Effects of orange winemaking variables on antioxidant activity and bioactive compounds

María del Carmen SCHVAB[1]*, María Mercedes FERREYRA[1], Cristina Verónica DAVIES[1], Andreina STEFANI[1], María Cristina CAYETANO[1], Liliana Mabel GERARD[1], Rodrigo Francisco GONZALEZ[1]

Abstract

Ascorbic acid, carotenoids and polyphenols stand out among the orange juice natural antioxidants. The winemaking process affects their bioavailability and bioactivity. Antioxidant activities (AA) were estimated in different process conditions to asses those properties. The AA and their correlation with ascorbic acid, total phenolics and carotenoids content were calculated. The variables and levels analyzed were: pasteurized and natural must (PJ and NJ), pH 3.5 and 4.0 and fermentation temperatures at 10°C and 20°C. Statistically significant differences (α=0.05) were found among bioactive compounds concentrations. Antioxidant compounds concentration was higher in raw material than in orange wine. Juice pasteurization caused the major losses while subsequent vinification affects them to a lesser extent. Highest antioxidants retention was measured in wines from JN fermented at pH 3.5 and 10 °C (JN-3.5-10) followed by wines from JP and fermented at the same conditions (JP-3.5-10). AA determined by DPPH showed a positive and close correlation with FRAP, while ABTS showed a low correlation with former assays. Juice pasteurization process and fermentation temperature influenced bioactive compound reduction which correlates with the AA variation.

Keywords: orange wines; antioxidant activity; process variables.

Practical Application: Adjustment of orange winemaking variables to preserve its antioxidant activity.

1 Introduction

Wine has always been the most important alcoholic beverage produced by man. Any fruit with enough fermentable sugars content is able to be used for winemaking applying similar technology as utilized with grapes. Therefore these products could be named as "wine of […]" (Brown et al., 1989; Ward, 1991; Varnam & Sutherland, 1997; Duarte et al., 2009). Fruit wines might be considered beneficial for health possibly related to their antioxidant activity (Nuengchamnong & Ingkaninan, 2010).

Antioxidants, including vitamin C and E, polyphenols, carotenoids and terpenoids among others are the most important bioactive compounds present in fruits (Bravo, 1998; Yen et al., 2002; Hensley et al., 2004; Stahl & Sies, 2005).

Antioxidant content in fruits as well as its associated antioxidant activity could be affected by physiological factors such as maturity and also by technological factors like processing and storage conditions (Lindley, 1998; Helyes & Lugasi, 2006).

Citric juices are characterized by important flavonoid and phenilpropanol content as well as ascorbic acid and carotenoids (Rapisarda et al., 1998; Hayat et al., 2010), all of them being responsible for health beneficial properties. Fruit juices pasteurization is a method commonly used for its preservation. Orange juice "partial sterilization" is one of the heat treatment objectives, trying to avoid altering to a lesser extent the nutritional compounds and its organoleptics properties. Besides, it reduces

the microbial number so that yeast starter (*S. cerevisiae)* has less competition to grow and perform the alcoholic fermentation.

Many new compounds are produced and others are transformed during winemaking, mainly influenced by processing conditions, such as temperature, pH and yeast strain used (Torrens, 2000; Del Pozo Bayón, 2011).

Of particular interest is the fruit winemaking at low temperatures (10°C-15°C) in order to produce and preserve the flavors volatile compounds and thus obtain a characteristic aromatic profile. However, these low temperatures may extend the process time (Torija et al., 2002, Beltran et al., 2008). Temperatures over than 20°C may contribute to the loss of some fruit primary aromas and desirable esters generated during fermentation, as well as the production of undesirable superior alcohols (Peynaud, 1981; Molina et al., 2007; Reddy & Reddy, 2011)

Low temperatures improves yeast ethanol tolerance so, it is suppose that must fermented at 10°C-20°C would maintain sensorial properties.

pH is an essential biological stability factor specially for lactic acid bacteria, so it can be considered as a selective parameter over species involved in wine microbiota (Nemeth et al., 2010) On the other hand, technological processes, alimentary and food processing habits considerably affect antioxidant compounds and their bioavailability and bioactivity (Tomás-Barberán, 2003).

