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EVOLUTION OF PHENOLIC COMPOUNDS DURING THE RED GRAPES FERMENTATION IN THE PRESENCE OF TRACE METALS

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Polyphenols are second metabolites originating from vegetable sources. They are of great importance due to their capacities of capturing free radicals which are known to be responsible for damaging cells and tissues. In grapes and subsequently in grape must, among other constituents, polyphenols, originating from grape skin, are found in good amount, especially in the red varieties.

In this article, the attention was focused on the influence of some added metals on the polyphenols content in grape must, during must fermentation process. The influence was studied through spectroscopic techniques UV-VIS and ^{1}H-NMR. The results showed that metals, added in a pre-fermentative stage of the grape must determine a more pronounced colour and a higher number of polyphenols in the final product: wine.

Keywords: fermentation, phenolic compounds, trace metals

1. Introduction

Nowadays, one important topic of research is related to the use of antioxidants compounds in the prevention or treatment of illnesses [1, 2]. Stress, genetic factors, or diets are the main factors for diabetes, cardiovascular or neurodegenerative diseases, the disorders of our decade. Researchers and nutritionists are striving to identify the natural, potential compounds which can act as antioxidants or health promoters reducing the oxidative stress by minimizing the content of free radicals [3, 4]. Besides fruits and vegetables, rich in vitamins and other biologically active compounds, wine contains 50-525 mg/L of polyphenols, essential compounds for the plant growth and prevention of diseases [5]. Polyphenols are a group of biologically active molecules, capable of catching free radicals, formed following oxidation reactions. These can be classified in two groups: flavonoids and non-flavonoids (including anthocyanins). It can exist in free forms, polymerized forms or linked to another compounds, being a colourless organic structure or a macromolecule. However, polyphenols are susceptible to

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oxidation, losing their biological function, all by damaging the heterocycle from its structure [6].

Researchers succeeded in prolonging the lifetime of polyphenols by adding different amount of metals, in wine, which act as chelators, protecting them from environmental conditions [7, 8]. Metals accumulate in wine not only through specific treatments required for its stabilisation, as bentonite or sulfiting procedures, but also by direct addition [9,10]. Literature data confirms interactions between polyphenols and low metal concentration leading to complexes.

In other alcoholic beverages, like whiskey, cognac, the presence of metals in limited quantities can emphasize the organoleptic properties, as flavour, odour or colour. In the same time, the specific quantity can be an useful tool in the identification process and recognition of pattern of different types of brandies or whiskey [11-14].

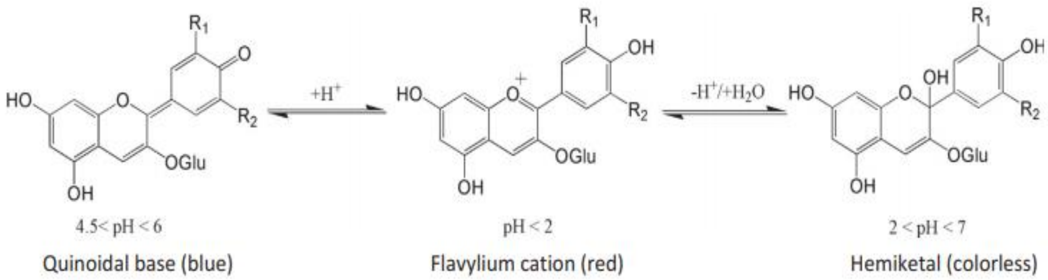

Fig. 1. Anthocyanins structure dependent on the pH of the solution, [20]

However, high quantities of these metals can lead to polyphenols reduction and colloidal instabilities of wine. During the harmful process, polyphenols, in presence of Fe(II) – Fe(III) and copper Cu(I) – Cu (II), as mediators, are oxidized losing their specific colour. At the beginning of the fermentation process, anthocyanins and tannins, major type of phenolic compounds is pruned, dependent on solution pH, see Fig.1, being the first oxidized. Various studies based on the interactions of anthocyanins with itself or with other phenolic compounds, show that oxygen exposure has a great influence on wine colour [15, 16]. Polyphenols can undergo chemical oxidation, due to the presence of metals, resulting in pigmented polymers or pyranoanthocyanins, more stable, over time.

