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Biochemical profiling of red and white wine varieties grown under tropical condition

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Abstract

Variation in yield parameter, biochemical profile like amino acid and sugar composition of different red and white grape wine varieties and their impact on quality of grapes was studied. The analysis showed difference in the concentration of total reducing sugar, phenol, protein, carbohydrate, starch, individual amino acid and individual sugar as the significance values less than 0.01. Total acidity was highest in the red variety of wine grape as compared to white variety of wine grape, however, reducing sugar and phenols were higher in the red variety. Similarly, sugar profile showed higher amounts of individual sugars like glucose and fructose in red variety of wine grape. Amino acid profile showed the concentration of different amino acids were high in white grape varieties as compared to the red grape varieties.

Key words:- Amino acid profile, Sugar profile, Yield parameters, Vitis vinifera.

Introduction

Grape (*Vitis vinifera L.)* is one of the major important commercial fruit crops of the country. It is grown on an area of about 136 thousand hectares with annual production of 2454 tons per hectare (FAO, 2015; Indian Horticulture Database). Maharashtra is the leading state in area and production followed by Karnataka, Tamil Nadu and some part of North India. Grape can be classified into four groups based on their final consumption i.e., table grapes, wine grapes, raisin and juice grapes (Patil et al. 1995). Out of the total grape production, approximately 1.5% grape is used for wine making (Indian Horticulture Database 2013).

The development and maturation of grape berries have received considerable scientific scrutiny because of both the uniqueness of such processes to plant biology and the importance of these fruits as a significant component of human diet and wine industry. For the winemaker, an outstanding attribute of *Vitis. vinifera* is its ability to store enormous quantities of sugar in its berries. The ripe phenotype is the summation of biochemical and physiological changes that occur during fruit development and make the organ edible (Giovannoni, 2001). Ripening stage of fruit affects physico-chemical parameters which ultimately affects the quality of processed products prepared from them. The fruit maturity is determined not only by 'sugar ripeness' but also by 'flavour ripeness' of the berries. The main maturity index is the sugar content, determined as the total soluble solids (TSS), also known as Soluble Solid Concentration (SSC) or °Brix. For certain specific cultivars and situations, titrable acidity (TA) and SSC-TA ratio are used as maturity indices (Guelfat-Reich and Safran, 1971).

The inquisitiveness of humans and the importance of wine colour, flavour, and astringency, the management of phenolic compounds are becoming important (Conde *et al.*, 2007). Phenols and related compounds can affect the appearance, taste, mouth feel, flavour and antimicrobial properties of wine. Free amino acids and ammonia make up the majority of nitrogen (N) containing compounds that are important in wine grapes for successful alcohol and/or malolactic fermentations (Bely, and Barre, 1990). However, free amino acid concentrations and their profiles within grapes, can vary depending upon cultivar, rootstock/scion combinations, vine nutrient management, vineyard site and growing season, (Gump, *et al*, 2002, Bell and Henschke, 2005). The total amino acid content and concentration of individual amino acids are important parameter in wine grapes, which ultimately influences the final quality of wine (Shiraishi *et al*., 1986). Several recent studies have been devoted to berry development, ripening and factors affecting berry composition. In this context, the present study was conducted to evaluate red and white wine grapes for quality, yield and significant biochemical parameters.

Materials and Methods

The study was conducted at ICAR-National Research Centre for Grapes, Pune (18.32 0N, 73.510E) in Mid-west Maharashtra (India) during 2012-13. Five white wine varieties (Chenin, Muscat, Sauvignon Blanc, Riesling and Colombard) and five red wine varieties (Cinsaut, Grenache, Tempranillo, Merlot and Cabernet Sauvignon) were selected for the study.

Berry and yield parameters

The yield parameter (100-berry weight, average bunch weight and yield/vine) were recorded at harvest time. The quality parameter (TSS, acidity, volatile acidity and pH) were recorded from must. The soluble solids concentration was determined from the juice using digital refractometer (model ERMA, Japan).

