TOTAL PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY OF WINE GRAPES GROWN IN ZACATECAS, MEXICO.

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RESUMEN:

La producción de uva para vinos (*Vitis vinifera* L.) en Zacatecas México ha aumentado en los últimos años, sin embargo existe poca información disponible acerca de su composición química. Se ha asociado a los polifenoles con la capacidad antioxidante de la uvas, debido a sus efectos beneficiosos para la salud. El objetivo del presente trabajo de investigación fue evaluar el contenido en fenoles totales (TPC) y la capacidad antioxidante (AC) de uvas producidas en Zacatecas, México. Se analizó la capacidad antioxidante y los fenoles totales de 4 variedades tintas y 4 variedades blancas. El diseño experimental fue completamente al azar con triplicado. Los datos fueron analizados mediante el análisis de la varianza (ANOVA) y mediante el test de Tukey ($p \leq 0.05$). Además se calculó la regresión lineal y correlación de Pearson para la actividad antioxidante y los fenoles totales. Los fenoles totales fueron significativamente más alto en la variedad tinta Rubired y en la Furmint en las variedades blancas. La AC (método ABTS) presentó una correlación positiva fuerte con los fenoles totales (R^2=0.8304). Los valores obtenidos en el presente estudio muestran que se pueden obtener uvas de gran calidad funcional en el estado de Zacatecas.

Palabras clave: Antioxidantes, *Vitis vinifera* L., Polifenoles, Fitoquímicos, Zacatecas, Nutrición.

ABSTRACT:

The production of wine grapes (*Vitis vinifera* L.) in Zacatecas, Mexico has increased in recent years; however, information about their chemical composition is not yet available. Polyphenols are associated with the antioxidant capacity of grapes due to their beneficial health effects. Therefore, the aim of this study was to evaluate the total phenolic content (TPC) and the antioxidant capacity (AC) of non-native wine grapes grown in Zacatecas, México. The AC and TPC of 4 white grape varieties and 4 red grape varieties were analyzed. Experimental design was completely randomized with three replications and the data was analyzed using ANOVA and compared with Tukey test ($p \leq 0.05$). Pearson's correlation and linear regression were calculated for the TPC and the AC. Total phenolic content was significantly higher in red varieties of Rubired and white varieties of Furmint. The AC (using the ABTS method) was significantly correlated with the TPC (R^2=0.8304). The values obtained in this study show that wine grapes with a high functional content can be harvested in the state of Zacatecas, non-native varieties resulted in higher capacities than the values reported in the country of origin for each respective variety.

Keywords: Antioxidants, *Vitis vinifera* L. Polyphenols, phytochemicals, Zacatecas, Nutrition.

INTRODUCCIÓN

Grape (*Vitis vinifera* L.) is a major fruit crop around the world with many cultivars varying in characteristics, such as flavor and colour, with or without seeds, which are associated to the content of phenolic compounds and consequently with antioxidant capacity. Grapes are one of the major sources of phenolic compounds, as compared with other fruits and vegetables (Mulero et al., 2015). Phenolic compounds are an integral part of the human diet and are considered to be non-nutrients with biologically active compounds (Subramani et al., 2002; Mulero et al., 2015) and they constitute one of the most numerous and widely distributed groups of natural products in the plant kingdom. They play a very important role in the composition of grapes and wines, contributing to principal sensory properties, such as colour, astringency and bitterness (Puértolas et al., 2010). These compounds were identified and quantified in several fruits and vegetables, and show a high correlation with antioxidant activity (Einbond et al., 2004). Polyphenols are classified into two main groups: non-flavonoids (hydroxybenzoic and hydroxycinnamic acids and their derivatives, stilbenes and phenolic alcohols) and flavonoids (anthocyanins, flavanols, flavonols and dihydroflavonols) (Fanzone et al., 2012).

Biological properties of polyphenols are attributed mainly to their powerful antioxidant, metal chelating and antiradical activities (Šeruga et al., 2011). Also, phenolic compounds have been reported to be able to reduce the risk of chronic diseases, eliminating free radicals that induce vascular relaxation, and they also exhibit anti-inflammatory, anti-cancer, antiviral and antibacterial properties (Gris et al., 2011).

Most of the data available in literature sources on phenolic composition and antioxidant activity of grapes stem from varieties produced in France, Italy, Spain, USA, Turkey, Chile, Brazil and Argentina. In Mexico, 75 % of grape production is allotted for grapes, 22 % goes towards wine production, juices and jams and 3 % for raisin production. In 2012, Sonora produced 80 % of all grapes in Mexico whereas Zacatecas followed in second place with 7.8 %; however, there is little information about nutritional content, total phenolic content and antioxidant activity of grapes produced in Zacatecas, Mexico. Therefore, the objective of this study was to determine the total phenolic content and antioxidant activity of non native wine grape varieties produced in the Department of Agriculture, Autonomous University of Zacatecas, México.

