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Influence of foliar riboflavin applications to vineyard on grape amino acid content

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ABSTRACT

Nitrogen is an important element for grapevine and winemaking, which affects plant development, grape juice fermentation and has a potential effect in modulating wine quality. The aim was to study the influence of foliar applications of riboflavin (vitamin B2) to vineyard on grape nitrogen composition. This vitamin has a reported capacity to protect different plant species, but its application to favor grape and grape juice quality had not previously been studied. This work reports the oenological properties and the effect on amino acid concentration of grape juices obtained from grapes treated with riboflavin at two different doses compared to control. Results showed that probable alcohol, malic acid, color intensity and hue had significant differences when the riboflavin treatments were applied. Most of the amino acids presented the highest concentrations when the lowest riboflavin dose was used. These results are promising in terms of fermentation development and grape juice nitrogen composition.

1. Introduction

Nitrogen is the most abundant soil-derived macronutrient in a grapevine, and plays a major role in many biological functions and processes of the plant as well as in fermentative microorganisms (Bell & Henschke, 2005). Thus, nitrogen concentration and composition of the grape and its juice can potentially affect wine quality and value. In this sense, fermentation kinetics and formation of flavor-active metabolites are affected by the nitrogen status of the grape juice.

Manipulation of grapevine nutrition has the potential to influence grape composition, helping to solve problems such as nitrogen deficiency, which is the leading cause of stuck and sluggish fermentations (Arias-Gil, Garde-Cerdán, & Ancín-Azpilicueta, 2007; Bely, Sablayrolles, & Barre, 1990). An increase in the concentration of the major nitrogenous compounds and thus the enhancement of yeast assimilable nitrogen (YAN) is a consistent effect of nitrogen application on grape in the vineyard (Bell & Henschke, 2005). Therefore, the type and amount of YAN have relevant implications for wine quality. Along these lines, previous studies have reported that different nitrogen sources and viticulture practices have an impact on the amino acid profile (Marschner, 2012; Scheible et al., 2004).

For example, it has been established that the use of pesticides, have been shown to have deleterious effects for the grape, such as the reduction of amino acid concentration (Oliva, Garde-Cerdán, Martínez-Gil, Salinas, & Barba, 2011). On the other hand, the excessive use of nitrogen fertilizers, which are normally added to the soil in order for it to be absorbed by plant roots, has led to ecological problems (Lasa et al., 2012). The main environmental problem derived from the excessive use of nitrogen fertilizers is the water contamination, besides the imbalances that harm the soil fertilization, polluting the environment. Therefore, the excessive use of nitrogen fertilizers provoke high nitrogen concentration in groundwater due to infiltrations, mainly of nitrates. Nitrates alter the water composition, or if are stored into the surface reservoirs, can be even toxic at certain levels. In these environmental media, nitrates also acts as fertilizers for aquatic vegetation, so, if they are concentrated, the eutrophication of the environment may occur. In an eutrophic environment, there is the proliferation of species such as algae and other green plants that cover the surface. This results in a high consumption of oxygen and its reduction in the aquatic environment, as well as hinders the incidence of solar radiation below the surface. These two phenomena produce a decrease in the self-depleting capacity of the medium and a reduction in the photosynthetic capacity of aquatic organisms. Foliar application of elicitors to vineyard is a delivery approach endowed with several advantages, which include fast and efficient assimilation of applied products by plant; hence, costs are reduced and, at the same time, it contributes to a sustainable and

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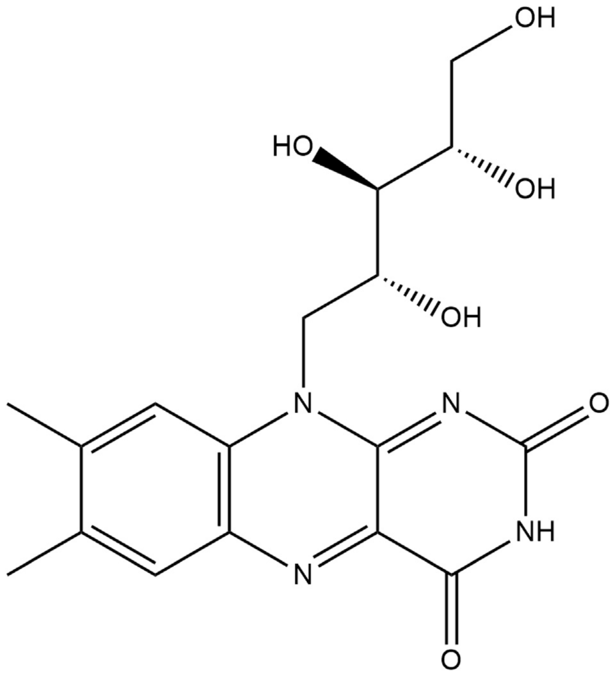

Riboflavin (vitamin B2)

Fig. 1. Structure of riboflavin (vitamin B2), the active compound studied in this work.

eco-friendly agriculture (Garde-Cerdán et al., 2014; Pérez-Álvarez, Garde-Cerdán, García-Escudero, & Martínez-Vidaurre, 2017).

Plants are subject to attacks by pathogens which can lead to yield losses and can affect the harvest quality. Elicitors are compounds that stimulate any type of defense response, promoting secondary metabolism to protect the cell and the whole plant (Delaunois et al., 2014). A better understanding of elicitors has allowed their application not only as a protective aid for plants against pathogens, but also as a biotechnological tool for the development of bioactive high added-value compounds (Ramirez-Estrada et al., 2016). Elicitors can also cause the final product to favor the production of secondary metabolites that can improve the final product nutritional quality in a cost-effective way, i.e. grape phenolic composition or amino acid content (Garde-Cerdán, Portu, López, & Santamaría, 2016; Garde-Cerdán et al., 2014).

