microbiological and sensory analysis

The total soluble solids contents of wine were recorded by using hand refractometer (Erma, Japan), while acidity and ascorbic acid contents were estimated as per the methods of Ranganna, (2000). The anti-oxidant property of wine in terms of FRAP values was determined as per Benzie and Strain, (1999). The amount of reducing sugars was determined by spectrophotometric according to Folin and Wu's method, (1920). The juice was analyzed by Thin layer Chromatography for sugars (Grace and Kwaku 2004). Ethanol concentration in the mulberry wine was determined spectrophotometrically (Caputi *et al.,* 1968). The phenolic content in mulberry wine was analyzed by High Performance Liquid Chromatography using method of Basha *et al*. (2004). Microbiological quality of mulberry wine was carried out as per method described by Speck, (1985).

For judging the sensory attributes of the wine, sensory evaluation was conducted by a panel of seven semi-trained judges. The attributes considered in the evaluation were colour, clarity, taste, tannin, astringency, sweetness and body of the product (Amerine *et al.,* 1980). The overall rating was obtained by calculating the average of the scores on a 9 point hedonic scale.

Statistical analysis

In this study, three replicates were kept and the experiment was laid in two factor CRD design and the data were subjected to statistical analysis using statistical package for agricultural workers developed by O.P. Sheoran of CCSHAU, Hisar.

RESULTS AND DISCUSSION

The biochemical analysis of extracted juice from mulberry fruit accessions MI-118, MI-497, MI-362 as depicted in Table 1, indicated high anthocyanin,

Table 1: Biochemical composition of mulberry fruit juice used for wine making

Variety	Fruit colour	TSS (°Brix)	Acidity (%)	Ascorbic acid (mg/100ml)	Anthocyanins (mg/100ml)	Phenolics (mg/100ml)	Pectin (%)	Protein (%)
MI-118	Red green	4.2	0.19	4.1	3.4	63	0.30	0.046
MI-497	Deep purple	5.1	0.26	4.1	68.5	207	0.42	0.184
MI-362	Red	5.4	0.36	4.1	6.9	94	0.33	0.077

phenolic and protein content in case of MI497 compared to other two accessions. The other characters including TSS, acidity, ascorbic acid content and pectin content were more or less the same.

The wines were analyzed for biochemical, microbiological and organoleptic quality parameters during one year of storage study. The microbiological analysis revealed no microbial growth up to one year of storage in all the treatments. The TSS content increased slightly in all the treatments during storage. Highest TSS content (7.8°B) was observed in MI-497 and MI-362 while lowest (7.6°B) in MI-118. Increase in TSS might be due to hydrolysis of polysaccharides into soluble saccharides (Mehta and Bajaj, 1983). The titratable acidity of wine increased during storage (Table 2). The highest acidity (0.79%) was observed in MI-362 while the lowest (0.34%) in MI-497. The increase in acidity might be due to the accelerated degradation of pectic substances (Conn and Stump, 1976). The total sugar content slightly decreased while, reducing sugar increased in mulberry wine during storage. It might be due to inversion of sucrose to glucose and fructose or breakdown of polysaccharides into the simple sugars (Rhiz-Nielo *et al.,* 1997**)**. Maximum anthocyanins content (9.17

Table 2: Biochemical changes in mulberry wines during storage

Parameters	Storage period (Months)	Treatments			C.D. at p=0.05		
		MI-118	MI-497	MI-362	Due to Mulberry accessions	Due to storage periods	Due to interaction
TSS (^{0}B)	0	6.8	7.0	7.2	NS	0.1	0.17
	3	7.2	7.4	7.6			
	6	7.6	7.8	7.8			
	9	7.6	7.8	7.8			
	12	7.6	7.8	7.8			
Titrable Acidity (%)	0	0.66	0.34	0.67	0.01	0.01	0.02
	3	0.69	0.37	0.71			
	6	0.73	0.51	0.78			
	9	0.74	0.52	0.79			
	12	0.74	0.53	0.79			
Reducing sugar (g/100 ml)	0	0.13	0.26	0.24	0.01	0.01	0.02
	3	0.19	0.29	0.41			
	6	0.25	0.34	0.49			
	9	0.27	0.35	0.51			
	12	0.28	0.36	0.52			
Total sugar (g/100 ml)	0	0.26	0.41	0.38	0.01	0.01	0.02
	3	0.25	0.34	0.32			
	6	0.21	0.30	0.29			
	9	0.19	0.29	0.29			
	12	0.19	0.28	0.28			
Alcohol (μ/v)	0	12.2	13.2	12.8	NS	0.1	0.1
	3	12.2	13.1	12.8			
	6	12.2	13.2	12.8			
	9	12.2	13.2	12.8			
	12	12.2	13.2	12.8			

CD = Critical Difference; NS = Non-significant

mg/100ml) was observed in MI-497 while the lowest (1.39 mg/100ml) in MI-118 (Fig. 1). The anthocyanins content decreased slightly during storage. Lopes *et al.* (2007) have also observed that anthocyanins might undergo complex reactions during aging, including oxidative degradation, resulting in a decrease.

