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RESEARCH PAPER

Optimization of Pre-fermentative Skin Treatment Parameters for Production of Quality Wines from Punjab Purple (syn-516) and H-144, Grape Cultivars of Punjab

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Abstract

A study was conducted to determine the effect of skin contact pretreatment on the visual, aromatic and phenolic properties of the grape juice from two cultivass viz., Punjab purple and H-144 cultivars. Response Surface Methodology (RSM) was used to optimize the three parameters i.e. skin weight (30-80%), temperature (10-20^{O}C) and KMS concentration (100-250 mg/L). Among the RSM combinations, 80% skin weight and 100 mg/L KMS concentration at 10^{O}C for 16h resulted in the maximum extraction of anthocyanins and phenolics from skin into the juice in both the cultivars. Overall, the pre-treatment process resulted in increasing the anthocyanins content from 20.5 to 43.5 mg/100 ml and total phenolics from 152.0 to 205.1 mg/100ml in Punjab Purple cultivar. However, in H-144 cultivar anthocyanin and phenolic contents increased from 0.70 to 1.9 mg/100ml, and 121.4 to 147.3 mg/100ml respectively.

Keywords: Anthocyanins, fermentation, grapes, maceration, phenolics, skinpretreatment, wine

A good quality wine is a combination of flavor and aroma coming from the fruit as well as fermentation process. Raw material i.e., grapes used for wine production contribute maximal to the flavor of the resulting wine as they contain the aromatic, phenolic and flavor compounds. Simple juice extraction from the grape berries does not lead to complete extraction of these compounds in to the juice because these compounds are present in the skin and seeds of the berries, and not in the pulp. Under Punjab conditions, where pre-monsoon rainfall damage to the grape crop is common, decrease in the quantity of these compounds has been observed. thus, to increase the grape juice quality for wine production, various methods and techniques like cold maceration, skin-contact, pressing and heat treatment of grape juice prior to fermentation have been followed to enhance the color, aromatic profile and nutrient content of the mature wine (Marais and Van, 1986). Skin maceration generally prompts increased concentration of most of the aroma components in the final wine, though the end-result is influenced by maceration conditions (time and temperature) as well as the fruit cultivar used (Sanchez *et al*, 2007; Selli *et al*, 2006). Phenolic compounds extracted during the maceration process improve the quality of wines and by modifying their color, flavor stability, and aging behavior. They also

contribute towards the sensory properties of wine, affecting parameters such as color, astringency, and bitterness (Esti and Tamborra, 2006; Dangles *et al*, 1992). However, skin contact increases the phenolic compounds of wines and in some cases may even cause more astringent and bitter taste (Cabaroglu *et al*, 1997). For this reason, maceration conditions must be carefully chosen.

Traditional winemaking techniques typically act by increasing the permeability of the envelopes of the grape skin cells to facilitate the release of valuable compounds. Infact, different enological techniques such as varied prefermentative maceration temperatures and times, thermovinification, must or grape freezing and the use of KMS and enzymes have been developed to enhance the extraction of phenolic compounds (Sanchez *et al*, 2007; Selli *et al*, 2006). Besides phenols, aroma compounds, aroma precursors, nitrogen compounds, polysaccharides and minerals are also released into the juice during the skin contact maceration thus, contributing to the final flavor and nutritive value of the juice. Therefore, optimization of the maceration process is essential to ensure a good balance between the wine components and the awareness of this phenomenon is essential for winemakers.

Keeping in consideration the significance of maceration in winemaking, the present study was aimed to optimize of three parameters (skin weight, temperature and potassium metabisulfite concentration) for the skin pretreatment and hence the enhanced extraction of phenolics and anthocyanins from the grape skin into the juice that improves overall quality of the wine produced.

Materials and Methods

The study was conducted at the Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab, India.

Extraction of juice

Healthy ripened grape berries [cultivars Punjab Purple (syn. H-516) and H-144, procured from the Department of Fruit Science, PAU, Ludhiana] were destemmed, washed with water (containing 0.1% KMS) and pressed mildly in a domestic fruit juicer to obtain juice that was immediately stored at 4^{O}C, till further use. Grape skin and seeds were stored separately in a deep freezer for further use in skin pretreatment.