Estimation of biochemical parameters

Estimation of carbohydrate and starch was done by Anthrone's method. Reducing sugar was estimated by dinitrosalicylic acid (DNSA) method. Total phenolic content was estimated using Folin-Ciocalteu reagent by measuring the absorbance of the reaction mixture at 650 nm (Singleton and Rossi, 1965). The results obtained were expressed as catechol equivalent (mg/g) of the crushed sample.

For protein estimation, 0.5g of crushed samples homogenized in 0.1 M phosphate buffer (pH 7.0) was used. The homogenate was centrifuged at 5000 rpm for 15 minutes at 40 °C and supernatant was used as a source for protein estimation. Proteins were estimated calorimetrically at 765nm as per (Lowry et al., 1951).

Analysis of Sugar

For sugar analysis, 1g of crushed sample was vortexed thoroughly with five ml of formic acid (0.1% in 20% methanol). After centrifugation (5000 rpm, 10 minutes) one ml of supernatant was drawn for further centrifugation at high speed (10,000 rpm, 4̊C, 10 min). The resultant supernatant extract was filtered (0.2 µm- membrane filter, Pall life sciences, India) and used for analysis.

Sugars were analyzed with an Evaporative Light Scattering Detector (ELSD). Chromatographic separation of sugars was performed using a phenomenex Luna column 5NH2 (250 mm × 4.6 mm) with a mobile phase of 70% acetonitrile and 30% HPLC grade water (degassed and ultra sonicated) at a flow rate of one mL/min, ELSD parameter temperature at 65 °C with gain six. The injection volume was 15 µL with the total run time of six minutes for a single run

Estimation of Amino acids by LC-MS Method

Reference standard

The certified reference standards of amino acid were purchased from S. D. Fine Chemicals (Mumbai). Standard stock solutions were prepared by dissolving 10 (± 0.1) mg reference standards in 10 mL water resulting in a final concentration of approximately 1000 µgml-1. A working standard mix of 10µgml-1 was prepared in water by mixing and diluting the individual standard stock solutions. Calibration standard solutions were prepared in the range of 10-1000 ngml-1 by diluting the 10 µgmL-1 solution. All the standard solutions used under the study were stored at 4°C.

LC-MS/MS analysis

The LC-MS/MS analysis was done with a Perkin Elmer series 200 coupled to an API 2000 (AB Sciex) mass spectrometer equipped with an electrospray ionization (ESI). The HPLC separation was carried out using an Atlantis dC18 column (100x2.1mmx5µm) from Waters India Pvt. Ltd., Bangalore. The mobile phase A composed of methanol:water (20:80) with 5mM ammonium formate and B composed of methanol: water (90:10) with 5mM ammonium formate. The column flow rate was 0.4mL min-1and column temperature 35°C. A gradient chromatography composed of 0-0.5 min 85% A, 0.5-6min 85-2% A, 6-11min 2% A, 11-12min 2-85% A and 12-20 min 85% A. The source parameters were set as nebulizer gas 40 psi, heater gas 60 psi, ion source temperature 550°C, ion spray voltage 5500V for positive polarity.

Extraction and sample preparation

Five gm. of crushed sample was taken into polypropylene tube containing 10ml of methanol with distilled water (1:1). After vortex, the mixture was centrifuged at 5000 rpm for five minutes. Two ml supernatant was taken in Eppendorf tube and again centrifuged at 10,000 rpm under 4oC for 10 min. A supernatant was filtered through 0.2µm-membrane filter (Pall life sciences, India) and the filtrate was used. 400 ml aqueous extract was taken in two ml eppendrof tubes for derivatization reaction followed by addition of 350 µl of borate buffer and 200 µl of 20mM FMOC-Cl reagent. The whole mixture was kept on an vortex for derivatization, after derivatisation 50 µl 20 % formic acid aad in eppendroff tube then vortex for 30 sec. Finally the extract was centrifuged at 10000 rpm for five min, pass through 0.2µm Nylon 66 membrane filter paper and 20µl extract injected to LC-MS/MS.