Antioxidant activity is a global and reliable estimation of the antioxidant capacity of foods. Besides, it has been described as an interesting parameter to value the dietary activity of the product (Acevedo et al., 2004).

Individual antioxidant compound quantification does not allow an accurate knowledge of the total antioxidant capacity of a mixture, compound or biological fluid because it is determined by synergistic interactions that could occur between them (Benzie & Strain, 1996) as well as each mode of action. Therefore it is necessary to combine methods to correctly assess a sample antioxidant capacity. During the last years many methods have been developed to evaluate antioxidant capacity of foods based on different aspects: ferric ion reducing antioxidant power (FRAP), oxygen radical absorption capacity (ORAC, TRAP), hydroxyl radical scavenging activity (deoxyribose assay), capture of organic molecules radicals (ABTS, DPPH), quantification of products generated by lipidic peroxidation (TBARs, oxidation of LDLs), among others (Frankel & Meyer, 2000; Sánchez Moreno, 2002; Aruoma, 2003).

It was not found any investigation that quantifies bioactive compounds and antioxidant activity in orange wines. Therefore the aim of this study was to determine antioxidant activity (ABTS, DPPH and FRAP methods), ascorbic acid, total phenolics and total carotenoids in those alcoholic fruit substrates in order to evaluate the influence of juice heat treatment, fermentation temperature and pH.

2 Materials and methods

2.1 Orange juice extraction

350 kg of Washington Navel oranges (2012 harvest) were washed under water circulation. The juice was obtained in a FMC extractor, FS BR 1 model (Brasil), and filtered through ASTM#18 sieves. The volume was divided in two fractions: one was reserved as "natural juice" (JN); the other was pasteurized (JP) to reduce native flora that could compete with inoculated yeasts during alcoholic fermentation, as well as to inactivate the pectinmethilesterase (PME) enzyme that could affects juice stability (Osorio et al., 2008; Maca et al., 2013). Heat treatment was performed at 90ºC for 30 s in a Figmay shell and tube heat exchanger (Argentina).

2.2 Analytical methods

Natural and pasteurized juice characterization

Titratable acidity: according to AOAC 9.135 method (Association of Analytical Communities, 1995), expressed as % anhydrous citric acid.

pH: potentiometric measurement, using TOA HM-30V pH meter.

Soluble solids: refractometer method, using Carl Zeiss, Jena, (Germany) refractometer, expressed as ºBrix.

Juice yield: expressed as mL juice per 100 g fruit (%).

15 oranges randomly selected were weighted, squeezed in manual cone and cup extractor and filtered through 1 mm sieves.

The volume obtained was measured in a graduated measuring cylinder. Results were expressed in percentage terms according to the Equation 1:

$$\text{Fruit yield, \%} = \left(\frac{\text{Volume, mL}}{\text{Weight, g.}} \right) \times 100 \qquad (1)$$

Direct reducing sugars: according to AOAC 1995, 16th ed., Official Method 923.09 (Association of Analytical Communities, 1995), expressed as g/100 mL juice.

Total reducing sugar: according to Lane-Eynon general volumetric method, AOAC, 1995, 16th ed., Official Method 923.09 (Association of Analytical Communities, 1995). Results were expressed as g/100 mL juice.

Antioxidant compounds determination in juices and wines

Carotenoids: reverse-phase HPLC method, using a Hewlett Packard 1100 and a UV detector. Stationary phase: Hewlett Packard, Hypersil AA-ODS, 5 μm, 2.1 × 200 mm; guard column: Hewlett Packard, ODS-Hypersil, 5 μm, 20 × 2.1 mm; mobile phase: methanol; flow rate: 1 mL/min; injection volume: 20 μL; peaks were identified at 473 nm (Yaping et al., 2002).

L-ascorbic acid: reverse-phase HPLC method using a Hewlett Packard 1100 and a UV detector. Stationary phase: Hewlett Packard, Hypersil BDS C 18 3 μm, 100 × 4.0 mm; guard column: Hewlett Packard, ODS-Hypersil C 18, 5 μm, 20 × 2.1 mm; mobile phase: 1 mM KH_2PO_4 aqueous solution (pH 3 with phosphoric acid), flow rate: 0.7 mL/min; injection volume: 20 μL; peaks were identified at 245 nm (Franke et al., 2004).

