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Studies on Biochemical Composition of Different Parts of Berries and Wine Quality of Wine Grape Varieties (*Vitis vinifera L.*)

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A B S T R A C T

The study was conducted on six red wine varieties (Syrah, Caladoc, Grenache Noir, Tempranillo, Cabernet Franc and Nielluccio) at the farm of ICAR-National Research Centre for Grapes, Pune. Five year old vines of these varieties were selected for the study. The grapes were harvested at about 23°Brix TSS. Anthocyanins, total phenols, tannins, reducing sugar, carbohydrate and protein were quantified in different parts of berries viz.; seed, skin and pulp. Among the different varieties, TSS and acidity showed non-significant effect while highest pH value (3.56) was found in Tempranillo and lowest (3.42) in Cabernet Franc. However, volatile acids were maximum in Grenache Noir (0.13) and minimum in Caladoc variety (0.10). The biochemical parameters like Anthocyanin in pulp and skin varied significantly among different varieties. In wine quality parameters, variety Caladoc recorded highest pH and ethanol % while Cabernet Franc, Grenache Noir, Syrah and Tempranillo showed highest volatile acids, total acids, mallic acid and glucose: fructose ratio respectively. The varieties Cabernet Franc and Syrah were found better for biochemical contents and wine quality.

Introduction

Wine is one of the most popular beverages prepared from grapes through fermentation under the controlled conditions. It comprises phenolic compounds mainly classified into flavonoids and non-flavonoids (Garrido and Borges, 2013). These compounds are considered to have antioxidant, anti-cancer and anti-inflammatory properties (Arranz *et al.*, 2012; Casas *et al.*, 2012) and they are also responsible for some of the sensory properties like colour, aroma, flavour, bitterness and astringency in grapes and wine (Del Rio *et al.*, 2013).

Tannin contains condensed form of polymerized flavan-3-ols and responsible for mouth feel, body and astringency of wine (Rice *et al.*, 2017). However, flavan-3-ols are originated from berry skin and seeds at varying concentrations depending on cultivars (Ribereau-Gayon *et al.*, 1982). These compounds are thought to be associated with bitterness and astringency in grape seed (Romeyer *et al.*, 1986). Tannins are responsible for mouth feel in wine (Manns *et al.,* 2013). However, the consumer gives less importance to wine with poor mouth feel.

Colour is the preference of consumer for red wine as it is predisposing their recognition or denial (Gonzalez-Neves *et al.,* 2014). The colour pigments are mainly found in berry skin with a concentration ranging from 200 to 5000 mg/kg of fresh grape (Jordao *et al.,* 1998). Among the different biochemicals, anthocyanin and tannins are responsible for colour in red wine. It also possesses antioxidant, anti-proliferative and immune modulatory properties (Mazue *et al.,* 2014).Consumers are more attracted towards the red wine, even though the colour is not contributor in taste or smell. Wine gets its colour mainly due to the presence of anthocyanin pigments carried from the berries (Boulton, 2001).Anthocyanin concentration in wine varies with the cultivar, region and the methods used during vinification. However, it is pH dependent, as the pH reduces the intensity of red colour becomes higher (Rice *et al.,* 2017). Casassa *et al.,* (2014) reported that anthocyanins develops during berry ripening (veraison) and reaches its maximum during berry maturation when the process of synthesis ends. Cultural practices followed during the season also play an important role in developing major biochemicals including anthocyanins in grape berries. The studies conducted by many researchers demonstrated that, vineyard management can be used to change the levels of compounds associated with wine quality. Guidoni *et al.,* (2002) suggested bunch thinning changes the concentration of anthocyanins in berries.

Ethanol plays an important role in wine aroma, taste and mouth feel. The concentration of ethanol is regulated by modifying sugar content in berries or harvesting at various fruit maturity level, as it was produced from sugar during fermentation (Scott *et al.,* 2017). During vinification process, the fermentation conditions influences contents of phenolic compounds which ultimately results in decrease in organoleptic and antioxidant properties of wine (Zhang *et al.,* 2017). The harvesting of grape is determined by checking the sugar level, acid content, colour etc. as high sugar, lower acid and rich colour are the indicating factors of harvesting (Boulton *et al.,* 1996).

