# Organic Acid Profile of White Wine Grapes at Different Berry Developmental Stages

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## Abstract

The organic acid composition of Chenin, Muscat, Sauvignon blanc, Viogner, Vermintino, Riesling, Colombard and Gewartztraminar white wine grape varieties at different berry developmental stages was quantified by HPLC. Significant differences were recorded for organic acids during berry developmental stages. Highest concentration of organic acid (655.91 gm/L) was recorded at 3-4 mm stage followed by (400.14 gm/L) 6-8 mm stage, while it was least at harvest stage (121.90 gm/L). Among different organic acids, malic acid concentration was found highest(364.25 gm/L) followed by tartaric acid (289.61 gm/L)and citric acid (2.05 gm/L)while, least concentration of lactic acid (0.00 gm/L)was found at 3-4 mm stage. Tartaric acid concentration was found to be increased over malic acid at 6-8 mm stage of berry development. At harvest stage, tartaric acid was found to be a predominant acid. Citric acid was found to be an minor acid in our study. Very less amount of lactic acid was recorded in Vermintino, Muscat and Colombard Grape wine varieties at harvest stage. In the present investigation Chenin Blanc showed highest concentration of total organic acid (207.93 g/L) followed by Sauvignon Blanc (185.84 gm/L) while least concentration of total organic acid was recorded for Muscat (152.91 gm/L). The variation in the concentrations of organic acid might be influenced by environmental factors, cultural practice and genetics of the varieties.
**KEYWORDS**: Organic acid, white wine grapes, berry development stages, HPLC, *Vitisvinifera L.*

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## Introduction:

Wine grape cultivation is gaining strong impetus in tropical climatic conditions of the world. Tropical viticulture has only been practiced commercially, since approximately 50 years. The grape cultivation is being shifted in the tropical condition. The production technology in the tropical regions differs significantly from the one employed in the traditional temperate regions.

In general that wine grapes require a temperate climate that includes predominantly winter rainfall, frost-free late spring, and warm to hot summers to ensure ripening, thus the global wine industry has been analysed predominantly in terms of Old World and New World wines from regions characterised by those criteria (McLennan, 1996). However, this largely ignores the nascent frontier of new climate wines, including the new altitude wines of tropical zones.

The production of quality wines requires a judicious balance between the sugar, acid and flavour components of wine, malic and tartaric acids are the most prominent organic acids in wine and play a crucial role in the winemaking process, including the organoleptic quality(flavour, colour and aroma) and the physical, biochemical and microbial stability of wine(Mato*et al* 2005).Being a constituent of the sourness group, each acid in wine conveys a characteristic flavour, aroma or taste(Herrero*et al* 1999).

In the case of wine, a common differentiation is made between acids which come directly from the grape (tartaric, malic and citric acids) and those that are originated, fundamentally, in the fermentation process (succinic, lactic and acetic acids). Also, there are small amounts of other acids like galacturonic, glucuronic, citramalic, dimethylglyceric, pyruvic, ketoglutaric, etc. (Berlitz &Grosch, 1992; Peynaud, 1999). These chemical composition of grape is influenced by a variety of factors such as maturity, variety, growing region and year (King *et al* 1988; Lamikanra*et al* 1995). In fruits, the total acid content generally reaches a maximum during growth and decreases duringripening(Eskin*et al*., 1971). Although having the same genotype, grapes, harvested from different climate, have different organic acid contents (Fuleki*et al*., 1993). During grape ripening, continuous warm conditions result in a lower acid content at maturity mostly due to increasing degradation of malic acid (Kanellis et al., 1993).Organic acids are mainlyused to determine fruit maturity (Lamikanra*et al*., 1995;Perez*et al*., 1997) and also have great importance in detection of wine alterations and/or illnesses, because they suppose a modification of acids content (Mato*et al* 2005).

In addition, they indicate the spoilage of fruit products (Gherardi, Saccani, Trifir, and Calza, 1995; Blanco, Quintanilla, Mangasand Gutierrez, 1996) and can be used as acidifiers in food industry (Blanco *et al*., 1996).Most of the available information related to flavor in grapes has been developed for grapes used for wine production.