Riboflavin (vitamin B2) is a water-soluble vitamin composed by a dimethylisoalloxazine ring substituted with a ribityl chain (Fig. 1), which is ubiquitously distributed in nature and found in a wide variety of foods (Powers, 2003). This vitamin was first identified and isolated as early as 1879 and named lactochrome due to its color and origin. During the following years its structure was elucidated, its role as a coenzyme in many physiological processes reported and its importance in maintaining human, animals and plants health clarified (Powers, 2003). Nowadays, riboflavin has been shown to be essential and to take part in key redox reactions across the human metabolism through its bioactive derivatives, i.e. flavin mononucleotide or flavin adenine dinucleotide (Barile, Giancaspero, Leone, Galluccio, & Indiveri, 2016; Powers, 2003). Hence, riboflavin deficiency is associated with health and nutritional problems in humans (Powers, 2003). However, the eliciting activity of riboflavin in plants was not reported until 2000 (Dong & Beer, 2000). More specifically, increased levels of riboflavin have been shown to activate the systemic acquired resistance via the induction of reactive oxygen species (ROS) and hormonal signaling transduction pathways, promoting the phenylpropanoid pathway and accumulation of phenolic compounds (Liu et al., 2010; Taheri & Tarighi, 2011). Thereafter, its role in enhancing disease resistance has been described in other plant species including grapes (Boubakri et al., 2013; Liu et al., 2010; Taheri & Tarighi, 2011).

Bearing in mind that riboflavin shows eliciting capacity, that it presents a nitrogenated structure which can also be assimilated by yeasts and that most vitamin synthesis is linked to plant nitrogen metabolism (Miret & Munné-Bosch, 2014) we hypothesized that riboflavin could be a potential candidate to enhance grape juice properties. Thus, the aim of this work was to study the influence of foliar application of riboflavin on grapevine, especially on nitrogen composition, a key factor not frequently monitored in detail when studying grapevine elicitors.

2. Materials and methods

2.1. Plant material and experimental layout

The study was carried out in 2016 in a vineyard of cv. Tempranillo (*Vitis vinifera* L.) grafted on Richter-110 rootstock, located in Logroño, La Rioja region, northern Spain (lat.: 42°26′3418″ N, long.: 2°30′5307″ W, elevation 465 m above sea level (m.a.s.l.)). Grapevines were planted in 1990 on a fine-loamy soil, classified as *Typic Haploxerepts* according to the United States Department of Agriculture soil classification (Keys to Soil Taxonomy, 2010). At the Ap horizon (0–20 cm), soil contained 230 g/kg of clay, 433 g/kg silt (2–50 µm), 337 g/kg sand, 9.3 g/kg organic matter and 149 g/kg carbonates, a pH of 8.62 (H_2O, 1:5) and an electrical conductivity of 0.17 dS/m. Grapevine rows were oriented north east to south west, and the planting density was 2900 plants/ha (grapevine and row spacings were 1.2 and 2.30 m, respectively). Grapevines were trained on a vertical shoot position system on a double Cordon Royat and were spur pruned to 12 buds per vine. Climatic data were recorded by a Riojan climatic service meteorological station (www.larioja.org/siar), placed in the same vineyard as the study. For 2016, the precipitation (from March to mid-October, the grapevines vegetative period) was 173 mm, average temperature was 16.45 °C, and potential evapotranspiration (FAO-Penman-Monteith) for the same period was 946.4 mm. Weather was characterized as semiarid Mediterranean, according to the UNESCO aridity index (UNESCO, 1979).

The experimental design consisted in a randomized complete block with three different treatments and three replicates per treatment. Each replicate had three adjacent grapevines. In each replicate, between treatments, there were two grapevines considered buffer and excluded in sampling to avoid edge effects. The Control and two different doses of foliar riboflavin (Sigma-Aldrich, Madrid, Spain) applications were the studied treatments. Control plants were foliar sprayed with a water solution of Tween 80 (Sigma-Aldrich, Madrid, Spain) alone, used as the wetting agent (1 mL/L). For each application, 200 mL/plant of each solution was sprayed over grapevine leaves. Riboflavin stock solutions of 0.5 and 1 mM were prepared – concentrations comparable to those used by Boubakri et al. (2013) – and applied with a water solution of Tween 80 on grapevine leaves at two different doses: 100 g/ha (Rf1) and 200 g/ha (Rf2). The foliar treatments were applied to the grapevines twice, at veraison (more than 50% of grapes colored) and one week later.

The experimental vineyard had not been nitrogen-fertilized, soil was mechanically tilled, and the rest of the vineyard management practices were similar in all treatments and managed according to standard practice for the region.

2.2. Grape sampling and analysis of grape juice oenological parameters

Grapes were harvested at their optimum technological maturity, and then were destemmed and crushed. The obtained grape juices were physico-chemically characterized by determining probable alcohol, pH, total acidity, color, hue and total polyphenol index (TPI) according to the ECC (1990). Malic acid and yeast assimilable nitrogen (YAN) were analyzed using a multi-analyzer Miura one (Tecnología Difusión Ibérica, Barcelona, Spain). The treatments were carried out in triplicate,