The aim of this paper was to investigate the influence of added metals in trace amounts, on the evolution of phenolic compounds present in red wine. During the red wine fermentation, due to the enhance amount of ethanol, the grape skins release a larger quantity of polyphenols in the grape must but diminishes the level of pigmentation. This amount could be influenced by the presence of specific metal ions capable to link or interfere with polyphenols, see Fig.2 [17, 18]. The experiments were conducted using different spectroscopic techniques, as UV-VIS and ^{1}H-NMR.

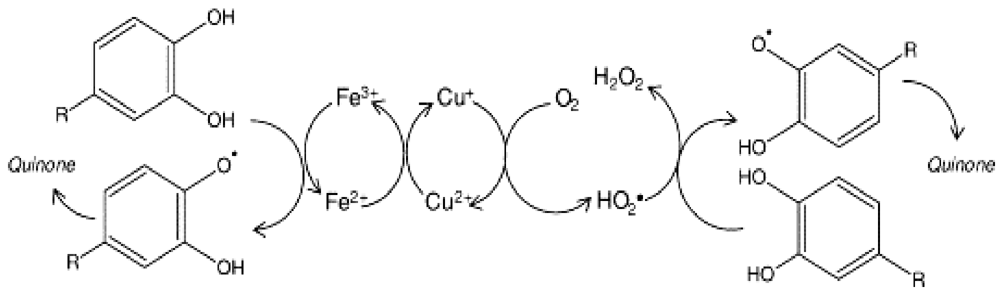

Fig. 2. Polyphenols oxidation in wine, in presence of Fe^{2+}/Fe^{3+} and Cu^{+}/Cu^{2+}, [18]

2. Experimental

The red grape samples were purchased from local market. It was processed for grape must extraction, by crushing and destemming the grapes. Must (300 mL) and grape skins (100 g) were introduced into the fermentation tank. A total of 6 fermentations were investigated: control (only grape must and skins), sample 1 and sample 3 (grape must, grape skin and a solution of $FeNH_4(SO_4)_2$), sample 2 and 4 (grape must, grape skin and a solution of $CuSO_4$,) as presented in table 1. The metal ions solutions were added at the beginning of the process, prior to fermentation (Fig.3). Each experiment was conducted in duplicate for each metal concentration.

Table 1. Concentration of metal solution contained in the samples, mg/L

Samples	Control samples	Sample 1 Fe^{2+}	Sample 2 Cu^{2+}	Sample 3 Fe^{2+}	Sample 4 Cu^{2+}
Concentration, mg/L	-	6	6	10^4	10^4

2.1. Materials and Reagents:

The reagents gallic acid, Na_2CO_3, $FeNH_4(SO_4)_2$, $CuSO_4$ used in the research were of analytical purity. Folin Ciocalteu reagent was purchased from Merck and kept at 4°C before use.

2.2. Methods:

Total polyphenol content analysis: The Folin Ciocalteu's method was applied for quantification of total polyphenol content: 0.1 mL of red must was contacted with 1 mL of distilled water and 1.5 mL of Na_2CO_3. After a period of 10 minutes of dark incubation, 0.5 mL of Folin Ciocalteu reagent was added. The sample was kept in a dark place for 30 minutes and then analysed by UV-VIS using a Thermo α Helios UV-VIS spectrometer.

Calibration curve was obtained using a standard gallic acid solution of 10^{-4} mg/L concentration.

The **wine colour intensity**: The method consists in measurement of absorbance at 3 wavelengths 420, 520 and 620nm, of red wine samples diluted 1:10 v/v.

The wine colour intensity results by adding together the 3 values for each sample:

$$I = A_{420} + A_{520} + A_{620}.$$

This method considers the contribution of 3 wavelengths: 420, 520 and 620 nm, specific for red, yellow, and blue components of wine [19].

^{1}H-NMR analysis: The sample preparation consisted in the dilution of wine (must) with deuterated water (9:1 v/v). The D_2O solution contains 10mmol/L TSP (deuterated trimethylsilyl propionate sodium salt) as internal standard. The samples were analysed on a Bruker Ascend 400Mhz using a 45º pulse, without power attenuation, with an acquisition time of 2,05 s, a spectral window of 6,4 KHz, 16 scans, with a number of recorded points of 26K, respectively d1 = 1 s (delay).

