The shikimic acid: an important metabolite for the *Aglianico del Vulture* wines

Pasquale Tamborra,[1] Dina Bolettieri,[2] Michele Latorraca,[2] Michela Tamborra,[1] Fiorella Paradiso,[2] Michele Savino[1]

[1]Consiglio per la Ricerca e la Sperimentazione in Agricoltura - Unità di Ricerca per l'Uva da Tavola e la Vitivinicoltura in Ambiente Mediterraneo - Cantina Sperimentale, Barletta (BAT); [2]Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università della Basilicata, Potenza, Italy

Abstract

Shikimic acid is a precursor for the biosynthesis of aromatic amino acids and flavonoids (anthocyanins, tannins and flavonols). In the pharmaceutical industry, it is obtained by extraction of star anise from China, and at a yield of 3-7% it is used for the production of antiviral drug, *e.g.* oseltamivir. Unlike flavonoids which are only present in the grape skins, shikimic acid is present in the juice together with hydroxycinnamil tartaric acids (caffeic, ferulic and p-coumaric acid). Therefore, their content in white wines may not be negligible and their presence may explain the epidemiological studies that showed a reduced incidence of cardiovascular diseases also in people with moderate white wine consumption. The content of shikimic acid has been used to characterize wines. In southern Italy it has been used to distinguish *Aglianico* grape, which holds medium-high content, from *Negroamaro*, *Primitivo* and *Uva di Troia* grapes who have rather lower levels. It could be useful also to distinguish *Fiano di Avellino* (high value) from *Fiano Minutolo* (low value). However, results of a recent work showed that the shikimic acid content decreases significantly during the ripening of the grapes and therefore its content in wine is strongly influenced by the harvest period. Finally, in a recent paper it was highlighted the increase in shikimic acid content at the end of fermentation in an *Aglianico del Vulture* wine, produced in the area of Rapolla (PZ, Italy) municipality during the 2013 harvest. These last experimental results explain why the values of shikimic acid were lower in grapes and surprisingly higher in wines produced in the 2011 and 2012 harvest.

Correspondence: Michele Savino, Consiglio per la Ricerca e la Sperimentazione in Agricoltura - Unità di Ricerca per l'Uva da Tavola e la Vitivinicoltura in Ambiente Mediterraneo - Cantina Sperimentale, via Vittorio Veneto 26, 76121 Barletta (BAT), Italy. Tel.: +39.0883.521346.
E-mail: michele.savino@entecra.it

Key words: ripening, shikimic acid, varietal marker.

Conference presentation: Meeting on *Environmental Sustainability and Food Security,* Potenza, Italy, 2014.

Received for publication: 21 July 2014.
Revision received: 7 November 2014.
Accepted for publication: 8 November 2014.

Italian Journal of Agronomy 2014; 9:615
doi:10.4081/ija.2014.615

Introduction

The shikimic acid is a biosynthetic precursor of the aromatic amino acids such as phenylalanine and tyrosine, and flavonoids (tannins, anthocyanins and flavonols). In the pharmaceutical industry, it is obtained from Chinese star anise with a yield of 3-7% of shikimic acid and it is used for the production of the antiviral oseltamivir.

In contrast to the flavonoids (anthocyanins, flavonols, tannins) present only in the skins of grapes, shikimic acid is present also in the juice along with the hydroxycinnamoyl tartaric acids (caffeic, paracoumaric and ferulic). Consequently, their content in white wines cannot be negligible and their presence may explain the epidemiological studies that have shown that the intake of white wine in moderate doses provides a reduced incidence of cardiovascular diseases in humans. Several studies have highlighted the biological properties of shikimic acid as an antiviral (Giovannini *et al*., 2008), anti-inflammatory (El-Seedi *et al*., 2003), inhibitor of platelet aggregation induced by ADP and collagen (Ma *et al*., 2000) and the ability to prevent the brain damage after focal ischemia-induced thrombosis (Ma *et al*., 1999).