The limited work has been carried out on quality and biochemical parameters for fresh grapes and wine under tropical condition. Considering these, the present study was carried out to evaluate red wine varieties for fruit quality and biochemical parameters of berries and wine.

Materials and Methods

The present study was conducted at the experimental farm of ICAR-National Research Centre for Grapes, Pune (18°32′N and 73°51′E) during 2016-17. Five year old vines of six different red wine varieties (Syrah, Caladoc, Grenache Noir, Tempranillo, Cabernet Franc and Nielluccio) grafted onto 110-R rootstock were selected for the study. The vines were spaced at distance of 2.66m X 1.33m trained to mini Y trellis with horizontal orientation of cordon having vertical shoot position.

In an annual growth period the vines are pruned twice i.e. first pruning is done during April (foundation pruning) while the second pruning in October (forward pruning).

Analysis of berry quality parameters

At harvest, the juice was extracted from grape berries and centrifuged at 500 rpm for 5 minutes. Total soluble solids was estimated using Oeno Foss (a FTIR based analyzer) and was expressed in °Brix while juice pH was estimated using pH meter. To measure volatile acids (g/L), titration method (0.1 N

NaOH) using phenolphthalein indicator was used.

Biochemical estimation of grape berries

Tannins from grape berries were determined using Folin-Denis method. Tannic acid was used as standard solution and the absorbance were recorded at 700 nm and was expressed in mg/g. Anthocyanin was estimated using pH differential method (pH 1 and pH 4.5) and absorbance was recorded at 520 and 700 nm. The phenols from the samples were determined using Folin-Ciocalteu method (Singleton and Rossi, 1965) using Gallic acid as standard and OD recorded at 765nm and was expressed in mg/g. Reducing sugar was estimated using Dinitrosalicylic acid (DNSA) method while, total carbohydrate was determined using Anthrone method with D-glucose as the standard and concentration of both were expressed in per cent. Protein estimation was done as per Lowry *et al.*, (1951) and the total protein content in fresh berries was expressed as Bovine Serum Albumin Fraction-V equivalent (%).

Wine preparation and analysis for quality parameters

The wine was prepared using standard protocol. Bunches from each variety were harvested after attaining the total soluble solids of around 23°Brix. The separated berries were crushed in Destemmer-cum-crusher and transferred into 20L stainless steel containers. To stop the activity of naturally occurring micro-organisms, potassium meta-bisulphite (KMS) was added (5mg/10 kg grape must). The prepared grape must was then exposed to cold shock at 5°C and the must was incubated with commercial yeast strain EC1118 (*Saccharomyces bayanus*) at 20 mg/L in the form of dry active yeast. During the fermentation period, the temperature was maintained below 22 ± 2°C with cold exchanger (Frozen water container). It took 11 days and the sugar level was less than 2g/L. Wine under each variety was separated from the skins and seeds manually. As soon as the racking and lees separation were completed, 60 ppm SO_2 was maintained and the bottles were kept in storage at 4°C for further analysis.

The wine quality parameters (pH, volatile acids, total acids, ethanol, and malic acid) were recorded on a FTIR based analyser called Oeno Foss. The wine samples were drown into falcon tube and centrifuged at 500rpm for 5 minutes and the readings were recorded.

Statistical analysis

The experiment was conducted in Randomized Block Design with six red wine varieties as treatments replicated three times. The data recorded on various parameters was tabulated using means of each treatment and was analysed using SAS version 9.3.