India is one of the leading countries in wine making; hence to maintain the quality of wine is very important. Organic acid significantly influenced the sensory and organoleptic properties of wine. Hence, the objective of this study was to characterize the changes in organic acid composition during berry developmental stages of wine varieties grown under tropical condition of India.

**Material and Methods:**

**Plant material:**

The research was carried out at the experimental farm of National Research Centre for Grapes, Pune (18.32°N and 73.51°E) during the year 2013-14. The vineyard is situated at mid-west Maharashtra state at an altitude of 559 m. Pune experiences tropical wet and dry climate with average temperature ranging between 20 to 28°C in grape season. Four-year-old white wine varieties of *Vitisvinifera* L. grafted onto 110-R rootstock was selected for study. The vines were planted in N-S direction in black soil (EC 0.7 and pH 7.8) spaced at 2.66 meter between the rows and 1.33 meter between the vines. The vines were trained to mini Y trellis with cordons placed horizontally so as the shoots were positioned vertically. The vines were drip irrigated during the period of berry development. In this region, the vines are pruned twice in a year i.e., double pruning and single cropping pattern is followed for grape cultivation. Normal cultural practices were followed for the production of healthy fruit during the season. The experiment was formulated in randomized block design with three replications consisting of ten vines under each replication.

**Sampling:**

Thirty healthy and fruitful vines in each variety were labelled immediately after berry set. From each variety, 100 berries were collected from 10 labelled vines under each replication, packed in sampling bags and brought to the laboratory in ice box and kept in cold conditions till further analysis. The berries were blended in mixer cum grinder. One gram on blended sample was taken into polypropylene tube containing 5ml of 1% formic acid in 19% methanol of 80% HPLC grade water. After vortexing for 5 min, the mixture was centrifuged at 5000 rpm for 5 minutes. One ml supernatant was then taken in Eppendorf tubes and again centrifuged at 10,000rpm at 4 °C for 10 min. Supernatant was filtered through 0.2 µm membrane filter (Pall Life Sciences, India) and the filtrate was used for analysis.

**Standards:**

The certified standards of organic acid (tartaric, malic, citric and lactic acid) were obtained from Aldrich Co. (Sigma Aldrich Chemi, Steinhein). The standard solution was prepared as 10 mg of individual acid in 10mL HPLC grade water. The stock solutions were then kept in freeze at 0°C.

**Analysis of organic acid:**

A HPLC 1260 series (Agilent Technologies, DE Germany) equipped with an inbuilt 4 channel degassing unit, standard auto-sampler, quaternary pump, a photodiode array detector (PDA) was used for the analysis of organic acids. The instrument control was performed using Agilent EZ chrome elite® software. The separation of organic acids was performed on Zorbax Eclipse plus $C_{18}$ column (100mm×4.6mm, 5 µm ) with column oven temperature maintained at 30 °C and 10 µL injection volume.

The mobile phase consisted of acidified water of pH 2 adjusted with orthophosphoric acid and 100% methanol (Volume ratio of 95:5) with an isocratic flow rate of 0.80 ml/minute. Prior to use, the solvent was filtered through vacuum filter and then sonicated for 5 to 10 min in an ultrasonic bath to remove air bubbles. The PDA was set at 210 nm for detection of all the organic acids. The total run time was 7 minutes. The chromatogram of standard organic acids with their intensity and retention time is shown in Figure 1.

**Statistical Analysis:**

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

**Results and Discussion:**

The data obtained for organic acids composition during berry developmental stages of white wine grape varieties are presented in Table 1.Among the different stages of berry development, the highest concentration of organic acid (655.91 gm/L) was recorded at 3- 4 mm stage followed by 6-8 mm stage (400.14 gm/L)while it was least at harvest stage (121.90 gm/L). The concentration of all the organic acid quantified in our study showed gradually decreases throughout the berry maturation period in all the varieties. Themalic acid concentration (364.25 gm/L) was significantly highest compared with tartaric acid (289.61 gm/L), citric acid (2.05 gm/L), lactic acid (0.00 gm/L) at 3 -4 mm stage of berry development. At this stage, Chenin White variety showed highest concentration of total organic acid (94.94 gm/L) followed by Sauvignon Blanc (90.19 gm/L) while least concentration of total organic acid was recorded for Viognier (63.43 gm/L).The highest concentration of organic acid composition in Chenin White due to the maximum accumulation of tartaric acid (40.16 gm/L), malic acid (54.48 gm/L) and citric acid (0.3 gm/L).

