



STUDY OF THE STABILITY OF THE ANTIOXIDANTS OF THE FLOR DE JAMAICA'S WINE (*Hibiscus sabdariffa* L) UNDER STORAGE

ESTUDIO DE LA ESTABILIDAD DE LOS ANTIOXIDANTES DEL VINO DE FLOR DE JAMAICA (*Hibiscus sabdariffa* L) EN EL ALMACENAMIENTO

Cristina López¹ , Carlos González-Gallardo^{2*} , M.J. Guerrero-Ochoa¹ ,
Gabriel Mariño³ , Belén Jácome⁴  and Elena Beltrán-Sinchiguano⁵ 

¹ Food engineering, Food Research Center, CIAL, Faculty of Engineering and Industry, Universidad Tecnológica Equinoccial, Quito, Ecuador

^{2*} Master in Food Quality of Animal Origin, Food Research Center, CIAL, Faculty of Engineering and Industry, Universidad Tecnológica Equinoccial, Quito, Ecuador

³ Master in Nanoscience and Nanotechnology, Food Research Center, CIAL, Faculty of Engineering and Industry, Universidad Tecnológica Equinoccial, Quito, Ecuador

⁴ Master in Operations and Industrial Security, Food Research Center, CIAL, Faculty of Engineering and Industry, Universidad Tecnológica Equinoccial, Quito, Ecuador

⁵ Master in Food Science, Food Research Center, CIAL, Faculty of Engineering and Industry, Universidad Tecnológica Equinoccial, Quito, Ecuador

*Corresponding author: carlosgonzalez@ute.edu.ec

Article received on July 8, 2018. Accepted, after review, on January 18, 2019. Published on March 1, 2019.

Resumen

Se ha determinado que la Flor de Jamaica es rica en compuestos fenólicos principalmente antocianinas y flavonoides como la delfinidina-3-sambubiosido, delfinidina-3-glucósido, cianidina-3-glucósido, cianidina-3-sambubiosido, quercetina y kaempferol. El objetivo de este trabajo fue estudiar la estabilidad de los antioxidantes del vino de flor de Jamaica (*Hibiscus sabdariffa* L) durante su almacenamiento. Para ello, el vino fue almacenado a una temperatura ambiente de 20°C y a temperatura de refrigeración de 6°C. A los 0, 7 y 14 días se determinó las características fisicoquímicas, turbidez, color, el contenido de polifenoles y capacidad antioxidante. Con respecto a las características fisicoquímicas (sólidos solubles, pH y acidez titulable) el vino no presentó diferencias entre muestras almacenadas en ambiente y refrigeración. El color de todas las muestras presentó altos de absorbancias en un rango de 515 y 520nm representativo de los pigmentos de color rojo, que corresponden a una alta concentración de antocianinas. La estabilidad del contenido de polifenoles no presentó diferencias significativas durante su almacenamiento, pero en refrigeración tuvieron un contenido fenólico menor, y menor porcentaje de inhibición del radical ABTS para las muestras sometidas a refrigeración. El almacenamiento del vino de flor de Jamaica a temperatura ambiente presenta mayor capacidad antioxidante, inhibiendo los radicales libres y disminuyendo la oxidación de compuestos fenólicos presentes en el vino,

alargando su vida útil.

Palabras clave: Antioxidante, ABTS, polifenol, vino, Flor de Jamaica.

Abstract

Flower of Jamaica is rich in phenolic compounds mainly anthocyanins and flavonoids such as delphinidin-3-sambubioside, delphinidin-3-glucoside, cyanidin-3-glucoside, cyanidin-3-sambubioside, quercetin and kaempferol. The objective of this work was to study the stability of the antioxidants of the wine of Jamaica flower (*Hibiscus sabdariffa* L) during its storage. For this, the wine was stored at an ambient temperature of 20°C and at a refrigeration temperature of 6°C. At 0, 7 and 14 days the physicochemical characteristics, turbidity, color, polyphenol content and antioxidant capacity were determined. Regarding the physicochemical characteristics (soluble solids, pH and titratable acidity) the wine did not present differences between samples stored in the environment and refrigeration. The color of all the samples showed high absorbances in a range between 515 and 520nm representative of the pigments that reflect the red color, which correspond to a high concentration of anthocyanins. The stability of the polyphenol content did not show significant differences during storage, however the samples subjected to refrigeration had a lower phenolic content and a lower inhibition percentage of the ABTS radical. Consequently, the storage of Jamaica flower wine at room temperature presents greater antioxidant capacity, inhibiting free radicals and decreasing the oxidation of phenolic compounds present in wine, extending its shelf life.

Keywords: Antioxidant, ABTS, polyphenol, wine, Jamaica flower.

Suggested citation: López, C., González, C., Guerrero, M.J., Mariño, G., Jácome, B. and Beltrán, E. (2019). Study of the Antioxidant Stability of Jamaica Flower Wine (*Hibiscus sabdariffa* L) Under Storage. *La Granja: Revista de Ciencias de la Vida*. Vol. 29(1):105-118. <http://doi.org/10.17163/lgr.n29.2019.09>.

Orcid IDs:

Cristina López: <https://orcid.org/0000-0002-6865-4014>

Carlos González-Gallardo: <https://orcid.org/0000-0001-5400-9439>

M.J. Guerrero-Ochoa: <https://orcid.org/0000-0002-4603-6098>

Gabriel Mariño: <https://orcid.org/0000-0003-1656-1438>

Belén Jácome: <https://orcid.org/0000-0002-5939-4660>

Elena Beltrán-Sinchiguano: <https://orcid.org/0000-0001-6146-5301>

1 Introduction

Currently, food studies containing bioactive compounds, including antioxidants, have increased by being molecules capable of interacting with reactive oxygen species (ERO) that include ion oxygen, free radicals and peroxides (Usoh et al., 2005) which are highly reactive by having in their structure an external layer with one or two valence electrons not paired. These elevated levels of free radicals in the cells are generators of damage to the proteins and lipids of the cell membrane and in nucleic acids, reason from which it is directly related with carcinogenic diseases (Ferretti et al., 2010) mutagenic, Alzheimer (Andzi Barhé and Feuya Tchouya, 2016), diabetes, hypertension, obesity and other metabolic symptoms (Chen et al., 2013). The antioxidant molecules transfer electrons to the outer layer with unmatched electrons of the free radicals, thus achieving their stability (Andzi Barhé and Feuya Tchouya, 2016), being capable of transferring their electrons avoiding the harmful effect in the cell, exerting a chelating mechanism and kidnapping the ERO (Sáyaigo Ayerdi and Goñi, 2010).

Jamaican flower (*Hibiscus sabdariffa* L.) has been one of the most studied species for its high content of antioxidant molecules such as vitamin E and C, phenolic compounds, mainly polyphenolic acids, flavonoids and anthocyanins (Cid-Ortega and Guerrero-Beltrán, 2012), these properties grant this flower anticancer protective effects, diuretics, anti-inflammatory, antimicrobial (Martínez Flórez et al., 2002), exerting a protective action of cell damage and lipid peroxidation (Galicia Flores et al., 2008). Studies carried out with extracts of chalice and hibiscus have determined that there is a high concentration of phenolic antioxidants of non-flavonoid type and simple or polymerized flavonoids in the tissues of these structures (Da-Costa Rocha et al., 2014). Within the group of flavonoids 5 subtypes are identified according to their structural characteristics: flavones, flavonols, flavanones and anthocyanidins (Martínez Flórez et al., 2002).