Results and Discussion

Quality parameters of grape berries

The volatile acids and pH varied significantly among the varieties while the differences for TSS and acidity were non-significant (Table 1). The highest pH value (3.56) was recorded in Tempranilo followed by Niellucio (3.55), while the least was in Cabernet Franc (3.42). The variation in juice pH might be due to varietal difference since all the varieties were grown under the same condition and the harvesting was also done at proper sugar level. The volatile acids in grape berries were higher in Grenache Noir (0.13 g/L) while Caladoc recorded lower concentration (0.10 g/L).For good wine stability, upper limit of pH for red wine should be 3.5 (Morris *et al.*, 1984). Suresh and Negi (1975) reported a pH range of 3.1-3.7 in thirty grape wine varieties in their must.

Biochemical contents of grape berries

Significant variation was recorded for tannin content in different berry part among the different varieties. The tannins content was higher in seed followed by skin while the concentration was less in berry pulp (Table 2). The same trend was also observed for phenol and anthocyanin content. In pulp, tannin content was higher in Cabernet Franc (0.57mg/g) while Tempranillo recorded less tannin (0.27mg/g).

In the grape berry skin, tannin content was higher in Syrah (19.50 mg/g) compared to lower in Cabernet Franc (9.54mg/g). However, Cabernet Franc recorded higher tannins in seed (43.00mg/g) as compared to the lowest in Grenache Noir (29.54mg/g). The biochemical contents in berries are the main source of wine compounds as they determine the wine quality. Sun *et al.,* (1999) reported remarkable share of tannins in grape seed in red wine. In contrast, Kilmister *et al.,* (2014) reported that higher anthocyanin content in berries is directly proportional to anthocyanin in wine.

The grape berries of Tempranillo and Cabernet Franc recorded less concentration of phenol in pulp and skin (0.24 mg/g and 8.59 mg/g respectively) as compared to higher concentration in Cabernet Franc and Syrah (0.51 mg/g and 17.74mg/g respectively). The seed of Cabernet Franc was higher in phenol (39.08 mg/g) and lowest in Grenache Noir (27.01 mg/g).Phenolic compounds and sugars are the two major parameters which has influence on the rheological properties such as density and viscosity and mouth feel sensations such as astringency oiliness and pungency (Neto *et al.,* 2015). Anthocyanin content in pulp and skin of berries in different varieties varied significantly. Berry skin recorded higher concentration of anthocyanin as compared to pulp. Syrah and Caladoc recorded higher anthocyanin in pulp and skin (33.58 mg/L and 93.24 mg/kg respectively), while Nielluccio variety recorded lower anthocyanin in pulp (5.64 mg/L) and skin (9.39 mg/kg) among different varieties. The bunch exposure to sunlight and also period required for colour development in wine varieties plays an important role in developing anthocyanin contents in grape berries. The changes in anthocyanin content also vary with the varieties. There are many factors which influence the accumulation of anthocyanin content in grape berries of them varieties and weather condition during the berry ripening are the dominant one. Torres *et al.,* (2016) reported that high temperature during ripening significantly declines the anthocyanin concentration in grape berries due to the inhibition of anthocyanin biosynthesis. Kilmister *et al.,* (2014) also concluded that anthocyanin concentration might be a key component for enhancing tannin solubility and extraction into wine.

Reducing sugar content in different parts of grape berries varied significantly among the different wine grape varieties studied. It was higher in the pulp followed by seeds and skin. The same trend was followed for carbohydrate and protein content. Syrah recorded highest reducing sugar in pulp (267.27 mg/g) and skin (127.93 mg/g) while in Tempranillo, reducing sugar in seed was higher (186.40 mg/g). The variety Nielluccio recorded lowest reducing sugar in pulp (248.27 mg/g) and skin (101.93 mg/g) compared to lowest reducing sugar in seed of Caladoc variety (168.90 mg/g). The Syrah recorded higher reducing sugar in pulp (267.27 mg/g) and skin while Nielluccio variety recorded lowest (101.93 mg/g) reducing sugar among the varieties. At harvest, sugar concentration in grape berries is directly proportional to the concentration of alcohol in wine; hence, it is necessary to ensure the adequate amount of sugar in grape berries before harvest. Xu *et al.,* (2015) also suggested that sugars accumulate in high levels in grape berries during ripening and control a range of vital processes such as synthesis and build-up of anthocyanins and aroma compounds. Several workers stated that even though the sugars are mainly accumulated in the pulp, the total sugar content in berry skin also rises during grape ripening and this has closed relationship with the anthocyanin biosynthesis in berries (He *et al.,* 2010). The grape grown under tropical condition generally produces high sugar and less acid. Harbertson *et al.,* (2013) reported that during the process of verification, the concentration of soluble sugars changes considerably from a high at harvesting, which further declines during alcoholic fermentation.

