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Preparation of an Alcoholic Beverage from Muskmelon (*Cucumis melo* L. var. Punjab Sunheri)

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A B S T R A C T

Muskmelon (cv. Punjab Sunheri) was evaluated for fermentative preparation of an alcoholic beverage. Pre-fermentative juice sterilization was optimized as a combination of potassium metabisulphite (0.1% v/v) + pasteurization which revealed decrease of more than 98.5% of fungal and 99% of bacterial counts. Pre-fermentative enzymatic treatment (50 units/100ml pectinase) resulted in 73.44% clarification of sterilized juice in 6 h at 45°C. Ethanolic fermentation by *Saccharomyces cerevisiae* MTCC 11815 was optimized with temperature (20°C), sugar concentration (21°B), inoculum size (6.25% v/v) and DAHP (0.15% w/v) that produced 12.8% (v/v) of ethanol in 5 days of fermentation. Post fermentative storage of wine revealed absence of yeast count after 30 days, a significant decrease in ethanol (13.1 to 12.4% v/v) and phenols (19.3 to 8.1 mg/100ml), total and free SO_2 of 102.4 and 10.24 ppm, respectively and retention of 50% of juice ascorbic acid (306.06 to 166.66 mg/100ml). Sensory analysis of the mature muskmelon-wine reflected it a standard wine with a sensory score of 60.1±5.84.

Introduction

Fruit wines are undistilled nutritive alcoholic beverages produced by fermentation of fruit juices either spontaneously or by known strain of microorganisms mainly a yeast species so as to develop a particular quality of wine. Among fruits, grapes have been used as the main raw material in the production of wines hence its name. However, a number of alternate fruits have been found suitable for wine production such as mango, banana, guava, apple, pear etc (Joshi and Attri, 2005; Durate *et al.*, 2010). Among these, muskmelon (*Cucumis melo* L.) (Family Cucurbitaceae) is one of the important fruits in India. Muskmelon, a sweet fruit of Punjab containing more than 90% water is also enriched with phytochemicals, making it a suitable substrate for wine preparation (Lester, 1997). This fruit is taken as table fruit and has a low shelf life even under refrigerated conditions (Dunlap *et al.*, 1990). However, Muskmelon with a TSS of 10-13% has a juice recovery of 85% but its juice pH of 5.7-6.7 makes it susceptible to bacterial contamination (Kim *et al.*, 2006). Hence there is an imperative need to increase shelf life of this fruit while retaining its useful components. Further, muskmelon juice is

hazy due to presence of pectin that makes a turbid wine after fermentation. Therefore it is imperative to standardize pre-fermentative and fermentative conditions for producing a good muskmelon-wine for which many statistical experimental design methods have been suggested. Earlier, we employed RSM and CCD to optimize fermentation conditions for production of muskmelon-wine as brix, temperature, inoculum size and DAHP concentration of 15^0 B, 20^0 C, 6.25% (v/v) and 0.15% (w/v) which produced an ethanol of 8.9% (v/v) (Jyoti, 2014). Hence for producing a wine of atleast 10% (v/v), the present study was conducted on producing melon wine by increasing the brix of wort using commercial sugar (sucrose) and evaluated its effect on quality of wine produced.

Materials and Methods

Muskmelon (*Cucumis melo* L.) cv. Punjab Sunheri was procured from Department of Vegetable Science, Punjab Agricultural University, Ludhiana. Healthy fruits were washed with boiled and cooled water containing 0.01% of KMS (Potassium metabisulphite). Juice was extracted by mechanical pressing followed by sieving and subjected to a combination of KMS (0.1% w/v) + Pasteurization (60-65˚C for 15-20 min) treatment. Thereafter, the juice was treated enzymatically with pectinase (50 units /100 ml) at 45˚C for 2-6 hours (Kocher and Pooja, 2011). The periodic samples were taken aseptically and evaluated for total viable count and % clarification.