Among the polyphenolic flavonoids compounds present in the chalice and flower leaves of Jamaica is the naringenin belonging to the class of flavones, the catechin, galocatechin gallate, epicatechin gallate and the galocatechin of the flavanol and the luteolin, tiliroside, sabdaritrin hydroxyflavone of the flavones class (Galicia Flores et al., 2008;

Da-Costa Rocha et al., 2014; Chen et al., 2013; Sindi et al., 2014). Also the chalice of hibiscus have shown to contain a high percentage of anthocyanins in their structural tissues, identifying the so-called Crisanteina and cyanidin-3-sambubioside or Gosipicianina (Da-Costa Rocha et al., 2014). These anthocyanins are natural pigments soluble in water, which are present in some vegetal tissues, being those responsible for the red, blue and purple coloration, the production of this compound is given in maturation and is stored inside the vacuoles in the plant cells (Yang et al., 2011).

In Ecuador, the production of hibiscus has spread in the Amazon region, due to its optimum temperature conditions ranging from 15 to 38°C, becoming an alternative to the sustainable economic development of its population (Meza Chavarría, 2012). However, it is a product with low industrialization as it is sold in bulk, also, there are few studies about the obtaining of products based on this raw material that provides bioactive compounds; therefore, this research was aimed at studying the stability of the antioxidants of the Jamaican flower wine (*Hibiscus sabdariffa* L.) during storage, in order to increase the by-products production of Jamaica flower.

2 Materials and methods

2.1 Wine elaboration

For the elaboration of the wine, hibiscus was used from the Macas parish, Morona Santiago province. Once conducted a physico-chemical and microbiological analysis in accordance with the provisions of the Mexican standard NMX-FF-115-SCFI-2010 (NMX, 2010) for agricultural products intended for human consumption: Flower (Chalice) of Jamaica, it was proceeded to make the must from the chalice with a 1:3 ratio (Jamaica chalice: water) until reaching a must of 23° Brix with a pH of 3 – 3,6; the fermentation was carried out during 29 days under anaerobic conditions at a temperature of 21° ± 1°C. The wine was subsequently submitted to a natural decanting process for 6 days, time in which samples were taken daily and in which soluble solids (° Brix), Ph, titratable acidity and turbidity were analyzed.

2.2 Treatments

The storing conditions of the Jamaican flower wine were for 14 days and were divided into two groups: (environmental temperature $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and refrigeration temperature 6°C), posterior samples were taken from each group during days 0, 7 and 14, following Gaculas staggered design for shelf life studies, using a single batch of product (or replicated lots) where samples are tested at zero time, and samples are taken at intervals determined by the expectation of survival probability (Kilcast and Subramaniam, 2000). After the samples were frozen, and once the storage stage was completed, the polyphenol content and antioxidant capacity were measured.

2.3 Methods of analysis

The analyses were carried out according to the official methods of the AOAC (2005a,b, 2012): for soluble solids (AOAC 932,12), pH (AOAC 960,19), titratable acidity (AOAC 962,12). Turbidity was measured according to the international method OIV-MA-AS2-08 (OIV, 2009) and Glories method described in the OIV-MA-BS-26 (OIV, 2014) was used for the intensity determination of the wine color, using a spectrophotometer whose absorbance measurements were performed in ranges 380 and 780 nm.

The polyphenol content was determined under the Folin-Ciocalteus protocol described by Zhen et al. (2016) and the antioxidant capacity was performed by the spectrophotometric method described by Chen et al. (2013); Re et al. (1999) based on the discoloration of the radical ABTS.

2.4 Experimental design

In this study a completely randomized experimental design was used, in which the results obtained were processed with an ANOVA variance analysis, and the means were compared using a Tukey test (HSD) with a level of 95% confidence.

3 Results and discussion

3.1 Soluble solids analysis ($^{\circ}$ Brix)

As shown in Figure 1, the storing effect of the Jamaican flower wine under two temperature conditions

over 14 days was not significant ($p > 0,05$) compared to the quantity of soluble solids ($^{\circ}$ Brix).

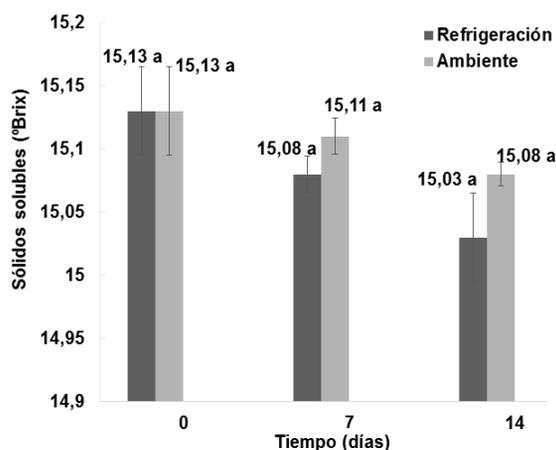


Figure 1. Quantity Behavior of soluble solids ($^{\circ}$ Brix) in the wines stored under two temperature conditions during 14 days. $N = 2 \pm$ standard deviation. Different letters among the means indicate significant differences according to Tukey test at $P < 0,05$.

It can be observed that the amount of soluble solids on day 0 of storage was $15,13 \pm 0,035^{\circ}$ Brix; after 7 days of storage at room temperature, there was a slight decrease at $15,11 \pm 0,014^{\circ}$ Brix, and finally at 14 days there was no a significant change, decreasing to $15,08 \pm 0,009^{\circ}$ Brix. Similar behavior was detected under storage conditions at refrigeration temperature, since after 7 days there was a slight decrease at $15,08 \pm 0,014^{\circ}$ Brix, and at 14 days there was $15,03 \pm 0,035^{\circ}$ Brix. In the physical-chemical analysis conducted by Mounigan and Badrie (2007), by submitting Jamaican flower wine to storage conditions at 23°C for 8 weeks, no significant differences were recorded in the amount of soluble solids between the samples, which were measured every 30 days, giving as results at 4 weeks of storage a value of $10,22^{\circ}$ Brix, and at 8 weeks a value of $10,17^{\circ}$ Brix.

Therefore, it can be observed that the amount of soluble solids in the Jamaican flower wine does not undergo significant changes with the time under environmental or refrigeration conditions during storage. The stability of soluble solids is because according to Blouin and Peynaud (2003) a suitable sulphured allows destroying the yeasts and bacteria present in the wine, which can continue with the slow consumption of substrate. On the other hand, the slight decrease without significant diffe-

rence in the quantity of soluble solids may be because although the wine went through a previous decanting process, during the storage time at rest the wine continues to decant slowly, settling certain glycosylated tannins, which can drag sugar crystals to the bottom of the bottle, causing a slight decrease in the ° Brix (Blouin and Peynaud, 2003; Mijares and Sáez, 2007; Bujan, 2002).