Total Flavonoids: spectrophotometer method, measured at a wavelength of 285 nm, with methanol as a blank and hesperidin standard solutions. Results were expressed as mg hesperidin/100 mL.

Total Phenolics: determination by the Folin-Ciocalteu method. The assay was performed as follows: X μL of the sample, (850 – X) μL of distilled water, 100 μL of sodium carbonate at 20% p/v and 50 μL of Folin-Ciocalteu reagent were agitated to homogenize. After 30 minutes of reaction at room temperature, absorbance was measured using a UV-visible SHIMADZU spectrophotometer (UV-1603) at 760 nm. The calibration curve was prepared with caffeic acid standard solution 0.1 mg/mL and sodium carbonate (20% p/v). Results were expressed as mM caffeic acid.

2.3 Orange winemaking

The volume of NJ was divided into two fractions: one was maintained at pH 3.5 (orange juice natural pH) and pH was adjusted to 4 in the other, as it is considered the optimal for *S. cerevisiae* growth (Fleet & Heard 1993). The pH adjust was made with solid $CaCO_3$ to facilitate the lees sedimentation at the end of alcoholic fermentation. Commercial sugar was added in sufficient quantity to reach 20 °Bx in order to obtain at least 80 g/L ethanol. In addition, 200 ppm KSO_3H was introduced as antiseptic. PJ was similarly treated.

S. cerevisiae native yeasts, isolated and selected from orange fermented juices (Hours Roque et al., 2005; Ferreyra et al. 2009) were used as pre-culture. It was incubated at 30±1 °C and yeast viable count was made in Petri plates with orange serum agar. When the count reached close to 5×10¹⁰ CFU /mL, the pre-culture was used to inoculate NJ and PJ and maintained at 10 °C y 20 °C. Yeasts optimal fermentation temperature is considered at 20°C and was selected because of this reason. Also, as yeasts demonstrated good tolerance at low temperatures, 10 °C was chosen expecting that more aromatic wines may be obtained in no longer time than in the previous case.

Each substrate was labelled as NJ and PJ, indicating the correspondent pH (3.5 or 4.0) and fermentation temperatures (10°C or 20°C).

2.4 Determination of antioxidant activity (AA)

DPPH radical-scavenging assay

The 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay is based on the scavenging of DPPH radicals (Brand-Williams et al., 1995). It was performed according to the Cavin et al. (1998) protocol: a methanol solution of DPPH· (20 mg/L) was adjusted to a constant absorbance value of 0.32 at 517 nm. Then, 7.5 µL of sample or references compounds were added, and diluted with 250 µL distilled water. Ascorbic acid and hesperidin were used as standard solutions. The decrease in absorbance was measured at 517 nm after incubation at room temperature in the dark for 30 minutes. Free radical scavenging capacity (% discolouration) was determined applying Equation 2:

$$\% \text{ discolouration} = \frac{\overline{A}_c - \overline{A}_s}{\overline{A}_c} \times 100 \tag{2}$$

where $\overline{A}_c$ is the control sample absorbance mean value and $\overline{A}_s$, the sample absorbance mean value.

According to the ∆A values obtained for the standard compounds concentrations, dilutions of the samples with distilled water were performed in order to produce an absorbance decrease within the expected range. Orange wine samples (NJ) were diluted 1:2 and 1:4 while (PJ) samples did not need dilutions.

ABTS radical-scavenging assay

2, 2-azino-bis-3(ethylbenzthiazoline-6-sulphonic acid) radical cation (ABTS·⁺) was produced by reacting ABTS (7 mM) and potassium pershulphate (2.45 mM) solutions in phosphate buffer solution pH 7.0, prepared 12 hours before use and kept in the dark at room temperature. The concentration of ABTS radical solution was adjusted to an absorbance of 0.700 units at 732 nm, with the buffer-solution. 990 µL ABTS·⁺ solution and 10 µL of the assayed solution were added to both samples and standard solutions (ascorbic acid) prepared to calibration curve. Absorbance decrease at 732 nm was measured when ABTS·⁺ solution reached equilibrium. The scavenging capacity of free radicals was calculated applying the former equation.