The amount of shikimic acid present in wines ranges from a few milligrams in the case of Pinot to 50 mg/L in Chardonnay and Trebbiano Toscano (Versini *et al*., 2003), although some wines has displayed higher values that exceed 100 mg/L. In Germany, the content of shikimic acid was used as a parameter to discriminate typical monovarietal Pinot wines purchased from Italy. In same case, Germany has dismissed a batch of Pinot wine with greater quantities of shikimic acid, in comparison to the amount considered normal for the variety. However, later, it was highlighted that a rather large variability of the content of shikimic acid is possible in Pinot: from a few milligrams per litre up to 35 mg/L. In southern Italy it has been used to distinguish *Aglianico* grape, which holds medium-high content, from *Negroamaro*, *Primitivo* and *Uva di Troia* grapes who have rather lower levels (Tamborra and Esti, 2010). It could be useful also to distinguish Fiano di Avellino (high value) from Fiano Minutolo (low value) (Tamborra *et al*., 2009). However, results of a recent work (Tamborra *et al*., 2012) showed that the shikimic acid content decreases significantly during the ripening of the grapes and therefore its content in wine is strongly influenced by the harvest period. Finally, in a recent paper (Paradiso *et al*., 2014) it was highlighted the increase in shikimic acid content at the end of fermentation in an *Aglianico del Vulture* wine, produced in the area of Rapolla (PZ, Italy) municipality during the 2013 harvest. These last experimental results explain why the values of shikimic acid were lower in grapes and surprisingly higher in wines produced in the 2011 and 2012 harvest. In this paper, we present the results of several studies conducted on the shikimic acid content in the grapes and wines.

Materials and methods

The analysis of organic acids present in grapes and wines is easily carried out by isocratic high-performance liquid chromatography (HPLC) using a Synergi 4 u Hydro-RP 80 A column (size 250×4.60 mm) of Phenomenex (Torrance, CA, USA) and phosphoric acid solution (1 mL of 85% H_3PO_4 in 1 L of water for HPLC) as mobile phase with flow of the 0.7 mL/min. The determination of small amounts of shikimic acid is facilitated by its high molar extinction coefficient at the wavelength of 210 nm.

Results and discussion

The results of the chemical analysis carried out on grapes and wines object of our experimentations, has shown that the content of shikimic acid is extremely variable, as shown in the Figure 1, ranging from a few mg/L to over 50 mg/L in order to variety.

In Table 1 are reported the average values of shikimic acid in relation to the different vintages and training systems.

In order to control the kinetics relative to the content of shikimic acid during maturation, 9 varieties of white grapes and 9 of black grapes, coming from the experimental vineyard Lamarossa of Rutigliano (BA), were monitored during the 2011 harvest.

Table 2 shows that the shikimic acid decreases significantly during the ripening of the grapes and therefore its content in wines depends on the time of harvest. However, it is important the dependence of the shikimic acid content from variety: lower in *Moscato b*., *Moscatello b*., *Minutolo b*., *Sangiovese n*., *Uva di Troia n*. and higher in *Bombino b*., *Pecorino b*., *Aglianico n*.

Even the different training systems (simple curtain, unilateral and bilateral cordon, unilateral and bilateral Guyot) have a certain influence on the content of shikimic acid. In these screenings it is possible to note the higher values of shikimic acid of wines from *Cabernet Sauvignon* in comparison to those from other cultivars (Table 3).

Shikimic acid in *Aglianico del Vulture*

Aglianico del Vulture has medium-high contents of shikimic acid (20-30 mg/L), however we found a very high value (96 mg/L) in a 2011 *Aglianico* wine, obtained in a vineyard of Rapolla (PZ, Italy) municipality with 3.94 g/L of malic acid at the date of 8 March 2012.

During the harvest in 2012, we compared *Aglianico del Vulture* of the same vineyard of Rapolla with *Aglianico del Vulture* of municipality of Venosa (PZ, Italy), that is from different protected designation of origin production area, detecting shikimic acid contents both in grapes and wines. The content of shikimic acid detected in grapes were 34 mg/L for Venosa and 17 mg/L for Rapolla. But the content of shikimic acid of wines (Table 4) were surprisingly very different: 29 mg/L for Venosa and 113 mg/L for Rapolla. This last data is very close to the value of the previous year (96 mg/L).

Also the content of succinic acid, an acid produced during alcoholic fermentation, is twice that of wine of Venosa.

Furthermore, during the vintage 2013 it was monitored the organic

Table 1. Shikimic acid contents in different years.

Variety	Vintage	Average±CV% (mg/L)	N. of sample
Primitivo	2005	11.1±13.6	5
Primitivo	2006	15.5±18.6	9
Primitivo	2008	6.9±10.7	23
Negroamaro	2006	10.7±6.8	17
Negroamaro	2008	4.8±17.2	38
Negroamaro	2009	4.4±11.4	27
Malvasia n.	2006	21.9±8.2	7
Montepulciano	2006	23.5±7.7	9
Cabernet S.	2005	78.7±6.1	5

Table 2. Evolution of shikimic acid content (mg/L±CV%) during ripening in different varieties.