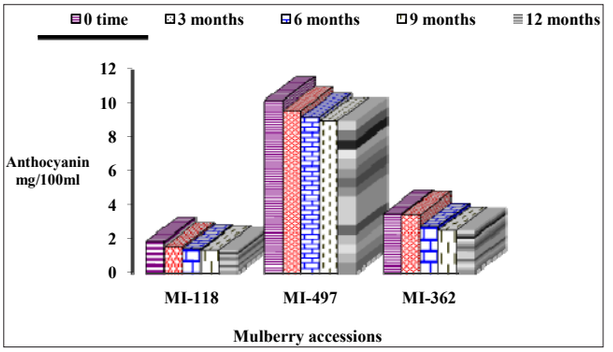

Fig. 1: Changes in anthocyanin content in mulberry wines during storage

The formation of insoluble polymeric pigments leading to loss of total anthocyanin and color strength in red wines have also been recorded (Mistry *et al.*, 1991). The data on antioxidant property measured in terms of FRAP values revealed that slight decrease in antioxidant activity in MI-497 and MI-362 took place but not in MI-118. The highest antioxidant content (2.89 mM/ml) was documented in MI-497 while the lowest (0.31 mM/ml) was present in MI-118, which decreased slightly in all wine samples (Fig. 2).

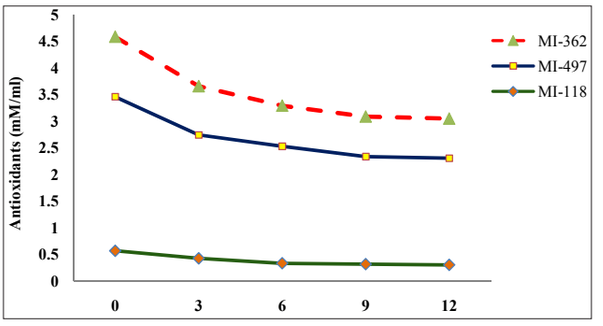

Fig. 2: Changes in antioxidant content of mulberry wines during storage

Arabshahi *et al.* (2007) reported a decrease of antioxidant activity with increasing storage temperature. Poiana *et al.* (2007) found a decrease in antioxidant activity with a decrease in anthocyanins content.

Ethanol is one of the most important constituents of any alcoholic beverage, since it imparts astringency and refreshing taste (Laing and Wilcox, 1983). The initial level of ethanol, up to 13%, remained almost constant throughout the storage period, revealing no fermentation activity in the samples during storage (Table 2). Duyen *et al.* (2013) have reported a fermentation duration of 96 h for naturally fermented Vietnamese style mulberry alcoholic beverage with specific flavor and 5% v/v alcohol content. Wang *et al.* (2013) have optimized the conditions for mulberry fermentation as pH 3.2, fermentation temperature 31.4°C, for getting 12% alcohol. Ezeronye, (2004) showed that the wines produced from the mulberry must have alcohol levels ranging from 10.6 to 12.6. Nguyen *et al.* (2013) have reported natural fermentation of mulberry juice to get 5% alcohol. However, the quality of wine might have varied due difference in type of yeast flora developed in fermentation.

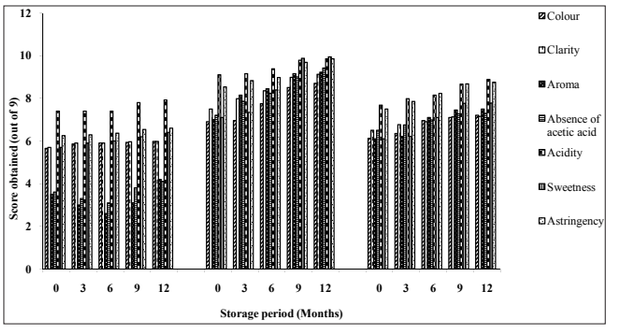

Fig. 3: Sensory evaluation of mulberry wines during storage

HPLC analysis (Fig. 4) reflected the presence of phenolics compounds *viz.* gallic acid, p –coumaric acid, catechins and kaempferol in all the mulberry wines while epi-catechin was additionally found in MI-497. All these phenolics have important health benefits (Jeong *et al.*, 2010). Goldberg *et al.* (1998) have reported that the concentrations of (+)-catechin and (-)-epicatechin were the highest in red Burgundy