The antioxidant activity expressed as ascorbic acid equivalents (AEAC) was obtained from the doses/response curve and

was defined as the acid concentration that produces the same antioxidant effect (absorbance decrease) as the sample.

Ferric ion reducing antioxidant power (FRAP)

FRAP reagent consists of acetic acid-sodium acetate buffer (pH 3.6), tripyridyl-triazine (TPTZ) (1 mM) and FeCl₃ (2 mM). This mixture produces TPTZ-Fe⁺³, a brown coloured complex. The standard calibration curve was made using FeSO₄.7 H₂O solutions ranging from 100 to 1000 µM. Both samples and the standard solutions were determined by adding 900 µL FRAP reactive, X µL sample or standard solution and (100 – X) µL of distilled water. The absorbance was read at 593 nm.

2.5 Statistical analysis

All data are presented as the mean ± SD (standard deviation) of three replicates of each sample. The statistical analysis was performed using Statgraphics Plus 2.0 software. Statistical differences among the groups were obtained by analysis of variance (ANOVA). α<0.05 differences were considered significant. Correlations between parameters were established using Pearson´s coefficient.

3 Results and discussion

Orange juice yield was 48 mL/100 g fruit. Values of the physicochemical parameters analyzed in NJ and in PJ are shown in Table 1.

One of the most important problems in citrus juice quality is the ascorbic acid reduction during heat treatment and storage (Polydera et al., 2003). As shown in Table 1, thermal processing caused a 20.60% vitamin C content decrease and was attributed to it losses due to high temperatures exposure and oxidation. Ravani & Joshi (2011) and Kurozawa et al. (2014).

After pasteurization, flavonoids and carotenoids also reduced their content in fresh juice (15.55% and 38.46%, respectively). Lee & Coates (2003) reported that carotenoids content of Valencia orange pasteurized juices was about 70% lesser than the natural one.

As mentioned above, ascorbic acid, carotenoids, flavonoids and other polyphenols contribute to orange juice antioxidant capacity, so quantification in wines obtained from oranges is also considered very important. It was observed that winemaking

Table 1. Mean and standard deviation of physicochemical parameters of natural (NJ) and pasteurized juice (PJ) obtained from Washington Navel oranges.

	NJ	PJ
pH	3.65±0.00	3.84±0.00
Soluble solids (°Brix)	11.00±0.01	11.20±0.01
Acidity (% anhydrous citric acid)	0.94±0.02	0.86±0.02
Ascorbic acid (mg/100 mL)	76.78±0.01	60.96±0.02
Direct reducing sugars (g/100 mL)	4.32±0.05	4.38±0.04
Total reducing sugars (g/100 mL)	8.71±0.03	8.92±0.02
Flavonoids (mg hesperidin/100 mL)	68.12±0.01	57.53±0.01
Carotenoids (mg β-carot. /100 mL)	1.17±0.03	0.72±0.04

process affected their concentration in all the samples evaluated, causing a significant decrease (Tables 1 and 2).

Ascorbic acid decreased between 25-48% in NJ wines and 14-71% for PJ ones during winemaking. When contrasting NJ wines, the highest ascorbic acid content retention (52-75%) was detected in samples fermented at 10°C and pH 3.5 (NJ-3.5-10). The same temperature and pH allowed for the highest ascorbic acid in PJ wines, that is PJ-3.5-10.

Carotenoids showed a great reduction of its initial content in juice. This is probably caused by lees precipitation and subsequent elimination after clarification. Retention was 7-14% and 4-10% in NJ and PJ referring to raw material, respectively.

Hesperidin, the main orange juice glucoside, also decreased its content probably due to an hydrolytic process during fermentation. The aglucon (hesperetin) formed after hesperidin total hydrolysis is highly insoluble, so it may precipitate with lees.

ANOVA showed significant differences between vitamin C, β-carotenes and hesperidin in both wines, NJ and PJ, at both fermentation temperatures and pH values with a 95% confidence level. Fermentation temperature is the factor that mostly affected these compounds retention while the other studied compounds exhibited an aleatory behavior, as it can be seen in Table 2.