3. Results and discussion

The fermentation in presence of high amount of metals was evaluated for 14 days. The polyphenols concentration in the first days of fermentation is increased due to mechanical forces applied for the destemming and pressing processes as it can be noticed in Fig. 4. Around the sixth day, a slight decrease can be noticed. The decrease could be caused by the assemblies formed between anthocyanins and macromolecular compounds, such as polysaccharides or proteins or even anthocyanins themselves, see Fig. 4. The assemblies could simply catch anthocyanins, making it impossible for analytical detection.

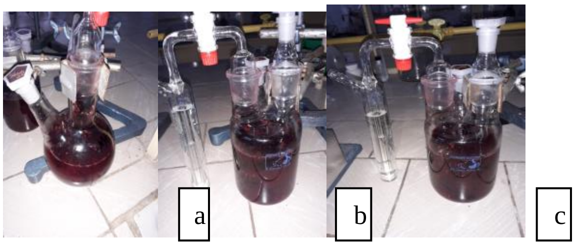

Fig. 3. Fermentation tanks: control fermenter (a), fermenter with iron (b) and fermenter with copper (c)

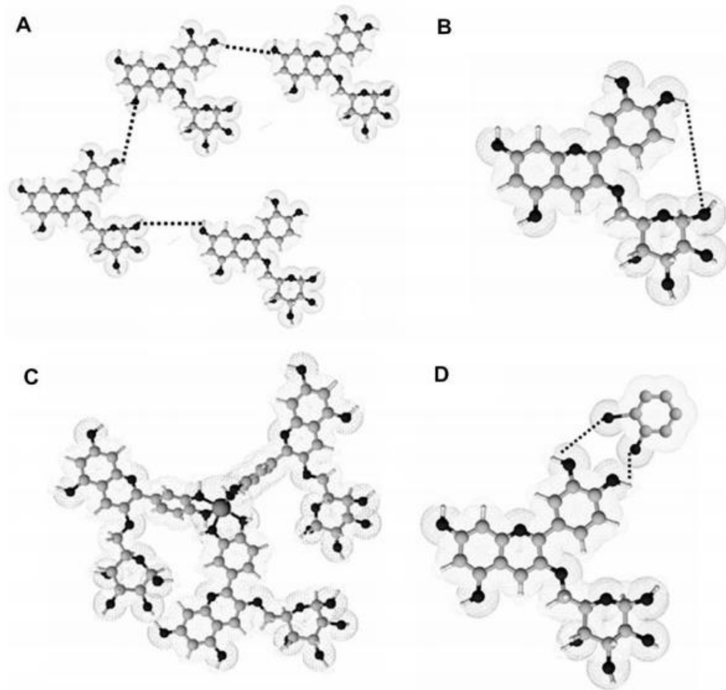

Fig. 4 . Types of interactions antocyanins can undergo (A – self association, B – intramolecular copigmentation, C – metal complexation and D – intermolecular copigmentation) [22]

However, following the fermentation process, along with the synthesis of a higher amount of ethanol content, the extraction of polyphenols, especially anthocyanins and tannins, from the grape skin is accelerated. During the next week of fermentation, the polyphenols content was strongly influenced by the presence of copper.

There were some distinctive phenomena observed when high and low amount of metal ions were added. When the added metal concentration was higher, cause of Fenton Reaction, Fig.1, polyphenols were oxidized to quinones, reactions mediated by ferric and ferrous states of the iron or cupric and cuprous state of copper [17]. The result was the minimization of their functional properties, even colour of the final product was altered, see 9th and 10th day from the table 3. In case of small amount of metal ions added, the situation was completely different, the colour of the final product was 7 time more intense than in the previous case (high amount of metal ions).

Table 2.

Colour Intensity of the samples for the 6 mg/L concentration of the metal solutions added

Days	I of Control sample	I of Fermenter with iron	I of Fermenter with copper
0	3.45	3.45	3.32
5	1.20	2.61	2.51
6	1.11	1.00	1.11
7	2.83	2.42	2.21
8	3.62	3.16	2.29
11	1.83	1.17	1.18
12	3.89	2.91	3.67
13	5.82	5.04	6.43

Table 3.