Pre-fermentation treatment

Pre-fermentation parameters (skin weight, temperature and concentration of KMS) were optimized by Response Surface Methodology (RSM), using Design Expert Software (DE-9) for both the grape cultivars. Response surface methodology was adopted in the experimental design as it emphasizes the modeling and analysis of the problem in which response of interest is influenced by several variables and the objective was to optimize this response.

A five-level three-factor central composite rotatable design (CCRD) was employed for the three factors namely skin weight, temperature and KMS concentration. CCRD was composed of a full 2^3 factorial design (eight points) augmented with six replications of the centre points (all factors at level 0) and six star points (points having for one factor an axial distance to the centre of $\pm \alpha$, whereas other two factors at level 0). The axial distance α was chosen to be 1 such that (-1) and (+1) actual values range between 30 and 80, 10 and and 20 and 100 and 200 for skin weight (%), temperature (^{O}C) and concentration of KMS (mg/L), respectively and a set of 20 experiments was planned (Tables 1 and 2).

Results were statistically analysed by Design expert software 'DE-9'. The variance for each factor assessed was partitioned into linear, quadratic and interactive components. The coefficients of the polynomial were represented by b_0 (constant), b_1, b_2, b_3 (linear effects); b_{12}, b_{13}, b_{23} (interaction effects); b_{11}, b_{22}, b_{33} (quadratic effects); and ε (random error). The significance of all the terms in the polynomial functions was assessed statistically using *F*-value at probability (*P*) of 0.05. The statistical analysis of the data and three-dimensional (3D) plotting were also performed using Design Expert software 'DE-9'. The second degree polynomial equation (Eq. 1) was calculated to estimate the response of the dependent variable. The response function (y) was related to the coded variables (x_i, i=1, 2, and 3) by a second degree polynomial equation as given below:

$$y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3 + b_{11} x_1^2 + b_{22} x_2^2 + b_{33} x_3^2 + \varepsilon \quad (1)$$

Grape juice viz. 300 ml was taken in the flask (500 ml capacity, autoclaved earlier and cotton plugged) for each of 20 combinations. According to the RSM design, grape skin and KMS were added to each flask and incubated at a required temperature for the 16h treatment. After the completion of the pre-treatment, each flask was taken out and the juice was filtered through the muslin cloth, spun at 5000 rpm for 5 minutes and analysed for the response parameters viz. total phenols (Malik and Singh, 1991), anthocyanins (Zoecklein, 2005) and color ($OD_{600\,nm}$).

Results and Discussion

Physico chemical characteristics of Grape juice

The comparative physico-chemical characteristics of grape juice evaluated on the basis of chemical analysis (Table 3) showed that H-144 cultivar had lower Brix and higher acidity than Punjab purple cultivar resulting in its low Brix-Acid ratio. Punjab Purple contained more of ascorbic acid, total phenols and anthocyanins (because of its red color) than the H-144 cultivar. Red grape cultivars contain higher content of phenolics, flavonoids and anthocyanins as compared to the white grape cultivars. ^{O}B-pH index and brix-acid ratio were in the desirable range that indicated that the both the cultivars are suitable for the wine production with respect to the Brix and pH values (Van and Archer, 2000).

Prefermentation treatment

Grape juice (300 ml juice for each combination) of both cultivars was subjected to the pre-fermentation skin maceration treatment according to the RSM plan. Both the cultivars, pre-treatment 80% skin weight (w/v), 10^{O}C temperature and 100 mg/L KMS concentration were optimum for the maximum extraction of phenolics and anthocyanins (Table 4 and 5).

All the models for the three responses phenols, color and anthocyanins were significant with the values of 135.01, 19.92 and 84.31 for skin Punjab purple pretreatment. Pretreatment of H-144 gave the values 133.68, 181.45 and 265.20 for these parameters, respectively (Table 6). However, the purple colored grapes of H-144 yielded white juice and the skin pretreatment process helped in the extraction of anthocyanins and phenolic compounds from its skin to increase their amount, thus increasing the antioxidant properties of the juice and of wine produced. Table 6 also showed that adjusted R^2 values for all models are in reasonable agreement with their predicted R^2 values.

