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OPTIMIZATION OF ETHANOL CONCENTRATION, GLYCEROL CONCENTRATION AND TEMPERATURE CONDITIONS OF GRAPE-MAHUA WINE TO MAXIMIZE THE QUALITY AND OVERALL ACCEPTABILITY

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ABSTRACT

Black grapes (*Vitis vinifera*) and mahua (*Madhuca longfolia*) extract was used in 90:10 grape-mahua ratio for fermentation for 15 days and subjected to clarification using bentonite and gelatin as fining agents. Ageing was allowed for three months and studies were conducted using response surface methodology to assess the effect of ethanol, glycerol and temperature on viscosity, color, specific gravity (SG), pH and overall acceptability. Experimental designs were conducted and 20 samples were prepared containing varying concentration of ethanol (7.55-13.44%), glycerol (6.19-18.8g/l) and temperature (5.6-22.4°C) respectively. The maximum desirability of 93% was obtained for wine under the optimized conditions 13.44% ethanol, 6.19g/l glycerol and 14°C temperature, having viscosity (efflux time), 12.9 s; color absorbance, 4.61; SG, 1.0012; pH, 3.34 and overall acceptability, 8.47.

Keywords: Ethanol, glycerol, mahua, optimization, quality, red wine

INTRODUCTION

The grape (*Vitis vinifera*) is one of the most ancient crops known to people and is one of the world's largest fruit crops with an annual production of 69.66 million metric tons, as per the FAO (2011) preliminary data. Grape cultivation has passed through a series of developments in terms of selection of agro-climatic regions, variety and cultural practices. Grape is largely utilized in the form of wine in the world. However, in India, out of total grape production, fresh table grapes accounts for 80 per cent followed by raisin (18%) and wine and juice (2%) (**Shikhamany, 2001**).

Wine is an alcoholic beverage made from anaerobic fermentation of fruits (mainly grapes). It is produced by fermenting crushed grapes using various types of yeast which consume the sugars found in the grapes and convert them into alcohol. Moderate consumption of wine can be beneficial in healthy individuals. Polyphenols, among them resveratrol, have generated a great amount of scientific research due to their in-vivo and in-vitro antioxidant capabilities (**Fehér and Drexler, 2001**). Among the various types of wines available, red wine, white wine and sparkling wines are the most popular light wines since they contain only 10–14% alcohol-content by volume. Wines are routinely categorized as being light, medium or full-bodied (**Gawel *et al.*, 2007**). Glycerol is a major product of yeast fermentation and is reported to range up to 9.9 g/L in Australian white table wines (**Rankine and Bidson, 1971**), and 9.36 g/L in South African dry white wines (**Nieuwoudt *et al.*, 2002**). However, **Noble and Bursick** (**1984**) estimated that an additional 26 g/L of glycerol is required before an increase in white wine viscosity is just noticeable. Based on this result, it is unlikely that glycerol concentration influences the perceived viscosity of dry white wine. **Nurgel and Pickering** (**2005**) reported enhanced perceived viscosity of a model wine upon increasing its glycerol concentration from 10 to 25 g/L. The contribution of ethanol to wine sensory properties extends beyond that of possibly enhancing fullness. Ethanol affects the headspace concentrations of many wine volatiles and also contributes to sweetness.

Mahua *(Madhuca longfolia)*, commonly known as mahwa or mahua, is an Indian tropical tree found largely in the central and northern plains and forests of India. The tree bears succulent, thick, cream-colored flowers, which are rich in sugar and also reported to contain minerals, proteins, cellulose, traces of fat and appreciable amount of vitamin C and vitamin B-complex (**Mande *et al.*, 1949**). Next to sugarcane, the mahua flowers are the most important source of raw materials for fermentation and production of alcohol and vinegar. Therefore, it could prove to be a good substitute for number of fermentation industries since it is quite cheap than sugar and easily available in bulk.