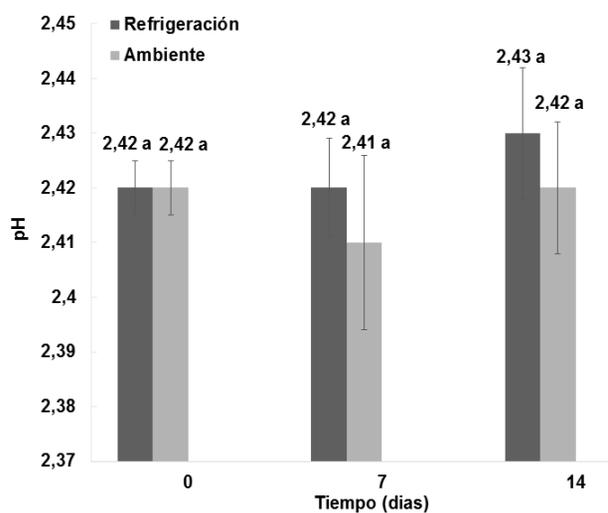


Figure 2. pH behavior in wines stored under two temperature conditions for 14 days. $N = 2 \pm$ standard deviation. Different letters among the means indicate significant differences according to Tukey test at $P < 0,05$.

3.2 pH analysis

As shown in Figure 2, the storing effect of Jamaican flower wine under two temperature conditions over 14 days was not significant ($p > 0,05$) compared to pH. It can be observed that on day 0 of storage, the ph level is $2,42 \pm 0,005$ and it has no significant differences over the course of 14 days and under two different storage conditions, where the environmental samples concluded with a pH of $2,42 \pm 0,012$ and refrigerated samples with a pH of $2,43 \pm 0,012$. Unlike the behavior presented by Mounigan and Badrie (2007), in which the storage process began with a pH of 2.61, during the course of 4 weeks it decreased to a pH of 2.43, and it was finally observed at 8 weeks an increment of the pH to 2.77. In the two storage studies made with Jamaica flower wine, the ph values coincide within the level recommended by Jackson (2011); Reeves (2010), who described that

the pH lower than 3.5 are favorable for the maintenance of the shelf life since the antiseptic power and antioxidant capacity of the sulphured increase, providing wines with a fresh flavor, reducing browning; causing coloring intensity and enriching the tone; thus, minimizing the concentration of the easiest oxidizable phenolic form of polyphenols.

3.3 Titratable acidity analysis

As shown in Figures 3 and 4, the storing effect Jamaican flower wine under two temperature conditions over 14 days was not significant ($P > 0.05$) with respect to titratable acidity in relation to citric acid and tartaric acid.

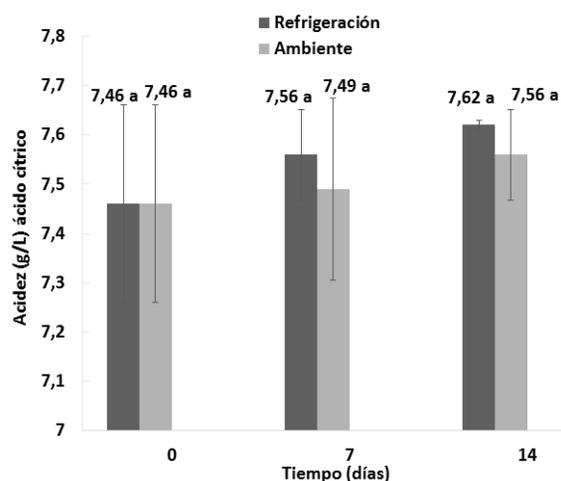


Figure 3. Titratable acidity behavior with respect to citric acid in wines stored under two temperature conditions for 14 days. $N = 2 \pm$ standard deviation. Different letters among the means indicate significant differences according to Tukey test at $P < 0,05$.

The wines stored under two temperature conditions during the course of 14 days did not present significant differences with respect to the quantities of citric acid and tartaric acid. The samples initiated the refrigeration storage process with a titratable acidity of 7,46 g/L of citric acid and 8,74 g/L of tartaric acid, and concluded at 14 days with an acidity of 7,62 g/L of citric acid and 8,94 g/L of tartaric acid. In the case of samples subjected to environmental conditions, they started the storage with an acidity of 7,46 g/L of citric acid and 8,74 g/L of tartaric acid, and finished at 14 days with an acidity of 7,56 g/L of citric acid and 8,86 g/L of tartaric acid. Similarly, Mounigan and Badrie (2007),

in their study did not report significant differences with respect to titratable acidity in relation to citric acid during the period of 8 weeks of storage, since the samples began with a acidity 4,2 g/L of citric acid, and concluded at 8 weeks with an acidity of 4,8 g/L of citric acid. According to Jackson (2011), the shelf life of all wines is reinforced by a desirable acid content and a low pH level, since the oxidation of phenolic compounds is favored at a low acidity level and the presence of the most easily oxidized state of phenolate also has a great influence on the degradation of fruit esters. When the acidity of the wine is undesirably low, it can be adjusted upward by adding tartaric acid or citric acid, since they are natural constituents of the fruits, but mainly because these acids are not metabolized adequately by most bacteria, thus reducing the likelihood of microbial deterioration.

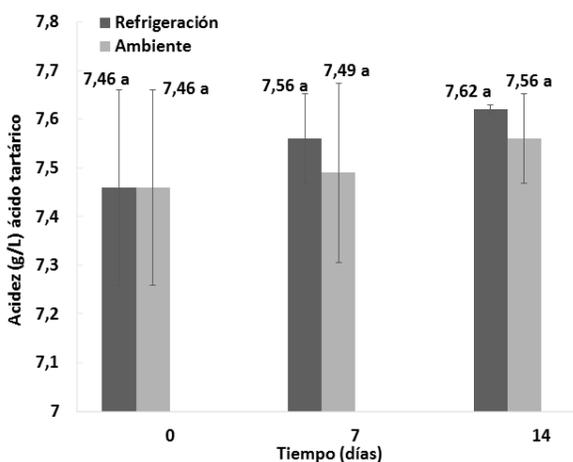


Figure 4. Performance of titratable acidity with respect to tartaric acid in wines stored under two temperature conditions for 14 days. $N = 2 \pm$ standard deviation. Different letters among the means indicate significant differences according to Tukey test at $P < 0,05$.