Phenolic degradation processes begin in the first stages of vinification and continue during storage (Gómez-Plaza & Cano-López, 2011). Ethanol content, temperature, homogenization process and added metabisulphite concentration are the main processing variables that affect phenolic concentration in wines (Garrido et al., 2013).

Researchers have reported identification and quantification of phenolics in orange juices and wines (Kelebek et al., 2008). They found two hydroxybenzoic acids (gallic and protocatechuic), five hydroxycinnamics acids (caffeic, chlorogenic, p-coumaric, ferulic and sinapic) as well as six flavanons (narirutin, naringin, hesperidin, neohesperidin, didimin and apigenin). According

to these results, caffeic acid was selected as the standard to express total phenolic content. As can be seen in Table 2, a 3-10% decrease occurs due to temperature effect, in NJ and PJ during alcoholic fermentation.

The aforementioned results indicate that it is necessary to combine at least two methods with different basis in order to evaluate the AA of a sample (Pérez-Jiménez & Saura-Calixto, 2007). In the present study two assays were used: one based on free radical scavenging capacity (DPPH y ABTS) and the other on ferric ion reducing antioxidant power (FRAP).

Ascorbic acid and hesperidin were used as standard compounds to plot the calibration curves for DPPH and ABTS assays and to calculate EC_{50} (antioxidant compound concentration necessary to cause a 50% decrease of the initial concentration of the coloured radical in the steady state). Ascorbic acid exhibited the highest antioxidant power. Results of DPPH· using both standards are shown in Table 3.

The data obtained using the three aforementioned AA methods (Table 4) showed which radical/ion is the most active, which is therefore providing the antioxidant profile of this new product.

The highest AA values were observed in DPPH assay results for all samples. A lower AA was observed in FRAP method although it was a high value, and the lowest was obtained according with ABTS.

NJ wines showed the AA highest levels, as expected, due to the principal bioactive compound concentration decrease during orange juice heat processing. This difference did not seem to relate to the method used, since NJ wines showed an AA 1- to 2-fold greater than the similar assay performed in PJ wines. Similar results were observed both in free radical scavenging and ferric ion reducing antioxidant power assays.

The correlation analysis (Pearson´s coefficient) between DPPH discolouration percentage and ascorbic acid, flavonoids, carotenoids and total phenolic content indicated more affinity

Table 2. Mean and standard deviation of antioxidant compounds concentrations present in orange wines produced from natural (NJ) and pasteurized juice (PJ).

Samples	Ascorbic acid (mg/100 mL)	Flavonoids (mg hesperidin/ 100 mL)	Total phenolics (mM caffeic acid)	Carotenoids (mg β-Carotene /100 mL)
NJ-3,5-10	57.60±0.12[a]	50.20±0.12[a]	11.9±0.1[a]	0.009±0.001[a]
NJ-3,5-20	55.55±0.13[b]	49.07±0.20[b]	10.1±0.3[b]	0.021±0.001[b]
NJ-4,0-10	52.80±0.15[c]	40.52±0.13[c]	11.9±0.5[a]	0.017±0.001[c]
NJ-4,0-20	39.60±0.12[d]	47.87±0.15[d]	8.6±0.3[c]	0.017±0.001[c]
PJ-3,5-10	52.46±0.15[e]	36.05±0.16[e]	7.1±0.2[d]	0.004±0.002[d]
PJ-3,5-20	25.52±0.13[f]	31.90±0.12[f]	7.1±0.1[d]	0.007±0.001[e]
PJ-4,0-10	32.92±0.13[g]	31.08±0.13[g]	8.0±0.3[e]	0.003±0.002[f]
PJ-4,0-20	29.55±0.16[h]	32.10±0.14[h]	6.9±0.2[f]	0.003±0.001[f]

Values in the same column followed by different superscript letters are significantly different using Duncan's multiple range test (α<0.05).

Table 3. EC_{50} for DPPH· using ascorbic acid and hesperidin as standards.

Compound	EC_{50} (mg/mL)
Hesperidin	143.77±0.17
Ascorbic acid	0.203±0.12

Table 4. Antioxidant activity determinations performed in orange wines.