There is grooving awareness about the red wine as a product in the domestic market due to the various health benefits it imparts. Attempts have been made to utilise the dry mahua flowers in the preparation of red grape wine which is well recognised for its anti-disease properties and as an important functional beverage. Ethanol, glycerol and temperature are the important parameters which directly influence the quality and overall acceptability of wine. **Gawel *et al.*** (**2007**) reported that ethanol and glycerol levels in realistic ranges had a small but inconsistent positive effect on the body and viscosity of Riesling wines. Glycerol is believed to be responsible for the mouth-feel characteristics that are often indicative of high-quality wines. Hence the present study was undertaken to optimize the ethanol, glycerol and temperature conditions of grape-mahua wine to maximize the quality and overall acceptability of wine by using Central Composite Rotatable Design (CCRD), Response Surface Methodology (RSM).

MATERIAL AND METHODS

Black grapes (*Vitis vinifera L.*) were purchased from the local market of Sangrur, Punjab, India. Mahua flowers (dry) were procured from a mahua growing district of Madhya Pradesh, India. Compressed yeast (*Saccharomyces cerevisiae var.*) was obtained from the local market of Sangrur. Bentonite and Gelatin in their pure form were used for the clarification of wine. All chemicals used in the study were either AR grade or extra pure.

Pre-treatment

The grapes were washed with tap water and their stem was removed. The de-stemmed grapes were kept immersed in water containing 100 ppm of potassium metabisulphite(KMS) solution. The grapes were crushed with hands so as to have a minimum of grinding and tearing of the grape tissues, without breaking the seeds. During crushing, sulfiting was done at the rate of 7-150 ppm to prevent bacterial fermentation of malic acid to lactic acid (**Patil *et al.*, 2012; Benda, 1981**). Hand pressing was done to separate the liquids from the solids and also to reduce the extraction of bitter and astringent materials (**Patil *et al.*, 2012**). The must was kept overnight to get maximum extraction of color from the skin.

Grape must fortification with mahua extract and fermentation

Fortification of grape must was done with 10% mahua extract (**Patil *et al.*, 2012**). Fermentation was carried out in amber coloured bottles for a period of 15 days. At the end of fermentation, the waste metabolites including lees were settled at the bottom of the bottle. The clear wine was separated from the lees by the process of racking and siphoning. The sample was further subjected to clarification and stabilization using bentonite and gelatin as fining agents. Bentonite and gelatin were added at a concentration of 0.02g/100g and 0.04g/100g (**Patil *et al.*, 2012**) for the clarification of wine. Samples were further subjected to ageing for a period of 3 months at ambient temperature.

Theoretical considerations and experimental design

Ethanol, glycerol and temperature are the important parameters which directly influence the quality and overall acceptability of wine. Glycerol is believed to be responsible for the mouth-feel characteristics that are often indicative of high-quality wines. Therefore, the effect of ethanol, glycerol concentration and temperature on the viscosity, colour, specific gravity, pH and overall acceptability of sample 'Z' (10% mahua extract) was assessed using Response surface methodology (RSM). Designed experiments were conducted to investigate the effect of ethanol concentration (x_1), glycerol concentration (x_2) and temperature (x_3) on product responses: viscosity (Y_1), colour (Y_2), specific gravity (Y_3), pH (Y_4) and overall acceptability (Y_5) of the wine obtained from the grapes and 10% mahua extract. The range of the parameters was carefully selected based on the literature available (**Taherzadeh *et al.*, 2002; Sachde *et al.*, 1980; Ough *et al.*, 1972**). The central value (zero level) chosen for experiment design were ethanol concentration 10.5 % (v/v), glycerol concentration 12.5g/l and temperature 14°C. Twenty different combinations of samples were prepared containing ethanol, glycerol and temperature in the range from 7-14% (v/v), 5-20g/L and 5-23°C respectively.

In developing the regression equation, the test factors were coded according to the equation.

$$X_i = (X'_i - X^x_i) / \Delta X_i \qquad (1)$$

Where X_i is the coded value of the i^{th} independent variable, X'_i is the natural value of the i^{th} independent variable, X^x_i is the natural value of the i^{th} independent variable at the center point and ΔX_i is the step change value or difference between maximum and minimum value. The range and the levels of the experiments variables used in the coded and uncoded form for centre, factorial and augmented point of design are summarized in Table 1.