The differences for organic acid composition among different grape varieties confirm the finding of (Kliewer*et al*. 1967). The maximum concentration of organic acid at 3 – 4 mm stage of berry development might be due to the rapid storage of malic and tartaric acid (Fillion*et al* 1999 andPratelli*et al* 2002). Both the acids accounting for more than 90% of the grape berries acidity (Boulton*et al* 1996). Although malic and tartaric acids have similar chemical structures, they are synthesised form glucose via different metabolic pathways in grapes berries. Malic acid is formed via glycolysis and TCA cycle, while ascorbic acid is the principle intermediary product of tartaric acid biosynthesis (Volschenk*et al*., 2006). Similar trends of organic acids composition were observed for 8–10mm stage of berry development. Among the different organic acids, tartaric acid composition was recorded at highest (227.61 gm/L) followed by malic acid (171.07 gm/L) while least concentration of citric acid (1.47 gm/L) was recorded at 8–10 mm stage of berry development. The highest concentration of organic acid of Cheningrapes was due to the highest level of tartaric acid (35.57 gm/L), malic acid (24.68 gm/L) and citric acid (0.24 gm/L) as compared to sauvignon blancvarity. The decrease in malic acid content in berry was diluted due to the influx of water during berry expansion in the second growth phase(Volschenk*et al* 2006).

At onset of ripening berry development, tartaric acid contents ranged from 16.97 gm/L to 15.54 g/L was highest in Chenin while least in Gewurztraminer. However, all other varieties ranged in between the concentration for tartaric acid composition. The highest concentration of malic acid (17.49 gm/L) was recorded for Chenin while least concentration (12.15 gm/L) was recorded for Viognier grapes. The total range of tartaric acid contents ranged from 0.18 gm/L to 0.08 gm/L with highest in Chenin White while least in Colombard grapes. The concentration of lactic acid was not detected at onset of ripening. The differences for the concentration of organic acid composition of the different white wine grapes might be due to the drastic changes in physical and biochemical of grape berries (Coombe, 1992; Davies and Robinson, 1996).

The rapid reduction of acidity coincides with changes in sugar composition of the berry at this stage. Atonset of ripening, the grape berries actively respire during the early stage of growth but the intensity of respiration slows down due to the availability of respiratory substrate, sucrose (via photosynthesis), become limited. The berries therefore force to shift its metabolism from sugar to L- malic acid respiration. Prior to the onset of onset of ripening*,* L-malic acid is the most abundant organic acidin the grape berry vacuole, resulting in the low internal pH of 2.5 of grapes (Ruffner, 1982; Ribereau-Gayon*etal,* 2000). Similarly, The maximum changes in tartaric and malic acid concentration in our study was recorded up toonset of ripening stage only confirms the results obtained by Possner and Kliewer(1985).The evaluation of organic acid contents in grapes is one of the most significant quality criteria as it indicates the suitability of cultivar for certain uses and it also reflects the berries metabolic activities during growth (Lamikanra*et al.* 1995).

The decrease in tartaric acid at each berry development stage was in proportion as compared to malic acid content while sudden decrease was observed from onset of ripening to harvest. The highest range of tartaric acid from Chenin(12.98 gm/L) to Riesling (10.16 gm/L)was obtained in our study. The malic acid composition showed similar pattern as tartaric acid for different wine varieties at harvest stage. The highest concentration of malic acid (4.78 g/L) was recorded in CheninWhite whereas the least concentration (1.78 gm/L) was recorded in Riesling grapes. Significant differences were recorded for citric acid composition among the different wine grape varieties at harvest stage and it was ranged from 0.1 gm/L in Chenin to 0.01 in Colombard grapes. At harvest stage, only the lactic acid was recorded in Colombard grapes (0.63 gm/L) followed by 0.41 gm/L in Muscat and 0.36 gm/L in Vermentino grapes. However, Viognier, Chenin, Sauvignon Blanc, Riesling, Gewurztraminer were did not recorded lactic acid contents.