3.4 Turbidity analysis

As shown in Figure 5, the storing effect of Jamaican flower wine under two temperature conditions over 14 days caused a significant ($P < 0,05$) compared to turbidity. It can be observed that the wine samples started the storage with a turbidity of $61,45 \pm 0,3$ NTU. In the case of samples that were subjected to environmental temperature at 21°C , turbidity decreased markedly towards day 7, reaching $47,62 \pm 0,2$ NTU and reducing up to 39,88 %, on day

14 was reported $36,94 \pm 0,3$ NTU. In the case of samples that were subjected to refrigeration temperature at 6°C , the turbidity decreased compared to the wines stored in environmental conditions, since by day 7 they reached $41,67 \pm 0,6$ NTU; and reducing up to 44,62 %, until day 14 in which was reported $34,03 \pm 0,7$ NTU. The wine samples subjected to refrigeration conditions presented a better response to the reduction in turbidity values over 14 days of storage, this is according to Blouin and Peynaud (2003), who described that the clarifying effect, whether by decanting or by flocculation, is more intense when it occurs at low temperatures or at refrigeration temperatures. However, during storage at rest, wines despite having been clarified in a previous decantation process, continued settling in the bottles. Turbidity is usually due to the presence of flavonols, tannins and fine crystals of quercetin and ellagic acid (Jackson, 2011), which are important polyphenols in wine that continue decanting with time; for this reason, samples subjected to two storage conditions had a noticeable decrease in turbidity, which is sensory favorable for wines since they acquire properties of transparency and limpidity (Blouin and Peynaud, 2003; Mijares and Sáez, 2007).

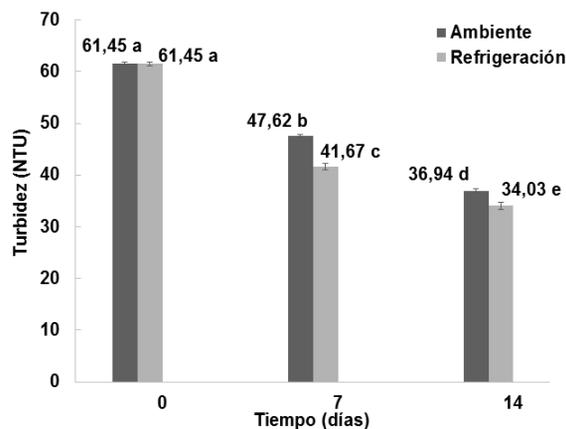


Figure 5. Turbidity behavior (NTU) in wines stored under two temperature conditions for 14 days. $N = 2 \pm$ standard deviation. Different letters among the means indicate significant differences according to Tukey test at $P < 0,05$.

3.5 Color analysis

In Figure 6, the means of absorbance measurements obtained by spectrophotometry are observed through the scanning of the electromagnetic spectrum

(380 to 780 nm) of each of the wine samples subjected to different storage conditions. It was verified that all samples had the highest absorbance value between 500 and 520 nm, located in the red region. Mariño et al. (2017), in the study of color in Mulberry wine of Castile (*Rubus Glaucus* Benth) reports a similar behavior of the absorbance, positioning

itself in a range between 515 and 520 nm. According to Jackson (2011); Reeves (2010), the results of both studies show similarities since the Jamaican flower and the Castile mulberry are raw materials rich in anthocyanins and other phenolic compounds that are the responsible for the red pigmentation in wines.

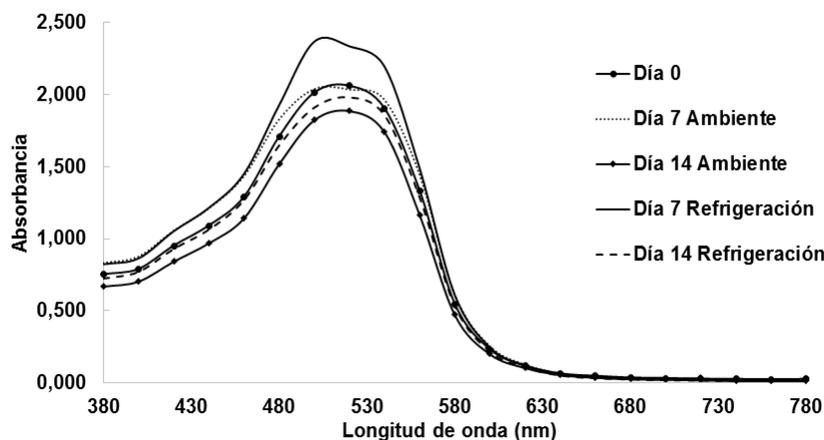


Figure 6. Absorbance behavior of wines stored under two temperature conditions for 14 days.

Glorious method allowed to determine the color intensity (IC), the tone (T), the yellow percentage, the red percentage and the blue percentage in the wine samples subjected to two different storage conditions. The coloring intensity is a chromatic characteristic related to the luminosity. In Figure 7 can be observed that the effect of storing the Jamaican flower wine under two temperature conditions over 14 days is significant ($p < 0.05$) compared to the coloring intensity. The wine subjected to storage conditions in refrigeration for 7 days presented the highest value of $3,5 \pm 0,005$ IC, suffering a slight decrease in day 14 to $3,01 \pm 0,001$ IC. In the case of wine subjected to environmental storage conditions on day 7, it presented a low value of $3,21 \pm 0,005$ CI, finally decreasing to 14 to $2,84 \pm 0,009$ IC.

According to Jackson (2011), low temperatures or refrigeration temperatures avoid the development of Maillard reactions that can generate compounds that affect the luminosity of wine; on the other hand, these slow the oxidation of phenolic flavonoids compounds, especially catechins in quinones that can modify the chromatic properties, generating pigments of brownish colors and providing opacity to the wine. It is for this reason that the wi-

nes stored at refrigeration temperatures have higher values of color intensity, compared to the samples stored at room temperature.

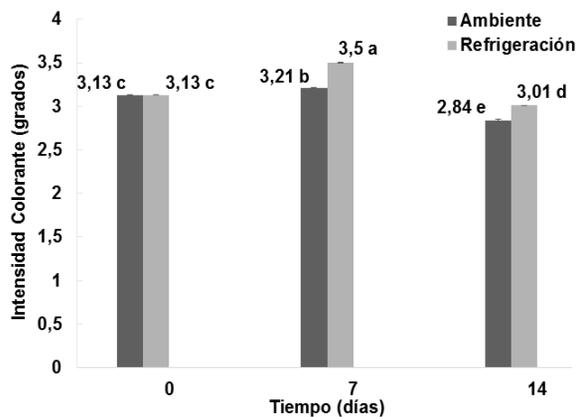


Figure 7. Behavior of the coloring intensity in wines stored under two temperature conditions during 14 days. $N = 2 \pm$ standard deviation. Different letters among the means indicate significant differences according to Tukey test at $P < 0,05$.

The hue or tone (T) is the quality factor of the color and corresponds to the dominant wavelength (yellow, red, blue) that characterizes the tonality of

the wine (García Barceló, 1990) and expresses the qualitative variation of the color in the sample (Iñiguez et al., 1995). Figure 8 shows that the storing effect of Jamaican flower wine under two temperature conditions over 14 days is significant ($p < 0.05$) with respect to tonality. Wine samples started the storage process with a tonality of $0,46 \pm 0,004$. Samples subjected to refrigeration storage conditions suffered slight variations in tone, culminating in day 14 with a value of $0,47 \pm 0,001$. Samples subjected to environmental storage conditions showed a more significant variation, being day 7 the highest with a value of $0,52 \pm 0,002$ for T, this superior value of the tone is closely related to the elevated percentage of red, which can be observed in Figure 9 for this sample.