Statistical Analysis

Analysis of variance was performed for each variable using the SAS statistical package 9.3 (SAS Institute, Cary, NC). Least Significant differences, coefficient of variance and significance of data among the treatments were calculated.

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Results and Discussion

Berry quality and yield

The data recorded on berry quality parameters such as TSS, acidity, pH, volatile acidity were presented in Table1. Amongst the white wine varieties, the TSS was highest in Chenin (24.30 °B) followed by Sauvignon (23.40 °B), Colombard (22.30 °B), Muscat (22.20 °B) and Riesling (21.50 °B). Interestingly, lowest acidity was found in the variety which had highest TSS and vice-versa, viz. 6.70 g/l, 6.00 g/l, 7.0 g/l, 6.10 and 7.60 g/l (Chenin, Sauvignon, Clolombard Muscat and Riesling respectively). The same trend was not similar in red wine varieties. In case of red wine variety, TSS was highest in Cabernet Sauvignon (24.00 °B) with highest acidity (7.70g/l), Tempranillo (23.50 °B) with 6.10g/l acidity, Merlot (23.10 °B with 6.50g/l acidity), Cinsaut (22.50 °B with7.30 g/l acidity) and least TSS (21.50 °B) and highest acidity (7.80g/l) was recorded in Grenache. The varying range of total soluble solids and total acidity reveal individual cultivars performance in the growth conditions rather than the range of harvest maturity. It has been reported that the accumulation of total soluble solids is genetically independent (Shiraishi et al, 2000).

The highest 100-berry weight was recorded in white wine variety Colombard (125.39g) followed by Chenin (124.12g) and Sauvignon (90.03g) as compared to the red wine variety Cinsaut (212.82g), Tempranillo (101.24g), and Grenache (92.59g). However, the Chenin showed highest bunch weight (118.29 g) while least bunch weight was recorded in Riesling variety (63.35 g). Among the red wine varieties, Tempranillo recorded higher bunch weight of 104.89g while the least bunch weight was recorded in Merlot (58.43g). However, the highest yield per vine was recorded in Colombard (7.57 kg per vine and Chenin (4.35 kg per vine, as compared to red wine variety Tempranillo (3.65kg) and Merlot (2.44kg per vine. It was observed that the white varieties of wine grapes recorded higher berry weight, bunch weight and yield per wine than that of red wine grape varieties.

Biochemical parameters

The data on various biochemical parameters from the berries are presented in Table 2. Significant differences were recorded for reducing sugar, protein, total phenols, starch and carbohydrate among white wine and red wine grapes. The highest carbohydrate was recorded in white wine variety viz. Chenin (170.51 mg/g) followed by Colombard (167.93mg/g) and Muscat (166.35mg/g) variety. Among the red wine varieties, Cabernet Sauvignon recorded higher carbohydrate concentration (162.46mg/g) followed by Grenache (157.85mg/g) and Tempranillo (155.90mg/g). The increase in carbohydrate content in these varieties might be due to the increase in canopy with shoot length that have resulted in highest photosynthetic rate which helps to store more carbohydrate in the sink. In tropical viticulture region, after fruit pruning, shoot density is maintained based upon the number of bunches retained. This is mainly required to nourish the developing bunch. The increased shoot length resulted in increased leaf area which might have contributed for better photosynthesis. These results are in accordance with a previous study where heavy canopy has been reported to cause active photosynthesis and store more carbohydrates (Gao and Cahoon, 1994). Similar results were also obtained by (Somkuwar et. al. 2014) who reported that potential of vine to produce carbohydrate to meet the demands of fruit production and vegetative growth based on effective leaf area, whereas proper crop load is important to achieve maximum yields of highest quality fruit without sacrificing vine capacity. Fruit production and shoot growth compete for available carbohydrates.