Sample	DPPH (% discol.)	ABTS (% discol.)	FRAP (FeSO$_4$ µg/mL)	DPPH (AEAC)	ABTS (AEAC)	FRAP (AEAC)
NJ-3,5-10	87.17	110.62	0.1412	36.80	2.76	28.23
NJ-3,5-20	72.82	101.81	0.1139	30.30	2.54	22.79
NJ-4,0-10	90.04	111.07	0.1296	38.00	2.77	25.92
NJ-4,0-20	92.65	116.06	0.1297	39.20	2.89	25.95
PJ-3,5-10	53.79	93.31	0.0895	19.60	2.32	17.71
PJ-3,5-20	33.02	94.12	0.0791	12.50	3.24	12.68
PJ-4,0-10	41.07	108.97	0.0850	18.30	2.54	15.09
PJ-4,0-20	45.85	112.79	0.0743	29.40	2.81	14.85

AEAC: ascorbic acid equivalents antioxidant activity, mg/100 mL; % discol.: % discolouration.

between flavonoids (0.8670), less with total phenolics (0.7900) and a weak correlation between carotenoids and ascorbic acid.

The ABTS results showed that Pearson´s coefficient varied from 0.3878 to 0.082 following the sequence: total phenolics, flavonoids, carotenoids and ascorbic acid. These low correlations indicated that samples presented higher activity with DPPH· than with ABTS·+.

Activity against TPTZ-Fe^{+3} (FRAP assay) exhibited the highest correlation coefficients with flavonoids and total phenolics (0.9026 and 0.8684, respectively), followed by ascorbic acid and total carotenoids (0.7460 and 0.7163, respectively).

These results are in agreement with many studies that reported that even though vitamin C content and AA in fruits are closely correlated (Yoo et al., 2004), this vitamin is not the only responsible for the AA but also other phytochemicals, such as phenolics, thiols, carotenoids or tocopherols (Sun et al., 2002; Guo et al., 2003). It has been pointed out that specifically flavonoids, within phenolics compounds, show AA (García-Alonso et al., 2004).

In addition, AA results expressed by the DPPH method are positively and closely correlated with FRAP assay (0.958), while using ABTS, they did not correlate with the previous methods significantly (0.548 and 0.463, respectively). Therefore, these results suggest that DPPH and FRAP are the recommended methods to asses AA in orange wines.

Ascorbic acid contribution to the AA by DPPH and FRAP assays in NJ wines was 72% and 51%, respectively. Similarly, PJ showed 60% and 56%, respectively. A lower ascorbic acid/AA ratio suggests that other components, different from this vitamin, such as phenolic compounds have more important antioxidant properties in orange wines.

4 Conclusion

The pasteurization process reduced ascorbic acid, carotenoids and flavonoids contents in orange wines. This fact correlates with the variations observed in NJ and PJ wines antioxidant activity.

Results showed that orange wines have greater AA against DPPH radical than ABTS radical. Consequently, a profile of their free radical scavenging activity may be obtained. Orange wines also reduced TPTZ-Fe^{+3} complex (FRAP assay) indicating its reducing capacity. The analyzed orange wines characteristics are attributed both to ascorbic acid as well as to phenolic and carotenoid contents.

The pasteurization process and the alcoholic fermentation temperature resulted as the most important influence both on the bioactive compound content and AA in the orange wines evaluated.

It is important to point out that the occurrence of bioactive compounds in food does not indicate that the food must exhibit AA, because positive and/or negative interactions can occur. The previously mentioned parameters quantified the ability of a sample to capture free radicals in a water solution. The methods performed intend a global characterization of the product, regardless of its individual composition.

References

Acevedo, B., Montiel, M., & Avanza, J. (2004). Estudio cinético de la degradación de la actividad antioxidante hidrosoluble de jugos cítricos por tratamiento térmico. *FACENA*, *20*, 91-95.

Aruoma, O. I. (2003). Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. *Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis*, *523-524*, 9-20. http://dx.doi.org/10.1016/S0027-5107(02)00317-2. PMid:12628499.

Association of Analytical Communities – AOAC International. (1995). *Official methods of analysis* (16th ed.). Maryland: AOAC International.