Experimental design for ethanolic fermentation of sugar chaptalized muskmelon juice

The granulated refined sugar was procured from local market and its syrup was prepared in small quantity of melon juice @ 250g/250 ml. The latter was used to chaptalize melon juice to raise the brix at 5 levels *viz.*, 15 to 23˚B and the above pretreated muskmelon juice (500 ml) was taken in 1000ml capacity Erlenmeyer flasks which were previously washed with boiled water and cotton plugged. The juice was fermented by inoculating a starter culture of *Saccharomyces cerevisiae* MTCC 11815 at already optimized fermentation conditions of brix, temperature, inoculum size and DAHP concentration of 15^0 B, 20^0 C, 6.25% (v/v) and 0.15% (w/v), respectively (Jyoti, 2014). The periodic samples were taken and analyzed for TSS (glass brixometer), pH (hand pH, Henna meter) and ethanol content (Caputi *et al.*, 1968). The fermentation efficiency of different treatments was calculated as:

$$\text{Fermentation efficiency: } \frac{\text{Actual ethanol produced (v/v)}}{\text{Theoretical ethanol (v/v)}} \times 100$$

Theoretical Ethanol % (v/v) = Sugar utilized (%) ×0.64

Sugar utilized = Available sugar- Sugar present after fermentation

Post-fermentative treatments

The bottles containing prepared muskmelon-wine were stored at 15^0 C and lees /debris was allowed to settle and the cleared wine was racked by siphoning. The racking was repeated after every 15 days till there was no further settling of debris. The clarified wine was stored in glass bottles (washed earlier with boiling water and capped) for up to 3 months. The refrigerated stored wine was analyzed for total microbial count using plate count method on GYE media, ethanol (% v/v), total phenols (mg/100ml) and ascorbic acid (mg/100ml) at different periods of time for up to 3 months. The clarified wine was also subjected to sensory analysis on the basis of Modified Davis Card (Amrine *et al* 1980) by a panel of 10 semi-trained judges.

The free and total sulphur dioxide in the stored wine was observed in muskmelon wine by Ripper Method (Ripper, 1898). The qualitative analysis of muskmelon wine with respect to amino acids carried out by Thin Layer Chromatography (Silica gel, Solvent system- Chloroform: Methanol: Acetic acid-65:25:4) was also studied (tera.chem.ut.ee;www.reachdevices.com).
The standard amino acids were also run on thin Layer Chromatograms and compared with those of muskmelon-wine.

Results and Discussion

Physicochemical characteristics of the juice

Physicochemical characteristics of the extracted juice from the Punjab Sunheri variety revealed a low brix (TSS 6.2 °B), acidity (0.14% w/v), pH (6.4), brix -acid ratio (43.69), ascorbic acid (306.06 mg/100ml) and phenols (35.1 mg/100ml). Earlier Pandey *et al.,* (2008) reported variety specific TSS of 13.45, 10.55, 9.48 and 10.18% in Kashi Madhu, NDM-18, NDM-21 and Punjab Sunheri, respectively. Similarly Parveen *et al.,* (2012) observed a TSS of 8-13% w/v and a total titratable acidity of 0.13-0.21% in muskmelon.

Augustin *et al.,* (1988); Beaulieu and Lee (2007) reported a pH range of 5.25-6.79 in muskmelon during storage of fruits harvested at maturity stage and a titrable acidity of 0.15-0.27%, respectively. As the brix-acid ratio was low, it was adjusted by chaptalizing sugar alongwith addition of citric acid (0.8 g/L) to adjust pH of the wort.

Pre-fermentation treatment

The results presented in table 1 revealed that the microbial count of fresh juice was high on nutrient agar (NA) than glucose yeast extract agar (GYE) media and thus more susceptible to bacterial contamination. KMS+ Pasteurization treatment of juice reduced microbial count to 1.0×102 on NA and 2.8×102on GYE which corresponded to a significant decrease of more than 98.5% of fungal counts and more than 99% of bacterial counts. In literature, there are no such reports (to be the best of our knowledge) on treatment of muskmelon juice for reducing native microflora. However, there are reports in other fruits and vegetables, e.g. Pasteurization for carrot and kunun-zaki (Kun *et al.,* 2008, Egbere *et al.,* 2009, respectively) and pasteurization in combination with preservatives like KMS and sodium benzoate in Sapota (Hiremath and Rokhade, 2012).