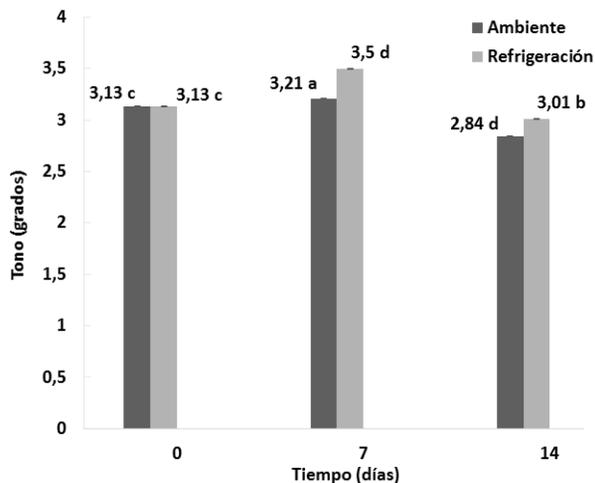


Figure 8. Tonal behavior in wines stored under two temperature conditions for 14 days. $N = 2 \pm$ standard deviation. Different letters among the means indicate significant differences according to Tukey test at $P < 0,05$.

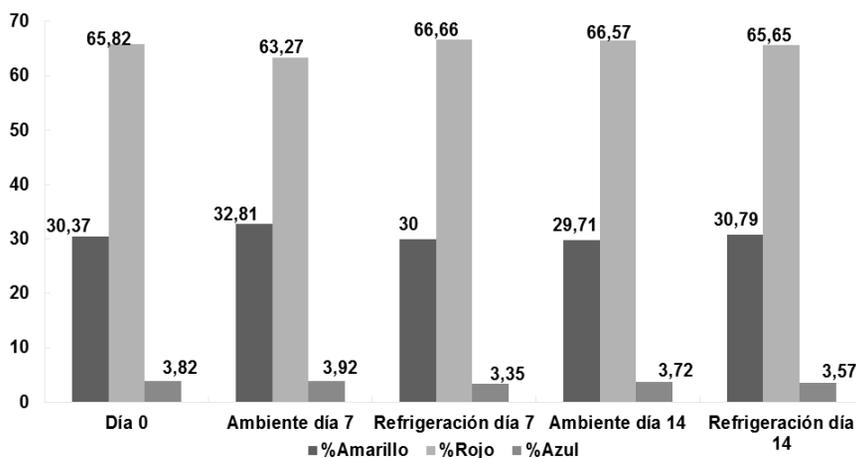


Figure 9. Behavior of the quantity of pigments in the wines stored under two temperature conditions for 14 days.

The polyphenolic components are the main substances that absorb the radiation in wines, and therefore are responsible for the colors they reflect (Iñiguez et al., 1995). In Figure 9, the different percentages of yellow, red and blue pigments present in the wine samples subjected to two storage conditions can be identified. All wines agree with a high percentage of red color of about 65%, according to Da-Costa Rocha et al. (2014), the Jamaican flower is rich in anthocyanins mainly in Delphinidin-3-Sambubiosido, Delphinidin-3-glucoside and Cyanidin-3-glucoside,

which correspond to flavylum cations, and a pH value between 1 and 3 is the responsible for reflecting the red color. In Figure 9 it is also possible to observe that all the wines under study have a content of about 30% of yellow, in agreement to the described by Galicia Flores et al. (2008), who report that the Jamaica flower is rich in phenolic compounds such as quercetin, quercetin-3-glucoside, quercetin-3-rutinoso and kaempferol, and according to Jackson (2011), these are responsible for presenting the yellow color. Likewise, it is observed, that all the samples have about 4% of blue which is a lot lo-

wer than the previous colors. According to Yang et al. (2011), certain anthocyanins of the quinonoides group present in the Jamaican flower are responsible for reflecting the blue color.

The significant variations ($p < 0.05$) with respect to the percentages of yellow, red and blue colors in wine samples subjected to different storage conditions for 14 days is according to the described by Jackson (2011) who in his study of color in wines said that color changes during storing conditions are considered normal and expected, and do not contribute to reducing the life of the product. Reeves (2010), describes that during the storage the glycosylated complexes formed by the anthocyanins can break, being sensitive to the oxidation that generates a degradation of the color, and that many other biochemical reactions succeeded during this stage generate a variety of pigments that give specific characteristics to each wine.

3.6 Analysis of the polyphenol content

3.6.1 Polyphenol content during the winemaking and decanting process of the Jamaican flower

Figure 10 shows the significant evolution of the polyphenol content from the raw material to the clarification of the wine. Polyphenol content in the fresh chalice of Jamaican flower was $204,02 \pm 0,4$ mg eq. gallic acid/100 g, with a similar value to those reported by Zhen et al. (2016); Sindi et al. (2014) of 189,8 mg eq. gallic acid/100 g and 216,7

mg eq. gallic acid/100 g, respectively. The differences in these values are due to the extraction method used by Zhen et al. (2016) and Sindi et al. (2014) who used methanol/water extractor solutions 70% v/v, in addition to the Jamaican flower variety used in each study and its maturation degree. The corrected initial wine wort showed a value lower than the polyphenols content of the fresh chalice of $79,13 \pm 3,9$ mg eq. gallic acid/100 mL sample, this is due to the 70% of water content that the wort has.

After the winemaking process, the quantity of polyphenols obtained in the wine is significantly higher than the initial must giving a value of $144,14 \pm 1,5$ mg eq. gallic acid/100 mL per sample. This noticeable increase in phenolic content after the winemaking is consistent with the study of the phenolic composition of grapes and wine (Flanzy, 2003). Additionally, during the fermentation other phenolic compounds are generated, resulting from the evolution of the native polyphenols of the raw material used for the elaboration of wine (Pérez Jiménez et al., 2010; Hidalgo T., 2011). Once the clarified wine was obtained, a value of $139,32 \pm 1,6$ mg eq. gallic acid/100 mL per sample was presented, without showing significant difference compared to the wine before decantation; this dismissal of polyphenols content is normal according to the described by Blouin and Peynaud (2003), because during the decanting process, the tannins eliminated during the clarification are scarce but are variable in concentrations from 50 to 300 mg/L.

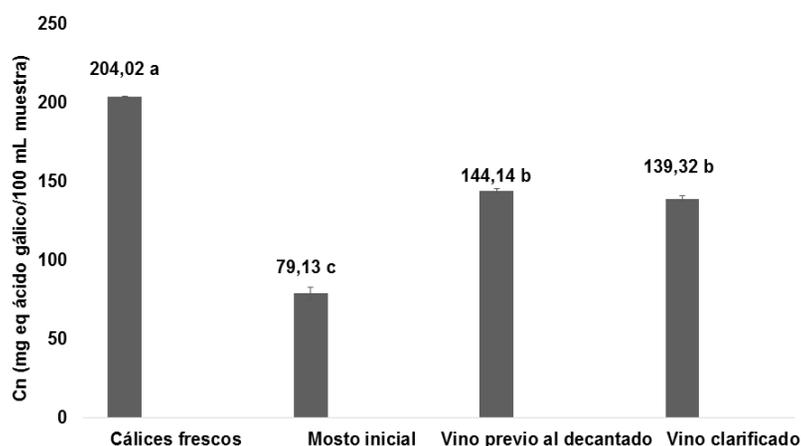


Figure 10. Polyphenols content behavior from the raw material to winemaking processes and decantation. $N = 2 \pm$ standard deviation. Different letters among the means indicate significant differences according to Tukey test at $P < 0,05$.