Table 1 The experimental design in coded and uncoded form for the optimization of variables using central composite rotatable design (CCRD)

Exp. No.	Coded			Uncoded		
	Ethanol (% v/v)	Glycerol (g/l)	Temp (°C)	Ethanol (% v/v)	Glycerol (g/l)	Temp. (°C)
1	1.68	0	0	13.44	12.5	14
2	0	0	0	10.5	12.5	14
3	-1	-1	-1	8.75	8.75	9
4	0	0	-1.68	10.5	12.5	5.59 (5.6)*
5	0	0	0	10.5	12.5	14
6	-1.68	0	0	7.55	12.5	14
7	0	0	0	10.5	12.5	14
8	1	1	1	12.25	16.25	19
9	0	0	0	10.5	12.5	14
10	0	-1.68	0	10.5	6.19	14
11	0	0	0	10.5	12.5	14
12	0	0	1.68	10.5	12.5	22.39 (22.4)*
13	-1	1	-1	8.75	16.25	9
14	0	1.68	0	10.5	18.8	14
15	-1	-1	1	8.75	8.75	19
16	-1	1	1	8.75	16.25	19
17	1	-1	1	12.25	8.75	19
18	0	0	0	10.5	12.5	14
19	1	-1	-1	12.25	8.75	9
20	1	1	-1	12.25	16.25	9

*The values shown in the parenthesis is the actual value considered for the experiment

Physicochemical and sensory analysis

Wine was subjected to various physicochemical parameters such as color (absorbance), specific gravity and pH by standard methods (**Ranganna, 2007**). Viscosity was measured in terms of efflux time for sample, expressed in seconds (**Sharma *et al.*, 2006**), Sensory analysis was performed by hedonic rating method (**Ranganna, 2007**). A panel of 20 semi-trained panelists was formed from the department of Food Engineering and Technology, SLIET, Longowal. Panelists evaluated the overall acceptability of the samples.

Statistical analysis

'Design expert – 6' software was used for regression and graphical analysis of the data obtained. The optimum values of the selected variables were obtained by solving the regression equation and also by analyzing the response surface graphs.

RESULTS AND DISCUSSION

The following quadratic response function was considered for the mathematical relationship between independent & dependent variables.

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1(j<i)}^{3} \sum_{j=1}^{3} \beta_{ij} X_i X_j \qquad (2)$$

Where Y is the measured response, βo, intercept term, βi, βij , and βii are the constant coefficients. The variable XiXj represents the first- order interactions between Xi and Xj for (j<i).

Viscosity

Viscosity was recorded in terms of time taken by wine to fall through the viscometer tube. The time varied from 12.58 to 14.8 seconds for the samples treated with different combinations of ethanol, glycerol and temperature (Table 2).

Table 2 Effect of ethanol concentration, glycerol concentration and temperature on viscosity, color, specific gravity, pH and overall acceptability of wine

Exp. no.	Uncoded			Dependent variables				
	Ethanol (% v/v)	Glycerol (g/l)	Temp. (°C)	Viscosity (efflux time, s)	Color	SG	pH	Overall Acceptability
1	13.44	12.5	14	14.28	2.4	1.00023	3.6	6.2
2	10.5	12.5	14	13.5	1.77	1.00237	3.7	6
3	8.75	8.75	9	14.24	1.48	1.00028	3.73	4.28
4	10.5	12.5	5.59 (5.6)*	14.08	1.08	1.00176	3.58	4.88
5	10.5	12.5	14	13.36	1.79	1.00233	3.72	5.75
6	7.55	12.5	14	13.32	1.2	1.00502	3.59	4
7	10.5	12.5	14	13.3	1.78	1.00269	3.68	5.58
8	12.25	16.25	19	14.06	2.02	1.00118	3.87	3.78
9	10.5	12.5	14	13.22	1.76	1.00399	3.7	5.46
10	10.5	6.19	14	13.93	2.7	1.00004	3.58	6.5
11	10.5	12.5	14	13.2	1.79	1.00279	3.65	5.32
12	10.5	12.5	22.39 (22.4)*	12.58	1.87	1.00554	3.66	4.67
13	8.75	16.25	9	13.5	1.8	1.00505	3.67	3
14	10.5	18.8	14	14.5	1.33	1.0061	3.84	3.47
15	8.75	8.75	19	13.67	1.67	1.00308	3.57	4.47
16	8.75	16.25	19	12.84	1.38	1.00626	3.59	3.42
17	12.25	8.75	19	12.74	3.2	1.00279	3.58	7
18	10.5	12.5	14	13.1	1.76	1.00269	3.71	5
19	12.25	8.75	9	13.18	3	1.00115	3.48	6.8
20	12.25	16.25	9	14.8	1.2	1.00309	3.64	3.6

