Acids in grapes

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Beyond water and sugars, acids are important components of grapes with essential implications for taste, wine- making process and preservation. Many acids were found in vine organs but, usually, almost the total content in grape juice is represented by tartaric, malic and citric acid. Their concentration depends on variety, maturation trends and pedo-climatic conditions. To assess the influence of these factors on the acidic content, for three years grape samples from different black and white grapes, cultivated in four different areas were analyzed to determine pH, titratable acidity and the acidic profile by HPLC. The organic acids content was influenced by the cultivar, the production areas and the vintage; however some varieties resulted less susceptible to different conditions.

Keywords: grape juice; organic acids; titratable acidity; HPLC

1. Introduction

Organic acids are characterized by the presence of a carboxyl group that determines their acidic function [1]. Carboxylic acids in plants can also be present as esters and salts and, generally, the vacuolar juice has a weak acidic reaction. Aliphatic organic acids are present in all plants and are responsible of important biological functions such as respiration processes. These acids may reach high concentrations in vegetal tissues. In this case they are related to other regulating system such as the acid-base balance, controlling the formation of cations and anions and creating a "buffer system" that controls, within certain limits, the pH of the medium. In fact, each organic acid, in presence of its salt, constitutes an elastic system designed to avoid any sudden change in the hydrogenation concentration [2].

Grapes acids affect the organoleptic characteristics of juices and wines and are useful in human nutrition stimulating saliva production and contributing to oral hygiene by reducing the number of bacteria responsible for dental caries and oral infections. They also promote the secretion of gastric juices and are slightly laxative and diuretic. When absorbed from the intestine, they pass into the bloodstream and, being weak acids, they have an alkalizing effect. In fact, they easily decompose into carbonic acid, forming sodium and potassium bicarbonates.

The complex of these new molecules is called "alkaline reserve," and constitutes the resource that the body uses to neutralize acids of different origin that are formed during the course of many pathologies. They are also employed for the production of phytocosmetics for skin purifying treatments [3].

When grapes reach the technological maturation, more than 95% of total acids is represented by tartaric, malic and citric acid [4, 5]. Grape must also contains mineral acids such as sulfuric, hydrochloric and phosphoric, which rarely exceed the concentration of 1 g/L. These strong acids are completely neutralized and are present in the form of ionized neutral salts such as calcium magnesium potassium sulfates, chlorides and phosphates, etc.

The concentration of the acidic functions of free and semi-salified acids forms the acidity of the musts, and it can be determined by titration with an alkaline solution up to pH 7. The titratable acidity is usually expressed as g/L of tartaric or sulfuric acid or milliequivalents per liter [6]. The salified fraction of organic acids requires a more complex procedure to be determined: the analysis of the alkalinity of ashes. The perceived acidity, when we taste a wine or a must, depends directly on the concentration of H^+ ions and then on the pH value. Tartaric acid is always present in small quantities in plants, except for a few species such as tamarind and grapevine, where it is the most abundant acid. In particular, grape tartaric acid is in the dextrorotatory optically active form with levo configuration (L(+) tartaric acid) [8] having the formula: $HOOC(CHOH)_2COOH$ and M.W. = 150,09.

It is the strongest acid in grapes and at technological ripeness its concentration ranges between 2 and 8 g/L, depending on the cultivar, on agronomic management of the vineyards and on pedo-climatic factors.