3.6.2 Study of the stability of the polyphenols content during the storing process of Jamaican flower wine

On day 0 the total polyphenols content was $139,32 \pm 1,6$ mg eq. gallic acid/100 ML (Figure 11) and showed no significant differences with respect to the values obtained during the course of the 14 days of study under two temperature conditions; therefore, it is observed that the quantity of phenolic compounds present in the samples of wine, remained stable despite the course of time and storage temperatures. Studies have stated that the Jamaican flower has an important phenolic content, among which are mainly identified flavonoids and anthocyanins, responsible for the color red of wine (Camussoni and Carnevali, 2004; Da-Costa Rocha et al., 2014; Chen et al., 2013; Zhen et al., 2016).

In spite of the fact that there is no significant difference between the two temperatures and the storage time, in Figure 11 it is observed that the samples subjected to refrigeration temperatures have a slightly lower phenolic content compared to the samples at environmental temperature, which explains that the wine during storage is constantly decanting and the cooling temperature favors the sedimentation of big polymerized tannins molecules (Blouin and Peynaud, 2003; Jackson, 2011).

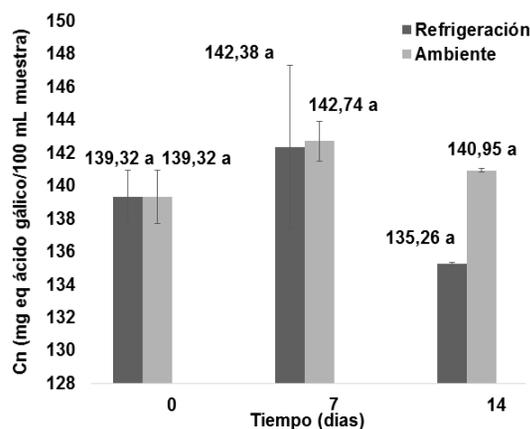


Figure 11. Behavior of polyphenols content in wines stored under two temperature conditions for 14 days. $N = 2 \pm$ standard deviation. Different letters among the means indicate significant differences according to Tukey test at $P < 0,05$.

3.7 Analysis of the antioxidant capacity

3.7.1 Antioxidant capacity during the winemaking and decanting processes of the Jamaican flower wine

Figure 12 indicates the significant differences in the antioxidant capacity during the winemaking process, in the extracts of the fresh chalice of Jamaican flower the antioxidant capacity was $13,74 \pm 0,1$ Equiv mM Trolox/100 g per sample, similar value to the reported by Zhen et al. (2016) of 15,25 Equiv MM Trolox/100 g, and superior to the reported by Sáyago Ayerdi and Goñi (2010) of 9,08 Equiv MM Trolox/100 g. The difference between these values may be due to the extraction method, since the antioxidant activity is dependent on the concentration of the extract, and the best responses obtained depend on the solvents applied (Kuskoski et al., 2005).

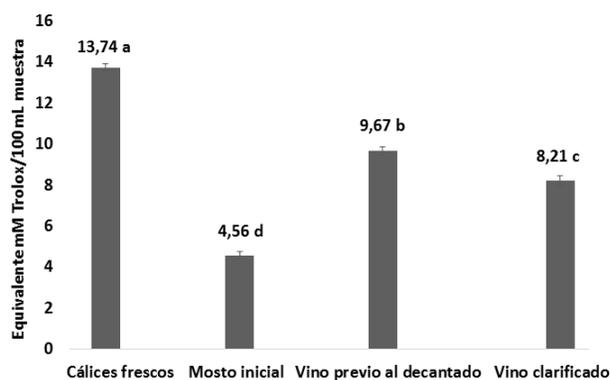


Figure 12. Behavior of the antioxidant capacity from the raw material to the winemaking and decantation processes. $N = 2 \pm$ standard deviation. Different letters among the means indicate significant differences according to Tukey test at $P < 0,05$.

It is observed in the must that there was a decrease in the antioxidant capacity presenting values of $4,56 \pm 0,1$ Equiv mM Trolox/100 ML per sample. In the comparison study of antioxidant activity in red and white wines was determined that there is a direct correlation between total polyphenols and antioxidant activity (Vinson and Hontz, 1995), reason for which it is explained that the initial must containing a quantity of total low polyphenols also had a low antioxidant capacity (Figure 12), since there is a linear relationship between the total polyphenols and the value of the ability to capture free radicals or antioxidant activity (Avalos Llano et al., 2003).

In the winemaking stage, the antioxidant capacity increases to $9,67 \pm 0,2$ Equiv mM Trolox/100 mL per sample; this noticeable increase in the antioxidant activity is related to the increase of phenolic content after the fermentation (Flanzy, 2003; Hidalgo T., 2011). Finally, the antioxidant activity in the clarified wine was $8,21 \pm 0,2$ Equiv mM Trolox/100 mL per sample. This decrease in the antioxidant activity is normal because during the decantation, tannins and other polymerized polyphenols are lost (Blouin and Peynaud, 2003).

3.7.2 Stability study of the antioxidant capacity during the storing process of the Jamaican flower wine

The antioxidant capacity of the Jamaican flower wine does not present significant differences between the samples and the time; however, the samples subjected to refrigeration have a slightly lower antioxidant capacity compared to the samples subjected to environmental temperature (Figure 13), which is explained because the sedimentation of the tannins happens faster at refrigerated temperatures (Blouin and Peynaud, 2003).

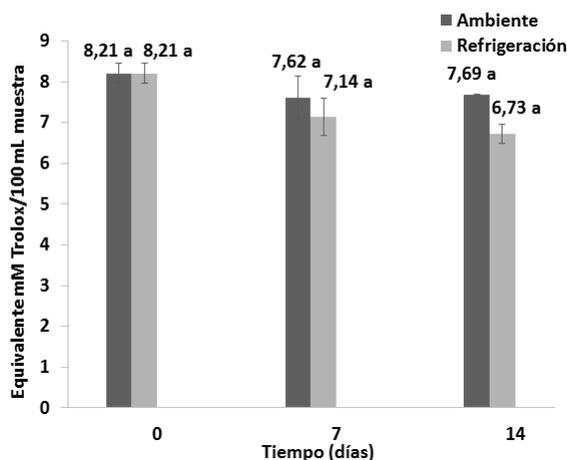


Figure 13. Behavior of the antioxidant capacity of the wines stored under two temperature conditions for 14 days. $N = 2 \pm$ standard deviation. Different letters among the means indicate significant differences according to Tukey test at $P < 0,05$.