Colour Intensity of the samples for the 10^4 mg/L concentration of the metal solutions added

Days	I of Control sample	I of Fermenter with iron	I of Fermenter with copper
1	1.18	1.18	1.2
2	1.22	1.21	1.21
3	1.15	1.12	1.13
4	1.20	1.13	1.09
5	0.89	1.07	1.05
8	1.00	0.97	0.93
9	0.90	0.89	0.84
10	0.84	0.79	1.04

Regarding the variation of colour in the fermenter containing the smaller amount of added metal, table 2, it can be noticed that in the 13th day, in the presence of copper, the intensity of the colour is the highest.

The total polyphenol measurements confirm the observation made previously, regarding the colour intensity. As shown in Fig. 5 the variation of the total polyphenol content in the samples fermented in presence of metal ions indicate a higher concentration of polyphenols in the obtained wine for the wine fermented with low amount of metal ions, as well a slight decrease of the total polyphenols in the presence of iron by comparison with coper.

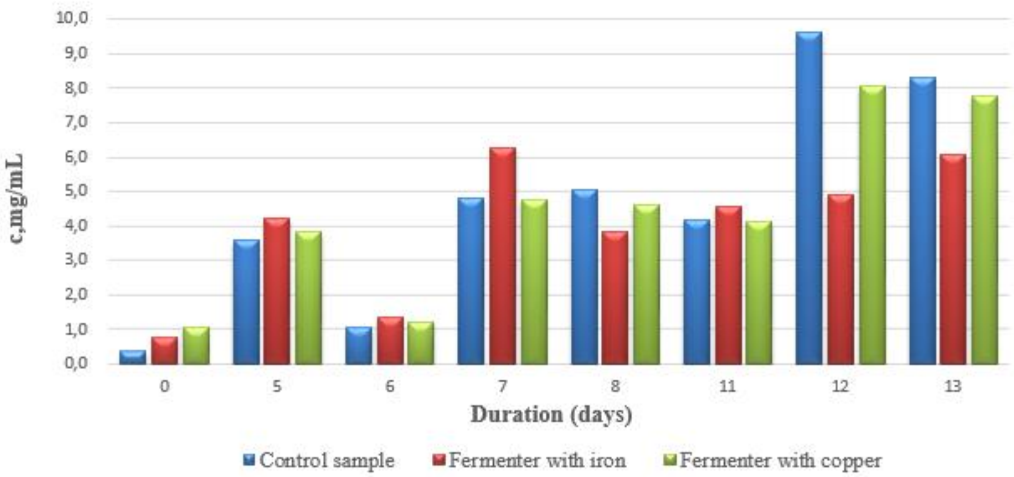

Fig. 5. Variation of polyphenols concentration with a metal solution concentration of 6 mg/L added.

Using [1]H-NMR analysis the fermentation process was monitored. In fig. 5 it is shown the NMR spectra of the grape must samples after 5 days of fermentation. It could be noticed that the sample with added iron ions (2) contains less ethanol in the same fermentation conditions as the one containing copper ions (3) or the control (1). In the same time the amount of polyphenols (visible in left side of Fig. 5, spectral area 10-6 ppm) is higher in the samples fermented with metal ions (2 and 3) by comparison with the one without added metal ions (1).

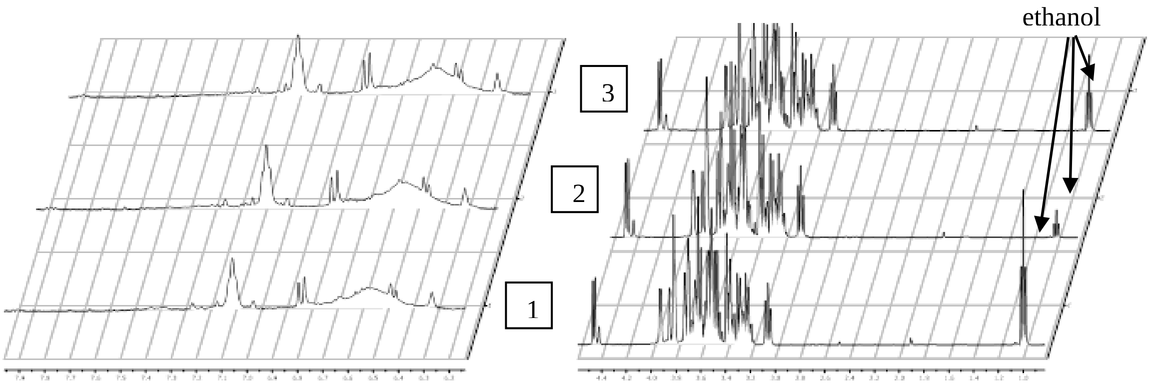

Fig. 5. NMR Spectra for must samples after 5 days of fermentation, (1- control, 2-with iron ions and 3- with cupper ions)