The concentration of reducing sugar in the berries was found to be significant in all the varieties studied. The reducing sugar increased with the increases in yield per vine. The concentration of sugar (299.53 mg/g) in Sauvignon Blanc was higher as compared to the red wine variety Cabernet Sauvignon (325.95mg/g) and Merlot (235.04mg/g). The changes in sugar content might be due to the changes in the photosynthetic activities of vine. The results of the present investigation is in confirms with the results obtained by (Somkuwar et. al., 2013) who found positive correlation between photosynthetic rate and reducing sugar.

The differences for starch, proteins and total phenols form the berries of wine varieties varied significantly. The highest proteins (12.38 mg/g) were recorded in Grenache variety while the least (4.29 mg/g) in Sauvignon Blanc. The results of the present investigation showed that the concentration of protein was higher in red wine varieties as compared to the white wine varieties. However, total phenolics were maximum in red wine variety Cabernet Sauvignon (5.73 mg/g) and minimum concentration recorded in white variety Sauvignon Blanc (2.19mg/g). These findings are similar with (Koes R.E.et al 1994, Firmin J.L. et al 1986) who reported that concentration of phenolics in red wine variety is much higher than that of white wine varieties. These compounds have a numerous roles including UV protection, pigmentation, disease resistance, and nodule protection.

Starch is a reserve compound in grapevine storage tissues such as, leaves, shoots and roots. The concentration of starch varied significantly in the berries. The least starch contents were found in white wine variety Chenin (10.97mg/g) followed by Muscat (9.79mg/g) and Riesling (9.58mg/g) as compared to red wine variety Tempranillo (12.60mg/g), Merlot (12.22mg/g), Grenache (10.35mg/g) and Cabernet sauvignon (9.53mg/g) respectively.

The differences in protein contents among the different varieties indicate the existence of wide range of variations. This might be due to the response of individual vines to environmental conditions. Several workers reported that various factors including cultivar, rootstock/scion combination, vine nutrient management, vineyard site and growing season affects the proteins and amino acid concentration within the grapes (Bell and Henschke, 2005; Gump et al., 2002). However, increased phenol content may help to reduce the disease incidence in grapevine. Somkuwar et al (2014) reported less incidence of anthracnose disease with higher phenol contents in leaf of grapevine, while working on Thompson Seedless grafted on Dogridge and 110-R rootstock.

Sugar profile

The differences amongst the sugar contents of different wine varieties are presented in Table 3. Fructose, glucose and sucrose as sugar components were determined in grapes and wine. The fructose sugar was in higher concentration than the other sugars. In the present study, the red varieties recorded high sugar concentration than the white wine varieties. Among the red varieties, Cabernet Sauvignon recorded higher sugar concentration (142.58 mg/lit) followed by Cinsaut (140.95mg/lit) the sugar composition is mainly determined by genotype, and sugar concentration is strongly affected by environmental and cultural practices (Dai et al., 2011). These results are in accordance with the (Rusjan et al. 2008) who obtained a higher amount of total sugar in red cultivars. However, the sucrose concentration is present in very negligible amount at harvest stage (below 5% of the total sugars). Moreover, the sucrose is absent in berries at harvest time or sometimes it may be present in 1 to 2.5 gm/lit. These results are similar to the observations recorded by (Kliewer1965) and recent work on wine grapes.

Amino acid

The data showed major differences in amino acid accumulation pattern among the red and white wine varieties. Major amino acids present were alanine, arginine, proline, and ornithine. It was observed that the amino acid content of white wine was higher as compared to the red wine varieties. The alanine content of Tempranillo wine was higher (8.74mg/lit) followed by Merlot (3.66 mg/lit), Cabernet Sauvignon (2.525g/lit). Arginine values were also found to be in higher concentration in white wine varieties Chenin (169.00mg/lit) and Muscat (159.05mg/lit) as compared to red wine varieties like Tempranillo (45.85mg/lit), Cinsaut (23.00mg/lit) and Grenache (11.85mg/lit). The results of the present investigation are similar to (Etiévant et al. 1988) who reported very low arginine values in French wines of Cabernet Sauvignon (on average 120 mgl-1 of primary amino acids). It was found that Merlot had substantially lower amounts of most amino acids. These results are in accordance with (Etiévant et al. 1988) who reported a mean value of 124 mgl-1 of amino acids for French Merlot wines.