It is a bi-acid and, in aqueous solution, has two dissociation constant: pK1=3,04 and pK2=4,37. It has two secondary alcohol groups which give it particular physico-chemical properties: the acid strength and the solubility in water of the acid and its salts. In particular, potassium bitartrate is not soluble in presence of ethanol and can cause undesired precipitates in wines. Malic acid, contrary to tartaric acid, is very common in the *Plantae* kingdom and it is present in all kind of fruits. It is particularly abundant in apples, plums, pears, peaches and apricots. Natural malic acid is the L (-) malic isomer, having the formula $HOOCCH_2CHOHCOOH$ and M.W. = 134,09. The malic acid content in ripe grapes can vary according to the grapevine variety, to seasonal trends and to temperatures during the last stages of maturation. Its average concentration ranges between 1,5 and 4 g/L. It is a bi-acid and has two dissociation constants in water, which are respectively: pK1 = 3,46 and pK2 = 5,13. The acid strength is lower than that of tartaric acid. It is always soluble in musts and wines but it can undergo microbiological transformations. Citric acid is a tri-acid having the formula HOC (COOH) $((CH_2)\ COOH)_2$ and M.W. = 192,13. It is present in many fruits and in particular in the citrus *genus*, in healthy grapes its concentration ranges between 150 and 500 mg/L, but it can reach higher values in case of *Botrytis cinerea* infections. Its dissociation constants are in order: 3,13, 4,76 and 6,40. In musts and wines conditions, with pH values between 3,20 and 3,90, it behaves like a bi-acid with an intermediate strength between tartaric and malic acid. It is always soluble in musts and wines but can undergo microbiological transformations. Due to its structure, it is able to form chelates with iron and copper ions, giving to musts and wines higher stability preventing the formation of hazes and precipitates known as iron and copper "casses".

Other acids that can be found in grapes only in little amounts or traces are: shikimic, ascorbic, aconitic, α-ketoglutaric, fumaric, galacturonic, glyceric, glycolic, isocitric, oxalic, oxaloacetic and pyruvic acids.

In this paper the content of malic, tartaric and citric acid in grapes from 8 different cultivar grown in 4 different areas of Tuscany (Italy) were compared to evaluate how different environmental conditions can affect the acids concentrations.

2. Materials and methods

The grapes were collected from four different areas of Tuscany (Italy), two from vineyards near the coast, in the provinces of Pisa (PI) and Grosseto (GR), and two from vineyards in the provinces of Arezzo (AR) and Lucca (LU). For the trial were chosen both local and international varieties, their names and abbreviations are listed in Table 1.

Table 1 List of white and black grape cultivars and their relative abbreviations.

Black grapes	White grapes
Cabernet sauvignon (Cab)	Manzoni bianco (Man)
Sangiovese (San)	Pinot bianco (PiB)
Pinot nero (PiN)	Sauvignon (Sau)
Nero d'Avola (NdA)	Fiano (Fia)

The vineyards had a density of 3600 plants per hectare, with 2,5 m of distance between rows and 1,1 m on the row. The vines, with a permanent unilateral cordon, were spur-pruned upward vertical position. Grapes were harvested and analyzed in 2014, 2015 and 2016 seasons. Titratable acidity and pH were measured following official EU methods (Official Methods of Wine Analysis, Reg. 440/2003). The concentration of malic, tartaric and citric acid were determined by HPLC as described by Flamini et al. [7]. Analysis of variance (ANOVA) was performed using Statgraphics Centurion (Ver.XV, StatPoint Technologies, Warrenton, VA).

3. Results

Table 2 F-Ratio and P-value for chemical parameters analyzed for grape samples.

	Titratable acidity		pH		Tartaric acid		Malic acid		Citric acid	
	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value
Factors										
A: Cultivar	16,4	< 0,001	62,3	< 0,001	25,7	< 0,001	8,5	< 0,001	3,9	0,002
B: Zone	2,3	n.s.	17,5	< 0,001	27,5	< 0,001	29,7	< 0,001	0,9	n.s.
C: Year	25,6	< 0,001	6,5	0,004	46,6	< 0,001	18,7	< 0,001	3,2	0,050
Interactions										
AxB	2,3	0,012	2,4	0,001	4,8	< 0,001	0,6	n.s.	1,3	n.s.
AxC	1,1	n.s.	1,8	n.s.	3,5	< 0,001	3,2	0,002	1,3	n.s.

The data on acidic composition of grapes were processed with the analysis of variance (ANOVA) considering as factors the cultivar, the year of production, the area of origin and their interactions.

The titratable acidity was mostly affected by the factors year of production (F = 25,6) and cultivar (F = 16,4). The values ranged between 5g/L and 7g/L and in 2014, a year characterized by low temperatures and frequent rains, the level of titratable acidity was higher than in 2015 and 2016. Although the production area did not have significant effects on the average values, the effect of its interaction with the cultivar was significant (P=0,001), showing the behaviour of the varieties in different pedo-climatic conditions. In details, the titratable acidity of PiN, PiB and Man grapes was constant in all experimental vineyards, whereas in other varieties, San, Sau and Cab, it was highly influenced by the Zone factor (Tab. 2, Fig. 1).