The KMS-pasteurization treated juice was subjected to pectinase action (50 units/100ml) at 45°C for 2-6h that revealed 73.44± 0.261 % clarification (due to pectin hydrolysis) in 6 h of incubation at 45^0 C (Table 1). Further, there was more decrease in number of bacterial and fungal counts which showed that during pectinase treatment, the juice did not undergo any microbial spoilage. Earlier, we optimized pectinase treatment (50 units/ 100ml) at a temperature of 45°C for 6 h for 47% clarity of guava juice (Nikhanj and Kocher, 2015). Saxena *et al.,* (2012) also standardized 0.09% (w/w) of pectinase for clarification of watermelon juice.

Ethanol fermentation of pretreated watermelon juice

The pectinase clarified muskmelon juice was chaptalized with sugar at five different brix levels of 15, 17, 19, 21 and 23°B and fermented using RSM optimized conditions of 20°C, 6.2% (v/v) and 0.15% (w/v) of temperature, inoculums size and DAHP, respectively (Bala and Kocher, 2012).

Table.1 Effect of pre-fermentation treatment of muskmelon juice

Growth Media	**cfu/ml of post treated muskmelon juice**		
Treatment	**KMS +Pasteurization**		
Nutrient agar (cfu/ml)	1.0×10^{2}		
% decrease	99.5		
Glucose yeast extract (cfu/ml)	2.8×10^{2}		
% decrease	98.6		
Time (h) ↓ \ cfu /ml	**Plate count (Nutrient agar (NA)/ Glucose yeast extract (GYE) after Pectinase treatment (**50 units/100ml, 6h,45^{0} C)		
0			
NA	2.5×10^{2}	2.2×10^{2}	1.0×10^{2}
GYE	3.0×10^{2}	3.5×10^{2}	2.8×10^{2}
2			
NA	1×10^{1}	4×10^{1}	2×10^{1}
GYE	4×10^{1}	2×10^{2}	1.2×10^{2}
%Clarification	72.21±0.190	71.67±0.07	71.55±0.296
4			
NA	2×10^{1}	5×10^{1}	4×10^{1}
GYE	3×10^{1}	3×10^{1}	5×10^{1}
%Clarification	72.44±0.155	72.35±0.777	71.82±0.268
6			
NA	4×10^{1}	6×10^{1}	5×10^{1}
GYE	4×10^{1}	1×10^{1}	3×10^{1}
%Clarification	73.44±0.261	72.03±0.735	71.75±0.325

*Plate counts in untreated Punjab Sunheri juice were 2.0×10^{4}cfu/ml in NA and 1.4×10^{4} cfu/ml in GYE.

*****Scale of treatment = 100ml

% decrease = $\frac{\text{Initial count- Final count}}{\text{Initial count}} \times 100$

Table.2 Effect of sugar chaptalization on ethanol content of pretreated muskmelon juice

Substrate concentration (%)	pH	*Brix (°B)	**Days	Ethanol %(v/v)	Reducing sugar (mg/100ml)	Fermentation efficiency (%)
15 (Control)	3.2± 0	2.5± 0.707	3	8.9± 0.141	0.335± 0.035	92.7
17	3.2± 0	3.4± 0.565	4	9.85± 0.071	0.425± 0.049	90.5
19	3.2± 0	4.0±0	5	11.5± 0.353	0.5± 0.014	94.5
21	3.0± 0	4.5± 0.707	5	12.8± 0.353	0.59± 0.021	95.2
23	3.0± 0	6.0± 0	5	12.5± 0.424	1.0± 0.212	84.9
CD (5%)		0.775				