Likewise, in the statistical analysis carried out for the determination of the inhibition percentage of the radical ABTS (Figure 14), the differences between the storage temperatures were evident, reason for which the samples subjected to refrigeration had an inhibition percentage slightly lower compared to

samples submitted at room temperature, explaining that the inhibition percentage is closely related to the decrease in the antioxidant capacity in the samples. The inhibition percentage of antioxidants contained in the Jamaican flower wines showed similar values to the grape wines of the evaluation study of the antioxidant activity subjected to different times of aging, in which were reported average values of 2,44 Equiv MM Trolox/100 ML and a inhibition percentage of 10,59% for Oporto wine; 45,04% for Barbera Malbec wine; 15,93 Equiv MM Trolox/100 ML and a inhibition percentage of 77,71% for Cabernet Sauvignon wine; 16,45 Equiv MM Trolox/100 ML and a inhibition percentage of 80,28% for Merlot wine (Muñoz Jáuregui et al., 2007). According to these comparisons, it can be determined that Jamaica flower wines subjected to the stability study during storage under two temperature conditions have an antioxidant capacity and an inhibition percentage of Intermediate free radicals compared to grape wines.

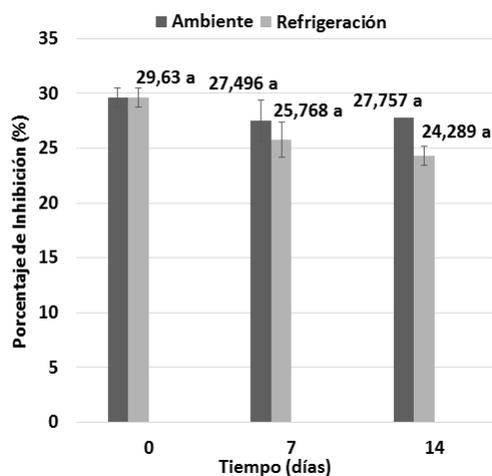


Figure 14. Inhibition percentage behavior of the ABTS of wines stored under two temperature conditions for 14 days. $N = 2 \pm$ standard deviation. Different letters among the means indicate significant differences according to Tukey test at $P < 0,05$.

4 Conclusions

The amount of soluble solids, pH and titratable acidity for citric acid and tartaric acid of wine, remained stable both in the refrigeration condition as in the environmental condition during 14 days of storage thanks to the adequate sulphured that all the wine samples received, eliminating the presence of

microorganisms, maintaining its sweetness and favoring the oxidation of phenolic compounds and the presence of the most easily oxidized state of the phenolate.

With regards to the turbidity analysis, significant differences were found between the two storage conditions, being the wine samples subjected to refrigeration for 14 days the ones that showed a higher decanting speed than the samples subjected to the environment conditions. With regards to the spectrophotometric sweep, it is concluded that all samples subjected to two storage conditions for 14 days presented their highest absorbance levels in a range between 515 and 520 nm representative of the pigments that reflect the red color, which correspond to a high concentration of anthocyanins. Glories' method allowed to identify a significant difference between the samples subjected to refrigeration, which had higher values of color intensity and tone than the samples submitted to the environment for 14 days, since at the refrigeration temperature prevents the development of Maillard reactions that can generate compounds that affect the luminosity of the wine and slows the oxidation of phenolic compounds such as flavonoids that can modify their chromatic characteristics. On the other hand, all wine samples agree with a high percentage of red color of about 65% that confirm that the Jamaican flower is rich in anthocyanins mainly in delphinidin-3-sambubiosido, delphinidin-3-glucoside and cyanidin-3-glucoside, which correspond to flavylum cations, and a pH value between 1 and 3 are responsible for reflecting the red color. In addition, all wines have a considerable content of about 30% of yellow, which is generated by polyphenols that reflect this color such as quercetin, quercetin-3-glucoside, quercetin-3-rutinoso and kaempferol.

The stability and antioxidant capacity of Jamaican flower wine is higher at room temperature, decreasing the likelihood of oxidation of phenolic compounds present in the wine. The polyphenols content is directly and linearly related to the antioxidant capacity, which is favorable for the maintenance of the useful life of the Jamaican flower wine.

According to the references consulted, the polyphenol content, the antioxidant capacity and the EROS inhibition percentage of the Jamaica flower wine is intermediate between the values reported in the different types of grape wines.

References

- Andzi Barhé, T. and Feuya Tchouya, G. (2016). Comparative study of the anti-oxidant activity of the total polyphenols extracted from *Hibiscus Sabdariffa* L., *Glycine max* L. Merr., yellow tea and red wine through reaction with dpph free radicals. *Arabian Journal of Chemistry*, 9(1):1 – 8. Online: <https://bit.ly/2Gob1Je>.
- AOAC (2005a). Official methods of analysis: Acidity (titratable) of wine. Quantitative Chemistry. American Society of Enologists-AOAC.
- AOAC (2005b). Official methods of analysis: Solids (soluble) in fruits and fruits products method 932.12. Quantitative Chemistry. American Society of Enologists-AOAC.
- AOAC (2012). Official methods of analysis: ph of wine. Quantitative Chemistry. American Society of Enologists-AOAC.
- Avalos Llano, K. R., Sgroppo, S. C., and Avanza, J. R. (2003). Actividad antioxidante y contenido en fenoles totales en vinos de origen nacional. *FACE-NA*, 19:11–19. Online: <https://bit.ly/2UV6r8q>.
- Blouin, J. and Peynaud, E. (2003). *Enología práctica: conocimiento y elaboración del vino*. Mundi-Prensa. Online: <https://bit.ly/2N4nCl3>, Barcelona, 4ta edition.
- Bujan, J. (2002). *Guía de la nueva cultura del vino: Introducción y práctica*. Freixenet, Barcelona, 1ra edition.
- Camussoni, G. and Carnevali, E. (2004). Determinación comparativa del contenido de polifenoles en vinos tintos de origen argentino. *Invenio*, 7(13):151–159. Online: <https://bit.ly/2TMqIN8>.
- Chen, J.-H., Wang, C.-J., Wang, C.-P., Sheu, J.-Y., Lin, C.-L., and Lin, H.-H. (2013). *Hibiscus sabdariffa* leaf polyphenolic extract inhibits ldl oxidation and foam cell formation involving up-regulation of *lxra/abca1* pathway. *Food Chemistry*, 141(1):397 – 406. Online: <https://bit.ly/2DvhBtu>.
- Cid-Ortega, S. and Guerrero-Beltrán, J. A. (2012). Propiedades funcionales de la jamaica (*Hibiscus sabdariffa* L.). *Temas Selectos de Ingeniería de Alimentos*, 6(2):47–63.