The organic acid decreases during berry maturation as generally attributed to transformation of acid to sugar (Winkler, *et al*., 1974). In the present investigation, malic acid concentration was higher than tartaric acid during unripe stage while the concentration was decreased proportionately after onset of ripening till the harvest. This was correlated with sugar development at harvest. Sabir*et al*. (2010) also reported that in juice of analysed cultivars, malic acid content was predominant and decreased with sugar accumulation. The results of the present study are in accordance with (Coombe 1987) who analysed changes in different chemical components at four different stages. Though the malic acid content was higher than the tartaric acid, the concentration was found to be decreased from onset of ripening to harvest. The malic acid content at harvest was almost 20% of the tartaric acid content at harvest. This rate of decrease in malic acid than the tartaric acid is agreeable with several workers (Sabir*et al*. 2010, Ruffner*et al*. 1983, PossnerandKliewer1985).

The differences among the varieties for major organic acid at different berry development stages might be due to the genotypic expression at a given environmental condition.

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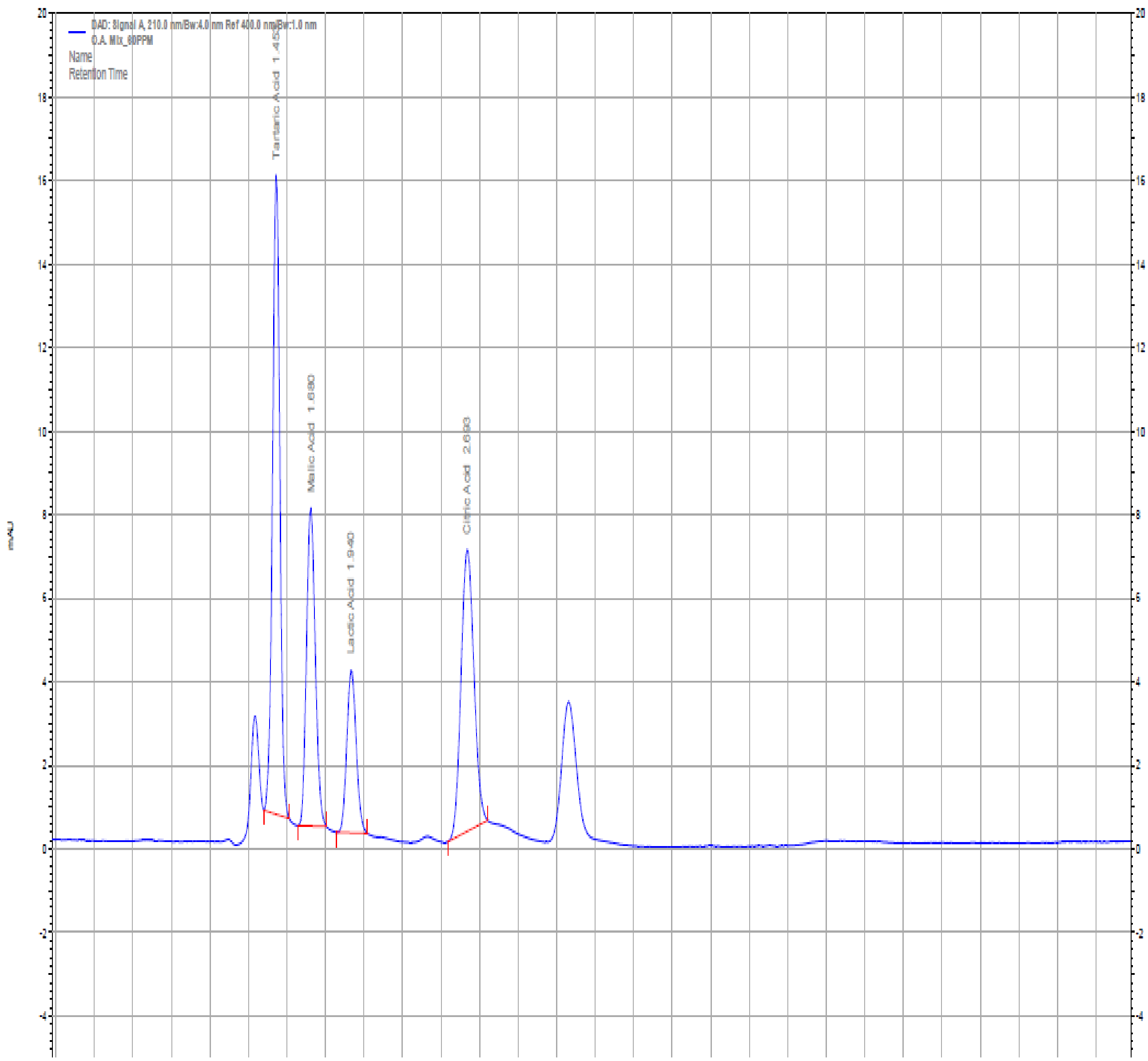