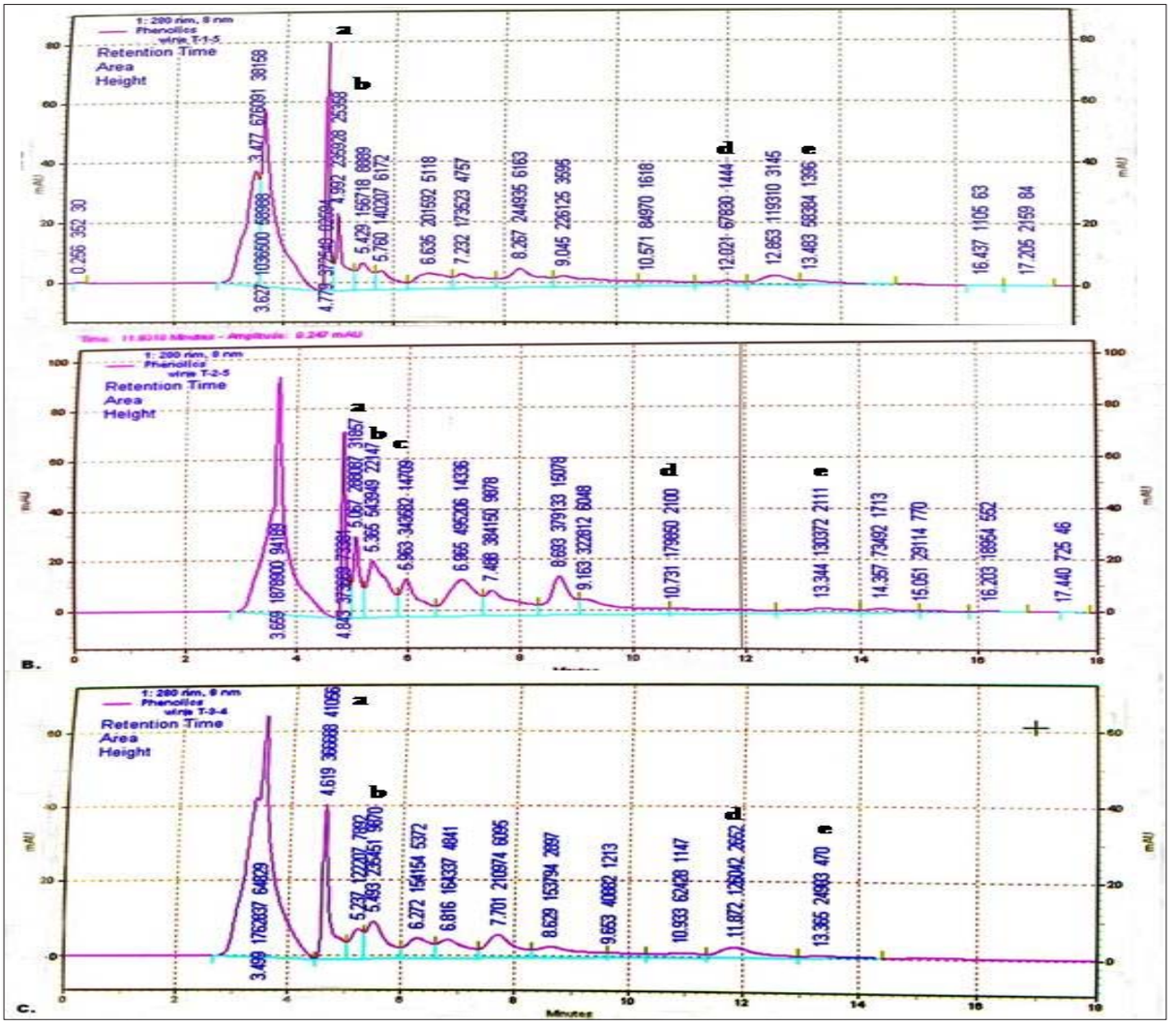

Fig. 4: HPLC chromatograms for Phenolics in wines made by different mulberry accessions (A)-MI. -118; (B) –MI-497; (C) – MI-362, alphabets denotes phenolics present in wines:

(a) Peak representing to Gallic acid, (b) Peak representing to Catechin, (c) Peak representing to Epi-catechin, (d) Peak representing to p-coumaric acid and (e) Peak representing to Kaempferol

and Canadian grape wines. Arts *et al*. (2000) reported the presence of (+)-catechin, (-)-epicatechin, (+)-gallocatechin, (-)-epigallocatechin, (-)-epicatechin gallate and (-)-epigallocatechin gallate in 18 types of red and white wines.

Sensory evaluation analysis during one year of storage study reflected wine prepared from MI-497 to be superior over those from other accessions on the basis of colour, taste, aroma and astringency and consumer acceptance. As shown in Fig. 3, the good red colour of the wine might be attributed to its high concentration of phytochemicals. However, high concentrations of phytochemicals are known to be responsible for the bitterness and astringency of red wine (Lesschaeve and Noble, 2005).

All the wines could be matured for one year without deterioration in quality and confirmed that the aging improved the acceptability of wine.

CONCLUSION

The data obtained generally indicated that the mulberry accessions MI-497 is suitable for wine production. Phenolics compounds viz. gallic acid, catechin, coumaric acid and kaempferol were detected in all three wines by HPLC while one compound epi-catechin was found in MI-497 only. The wine has attractive deep red colour, high nutraceutical value and significant domestic and export potential. This study will be helpful in exploring better post harvest use of mulberry fruit.

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