The data were put in equation 1 and the resulting regression second polynomial equations (in terms of coded factors) with significant factors for the three responses (Punjab Purple eqn. 2, 3, 4 and H-144 eqn. 5, 6, 7) of both the cultivars are as shown below:

Punjab Purple

$$\text{Phenols} = +154.48 + 19.33*A - 1.62*C - 1.75*A*C - 5.81*A^2 - 2.78*B2 - 2.52*C^2 \quad (2)$$

$$\text{Color} = +0.086 + 0.088*A - 0.069*B - 0.073*A*B + 0.025*A^2 + 0.051*B^2 \quad (3)$$

$$\text{Anthocyanins} = +26.86 + 4.75*A + 0.48*B + 0.49*C + 0.79*A*B + 2.89*A^2 \quad (4)$$

H-144

$$\text{Phenols} = +134.47 + 19.22*A - 1.72*C - 5.74*A^2 - 2.84*B^2 - 2.45*C^2 \quad (5)$$

$$\text{Color} = +0.022 + 0.020*A + 5.012E\text{-}003*A^2 \quad (6)$$

$$\text{Anthocyanins} = +1.65 + 0.38*A - 0.089*A^2 - 2.514E\text{-}003*B^2 \quad (7)$$

The ANOVA analysis of the optimization study indicated that the model term A (skin weight) was significant ($p < 0.05$) for all the responses for both the cultivars. However, the interactions between the skin weight and KMS concentration (AC) for the phenols response and interactions between skin weight and temperature (AB) were found to be significant for the color and anthocyanins responses for the Punjab purple cultivar (Fig. 1a, 1b and 1c). In contrast, no interaction was found to be significant in the pretreatment of H-144 cultivar juice.

Figure 1a showed the interaction between the skin weight and KMS concentration for the phenol response that indicates increase in the phenol content with the increase in the skin weight used for the treatment. However, phenol content decreased with increase in the KMS concentration. Similarly, in the Fig. 1b and 1c with the increase in the skin weight and temperature upto 15^{O}C, color and anthocyanin contents increased initially and then declined. A study on effect of different maceration temperature and time on color and sensory properties of Cabernet Sauvignon wines have show that the maceration period of 6 days at 15^{O}C resulted in high sensory score of resulting wine produced (Sener and Yildirim, 2013). Elsewhere in the literature, Beer *et al.,* (2006) found that pre-fermentation maceration of pinot grapes have potential to improve the color of the wine by extracting anthocyanins from the grape skin along with phenolics and flavonoids. In fact, the differences in mean values of the total phenolic were related to the juice processing bydifferent methods at industrial scale juice processing (Sautter *et al*, 2006). Thus, the present study optimized the conditions for skin pretreatment of both the cultivars (Punjab pink and H-144) w.r.t. skin weight - 80%, temperature - 10^{O}C and KMS concentration - 100 mg/L (statistically analyzed with DE-9 software) that were further validated at 1 litre scale. The results presented in table 7 showed increase in anthocyanins, color and total phenols in juice and a concomitant decrease in all the three parameters in the skin post-treatment for both the varieties.

Table 1: Experimental codes, ranges and levels of the independent variables for response surface methodological experiment

Variables	Units	Symbol code	Levels				
			-1.682	-1	0	+1	+1.682
Skin weight	%	A	12.96	30	55	80	97.04
Temperature	°C	B	6.59	10	15	20	23.41
KMS concentration	mg/L	C	48.87	100	175	200	301.13

Table 2: Experimental design for the skin pre-treatment of grape cultivars according to the RSM

Run	A:skin weight (%)	B:temp (°C)	C:KMS conc. (mg/L)
1	55	23.41	175
2	55	6.59	175
3	80	20	250
4	30	20	100
5	55	15	175
6	55	15	48.87
7	55	15	301.13
8	80	20	100
9	30	10	100
10	55	15	175
11	55	15	175
12	97.04	15	175
13	55	15	175
14	12.96	15	175
15	55	15	175
16	55	15	175
17	30	20	250
18	80	10	100
19	30	10	250
20	80	10	250

Table 3: Physiochemical characteristics of Grape juice (Punjab purple and H-144)