**Fig.1 chromatogram of organic acid standards (Tartaric acid, Malic acid, Lactic acid and Citric acid).**

**Table 1: Organic acid profile at different berry developmental stages of white wine varieties**

| | | 3-4 mm | | | | 8- 10 mm | | | | Onset of ripening | | | | Harvest | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sr no. | Variety name | Tartaric acid gm/lit | Malic acid gm/lit | Lactic acid gm/lit | Citric acid gm/lit | Tartaric acid gm/lit | Malic acid gm/lit | Lactic acid gm/lit | Citric acid gm/lit | Tartaric acid gm/lit | Malic acid gm/lit | Lactic acid gm/lit | Citric acid gm/lit | Tartaric acid gm/lit | Malic acid gm/lit | Lactic acid gm/lit | Citric acid gm/lit |
| 1 | Viognier | 29.62$^{d}$ | 33.57$^{g}$ | NA | 0.27$^{cd}$ | 23.52$^{e}$ | 21.45$^{cd}$ | NA | 0.19$^{d}$ | 16.05$^{bc}$ | 12.15$^{d}$ | NA | 0.11$^{d}$ | 11.74$^{c}$ | 4.56$^{a}$ | 0$^{d}$ | 0.08$^{c}$ |
| 2 | Vermentino | 33.39$^{c}$ | 51.4$^{b}$ | NA | 0.28$^{bc}$ | 21.75$^{f}$ | 23.53$^{b}$ | NA | 0.18$^{e}$ | 15.68$^{bc}$ | 14.38$^{c}$ | NA | 0.1$^{e}$ | 10.8$^{e}$ | 3.93$^{b}$ | 0.36$^{c}$ | 0.06$^{e}$ |
| 3 | Chenin | 40.16$^{a}$ | 54.48$^{a}$ | NA | 0.3$^{a}$ | 35.57$^{a}$ | 24.68$^{a}$ | NA | 0.24$^{a}$ | 16.97$^{a}$ | 17.49$^{a}$ | NA | 0.18$^{a}$ | 12.98$^{a}$ | 4.78$^{a}$ | 0$^{d}$ | 0.1$^{a}$ |
| 4 | Muscat | 37.86$^{b}$ | 38.31$^{f}$ | NA | 0.25$^{e}$ | 26.69$^{d}$ | 12.36$^{e}$ | NA | 0.2$^{c}$ | 14.56$^{d}$ | 6.1$^{f}$ | NA | 0.15$^{c}$ | 11.83$^{c}$ | 4.12$^{b}$ | 0.41$^{b}$ | 0.07$^{d}$ |
| 5 | Sauvignon | 39.51$^{a}$ | 50.39$^{bc}$ | NA | 0.29$^{ab}$ | 32.42$^{b}$ | 21.56$^{cd}$ | NA | 0.22$^{b}$ | 16.8$^{a}$ | 9.47$^{e}$ | NA | 0.16$^{b}$ | 12.36$^{b}$ | 2.57$^{d}$ | 0$^{d}$ | 0.09$^{b}$ |
| 6 | Riesling | 37.87$^{b}$ | 40.67$^{e}$ | NA | 0.2$^{f}$ | 31.56$^{b}$ | 21.24$^{d}$ | NA | 0.14$^{g}$ | 15.88$^{bc}$ | 15.8$^{b}$ | NA | 0.09$^{f}$ | 10.16$^{f}$ | 1.78$^{e}$ | 0$^{d}$ | 0.04$^{f}$ |
| 7 | Gewurztraminer | 32.48$^{c}$ | 48.44$^{cd}$ | NA | 0.26$^{de}$ | 27.49$^{cd}$ | 23.79$^{ab}$ | NA | 0.15$^{f}$ | 15.54$^{c}$ | 11.46$^{d}$ | NA | 0.1$^{e}$ | 11.28$^{d}$ | 1.79$^{e}$ | 0$^{d}$ | 0.02$^{g}$ |
| 8 | Colombard | 38.72$^{ab}$ | 46.99$^{d}$ | NA | 0.2$^{f}$ | 28.61$^{c}$ | 22.46$^{c}$ | NA | 0.14$^{g}$ | 16.08$^{b}$ | 15.55$^{b}$ | NA | 0.08$^{g}$ | 12.07$^{bc}$ | 3.28$^{c}$ | 0.63$^{a}$ | 0.01$^{h}$ |
| 9 | CV% | 3.85 | 7.25 | | 0.04 | 4.62 | 3.85 | | 0.04 | 0.75 | 3.77 | | 0.04 | 0.89 | 1.19 | 0.26 | 0.03 |
| 10 | LSD | 1.484 | 2.179 | | 0.013 | 1.450 | 1.032 | | 0.009 | 0.526 | 0.935 | | 0.008 | 0.393 | 0.227 | 0.049 | 0.009 |
| 11 | Significance(<0.01) | ** | ** | | ** | ** | ** | | ** | ** | ** | | ** | ** | ** | ** | ** |