MATERIALES Y MÉTODOS

White grape varieties of Furmint, Palomino, Semillon, Sauvignon Vert, and red grape varieties Cabernet Sauvignon, Merlot, Rubired and Petite Syrah were harvested and used for the experiments. The plantation design allowed 3 meters of separation between rows and 1.5 meters between plants, obtaining a density of 2600 plants per unit of production. In addition, using a system of bilateral cordon permitted optimal sun exposure. This plantation design was used for both red and white varieties. The growing season began in March 2015 and ended in August 2015. All grapes were stored in a refrigerator (up to 12 h), were rinsed with distilled water and dried with paper towels before handling.

Analysis

Extraction of Phenolic compounds

Extracting the phenolic compounds (Tomás-Barberán et al., 2001) consisted of homogenising 35 g of the grape sample for 10 min with 40 mL of methanol, 10 mL of HCl 6N and 2mg of NaF to inactivate the polyphenol oxidases and prevent phenolic degradation. After extraction, the mixture was centrifuged (2701 x g; 4 °C) for 10 min. The supernatant obtained was stored (24 h) in opaque vials at 4 °C until analyzed.

Determination of total phenolic content (TPC)

Total phenolic content (TPC) was quantified using the Folin–Ciocalteu test (Li et al., 2006). 250 µL of extract was mixed with 15 mL deionized water and 1.25 mL of Folin-Ciocalteu phenol reagent. After 5 minutes, 3.75 mL of Na2CO3 (7.5 %) is added and leveled to 25 mL with deionized water. Absorbance was measured at 765 nm in a spectrophotometer UV-Vis (Termo Scientifc 10S, Termo Fisher Scientifc Inc, USA). The results were reported as milligrams of gallic acid (mg GAE 100 g^{-1} fresh grape).

Antioxidant capacity (AC) ABTS + scavenging ability

The same extract obtained for TPC quantification was used for the determination of antioxidant capacity (AC). The AC was determined through a modification of the spectrophotometric technique developed by Re et al. (1999), using the ABTS + radical (Sigma) generated by 2.45 mM potassium persulfate ($K_2S_2O_8$). The mixture was allowed to stand in the dark at room temperature (~20° C) for 16 h before use, and then the ABTS + solution was diluted to give an absorbance of 0.7 ± 0.1 at 734 nm. Following this, 100 µL of grape extract was mixed with 900 µL of the ABTS + diluted solution, and the absorbance was measured at 734 nm. The results were expressed as antioxidant activity equivalent to µmol units of Trolox (TEAC) 100 g-1 fresh sample. All the experiments were replicated thrice.

Experimental design and Statistical analysis

Experimental design was completely randomized with three replications. All analyses were carried out in triplicate and the results expressed as mean values ± standard deviation. To determine whether there were statistically significant differences between the data of the variables in the grape varieties, a one-way ANOVA was carried out. If ANOVA was significant, a Tukey test was applied ($p \leq 0.05$). The values of antioxidant activity and total phenol content were analyzed using Pearson correlation and linear regression. All statistical analyses were performed using Statgraphics® Centurion XV (Statpoint Technologies Inc., Warrenton, VA, USA).

RESULTADOS Y DISCUSIÓN

Red grapes showed higher values of TPC as compared to white grapes and the highest concentration was found in Rubired grapes (607.6 mg GAE 100 g^{-1}), followed by Merlot (537.1 mg GAE 100 g^{-1}), Petite Syrah (488.8mg GAE 100 g^{-1}) and Cabernet Sauvignon (321.9 mg of GAE 100 g^{-1}). Out of the white grape varieties, the Furmint grape had the highest TPC (218 mg GAE 100 g^{-1}) followed by Palomino (148.5 mg GAE 100 g^{-1}), Semillon (142 mg GAE 100 g^{-1}) and Sauvignon Vert (112.7 mg GAE 100 g^{-1}) (Figure 1).

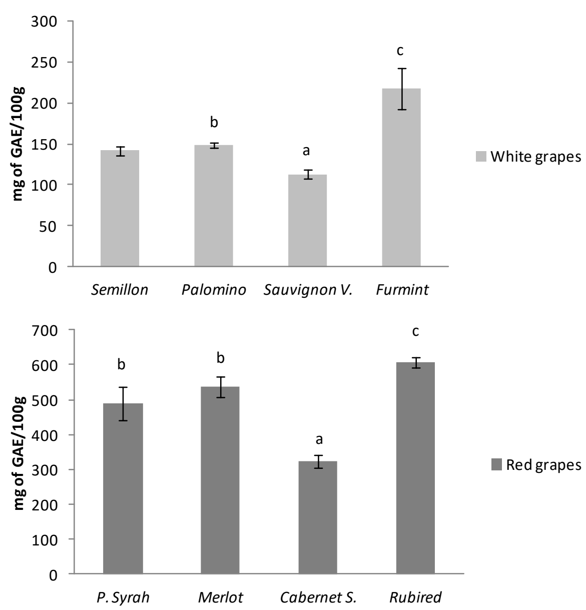

Figure 1. Values and standard deviation of the total phenol content (mg GAE 100g-1) in white and red wine grape varieties.