*With Hydrometer, brix was zero in all different substrate concentrations treatments
**Days for fermentation
Volume of wort : 300ml, Inoculum size: 6.25% v/v, DAHP: 0.15% w/v, Temperature: 20°C

Table.3 Validation of Optimized fermentation conditions of Punjab Sunheri

Fermentation time (Days)	Brix (°B)		pH		Reducing sugar (mg/100ml)		Ethanol (% v/v)	
	Pectinase treated	Control*	Pectinase treated	Control*	Pectinase treated	Control*	Pectinase treated	Control*
0	21.0±0.13	21±0.20	3.8±0.071	3.8±0.071	985±7.07	985±6.45	0.0±0.06	0.0±0.03
1	19.0±0.15	20±0.71	3.7±0.0	3.8±0.0	785±7.77	869.01±6.81	0.525±0.16	0.32±0.08
2	16.5±0.71	16.9±0.07	3.7±0.071	3.5±0.071	645±5.65	800.9±4.21	3.8±0.14	2.96±0.11
3	12.5±0.71	14±0.71	3.5±0.071	3.4±0.0	61±1.41	577.6±1.89	9.15±0.07	4.2±0.14
4	9.0±0.05	12.1±0.14	3.3±0.0	3.0±0.071	32±1.41	402.02±0.29	9.9±0.14	4.4±0.14
5	0.0±0.10	8.2±0.35	3.2±0.071	3.0±0.0	4.8±0.92	311.2±0.35	12.7±0.353	5.1±0.21
6	-	5±0.07	-	3.0±0.0	-	22.8±0.57	-	6.83±0.13
7	-	0±0.04	-	3.0±0.0	-	11.2±0.14	-	8.2±0.14

pectinase treated juice

Culture conditions: Scale of fermentation: 5L
Brix : 21^0 B Inoculum size : 6.25% v/v
Temperature : 20^0 C DAHP : 0.15g w/v

Table.4 Effect of storage time on microbiological and physicochemical properties of Muskmelon wine cv. Punjab Sunheri

Storage Time (Days)	Parameters		
	% Ethanol (v/v)	Phenol (mg/100ml)	Total yeast count (cfu/ml)
0	13.1± 0.212	19.3 ± 0.019	3.2×10^2
15	12.9± 0.282	18.1 ±0.036	1.9×10^2
30	12.7± 0.353	15.2 ±0.048	6×10^1
45	12.6± 0.494	13.9 ±0.036	0.0
60	12.4± 0.565	8.1 ± 0.022	0.0
CD (5%)	CD (days)-0.195 CD(ethano)-0.309	CD (days)-1.56 CD(phenol)-2.46	-
Sensory score		60.1±5.84	

Total SO_2 = 102.4 ppm
Free SO_2 = 10.24 ppm
Ascorbic acid = 166.66 mg/100ml (Ascorbic acid in juice: 306.06mg/100ml)

The results presented in table 2 revealed that all the treatments had significantly different ethanol production and the juice having a Brix level of 21°B produced a maximum of 12.8% (v/v) ethanol in 5 days of fermentation. Elsewhere, studies on muskmelon fermentation have revealed low ethanol contents of 4.2% and 6.5%, respectively (Hernandez-Gomez *et al.,* 2005; Shukla *et al.,* 1991). However, muskmelon juice if chaptalised with sugar content of 21%, pH of 3.8 at 24^0 C produced muskmelon wine with ethanol content of 11% (v/v).

The authors optimized 21% & 22% (w/v) as initial sugar concentration to produce 11% (v/v) ethanol in muskmelon-wine and watermelon-wine production, respectively (Yang, 2007; Yang, 2008). Such reports for alternate fruits like pineapple, mango, grapes, guava etc. are also available. Earlier, we also optimized 9.0 and 5.0% (v/v) inoculum sizes of Saccharomyces cerevisiae for wine production from guava and grapes, respectively (Kocher and Pooja, 2015).