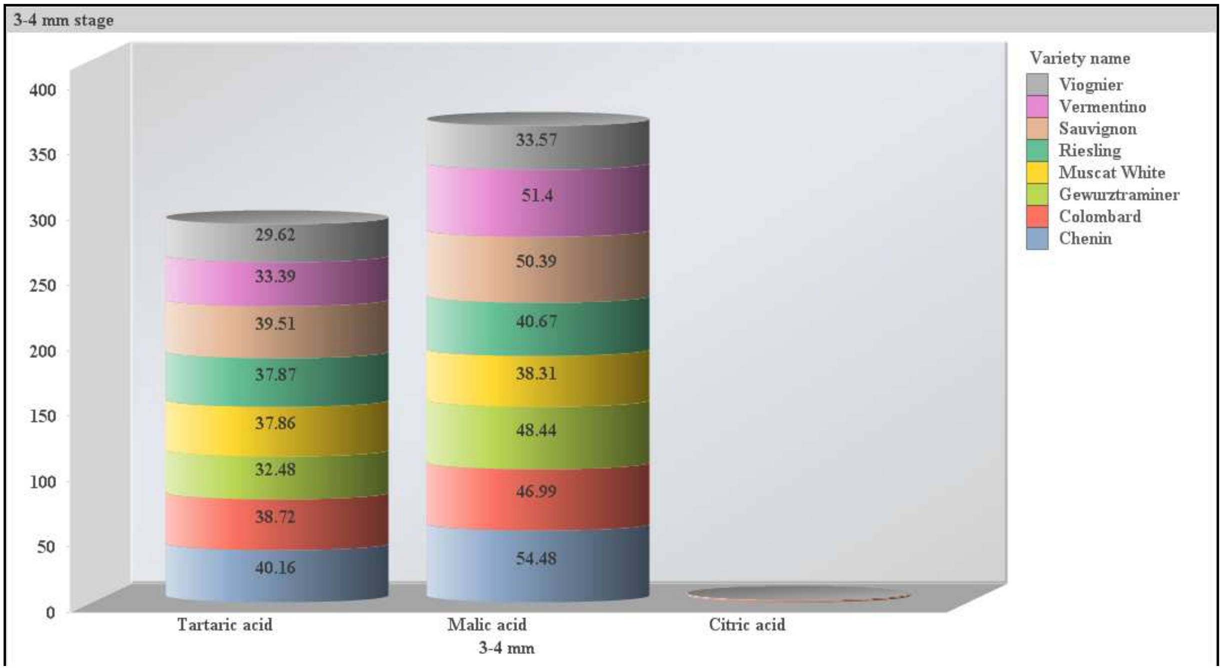


**Fig 2:- Graph showing organic acid at 3-4 mm development stage**

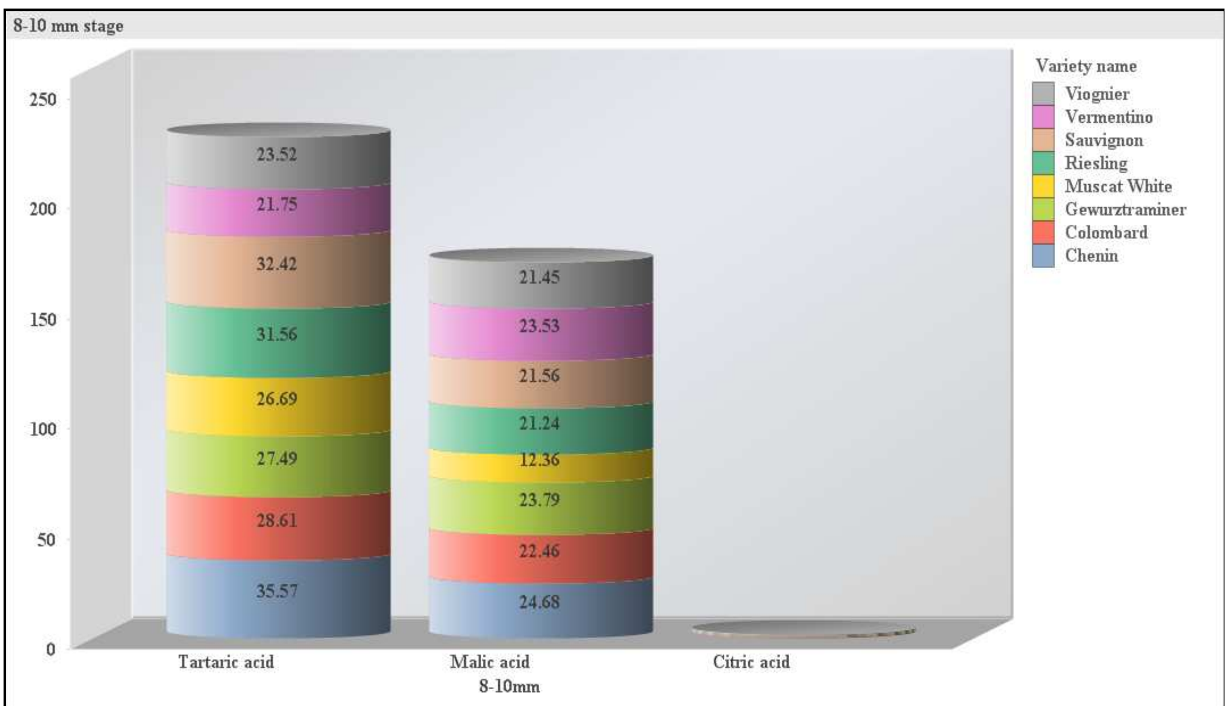