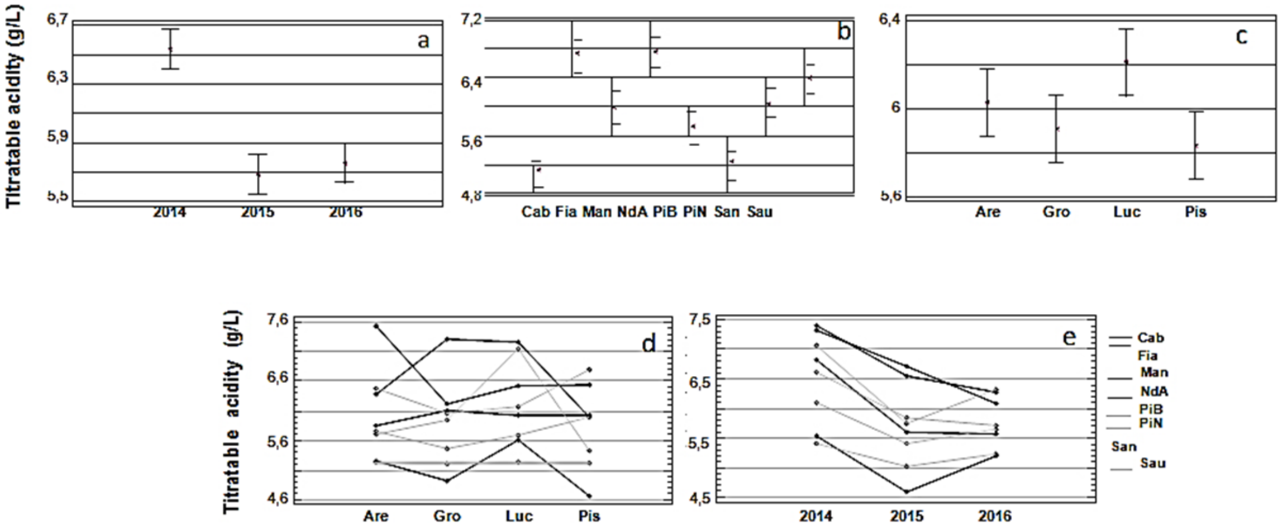

Fig. 1 Average values of titratable acidity, as g/L of tartaric acid, per factors (a: Year, b: Cultivar, c: Zone) and interaction plot of Variety with Zone (d) and Year (e).

The average pH values in the three-year period showed small variations with lower levels in 2015 and in the zone LU. Considering the cultivars, the pH varied approximately from 3,5 in Cab and PiN and 3,0 in Fia. The interaction between cultivar and zone resulted significant and confirmed the trends observed for titratable acidity (Fig. 2).