Variety	Sampling			
	Aug 20	Aug 31	Sep 13	Sep 19
Moscato b.	5.5±7.4	2.6±4.6	1.9±5.6	1.3±8.9
Moscatello b.	6.4±12.3	3.3±8.8	1.3±12.5	1.6±6.6
Grillo b.	10.3±8.7	9.0±6.5	4.8±7.6	3.9±11.1
Montepulciano n.	26.7±5.5	4.7±112.3	17.7±10.4	1.3±6.7
Sangiovese n.	n.d.	13.5±9.1	1.6±8.6	1.9±5.2
Malvasia n.	5.8±6.4	2.4±7.7	1.9±9.3	2.9±13.6
Negroamaro n. (Early)	18.0±7.5	28.0±9.2	15.1±1.64	4.2±14.7
Uva di Troia n.	17.9±11.3	12.6±6.4	5.0±9.7	5.0±10.0
Minutolo b.	9.0±4.8	15.1±5.9	3.1±5.3	6.3±8.7
Aglianicone n.	12.9±9.9	11.3±9.6	4.8±11.5	5.5±7.8
Fiano b.	n.d.	22.3±12.3	12.0±6.9	9.4±6.3
Susumaniello n.	33.0±8.9	26.1±10.4	15.7±6.4	14.2±6.9
Mantonico b.	13.9±10.1	15.5±7.6	11.3±10.2	16.4±5.7
Bombino b.	52.2±13.5	34.8±13.8	29.3±15.3	15.5±12.3
Primitivo n.	n.d.	18.2±7.8	19.2±18.5	28.0±6.8
Greco b.	27.1±12.0	38.5±11.4	24.8±12.6	30.4±4.2
Pecorino b.	68.3±11.2	75.3±9.0	43.8±7.5	37.5±8.8
Aglianico n.	n.d.	59.2±8.8	44.1±9.9	36.4±9.5

n.d., not detected.

Table 3. Influence of training systems on shikimic acid content (mg/L±CV%).

Wines 2006	Simple curtain	Unilateral cordon	Bilateral cordon	Unilateral Guyot	Bilateral Guyot
Primitivo	18.4±5.6	15.0±4.8	23.4±7.9	16.5±5.3	24.0±8.8
Malvasia	nd	20.8±4.2	23.5±6.6	20.0±7.6	24.6±5.1
Montepulciano	24.1±7.8	19.8±5.0	24.9±9.3	22.1±8.0	25.0±5.0
Negramaro	9.5±4.4	9.0±8.6	10.4±8.7	9.8±4.9	11.3±4.3
Cabernet s.	72.9±6.1	77.1±6.4	83.0±8.8	74.2±6.5	86.6±7.4

nd, not detected.

acids composition of the *Aglianico del Vulture* produced in the same areas of Venosa and Rapolla. An aliquot of must of both areas have been transported and analysed during the fermentation at the Experimental Cellar of Barletta (CRA-UTV). It emerged (Figure 2) that the two samples of Venosa, inoculated with selected yeasts, showed no variations of the content of shikimic acid. Instead, the two samples of Rapolla have shown a significant increase in the content of shikimic acid up to 50 mg/L. To check whether such increase of shikimic acid was linked to the hydrolysis of its possible precursors, the juice of *Aglianico* of Venosa and Rapolla were subjected to different types of hydrolysis (basic, acid and enzymatic). The results showed no changes in the content of shikimic acid. Therefore, it remains to investigate the hypothesis of a possible microbial process responsible for the increase of shikimic acid. Recently (Tripathi *et al*., 2013) have shown that strains of bacterium *Citrobacter freundii* can produce 9.11 g/L of shikimic acid from 20 g/L of glucose in suitable conditions of fermentation. However, a preliminary microbial analysis of the fermenting must in a laboratory has not revealed the presence of this organism.

Table 4. Average±CV% of organic acid content in the *Aglianico* wines 2012.

		Rapolla area	Venosa area
Tartaric acid	g/L	2.78±11.4	3.26±13.7
Malic acid	g/L	3.76±7.3	1.02±6.4
Shikimic acid	mg/L	113.0±5.5	29.0±7.0
Lactic acid	g/L	0.32±8.8	0.27±7.9
Citric acid	g/L	0.34±12.6	0.46±15.4
Succinic acid	g/L	2.14±6.9	1.08±8.0

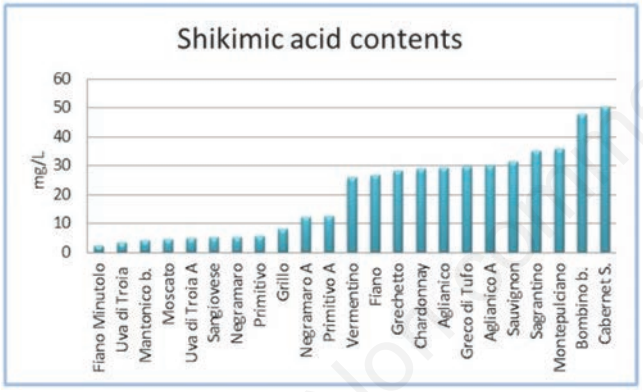

Figure 1. Shikimic acid contents in different grape varieties.