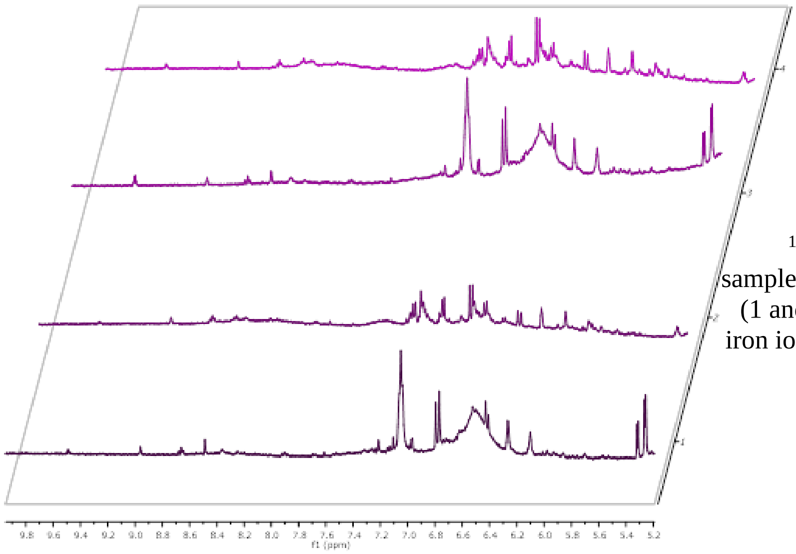

Fig. 6. Area of 5.2-10 ppm of ^{1}H-NMR spectra for must/wine samples after 30 days of fermentation (1 and 2- must and wine with added iron ions and 3 and 4 - with must and wine with added cupper ions)

When the fermentation occurs in the presence of metal ions the profile of the polyphenols present in the final product (wine) is similar (fig. 6). It ca be noticed on the other hand that in the case of fermentation with added cupper ions (fig.6, spectrum 4) by comparison with the fermentation in the presence of iron ions (fig.6, spectrum 2) the amount of the polyphenols in the obtained wine is slightly higher. Therefore, the results obtained by the two previous methods (total polyphenols and wine colour intensity) are confirmed by ^{1}H-NMR analysis.

6. Conclusions

Adding small amounts of solutions containing metal ions, iron and copper, prior to fermentation, could lead to assemblies of anthocyanins with polyphenols, protecting them from environmental oxidation. Therefore, the presence of iron and copper ions in the fermentation process impact the progress of the fermentation process, from the formation of ethanol to the amount of polyphenols and anthocyanins in the obtained wines.

In addition, the colour of the wine is strongly influenced by the presence of phenolic compounds, anthocyanins at the beginning of the fermentation and condensed tannins and copigments formed between pigments and organic molecular structure, colourless, during the fermentation and the weeks, following this process. Oxygen exposure is of great importance, it being capable of intensifying the pigments extraction during the maceration process and to generate colourful compounds.

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R E F E R E N C E S

[1] *Ronald Ross W., Victor R. P., Sherma Z.,* Polyphenols: Mechanisms of Action in Human Health and Disease, Academic Press, **vol. 1**, 2018.

[2] *Cooper KA, Chopra M, Thurnham DI.,* Wine polyphenols and promotion of cardiac health, Nutrition Research Reviews, **vol.17**, p.111-130, 2004.

[3] *Rong T.*, Chemistry and Biochemistry of Dietary Polyphenols, Nutrients, **vol.2**, p.1231-1246, 2010.

[4] *Du Y, Guo H, Lou H.*, Grape seed polyphenols protect cardiac cells from apoptosis via induction of endogenous antioxidant enzymes, Journal of Agricultural and Food Chemistry, **vol.5**, p.1695-1701, 2007.