Proline concentration was dominant among the white wine varieties like Chenin (260.05 mg/lit) and Colombard (227.24 mg/lit) as compared to red wine varieties viz., Merlot (247.50 mg/lit), and Cabernet sauvignon (174.50 mg/lit). Similar results were also obtained by (Soufleros et al. 2002). The results on amino acid contents in the present study also confirm the results of (Millery et al. 1986). Similar results were also obtained for ornithine.

Conclusion

Certain aspects of grape berry growth and ripening, particularly the rapid changes in acid and sugar levels during ripening, have been studied in the past. The results of the present study provided considerable information to understand the sugar, amino acid and biochemistry of five commercially important red and white grape cultivars at the berry harvest period. The findings of the present study are anticipated to aid grape growers worldwide, as well as provide relevant data for future studies on the biochemistry of grape berries. The results will also be useful for future research on the analysis of the chemical composition of grape juices.

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Fig.1 Individual sugar standard chromatogram.

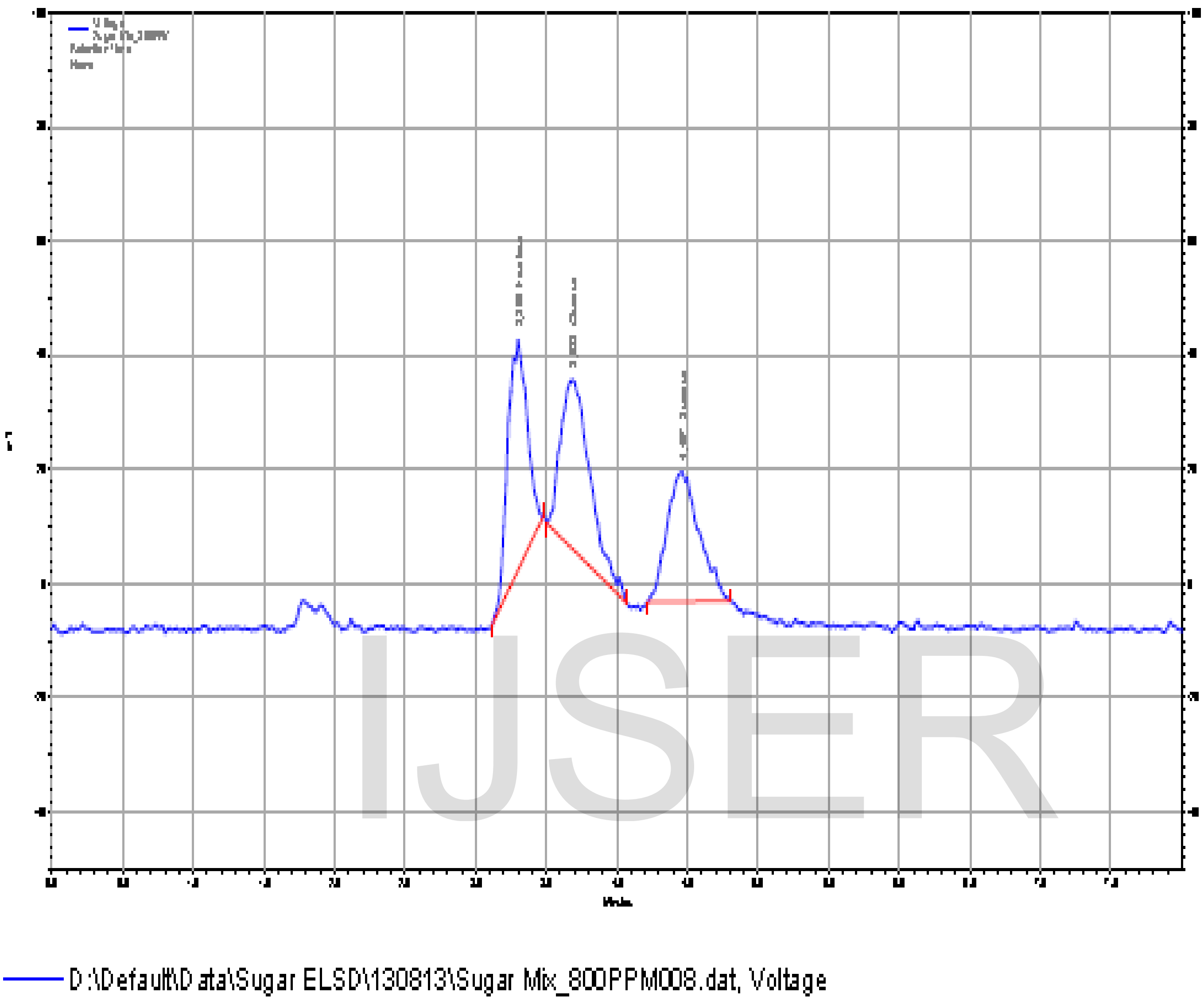

Fig.2 Amino acid standard chromatogram.