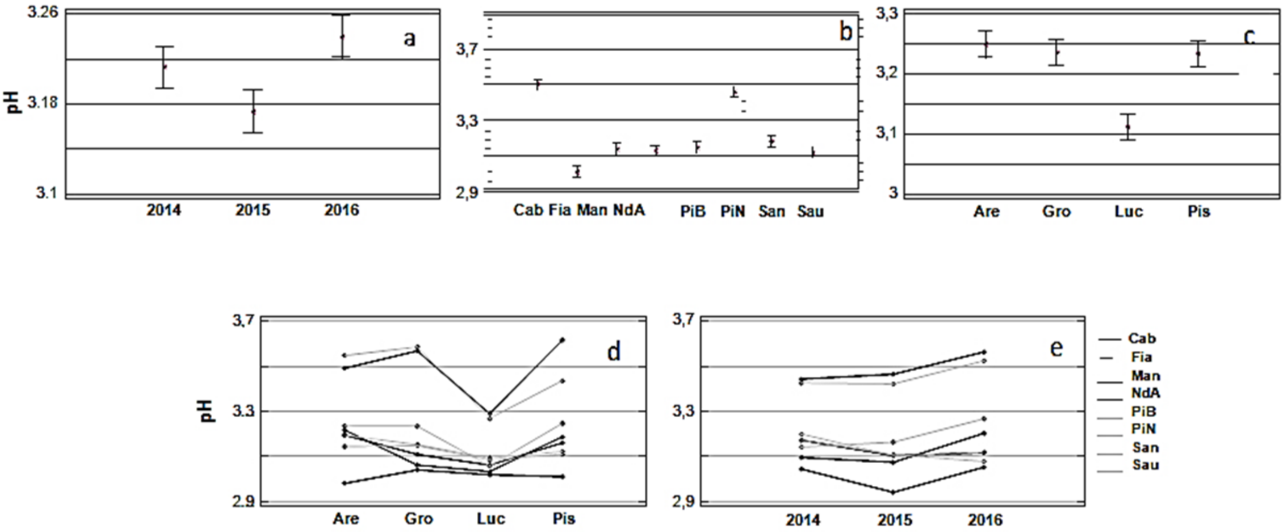

Fig. 2 Average values of titratable pH per factors (a: Year, b: Cultivar, c: Zone) and interaction plot of Variety with Zone (d) and Year (e).

The concentration of tartaric acid was strongly influenced by all the factors, and the interactions were also significant (Tab. 2). The average values ranged from about 3 g/L in the Arezzo and Pisa area and 3,7 g/L of Lucca (Fig. 3). Regarding the cultivar, the values were between 2,5 g/L of Cab, PiN and PiB and 4 g/L of NdA. During the three years of observation, mean values dropped from 3,7 g/L in 2014 to 2,9 g/L in 2016. Each variety had constant concentrations in all the vineyards except for Sangiovese and Nero d'Avola grown in the Lucca hills, that produced grapes with higher tartaric acid content than in other zone.

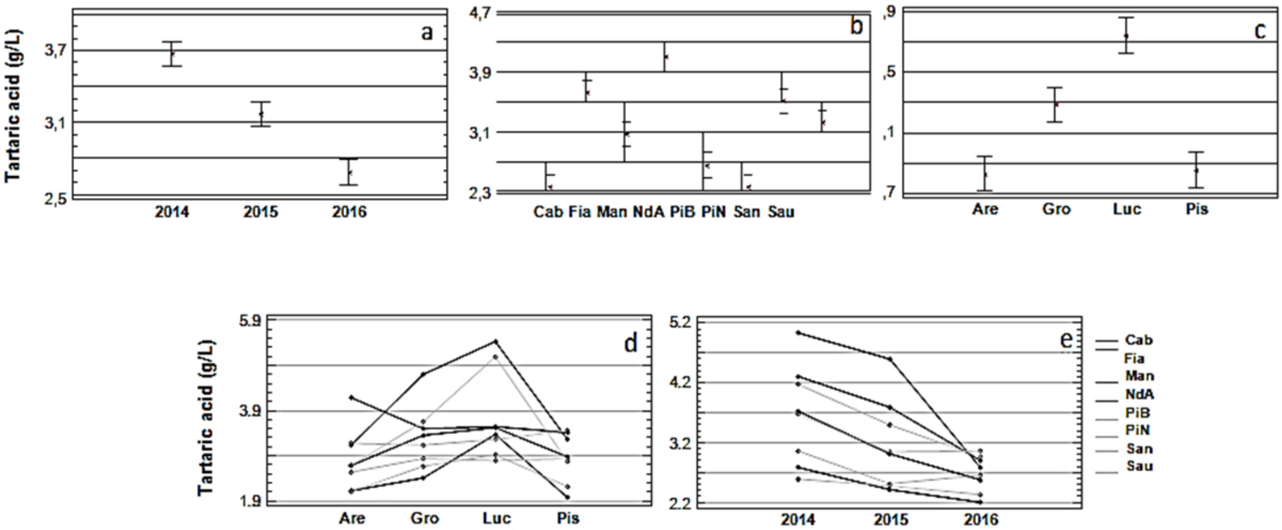

Fig. 3 Average values of tartaric acid per factors (a: Year, b: Cultivar, c: Zone) and interaction plot of Variety with Zone (d) and Year (e).