Therefore, the optimized studies of fermentation parameters revealed sugar concentration of 21°B, temperature 20°C, inoculum level 6.25% (v/v) and DAHP 0.15% (w/v) as optimum for 'Punjab Sunheri' fermentation. These conditions were also validated on pretreated juice at 5L scale. The results presented in table 3 revealed that pectinase treated and untreated wort fermentation at 5L validated the optimized fermentation conditions with ethanol production of 12.7±0.353 % (v/v) and 8.2% (v/v), respectively.

The pH of fermenting juice decreased from 3.8 to 3.2 and 3.8 to 3.0 and reducing sugars decreased from 985±7.07 to 4.8±0.92 mg/100ml in 5 days of fermentation and 985-11.2 mg/100ml in 7 days of fermentation, respectively.

Post- fermentation treatment

The results of different physicochemical and microbiological parameters studied during racking (Table 4) revealed that the yeast cells were undetectable after 30 days of storage in muskmelon wine. Ethanol content though significantly decreased over the storage period of 60 days to a final % ethanol (at 60 days) of 12.4% (v/v) was still sufficient to be named a wine. Total phenols also decreased significantly from 19.3 to 8.1mg/100ml during storage and ascorbic acid decreased from 306.06 to166.66mg/100ml during storage period.

Earlier, literature reports up to 90% decrease of anthocyanins with no change in flavanol content of red wines during storage. It was also reported that the decrease in phenols stabilized after 90-120 days of storage (Zafrilla *et al.,* 2003) and volatile compounds in red wine were significantly decreased by increasing storage time (Perez-Prieto *et al.,* 2003). Decrease in total phenols upto 6 months of storage in white wine was also observed by Kallithraka *et al* (2009). The storage of wine in oak wood barrels for a month improved its quality and led to the reduction in undesirable components such as n-propanol, n-butanol, iso-butanol, isoamyl alcohols (Soni *et al.,* 2009).

The free and total sulphur dioxide in the stored wine was also observed. The muskmelon-wine contained 102.4 ppm of total SO_2 and 10.24 ppm of free SO_2 at 60days of storage against maximum limits of 200 ppm and 50 ppm, respectively. It has been reported that free sulfur dioxide levels higher than 25 ppm severely bleached the color of red muscadine wine and lessened browning in high pH wine only (Sims and Moris, 1984). High SO_2 levels also lessened browning of wine stored at 20°C, but not at higher storage temperatures. The muskmelon-

wine samples were also subjected to sensory analysis to find out their acceptability among the semi-trained tasting panelists. The wine prepared from Punjab Sunheri variety of muskmelon under the optimized conditions having 12.7±0.353 % ethanol (v/v) was found to be of standard quality with a mean score of 60.1±5.84 (Table 4). The semi-trained panelists however differed significantly in their taste opinions.

Earlier, it has been reported that melon wine with initial sugar concentration of 25°B presented the best attributes of color, smell, taste, limpidity and appearance on the basis of sensory evaluation (Padin *et al.,* 2012). It was also observed that muskmelon wine was light yellow, good in mouth feel, luster-transparent and unique in flavor (Yang, 2007).

The qualitative analysis of wine with respect to amino acids carried out by Thin Layer Chromatography revealed amino acid spots showing Rf values of 0.12, 0.146, 0.18, 0.32, 0.46, 0.56, respectively. The standard amino acids were also run on Thin Layer Chromatograms and compared with the spots of wine Thin Layer Chromatograms. Based on comparison and the available literature (tera.chem.ut.ee;www. reachdevices.com), the Rf values were designated to the presence of histidine, arginine, lysine, proline, threonine, methionine, alanine, valine, tyrosine and tryptophan in muskmelon-wine (http://tera.chem.ut.ee).

The present study thus revealed preparation of an alcoholic beverage from muskmelon var. Punjab Sunheri. Besides optimization of pre-fermentation, fermentation and post-fermentation parameters, the muskmelon wine was tested for the quality parameters for up to 60 days. It may be concluded that muskmelon-wine is a nutritive alcoholic beverage having phenols and amino acids.

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