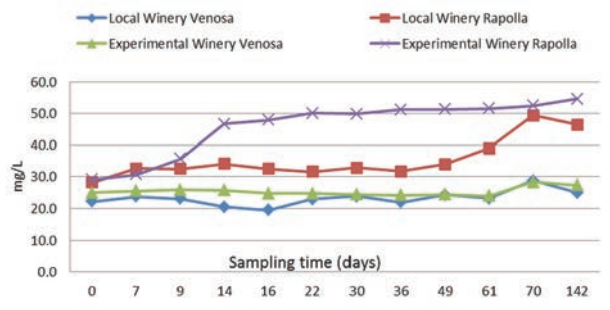

Figure 2. Shikimic acid kinetic of the *Aglianico* wines 2013.

Conclusions

The shikimic acid, a precursor of aromatic amino acids and of flavonoids with a strong anti-inflammatory activity, was used as a varietal marker to distinguish *Aglianico del Vulture* from other varieties of black grapes of Apulia. The varietal dependence is confirmed by data related to the research project *Vitivin-Valut. Primitivo and Negroamaro* confirmed low shikimic acid content, as well as the *Moltepulciano* and *Cabernet Sauvignon* confirmed high values. However, shikimic acid decreases significantly during the ripening of the grapes and therefore its content in wines strongly depends on the time of harvest.

Moreover, three years of investigations have highlighted a surprising increase of shikimic acid during winemaking processes in a cru *Aglianico del Vulture* (Rapolla). Its content can exceed 100 mg/L. Further investigations are necessary to explain this phenomenon.

References

El-Seedi HR, Ringbom T, Torsell K, Bohlin L, 2003. Constituents of Hypericum laricifolium and their cyclooxygenase (COX) enzyme activities. Chem. Pharm. Bull. 51:1439-40.

Giovannini L, Guidi A, Settimini L, Toti L, Mannari C, Flak WY, Bertelli A, 2008. Immunomodulatory activity of shikimic acid and quercetin, two compounds present in white wine, in a in vitro experimental model. Proc. 31st World Congress of Vine and Wine, June 15-20, Verona, Italy.

Ma Y, Sun JN, Xu QP, Guo YJ, 2000. Inhibitory effects of shikimic acid on platelet aggregation and blood coagulation. Acta Pharm. Sinica 35:1-4.

Ma Y, Xu QP, Sun JN, Bai LM, Guo YJ, Niu JZ, 1999. Antagonistic effects of shikimic acid against focal cerebral ischemia injury in rats sujected to middle cerebral artery thrombosis. Acta Pharm. Sinica. 20:701-4.

Paradiso F, Bolettieri D, Savino M, Latorraca M, Tamborra P, 2014. L'acide shikimique: un métabolite important pour le cépage Aglianico del Vulture. Proc. 3rd Edition of Wine Active Compounds, Oenoplurimédia, France.

Tamborra P, Esti M, 2010. Authenticity markers in Aglianico, Uva di Troia, Negroamaro and Primitivo grapes. Analyt. Chim. Acta 660:221-6.

Tamborra P, Piracci A, Coletta A, Esti M, 2009. Winemaking technique optimization for enancing aroma in Fiano wine. Atti Accademia Italiana della Vite e del Vino, Relazione n. 57, Tornata 26.

Tamborra P, Suriano S, Caputo AR, Toci AT, Giurato C, Pichierri A, Mazzone F, Antonacci D, 2012. Influenza delle tecniche culturali e del grado di maturazione sul contenuto di acido scichimico. Proc. 35th World Congress of Vine and Wine, June 2012, Izmir, Turkey.

Tripathi P, Rawat G, Yadav S, Saxena RK, 2013. Fermentative production of shikimic acid: a paradigm shift of production concept from plant route to microbial route. Bioprocess Biosyst. Eng. 36:1665-73.

Versini G, Mattivi F, Moser S, Pisoni A, Volunteer G, 2003. Shikimic acid quantification in experimental mono-varietal white wines produced in Italy. pp 166-169 in Proc. 7th Int. Symp. of Oenology, Lavoisier-Tec & Doc, Paris, France.