Carbohydrate content in skin varied significantly while in pulp and seed it showed non-significant effect. The variety Nielluccio recorded higher carbohydrate content in skin (131.87mg/g) while Caladoc recorded lower concentration (119.40mg/g). Dreier *et al.,* (2000) reported that berry growth rate is significantly correlated with increased carbohydrate concentration and water availability (Table 3).

The protein content in different parts of berries varied significantly. Higher protein content was recorded in pulp of Tempranillo (28.21 mg/g), berry skin of Grenache Noir (46.22 mg/g) and seed of Caladoc (70.23 mg/g). The lowest protein content was recorded in pulp of Caladoc (14.67mg/g), berry skin of Nielluccio (24.74mg/g) and seeds of Tempranillo (47.66mg/g). Vincenzi *et al.,* (2013) in their studies reported that grape seed protein extract is being used as a valuable fining agent for wine. However, most of the grape seeds are treated as a waste material.

Wine quality

Significant differences were recorded among the different varieties for wine quality parameters studied (Table 4). The wine made from Cabernet Franc recorded lowest pH (3.40) followed by Syrah (3.42) while the variety Niellucio and Caladoc recorded higher pH of 3.50 each respectively. Pan *et al.,* (2011) concluded that pH value regulates the degradation of glucose and fructose as lower the pH value, slow will be the degradation. It is also playing a modulating role in wine haze formation, which diminishes or overthrows the commercial value of wine (Lambri *et al.,* 2013).

The concentration of volatile acid was higher in wine made from Cabernet Franc (0.36g/L) followed by Niellucio (0.27g/L) while the variety Syrah recorded least volatile acids (0.22g/L). Total acid was higher in Grenache Noir (4.35g/L) followed by Tempranilo (4.25g/L) and least in Caladoc (4.15g/L).Volatile acid plays an important role in fermentation process as it delivers information about the degree of improper fermentation processes occurring during winemaking (Mateo *et al.,* 2014) while acids, ethanol and tannins are the primary factors that determine the wine aroma, taste and mouth feel in red wine (Scott *et al.,* 2017).

The wine made from Caladoc variety recorded higher concentration of ethanol (13.20%) followed by Cabernet Franc (12.80%) while the lower quantity of ethanol was recorded in Grenache Noir (12.20%). The concentration of ethanol (14-16%) was considered to be a fundamental requirement for the wine quality as it is linked to sugar content of grape berries, which affect the overall flavour of wine (Meillon *et al.,* 2010).

However, it decreases astringency and also increases the bitterness of wine (Fontoin *et al.,* 2008).

Malic acid concentration was higher in wine made from Syrah (2.90 g/L) followed by Niellucio (2.75 g/L) while it was less in Caladoc (2.10 g/L). During the wine making process, malic acid influences fermentation. Bovo *et al.,* (2016) reported that at high concentration of malic acid, all strains of *Saccharomyces* yeasts were positive that enhanced the rate of fermentation process consuming all the sugar. Van Leeuw *et al.,* (2014) reported the variation due to influence of grape cultivar on the taste and colour of wine while Zeravik *et al.,* (2016) reported role of regional factors for the malic acid concentration in wine.