The phenolic composition of grape varieties depends on several factors, such as the intrinsic potential of each grape variety (Rodríguez Montealegre et al., 2006; Ruberto et al., 2007; Obreque-Slier et al., 2010; Costa et al., 2015). Due to variations among cultivars, different species of grapes show diverse phenol content (Rebello et al., 2013). The climate conditions are particularly important for grapevine cultivation, in which heat, drought and light intensity are just some environmental factors that have an impact on the phenolic metabolism as well as grape development and chemical composition (Teixeira et al., 2013). In our study, the differences between the grapes of the same colour can be attributed to grape variety because there were no climatic and geographical differences.

According to Mazza et al. (1999), varietal factors determine concentration, distribution and accumulation of polyphenols in grapes. Besides, total polyphenols in white grape varieties are lower than in red grapes, since white varieties do not synthesize anthocyanins and polyphenols; synthesis in grapes is influenced by factors such as temperature, water availability, sun exposure of the clusters, and time of the vintage during the berry ripening (Ivanova et al., 2011). In warmer climates, high light exposure can increase the concentration of phenolics and anthocyanins (Roubelakis-Angelakis and Kliewer, 1986). Sun exposure is generally considered to be of primary importance for high quality wine production. However, it is not clear whether the effect on fruit composition is due to visible light or ultraviolet light or both (Keller and Torres-Martinez, 2004; Schreimer et al., 2012). In the vineyard, e.g., any cultural practices that favor the exposure of grape brunches to sunlight boost flavonol accumulation. This occurs equally in white and red grapes (Teixeira et al., 2013).

Merlot was 37 % higher in GAE compared with those reported by Abe et al. (2007) who found 337 mg GAE 100 g-1. Panceri et al. (2013) reported 89% less (59.28 mg GAE 100g-1) and Burin et al. (2014) 85 % less (83.1 mg GAE 100 g-1). Also for the Cabernet Sauvignon variety a difference of 79.1 % and 76 % was observed in total phenol content compared with those reported by Panceri et al. (2013) and Burin et al. (2014), who reported 67.4 and 76.5 mg GAE 100 g-1, respectively. Regarding Petite Syrah grape, the results in our study differ by 21 % with those published by Abe et al. (2006) (388 mg GAE 100 g-1).

Table I. Values and standard deviation of antioxidant capacity in white and red wine grapes varieties.

	ABTS µmol of TEAC $100g^{-1}$
White Grapes	
Semillon	4.309 (0.003) a[1]
Palomino	4.377 (0.014) b
Sauvignon Vert	4.373 (0.007) b
Furmint	4.310 (0.018) a
Red grapes	
P. Syrah	152.851 (3.843) c
Merlot	121.932 (13.793) a
Cabernet S.	124.406 (5.572) a,b
Rubired	148.956 (12.613) b,c

For AC, red grapes showed a higher capacity (µmol TEAC 100 g-1) than white grapes (Table 1). Among the red grapes, Petite Syrah showed the highest AC (µmol TEAC 100 g^{-1}), followed by Merlot, Cabernet Sauvignon y Rubired varieties. In white grapes, Palomino showed the highest AC.

For Cabernet Sauvignon, AC values were 213 µmol TEAC $100g^{-1}$ with the ABTS assay (Burin et al., 2014), whereas Panceri et al. (2013) reported 195 µmol TEAC 100 g^{-1} with ABTS. For Merlot, AC values were 231.6 µmol TEAC 100 g^{-1} with ABTS (Panceri et al., 2013).

In our study, AC with the ABTS method shows a stronger correlation (R^2 = 0.8304) with total phenol content, this is shown in Figure 2 in which the mathematical model proved well adjusted for TPC and AC. This goes along with the results of other authors who verified that the antioxidant capacity of grapes is dependent on the phenolic composition (Tagliazucchi et al., 2010).

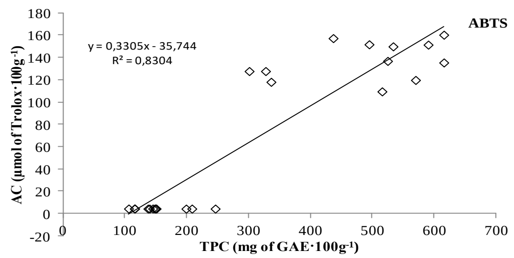

Figure 2. Relationship between antioxidant capacity (µmol Trolox 100 g^{-1}) and total phenolic compounds (mg GAE 100 g^{-1}) of white and red wine grape varieties.

The biological properties of phenolic compounds include antibacterial, antifungal effects, as well as cardioprotective, anticancer actions. The composition of phenolics in grapes vary with variety, species, season, environmental and management factors such as soil conditions, climate and crop load. The AC of grape-derived products is influenced by their total phenol content, which is influenced mainly by the grape variety (Burin et al., 2014). However, Nixdorf y Hermosín-Gutiérrez (2010) observed just the opposite, a lack of correlation and a negative correlation between total phenol content and AC.

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