- Da-Costa Rocha, I., Bonnlaender, B., Sievers, H., Pischel, I., and Heinrich, M. (2014). *Hibiscus sabdariffa* L. – a phytochemical and pharmacological review. *Food Chemistry*, 165:424 – 443. Online: <https://bit.ly/2S0oQyE>.
- Ferretti, G., Bacchetti, T., Belleggia, A., and Neri, D. (2010). Cherry antioxidants: From farm to table. *Molecules*, 15(10):6993–7005. Online: <https://bit.ly/2GkZbQ9>.
- Flanzy, C. (2003). *Enología : fundamentos científicos y tecnológicos*. Mundi-Prensa, Madrid, 2da edition.
- Galicia Flores, L. A., Salinas Moreno, Y., Espinoza García, B. M., and Sánchez Fera, C. (2008). Caracterización fisicoquímica y actividad antioxidante de extractos de jamaica (*Hibiscus sabdariffa* L.) nacional e importada. *Revista Chapingo. Serie horticultura*, 14(2):121 – 129. Online: <https://bit.ly/2Stp2LT>.
- García Barceló, J. (1990). *Técnicas Analíticas para vinos*. GAB System. Online: <https://bit.ly/2UUwz37>, Barcelona, 1ra edition.
- Hidalgo T., J. (2011). *Tratado de Enología. Tomo I, volume I*. Mundi-Prensa., Madrid, 2da edition.
- Iñiguez, M., Ortega, A. P., Rosales, A., Ayala, R., and Puras, P. (1995). Estudio de color de los vinos tintos de la d.o.c. rioja. *Dialnet*, (7):167–186. Online: <https://bit.ly/2GCJAdW>.
- Jackson, R. S. (2011). *Food and Beverage Stability and Shelf Life*, chapter Shelf life of wine, pages 540–570. Woodhead Publishing Series in Food Science, Technology and Nutrition. Ltd. Oxford, UK.
- Kilcast, D. and Subramaniam, P. (2000). *The Stability and Shelf-Life of Food*. Woodhead Publishing, Boston, 1st edition.
- Kuskoski, M. E., Asuero, A. G., Troncoso, A. M., Mancini-Filho, J., and Fett, R. (2005). Aplicación de diversos métodos químicos para determinar actividad antioxidante en pulpa de frutos. *Ciência e Tecnologia de Alimentos*, 25(4):726 – 732. Online: <https://bit.ly/2DvSu9W>.
- Mariño, G., Coronel, M., González, C., and Beltrán, E. (2017). Uso de bentonita sódica como pretratamiento a la microfiltración tangencial de vino de mora de castilla rubus glaucus benth. *Enfoque UTE*, 8(5):53 – 66. Online: <https://bit.ly/2IaNP2V>.
- Martínez Flórez, S., González Gallego, J., Culebras, J., and Tuñón, J. (2002). Los flavonoides: propiedades y acciones antioxidantes. *Nutrición Hospitalaria*, 17(6):271–278. Online: <https://bit.ly/2BAIU6u>.
- Meza Chavarría, P. (2012). *Guía: flor de jamaica (Hibiscus sabdariffa L.) e (Hibiscus cruentus Bertol)*, asociación para el desarrollo eco-sostenible (adees). online: <https://bit.ly/2N09Obx> edition.
- Mijares, M. I. and Sáez, J. A. (2007). *El Vino: de la cepa a la copa*. Mundi-Prensa. Online: <https://bit.ly/2Ib2CKG>, Madrid, 4ta edition.
- Mounigan, P. and Badrie, N. (2007). Physicochemical and sensory quality of wines from red sorrel/roselle (*Hibiscus sabdariffa* L.) calyces: effects of pretreatments of pectolase and temperature/time. *International Journal of Food Science & Technology*, 42(4):469–475. Online: <https://bit.ly/2E5IvJL>.
- Muñoz Jáuregui, A. M., Fernández Giusti, A., Ramos Escudero, F., and Alvarado Ortiz, C. (2007). Evaluación de la actividad antioxidante y contenido de compuestos fenólicos en vinos producidos en Perú. *Revista de la Sociedad Química del Perú*, 73(1):30 – 40. Online: <https://bit.ly/2E5uX1d>.
- NMX (2010). *Productos agrícolas destinados para consumo humano-Flor (cáliz) de jamaica (Hibiscus sabdariffa L.)-Especificaciones y métodos de prueba*. Mexico, norma mexicana, nmx-ff-115-scfi-2010. online: <https://bit.ly/2GCQHTD> edition.
- OIV (2009). *Compendium of International Methods of Analysis: Wine turbidity. Method OIV-MA-AS2-08*. International organisation of vine and wine. Online:<https://bit.ly/2NC7ZF6>, oiv edition.
- OIV (2014). *Compendium of international Methods of Analysis of spirituous beverages of vitivinicultural origin: Measurement of colour intensity*. International organisation of vine and wine. Online:<https://bit.ly/2E8Ovlj>, oiv edition.
- Pérez Jiménez, J., Neveu, V., Vos, F., and Scalbert, A. (2010). Systematic analysis of the content of 502 polyphenols in 452 foods and beverages: An application of the phenol-explorer database. *Journal of Agricultural and Food Chemistry*, 58(8):4959–4969. Online: <https://bit.ly/2tj2EWF>.

- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., and Rice-Evans, C. (1999). Antioxidant activity applying an improved abts radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9):1231 – 1237. Online: <https://bit.ly/2DxocU7>.
- Reeves, M. J. (2010). *Food Packaging and Shelf Life. A Practical Guide*, chapter Packaging and the Shelf Life of Wine, pages 231–259. Online:<https://bit.ly/2DwRHFM>. CRC Press, Taylor & Francis Group.
- Sáyago Ayerdi, S. G. and Goñi, I. (2010). *Hibiscus sabdariffa L.*: Fuente de fibra antioxidante. *Archivos Latinoamericanos de Nutrición*, 60(1):Online:<https://bit.ly/2TNEaka>.
- Sindi, H. A., Marshall, L. J., and Morgan, M. R. (2014). Comparative chemical and biochemical analysis of extracts of hibiscus sabdariffa. *Food Chemistry*, 164:23 – 29. Online:<https://bit.ly/2Byuxi6>.
- Usoh, I., Akpan, E., Etim, E., and Farombi, E. (2005). Antioxidant actions of dried flower extracts of *Hibiscus sabdariffa L.* on sodium arsenite - induced oxidative stress in rats. *Pakistan Journal of Nutrition*, 4(3):135–141. Online:<https://bit.ly/2WZK43F>.
- Vinson, J. A. and Hontz, B. A. (1995). Phenol antioxidant index: Comparative antioxidant effectiveness of red and white wines. *Journal of Agricultural and Food Chemistry*, 43(2):401–403. Online:<https://bit.ly/2TTgric>.
- Yang, M., Koo, S., Song, W., and Chun, O. (2011). Food matrix affecting anthocyanin bioavailability: Review. *Current Medicinal Chemistry*, 18(2):291–300. Online: <https://bit.ly/2GJF6SC>.
- Zhen, J., Villani, T. S., Guo, Y., Qi, Y., Chin, K., Pan, M.-H., Ho, C.-T., Simon, J. E., and Wu, Q. (2016). Phytochemistry, antioxidant capacity, total phenolic content and anti-inflammatory activity of *Hibiscus sabdariffa* leaves. *Food Chemistry*, 190:673 – 680. Online: <https://bit.ly/2TMSzgc>.