Beltran, G., Novo, M., Guillamón, J. M., Mas, A., & Rozès, N. (2008). Effect of fermentation temperature and culture media on the yeast lipid composition and wine volatile compounds. *International Journal of Food Microbiology*, *121*(2), 169-177. http://dx.doi.org/10.1016/j.ijfoodmicro.2007.11.030. PMid:18068842.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, *239*(1), 70-76. http://dx.doi.org/10.1006/abio.1996.0292. PMid:8660627.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food*

Science and Technology, *28*(1), 25-30. http://dx.doi.org/10.1016/S0023-6438(95)80008-5.

Bravo, L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews*, *56*(11), 317-333. http://dx.doi.org/10.1111/j.1753-4887.1998.tb01670.x. PMid:9838798.

Brown, C. M., Campbell, I., & Priest, F. G. (1989). *Introducción a la biotecnología*. Zaragoza: Editorial Acribia.

Cavin, A., Hostettmann, K., Dyatmyko, W., & Potterat, O. (1998). Antioxidant and lipophilic constituents of Tinospora crispa. *Planta Medica*, *64*(5), 393-396. http://dx.doi.org/10.1055/s-2006-957466. PMid:17253260.

Del Pozo Bayón, M. (2011). Descifrando las claves químicas que explican el aroma del vino. *ACE: Revista de Enología*, (127), (n.d.).

Duarte, W. F., Dias, D. R., Melo Pereira, G. V., Gervásio, I. M., & Schwan, R. F. (2009). Indigenous and inoculated yeast fermentation of gabiroba (Campomanesia pubescens) pulp for fruit wine production. *Journal of Industrial Microbiology & Biotechnology*, *36*(4), 557-569. http://dx.doi.org/10.1007/s10295-009-0526-y. PMid:19190949.

Ferreyra, M. M., Schvab, M. C., Gerard, L. M., Zapata, L. M., Davies, C. V., &, Hours Roque, A. (2009). Alcoholic fermentation of orange juice with S. Cerevisiae. *Ciencia, Docencia y Technologia*, *39*, 143-158.

Fleet, G. H., & Heard, G. M. (1993). Yeasts: growth during fermentation. In G. H. Fleet (Ed.), *Wine microbiology and biotechnology* (pp. 27-54). Chur: Harwood Academic.

Franke, A. A., Custer, L. J., Arakaki, C., & Murphy, P. S. (2004). Vitamin C and flavonoid levels of fruits and vegetables consumed in Hawaii. *Journal of Food Composition and Analysis*, *17*(1), 1-35. http://dx.doi.org/10.1016/S0889-1575(03)00066-8.

Frankel, E. N., & Meyer, A. S. (2000). The problems of using one dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture*, *80*(13), 1925-1941. http://dx.doi.org/10.1002/1097-0010(200010)80:13<1925::AID-JSFA714>3.0.CO;2-4.

García-Alonso, M., Pascual-Teresa, S., Santos-Buelga, C., & Rivas-Gonzalo, J. C. (2004). Evaluation of the antioxidant properties of fruit. *Food Chemistry*, *84*(1), 13-18. http://dx.doi.org/10.1016/S0308-8146(03)00160-2.

Garrido, G., Ortiz, M., & Pozo, P. (2013). Fenoles y flavonoides totales y actividad antioxidante de extractos de hojas de Lampaya medicinalis F. Phil. *Journal of Pharmacy & Pharmacognosy Research*, *1*(1), 30-38.

Gómez-Plaza, E., & Cano-López, M. (2011). A review on micro-oxygenation of red wines: claims, benefits and the underlying chemistry. *Food Chemistry*, *125*(4), 1131-1140. http://dx.doi.org/10.1016/j.foodchem.2010.10.034.

Guo, C., Yang, J., Wei, J., Li, Y., Xu, J., & Jiang, Y. (2003). Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. *Nutrition Research*, *23*(12), 1719-1726. http://dx.doi.org/10.1016/j.nutres.2003.08.005.