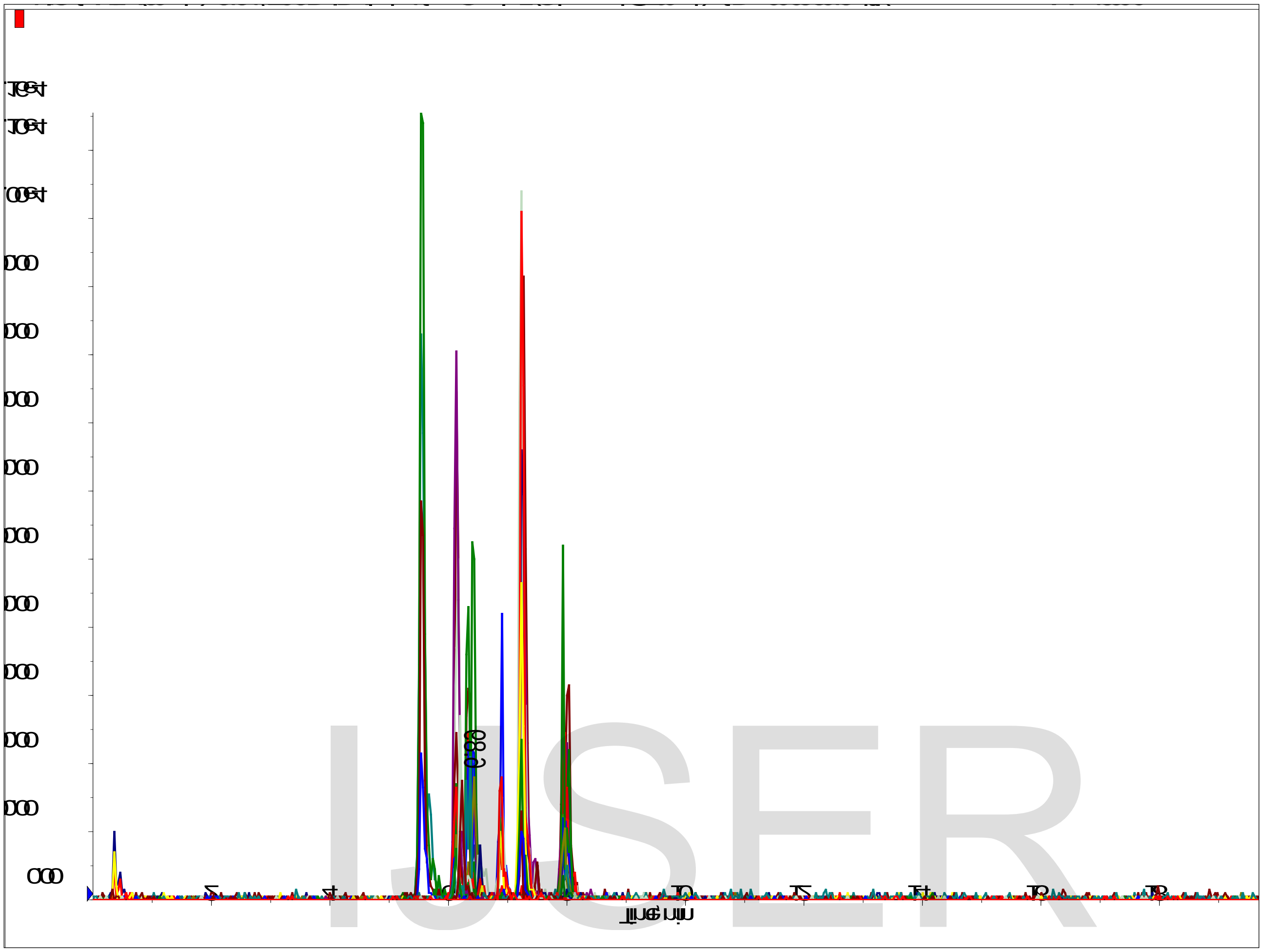

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Table no.1:- Berry and Yield parameters of white and red wine varieties.

Name of variety	100 berry wt (g)	Average bunch wt (g)	juice % recovery	Yield/vine (kg)	TSS (°Brix)	pH	TA g/l	VA g/l
White varieties								
Colombard	125.39a	100.72b	70.50b	7.57a	22.30b	3.12e	7.00b	0.07c
Riesling	73.26d	63.35e	56.50d	3.88 d	21.50d	3.30d	7.60a	0.09a
Sauvignon Blanc	90.03b	91.67c	61.50c	4.20c	23.40b	3.76a	6.00d	0.06d
Muscat	80.47c	79.82d	62.50c	2.50e	22.20c	3.67b	6.10d	0.07c
Chenin	124.12a	118.29a	72.50a	4.35b	24.30a	3.52c	6.70c	0.08b
LSD at 5%	2.71	2.75	1.43	0.129	0.55	0.08	0.13	0.005
Cv%	2.05	2.26	1.65	2.13	1.81	1.81	1.49	1.58
Significance	**	**	**	**	**	**	**	*
Red varieties								
Cabernet Sauvignon	77.38d	76.8b	64.50b	3.58ab	24.00a	3.46c	7.70a	0.10a
Merlot	60.39e	58.43c	60.50c	2.44d	23.10b	3.67b	6.50c	0.06d
Tempranillo	101.24b	104.89a	64.00b	3.65a	23.50b	3.91a	6.10d	0.07c
Grenache	92.59c	102.24a	71.00a	3.55b	21.50c	3.20d	7.80a	0.04e