Malic acid showed average values below 3 g/L and it was always lower than tartaric acid. As it was observed for tartaric acid, in 2014 malic acid concentrations were higher than in the 2015 and 2016 vintages that resulted, in this case, similar to each other. The vineyards of Grosseto and Lucca, despite the grapes showed higher tartaric acid values than the others, resulted the areas where the lowest malic acid concentrations were measured. White berry grapes were richer in malic acid; the values ranged between 2,3 g/L of Sauvignon and 1,7 g/L of Sangiovese and Nero d'Avola. In 2015, differently than the other varieties, Nero d'Avola and Cabernet sauvignon incurred in a decrease of the malic acid average concentration. Finally, all the variations were influenced in the same way in the different cultivation areas, the interactions between Variety and Zone, in fact, were not significant (Tab. 2, Fig. 4).

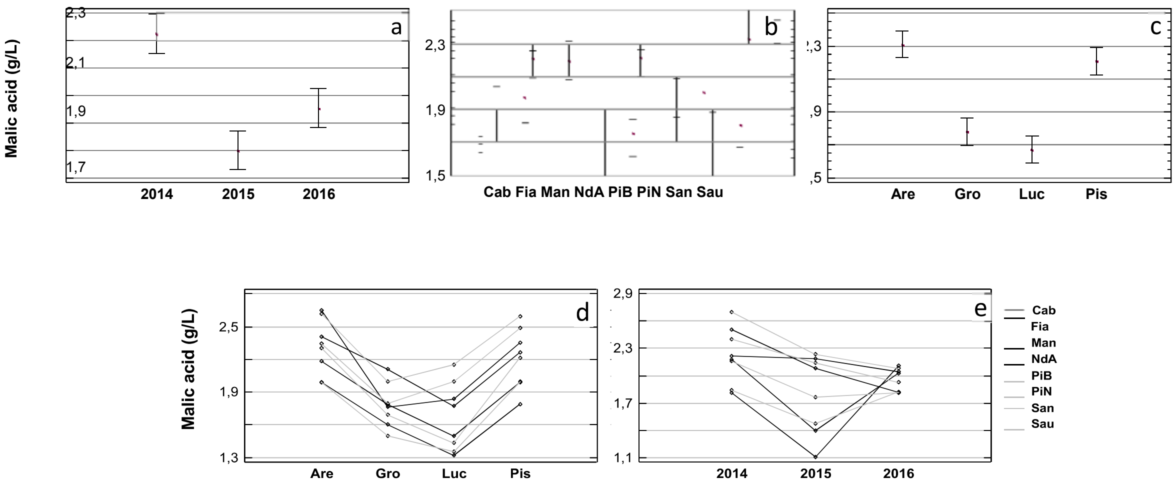

Fig. 4 Average values of malic acid per factors (a: Year, b: Cultivar, c: Zone) and interaction plot of Variety with Zone (d) and Year (e).

Citric acid, as expected, was the minor acid in all the examined grapes, and it was always lower than 0,5 g/L. The data varied within 0,41 g/L of Manzoni bianco and 0,31 g/L of Nero d'Avola and Fiano. The factors Year and Zone had little effect on citric acid concentrations; the interactions between the factors do not reveal significant variations and the differences were modest (Tab. 2, Fig. 5).