**Fig 3:- Graph showing organic acid at 8-10 mm development stage**

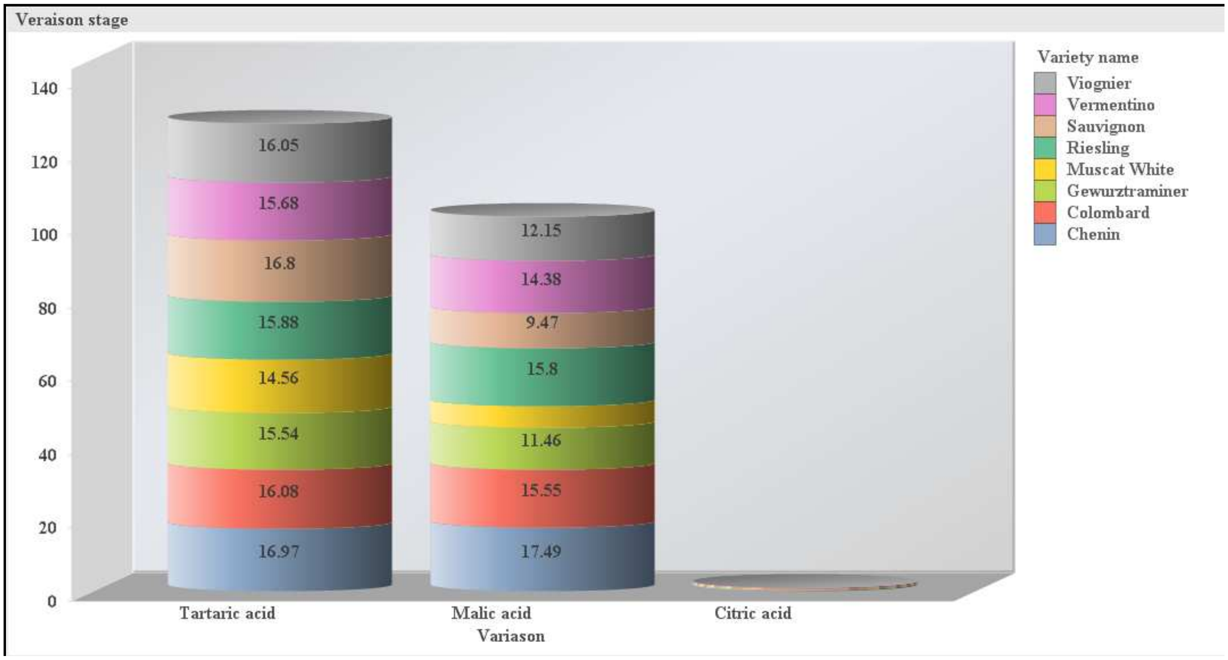


**Fig 4:- Graph showing organic acid at onset of ripening