[5] *Emilio B., Katia C., Paula Z. C., Mateus da Silveira P., Marlise Nara C. and Alberto Fontanella B.,* Total polyphenols contents in different grapevine varieties in highlands of southern brazil, BIO Web Conference, 40th World Congress of Vine and Wine, **vol. 9,** 2017.

[6] *M. Ren , X. Wang, G. Du, C. Tian, J. Zhang, X. Song, D. Zhu,* Influence of Different Phenolic Fractions on Red Wine Astringency Based on Polyphenol/Protein Binding, South Africa Journal of Enology, **vol.38**, 2017.

[7] *Lurdes M., M. Tereza F.,, Marta S., Rui R,, M. Helena F., Keith R. J.*, Interactions of Flavonoids with Iron and Copper Ions: A Mechanism for their Antioxidant Activity, Free Radical Research, **vol.36**, p. 1199–1208, 2002.

[8] *Santosh K., Richard. K. O. A.,* Iron binding characteristics of phenolic compounds: some tentative structure–activity relations, Analytical, Nutritional and Clinical Methods, **vol.81**, p.133–140, 2003.

[9] *Sergio L.C. F., Hadla S. F., Robson M. de J., Joao V.S. S., Geovani C. B., Anderson S. S.*, Development of method for the speciation of inorganic iron in wine samples, Analytica Chemica Acta, **vol. 602**, p.89-93, 2007.

[10] *Nikolaos K., Mark S. , Anque G. , Paul A. S., Geoffrey R. S. , Eric N. W., Andrew C. C.*, The impact of wine components on fractionation of Cu and Fe in model wine systems: Macromolecules, phenolic and sulphur compounds, Food Research International, **vol.98**, p.95-102, 2007.

[11] *Jorge G. Ibanez, Alejandra C-A., Maximiliano B-S., Norberto C.* Metals in alcoholic beverages: A review of sources, effects, concentrations, removal, speciation, and analysis, Journal of Food Composition and Analysis, **vol. 21**, p. 672– 683, 2008.

[12] *Tariba B.*, Metals in wine--impact on wine quality and health outcomes, Biological Trace Element Research, vol.144, p.143-156, 2011.

[13] *Pawel P.,* What do metals tell us about wine?, TrAC Trends in Analytical Chemistry, **vol.26**, p. 941-949, 2007.

[14] *Krystyna P.,* Chemical speciation and fractionation of metals in wine, Chemical Speciation and Bioavailability, **vol.19**, p.1-8, 2007.

[15] *J. Heras-Roger, O. Alonso-Alonso, A. Gallo-Montesdeoca, C. Díaz-Romero, J. Darias-Martín*, Influence of copigmentation and phenolic composition on wine colour, Journal Food Science Technology, **vol. 53**, p. 2540–2547, 2016.
[16] *Roger B.*, The copigmentation of anthocyanins and its role inthe color of red wine: a critical review, American Journal EnologyViticulture, **vol. 52**, p.67–87, 2001.
[17] *Nathan R. P. and Julia L. B.*, A Review of the Antioxidant Mechanisms of Polyphenol Compounds Related to Iron Binding, Cell Biochemistry and Biophysics, **vol. 53**, p. 75–100, 2009.
[18] *Wessel du T., J. Marais , Isak S. P., Maret Du T.*, Oxygen in Must and Wine: A review, South African Journal for Enology and Viticulture, **vol.27**, p.76-94, 2006.
[19] *Ljiljana M. B., Dejan M. G., Ranko M. S.*, Spectrophotometric characterization of red wine color from the vineyard region of Metohia, Journal of Agricultural Sciences, **vol.61**, p.281-290, 2016.
[20] *Fei H., Na-Na L., Lin M. , Qiu-Hong P. , Jun Wa, Malcolm J. R., Chang-Qing D.*, Anthocyanins and Their Variation in Red Wines I. Monomeric Anthocyanins and Their Colour Expression, Molecules, **vol.17**, p.1571 – 1601, 2012.
[21] *John C.D.*, Review of Oxidative Processes in Wine and Value of Reduction Potentials in Enology, American Journal of Enology and Viticulture, **vol.63**, p.1-10, 2012.
[22] *Araceli C.-O, Ma. de Lourdes P.-H., Ma. E. P. Hernández, J. A. Rodríguez, C. A. Galán-Vidal*, Chemical studies of anthocyanins: A review, Food Chemistry, **vol.113**, p.859-871, 2009