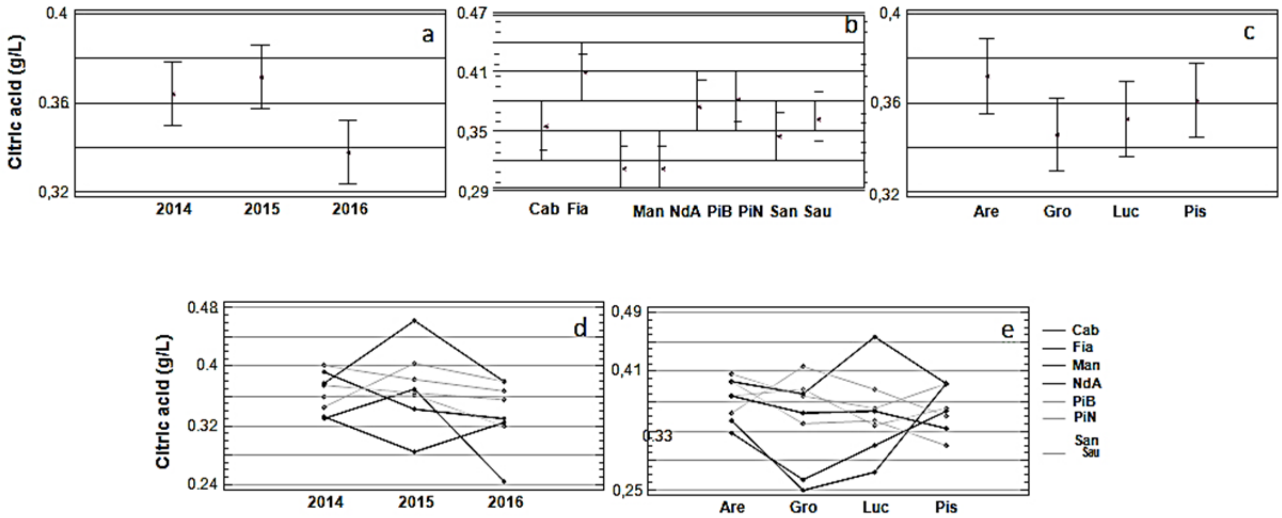

Fig. 5 Average values of citric acid per factors (a: Year, b: Cultivar, c: Zone) and interaction plot of Variety with Zone (d) and Year (e).

4. Conclusions

The three-year analysis showed that titratable acidity was higher in 2014, a year characterized, in Tuscany, by a fresh and rainy summer that caused a delayed maturation of fruits and provoked, consequently, an increase of the concentration of the major acids.

The grapevine varieties considered showed differences in acidic composition. Cabernet Sauvignon, Pinot n. and Pinot b. had the lowest levels of tartaric acid, while higher contents were detected in Nero d'Avola and Sangiovese, which at the same time showed lower levels of malic acid.

From the results emerged that some grape varieties have a more steady behaviour and are less dependent on the factors Year and Zone (PiB, PiN and Man), while others are much more unstable (NdA and San). Tartaric acid content was influenced by all the factors considered, but a more pronounced effect was caused by the Year. Malic acid is also more affected by environmental factors (Zone and Year). The concentrations of citric acid were only marginally conditioned by pedo-climatic conditions, while it seems to be typical of the cultivar.

Since organic acids play a fundamental role in the taste and nutritional properties of grapes and wines, it is important to know which mechanisms regulate the accumulation in the berries.

This work has highlighted how the variability in organic acid content in grapes can be strictly related to the response of the different varieties to the environmental factors. Only with comparative studies on many cultivars it can be determined which are the specific factors that actually affect the quality of the grapes. This preliminary work represents only a small example and, in perspective, it will be repeated increasing the number of variables and grape varieties involved, and expanding the analytical spectrum of the monitored metabolites, such as the acids that usually are present in grapes in smaller quantities or in trace amounts.

References

[1] Ternay A.L., Contemporary organic chemistry. W.B. Saunders company. Philadelphia; 1979. p. 769-778.
[2] Cultrera R., Chimica agraria. Ed. Cedam Padova 1977; Vol I, p. 129-156.
[3] Various Authors, Enciclopedia della salute. Ed. Librex, Milano; 1971 . Vol. VI, p. 1642-1644.
[4] Cotea V.D., Tratat de oenologie. Ed. Ceres, Bucarest 1985; Vol II, p. 197-215.
[5] Wen Y., Cui J., Zhang Y., Duan C., Pan Q. 2014. Comparison of organic acids levels and L-IdnDH expression in Chinese-type and European-type grapes. Euphytica 196: 63-76.
[6] Blouin J., Guimbertau G., Maturation et Maturité des raisins. Ed. Feret, Bordeaux; 2000. p.55-65.
[7] Flamini R., Hyphenated techniques in grape and wine chemistry. J.Wiley & Sons Ltd Chicester England; 2008. p. 1-11
[8] Usseglio-Tomasset L. Chimica enologica. Edizioni AEB Brescia 1978; p. 31-37.