stage**

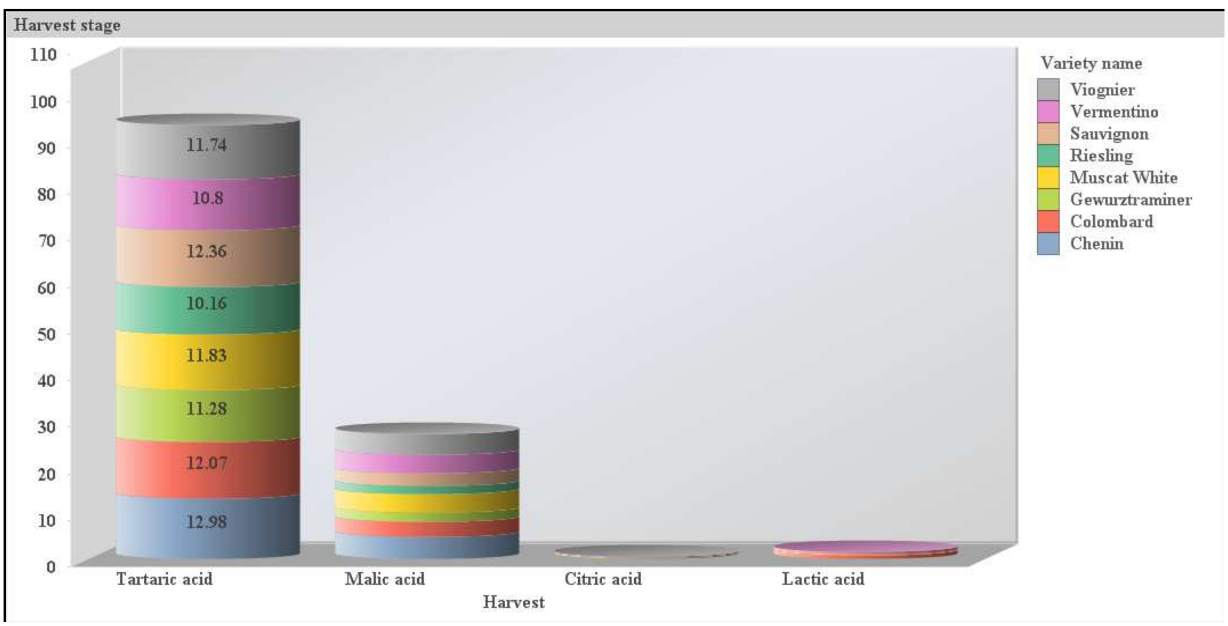


**Fig 5:- Graph showing organic acid at Harvest stage**

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