*The values shown in the parenthesis is the actual value considered for the experiment

The maximum viscosity was obtained when the ethanol concentration, glycerol concentration and temperature were 12.25% (v/v), 16.25 g/l, 9°C while minimum was at 10.5% (v/v), 12.5 g/l, 22.4°C respectively (Table 2). Regression model fitted to the experimental results of viscosity (Table 3) shows the validity of the model. F value, 16.59, coefficient of determination R^2, 0.937 and the Adj R^2, 0.880 also indicated the fitness of the model in prediction of the results. Lack-of-fit (LoF) was found to be non-significant.

Table 3 Regression coefficients of the second order polynomial and their significance

Coefficients	Viscosity	Color	SG	pH	Overall acceptability
β_0	13.2859***	1.7683***	1.0028***	3.69325***	5.5304***
β_1	0.1570**	0.3740***	-0.0010***	0.00196	0.7109***
β_2	0.1705**	-0.3847***	0.0013***	0.06204***	-1.0138***
β_3	-0.3611***	0.1551**	0.0007**	0.01644**	0.0466
β_{11}	0.1451**	0.0523	-0.0001	-0.0342***	-0.2271**
β_{22}	0.2918***	0.1283**	3.42085E-05	0.00647	-0.2677**
β_{33}	-0.0210	-0.0626	0.0002	-0.0254**	-0.3420**
β_{12}	0.5637***	-0.3762***	-0.0009**	0.06125***	-0.5112**
β_{13}	0.0062	0.1562	-0.0005	0.07125***	-0.0287
β_{23}	-0.0487	0.00125	-0.0006*	0.02625**	0.0262
R^2, %	93.72	93.76	89.57	96.20	95.13
F	16.59	16.71	9.55	28.16	21.74
Adeq. precision	16.04	15.26	11.93	23.87	15.02
Adj. R^2, %	88.07	88.15	80.19	92.78	90.76
Pred R^2, %	60.86	48.14	36.19	82.58	76.65
LoF	No	No	No	No	No

*Significant at 10%, **Significant at 5%, ***Significant at 1%, LoF, Lack of Fit

The 3D graph (Figure 1) shows that viscosity increased with increasing concentration of glycerol whereas it decreased with the increasing concentration of ethanol. With increase in glycerol, the viscosity also increased. **Gawel *et al.*** (**2007**) found that increased concentration of ethanol and glycerol contributed to increase in viscosity of wine, whereas, an increase in temperature resulted in decrease in viscosity of wine.

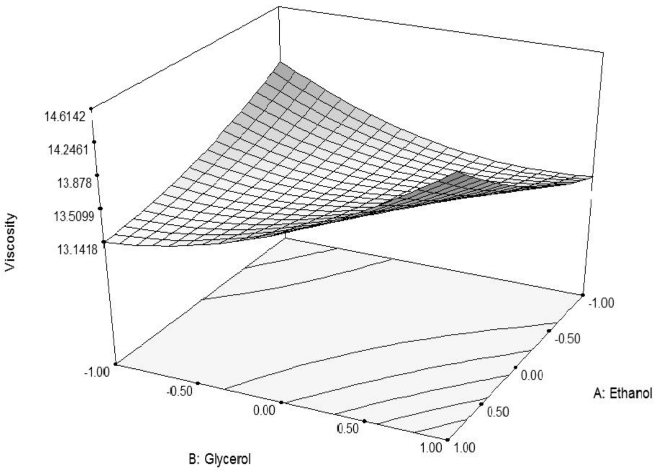

Figure 1 3D plot for the effect of ethanol concentration (A) and glycerol concentration (B) at constant temperature (14°C) on viscosity of wine