Cinsaut	212.82a	102.31a	63.50b	2.64c	22.50a	3.61b	7.30b	0.08b
LSD at 5%	5.83	2.80	1.26	0.07	0.55	0.09	0.11	0.005
Cv%	3.99	2.35	1.46	1.85	1.79	1.92	1.21	2.21
Significance	*	**	**	**	**	**	**	*

Table no 2:- Biochemical analysis of white and red wine varieties.

Name of Variety	Reducing Sugar	Protein	Phenols	Starch	Carbohydrate
White variety					
Colombard	296.86ab	1.78e	1.33c	8.13c	167.93ab
Riesling	252.38d	3.47b	2.15a	9.58b	161.94c
Sauvignon Blanc	258.19c	4.29a	2.19a	4.42d	164.45bc
Muscat	292.50b	2.67c	1.85b	9.79b	166.35b
Chenin	299.53a	2.54d	1.87b	10.97a	170.51a
LSD	5.40	0.12	0.05	0.21	3.52
Cv%	1.45	3.14	2.25	1.84	1.57
significance	**	**	**	**	*
Red variety					
Cabernet Sauvignon	325.95a	10.37b	5.73a	9.53d	162.46a
Merlot	235.04e	8.16c	4.51b	12.22b	143.26c
Tempranillo	257.80d	8.12c	3.95d	12.60a	155.90b
Grenache	285.53c	12.38a	4.40c	10.35c	157.85b
Cinsaut	298.25b	4.12d	3.82e	8.72e	136.33d
LSD	5.50	0.15	0.07	0.30	3.06
Cv%	1.46	1.37	1.18	2.09	1.51
significance	**	**	**	**	**