Parameters	Grape cultivar	
	Punjab purple	H-144
TSS (°Brix)	17°B	15.6°B
pH	3.8	4.0
B-pH index (200-270)*	245.48	249.60
Acidity	0.50	0.52
Brix-acid ratio (27-34)*	34.0	30.0
Ascorbic acid (mg/100ml)	1470.2	1290.4
Total phenols (mg/100ml)	152.0	121.4
Anthocyanins (mg/100ml)	20.5	0.70

*Desirability level; Index- $(pH)^2$ X Brix; Ratio- Brix/Acidity

Table 4: Experimental Response profile for RSM process variables combinations and responses in Punjab Purple

Run	A*	B*	C*	R1*	R2*	R3*
1	55	23.41	175	150.3	0.122	29.3
2	55	6.59	175	150	0.331	26.06
3	80	20	250	206	0.125	36.8
4	30	20	100	135.5	0.079	25.1
5	55	15	175	150.9	0.151	26.73
6	55	15	48.87	151	0.116	26.75
7	55	15	301.13	150.2	0.111	28.9
8	80	20	100	202.7	0.139	36.4
9	30	10	100	136.3	0.075	25.91
10	55	15	175	150.3	0.047	26.7
11	55	15	175	151.4	0.063	26.16
12	97.04	15	175	201.2	0.269	42.1
13	55	15	175	150.2	0.134	27.3
14	12.96	15	175	138.5	0.034	27.62
15	55	15	175	150.2	0.079	26.91
16	55	15	175	150.8	0.045	27.4
17	30	20	250	136.8	0.071	24.7
18	**80**	**10**	**100**	**205.1**	**0.409**	**43.5**
19	30	10	250	136.5	0.077	26.52
20	80	10	250	200.8	0.438	36

***A**-Skin weight (%); **B**-Temperature (°C); **C**- KMS concentration (mg/L); **R1**- Phenols (mg/100ml); **R2**- Color (OD_{600nm}); **R3**- Anthocyanins (mg/100ml)

Table 5: Experimental Response profile for RSM process variables combinations and responses in H-144

Run	A*	B*	C*	R1*	R2*	R3*
1	55	23.41	175	129.2	0.025	1.61
2	55	6.59	175	122.2	0.029	1.66
3	80	20	250	142.8	0.051	1.88
4	30	20	100	101.4	0.008	1.16
5	55	15	175	132	0.023	1.6
6	55	15	48.87	131.3	0.02	1.64
7	55	15	301.13	122.3	0.021	1.6
8	80	20	100	145	0.049	1.91
9	30	10	100	106.3	0.007	1.16
10	55	15	175	135.1	0.02	1.67
11	55	15	175	136	0.022	1.66
12	97.04	15	175	149	0.067	1.99
13	55	15	175	135.1	0.022	1.62
14	12.96	15	175	86	0.004	0.79
15	55	15	175	134.9	0.021	1.66
16	55	15	175	134	0.023	1.67
17	30	20	250	104.8	0.009	1.17
18	**80**	**10**	**100**	**147.3**	**0.05**	**1.9**
19	30	10	250	105	0.007	1.15
20	80	10	250	139	0.05	1.98

***A**-Skin weight (%); **B**-Temperature (°C); **C**- KMS concentration (mg/L); **R1**- Phenols (mg/100ml); **R2**- Color (OD_{600nm}); **R3**- Anthocyanins (mg/100ml)

Table 6: Adjusted R^2 and Predicted R^2 values of model responses for both grape cultivars

Cultivar	Response	Model F-value	p-value*	Adjusted R^2 value**	Predicted R^2 value
Punjab Purple	Anthocyanins	84.31	<0.001	0.9753	0.9147
	Color	19.92	<0.001	0.8996	0.8322
	Phenols	135.01	<0.001	0.9845	0.9482
H-144	Anthocyanins	265.20	<0.001	0.9921	0.9811
	Color	181.45	<0.001	0.9884	0.9608
	Phenols	133.68	<0.001	0.9843	0.9476

*p-value < 0.05 and **R^2 value of > 0.75 indicates good fitness of the model

Table 7: Validation results of the optimized parameters at 1 lt. scale and analysis of grape skin