The response surface plot (Figure 2) showed that there was a decrease in viscosity with an increase in temperature. In the figure, the interactive effect of ethanol and temperature, shows dominating effect of temperature as compared to ethanol concentration. **Yanniotis *et al.*** (**2007**) described decrease in the viscosity of wine with increasing temperature using Arrhenius equation. In its pure form, glycerol is a viscous liquid at room temperature. Therefore it is reasonable to assume that it contributes to the perceived viscosity and fullness of dry wines. However, **Noble and Bursick** (**1984**) estimated that an additional 26 g/L of glycerol is required before an increase in wine viscosity. Based on this result it is unlikely that glycerol concentration influences the perceived viscosity of dry table wine. **Nurgal and Pickering** (**2005**) reported enhanced perceived viscosity of wine upon increasing its glycerol concentration from 10-25g/L and increasing ethanol from 0-15%.

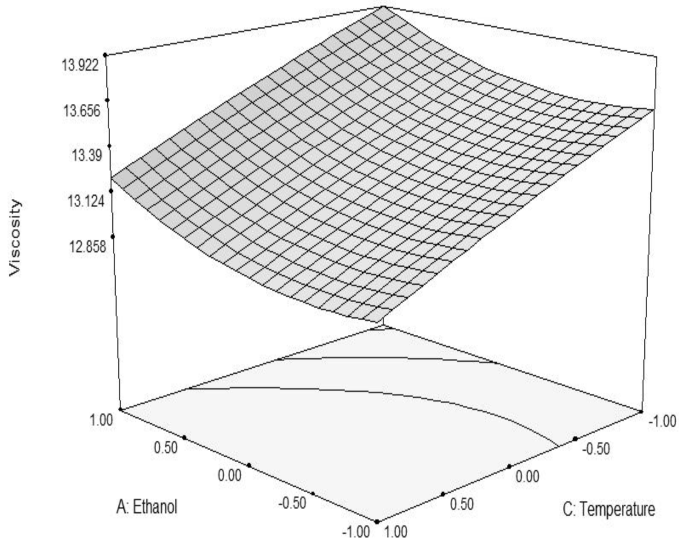

Figure 2 3D plot for the effect of ethanol concentration (A) and temperature (C) at constant glycerol concentration of 12.5 g/l on viscosity of wine

Color

Color absorbance varied from 1.08 to 3.2 for the samples treated with different combinations of ethanol, glycerol and temperature (Table 2). The maximum color was obtained when the ethanol concentration, glycerol concentration and temperature were 12.25% (v/v), 8.75 g/l, 19°C while minimum was at 10.5% (v/v), 12.5 g/l, 5.6°C respectively (Table 2). Regression model fitted to experimental results of color (Table 3) shows the validity of the model. The value of the coefficient of determination R^2, 0.937 and the Adj R^2, 0.881 also indicated the fitness of the model in prediction of the data. **Mutanen *et al.*** (**2007**) carried out a study to characterize the optical properties of red wine using color as one of the important parameters. **Joshi and Sandhu** (**2000**) reported that increasing ethanol content of wine decreases the color units and increase the viscosity. **Canals *et al.*** (**2005**) reported that the presence of ethanol in wine facilitates anthocyanin and especially pro-anthocyanidin extraction, but it also decreases co-pigmentation phenomena, which can decrease the color intensity.