Table no 3:- Sugar profile of white and red varieties

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Name of variety	Fructose	Glucose	Sucrose	Ratio (Glu/Fru)
White variety				
Colombard	116.37b	115.07b	0.01c	0.98b
Riesling	113.43c	108.36c	0.02c	0.96c
Sauvignon Blanc	109.73d	99.26d	0.01c	0.90e
Muscat	76.80e	70.77e	1.54b	0.92d
Chenin	120.46a	118.47a	2.09a	0.99a
LSD @5%	0.02	0.04	0.03	0.01
Cv%	1.2	2.1	1.4	3.2
significance	**	**	**	**
Red variety				
Cabernet Sauvignon	142.58	140.95	0.00	0.99
Merlot	127.36	124.05	0.85	0.97
Tempranillo	108.46	102.99	1.61	0.95
Grenache	115.59	110.35	0.00	0.95
Cinsaut	140.44	136.88	0.00	0.97
LSD @5%	0.03	0.05	0.06	0.04
Cv%	1.40	2.3	3.7	1.7
significance	**	**	**	**

Table no: - 4 Amino acid profile of white and red varieties.

Sample Name	Alanin	Alanin NH4	Arginine	Aspartic acid	Glutamic acid	Glutamic acid 18	Glycine	Hy.Proline	Isoleucine	Norleucine	Ornithine	Proline
White varieties												
Colombard	4.44	2.56	4.51	0.08	2.26	0.80	2.05	4.26	0.37	0.31	241.1	227.24
Riesling	6.40	5.58	17.90	0.28	6.00	6.37	2.19	1.71	0.75	0.84	51.00	78.8
Sauvignon Blanc	3.35	1.67	42.80	0.08	1.35	1.87	1.14	0.85	0.23	0.48	63.50	114.5
Muscat	1.80	1.01	159.05	0.34	1.89	1.68	1.10	0.56	0.25	0.38	42.15	49
Chenin	27.44	2.54	169.00	1.73	5.83	5.99	5.09	4.71	2.31	1.95	168.9	260.05
LSD @ 5 %	0.04	0.03	0.05	0.07	0.12	0.46	0.38	0.90	0.38	0.47	0.48	17.59
CV %	1.37	1.78	2.59	1.47	3.57	2.90	3.28	3.18	4.78	2.90	4.67	6.40
significance	**	**	**	**	**	**	**	**	**	**	**	**
Red varieties												
Cabernet Sauvignon	2.52	0.70	4.58	0.30	0.61	0.59	0.71	3.82	0.20	0.29	24.50	174.5
Merlot	3.66	1.32	6.48	0.00	1.39	1.24	0.63	1.67	0	0.39	20.35	247.5
Tempranillo	8.74	7.11	45.85	0.81	6.04	7.43	4.05	1.51	1.52	0.53	156.5	172.5
Grenache	0.31	0.22	11.85	0.04	0.20	0.17	0.41	0.15	0.14	0.00	16.45	32.15
Cinsaut	0.75	0.72	23.00	0.00	0.36	0.43	0.47	0.51	0.15	0.00	27.5	38.25
LSD @ 5 %	1.48	2.40	2.49	3.18	4.39	2.58	2.6	1.62	2.49	1.5	2.39	17.11
CV %	3.59	4.29	5.90	4.28	3.19	2.68	4.29	2.69	1.9	2.7	1.69	6.83
significance	**	**	**	**	**	**	**	**	**	**	**	**

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