Parameters	Punjab Purple				H-144			
	J1*	J2*	S1*	S2*	J3**	J4**	S3**	S4**
Anthocyanins (mg/100ml)	20.5	43.5	54.9	30.1	0.70	1.9	2.1	0.8
Color ($OD_{600\ nm}$)	0.276	0.409	—	—	0.03	0.05	—	—
Total phenols (mg/100ml)	152.0	205.1	237.3	181.9	121.4	147.3	178.2	149.9

J1*- Punjab purple juice before treatment; J2*- Punjab purple juice after treatment; S1*- Punjab purple skin before treatment; S2*- Punjab purple skin after treatment; J3**-H-144juice before treatment; J4**- H-144 juice after treatment; S3**- H-144 skin before treatment; S4**- H-144 skin after treatment

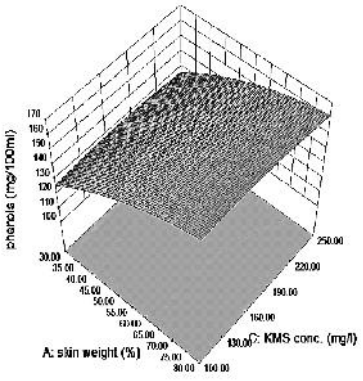

Fig. 1a

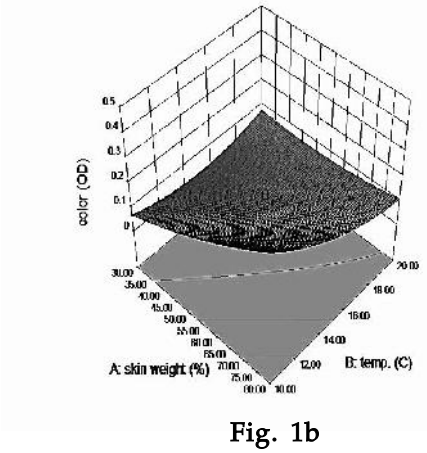

Fig. 1b

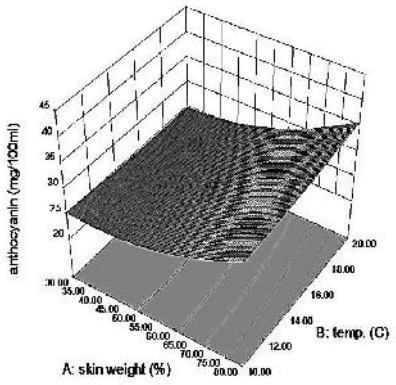

Fig. 1c

Fig. 1: 3D surface plots representing interaction between significant prefermentative parameters. **1(a)** Skin weight and KMS concentration for the response phenols; **1(b)** Skin weight and temperature for the response color; **1(c)** Skin weight and temperature for the response anthocyanins of the Punjab purple cultivar

Elsewhere, Frankel and Meyer (1998) also determined the concentrations of total phenolics in commercial juices produced from grapes belonging to 'Concord' cultivar and mixtures with other grape cultivars as 1.79 and 1.47 g L^{-1} gallic acid equivalents, respectively which showed that skin pretreatment is a cultivar specific process. This study thus standardized grape juice skin pretreatment conditions with 80% skin weight, 100mg/L KMS concentration at 10^{O}C for 16h for both the cultivars viz. Punjab purple and H-144 . Further, Samappito and Butkhup (2010) also reported that mulberry wines made with skin contact treatments had higher values for total flavonoids and total phenolics contents than the control (untreated) wine. It has been observed in this study that skin contact not only increased some aroma attributes but there was also a concomitant increase of alcohols and ester concentrations in skin contact wines than the untreated wines as documented earlier also (Kaur *et al*, 2012) which will help in preparing quality wines from Punjab purple and H-144 grape cultivars. Marais and Rapp (1988) also studied the effect of skin-contact time and temperature on juice and wine composition which resulted in overall improvement in flavor as well as nutritive value of wine produced from skin pretreated juice. It is concluded that the grape juice pre-treated with skin had higher values for total phenolics and anthocyanins contents with enhanced color than the untreated juice (control) that are known to contribute towards flavor, aroma and nutritive value of the wine produced from it.

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