Specific gravity and pH

Specific gravity varied from 1.00004 to 1.00626 for the samples treated with different combinations of ethanol, glycerol and temperature (Table 2). The maximum specific gravity was obtained when the ethanol concentration, glycerol concentration and temperature were 8.75% (v/v), 16.25g/l, 19°C while minimum was at 10.5% (v/v), 6.19 g/l, 14°C respectively (Table 2). The F value, lack-of-fit, R^2 and the Adj R^2 values indicated the fitness of the model in prediction of the results (Table 3). Response surface plot (Figure 3) shows that specific gravity increased with increase in glycerol concentration and decrease in ethanol concentration. The pH of the samples treated with different combinations of ethanol, glycerol and temperature (Table 2) varied from 3.48 to 3.87. **Sachde *et al.*** (**1980**) reported that red wine contains pH in the range 3.4 to 4.2. The maximum pH was obtained when the ethanol concentration , glycerol concentration and temperature were 12.25% (v/v), 16.25 g/l, 19°C while minimum was at 12.25% (v/v), 8.75 g/l, 9°C respectively (Table 2). Regression model fitted to experimental results of pH (Table 3) shows the validity of the model.

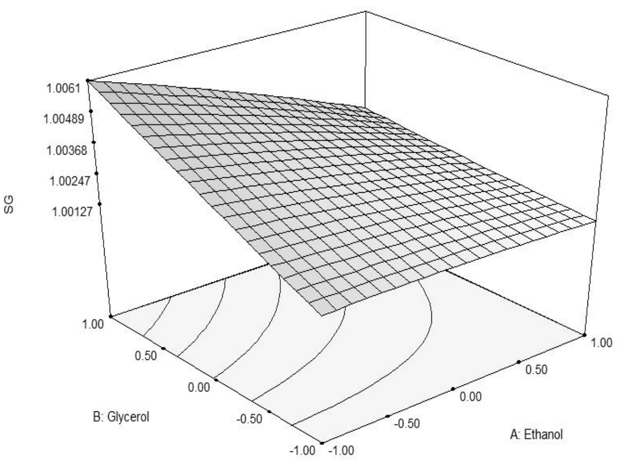

Figure 3 3D plots for the effect of ethanol concentration (A) and glycerol concentration (B) at constant temperature (14°C) on specific gravity of wine

Overall acceptability

Overall acceptability was assesed using 9-point Hedonic scale rating. Hedonic scale result shows overall acceptability from 3.0 to 7.0 for the samples treated with different combinations of ethanol, glycerol and temperature (Table 2). The overall acceptability was found to be best for the sample containing 12.25%v/v ethanol, 8.75g/l glycerol concentration and 19°C temperature while it was found to be least for sample containing 8.75% (v/v) ethanol, 16.25 g/l glycerol and 9°C temperature respectively (Table 2). Regression model was fitted to experimental results of overall acceptability (Table 3).

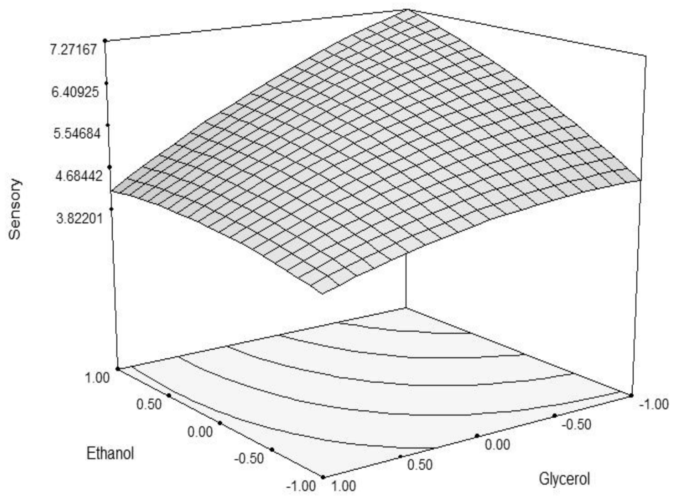

Figure 4 3D plots for the effect of ethanol concentration and glycerol concentration at constant temperature (14°C) on the overall acceptability of wine

3D plot (Figure 4) shows that there was a decrease in overall acceptability with decreasing concentration of ethanol and increasing concentration of glycerol. Overall acceptability increased with increasing the ethanol concentration while it decreased with increase in glycerol concentration of wine (Figure 5). Glycerol is the major fermentation by-product of *Saccharomyces cerevisiae* after ethanol and CO_2, which indirectly contributes to the overall acceptability of wine. Glycerol is believed to be responsible for the mouth-feel characteristics that are often indicative of high-quality wines (**Fauhl *et al.*, 2004**). **Joshi and Sandhu** (**2000**) reported that with increase in the alcohol content of wine, there was an increase in the sensory score of most wines.