Table.1 Grape berries biochemical composition of different wine varieties

Varieties	Berry analysis			
	TSS (^{0}B)	Acidity (g/L)	Juice pH	VA (g/L)
Syrah	22.97	5.53	3.54	0.12
Caladoc	23.00	5.33	3.46	0.10
Grenache Noir	22.77	5.53	3.51	0.13
Tempranillo	23.30	5.57	3.56	0.11
Cabernet Franc	23.70	5.50	3.42	0.12
Nielluccio	22.90	5.37	3.55	0.12
C V %	2.59	4.64	1.38	6.16
LSD 5%	1.09	0.46	0.09	0.01
Significance	NS	NS	*	*

Table.2 Changes in biochemical parameters of grape berry in different wine varieties

Varieties	Tannin (mg/g)			Phenol (mg/g)			Anthocyanin	
	Pulp	Skin	Seed	Pulp	Skin	Seed	Pulp (mg/L)	Skin (mg/kg)
Syrah	0.54	19.50	36.42	0.48	17.74	32.33	33.58	74.68
Caladoc	0.54	19.09	31.49	0.48	17.36	28.81	23.49	93.24
Grenache Noir	0.34	14.22	29.54	0.30	12.89	27.01	16.44	22.78
Tempranillo	0.27	17.43	35.30	0.24	15.84	32.29	7.75	34.52
Cabernet Franc	0.57	9.54	43.00	0.51	8.59	39.08	8.92	15.74
Nielluccio	0.30	11.89	31.62	0.27	10.75	28.92	5.64	9.39
C V %	1.52	7.50	3.10	1.52	3.04	2.36	6.88	1.22
LSD 5%	0.01	2.08	1.95	0.01	0.77	1.35	2.00	0.93
Significance	**	**	**	**	**	**	**	**

*$p \leq 0.05$; **$p \leq 0.001$; NS- No significant differences

Table.3 Status of biochemical parameters of grape berry in different wine varieties

Varieties	Reducing sugar (mg/g)			Carbohydrate (mg/g)			Protein (mg/g)		
	Pulp	Skin	Seed	Pulp	Skin	Seed	Pulp	Skin	Seed
Syrah	267.27	127.93	179.90	269.25	130.58	196.13	19.35	38.56	64.36
Caladoc	263.27	112.93	168.90	277.85	119.40	174.62	14.67	42.50	70.23
Grenache Noir	259.60	125.60	180.90	273.55	127.57	191.83	27.85	46.22	52.04
Tempranillo	266.27	109.93	186.40	290.75	111.66	187.53	28.21	37.03	47.66
Cabernet Franc	249.27	114.60	182.40	273.55	119.83	183.23	16.04	32.60	54.65
Nielluccio	248.27	101.93	180.40	277.85	131.87	178.92	15.66	24.74	47.86
C V %	1.49	2.01	1.95	3.57	1.03	9.25	3.17	3.32	2.07
LSD 5%	7.04	4.22	6.37	18.01	2.31	31.20	1.17	2.23	2.12
Significance	**	**	**	NS	**	NS	**	**	**

Table.4 Effect of different wine varieties on wine quality

Varieties	Wine analysis					
	pH	VA (g/L)	Total acid (g/L)	Ethanol %	Mallic acid (g/L)	Glucose: Fructose ratio
Syrah	3.42	0.22	4.20	12.60	2.90	0.75
Caladoc	3.50	0.23	4.15	13.20	2.10	0.80
Grenache Noir	3.45	0.25	4.35	12.20	2.50	0.95
Tempranillo	3.46	0.24	4.25	12.50	2.40	2.05
Cabernet Franc	3.40	0.36	4.20	12.80	2.60	0.97
Nielluccio	3.50	0.27	4.22	13.00	2.75	0.85
C V %	1.01	4.63	1.47	1.51	3.34	2.41
LSD 5%	0.06	0.02	0.11	0.35	0.15	0.05
Significance	*	**	*	**	**	**

The wine made from Tempranilo recorded extremely high concentration of glucose: fructose (2.05g/L) followed by Cabernet Franc (0.97g/L) and lowest concentration in the wine made from Syrah (0.75g/L). Glucose concentration strongly influences the process of verification (Bovo *et al.*, 2016).Considering the results obtained in the present investigation, the varieties Cabernet Franc and Syrah were found better for biochemical contents and wine quality.

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