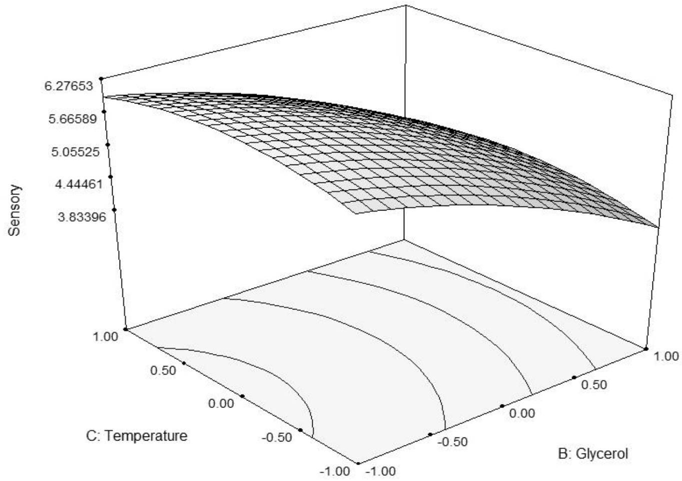

Figure 5 3D graph plot for the effect of glycerol concentration (B) and temperature (C) at constant ethanol concentration of 10.5% (v/v) on the overall acceptability of wine

Optimization of parameters

A numerical multi-response optimization technique was used to optimize the effect of ethanol concentration, glycerol concentration and temperature on viscosity, specific gravity, pH, color and sensory perception (overall acceptability) of wine. The software uses second order model to optimize the responses. Under the selected range, the optimum conditions were found as 13.44% (v/v) ethanol concentration, 6.19g/l glycerol concentration and 14°C temperature with the desirability of 93%. It was observed that experimental value of viscosity (efflux time, 13.2), specific gravity (1.0029) and overall acceptability (8.6) was fractional higher while the color absorbance (4.23) and pH (3.31) was slightly lower than predicted values i.e. Viscosity (efflux time),12.9; colour absorption, 4.61; specific gravity, 1.0012; pH, 3.34 and overall acceptability, 8.47. Thus, there was a little variation in the predicted (in range) values and the actual experimental values. The difference in the predicted and actual experimental values was statistically assessed by determining the coefficient of variation (COV). The coefficient of variation was found to be 1.625 for viscosity, 6.063 for color, 0.1199 for specific gravity, 0.637 for pH and 1.077 for overall acceptability respectively.

CONCLUSION

Viscosity (efflux time) of wine increased with increase in glycerol concentration while decreased with increase in temperature and ethanol concentration. No significant effect of glycerol, ethanol and temperature was found on the colour of wine. Specific gravity increased with increasing glycerol concentration and decreasing ethanol concentration. The pH increased with increasing ethanol and glycerol concentration of wine. Overall acceptability increased with increasing ethanol concentration and decreasing glycerol concentration. No significant effect of temperature was observed except overall acceptability of wine. The maximum desirability of 93% was obtained for wine under the optimum conditions of 13.44% ethanol, 6.19g/l glycerol and 14°C temperature having viscosity (efflux time) of 12.9 s, color absorbance 4.61, SG 1.0012, pH 3.34 and overall acceptability 8.47. The difference in coefficient of variation was found to be 1.625 for viscosity, 6.063 for colour, 0.1199 for specific gravity, 0.637 for pH and 1.077 for overall acceptability, when compared with the experimental to the predicted results under the optimum conditions of 13.44% ethanol, 6.19g/l glycerol and 14°C temperature respectively.

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