Hayat, K., Zhang, X., Farooq, U., Abbas, S., Xia, S., Jia, C. H., Zhong, F., & Zhang, J. (2010). Effect of microwave treatment on phenolic content and antioxidant activity of citrus mandarin pomace. *Food Chemistry*, *123*(2), 423-429. http://dx.doi.org/10.1016/j.foodchem.2010.04.060.

Helyes, L., & Lugasi, A. (2006). Formation of certain compounds having technological and nutritional importance in tomato fruits during maturation. *Acta Alimentaria*, *35*(2), 183-193. http://dx.doi.org/10.1556/AAlim.35.2006.2.5.

Hensley, K., Benaksas, E. J., Bolli, R., Comp, P., Grammas, P., Hamdheydari, L., Mou, S., Pye, Q. N., Stoddard, M. F., Wallis, G., Williamson, K. S., West, M., Wechter, W. J., & Floyd, R. A. (2004). New perspectives on vitamin E: γ-tocopherol and carboxyelthylhydroxychroman metabolites in biology and medicine. *Free Radical Biology & Medicine*, *36*(1), 1-15. http://dx.doi.org/10.1016/j.freeradbiomed.2003.10.009. PMid:14732286.

Hours Roque, A., Ferreyra, M. M., Schvab, M. C., Gerard, L. M., Zapata, L. M., & Davies, C. V. (2005). Caracterización fisicoquímica y microbiológica de jugos de naranja destinados a vinificación. *Ciencia, Docencia y Technologia*, *31*, 219-239.

Kelebek, H., Canbas, A., & Selli, S. (2008). Determination of phenolic composition and antioxidant capacity of blood orange juices obtained from cvs. Moro and Sanguinello (Citrus sinensis (L.) Osbeck) grown in Turkey. *Food Chemistry*, *107*(4), 1710-1716. http://dx.doi.org/10.1016/j.foodchem.2007.10.004.

Kurozawa, L., Terng, I., Hubinger, M., & Park, K. (2014). Ascorbic acid degradation of papaya during drying: effect of process conditions and glass transition phenomenon. *Journal of Food Engineering*, *123*, 157-164. http://dx.doi.org/10.1016/j.jfoodeng.2013.08.039.

Lee, H. S. & Coates, G. A. (2003). Effect of thermal pasteurization on Valencia orange juice color and pigments. *LWT - Food Science and Technology*, *36*(1), 153-156. http://dx.doi.org/10.1016/S0023-6438(02)00087-7.

Lindley, M. G. (1998). The impact of food processing on antioxidants in vegetable oils, fruits and vegetables. *Trends in Food Science & Technology*, *9*(8-9), 336-340. http://dx.doi.org/10.1016/S0924-2244(98)00050-8.

Maca, M. P., Osorio, O., & Mejía-España, D. F. (2013). Inactivación térmica de pectinmetilesterasa en tomate de árbol (Solanum betaceum). *Información Tecnológica*, *24*(3), 37-41.

Molina, A. M., Swiegers, J. H., Varela, C., Pretorius, I. S., & Agosin, E. (2007). Influence of wine fermentation temperature on the synthesis of yeast-derived volatile aroma compounds. *Applied Microbiology and Biotechnology*, *77*(3), 675-687. http://dx.doi.org/10.1007/s00253-007-1194-3. PMid:17938912.

Nemeth, A. H., González, C. P., Pérez, B. R., Petit, W. B., Motzezak, R. H., & Granadillo, I. L. (2010). Evaluación química del vino de semeruco (Malpighia spp.) producido en el estado Falcón, Venezuela. *Multiciencias*, *10*(3), 234-240.

Nuengchamnong, N., & Ingkaninan, K. (2010). On-line HPLC–MS–DPPH assay for the analysis of phenolic antioxidant compounds in fruit wine: Antidesma thwaitesianum Muell. *Food Chemistry*, *118*(1), 147-152. http://dx.doi.org/10.1016/j.foodchem.2009.04.069.

Osorio, O., Martinez-Navarrete, N., Moraga, G., & Carbonell, J. V. (2008). Effect of thermal treatment on enzymatic activity and rheological and sensory properties of strawberry purees. *Food Science & Technology International*, *14*(5 Suppl), 103-108. http://dx.doi.org/10.1177/1082013208095328.

Pérez-Jiménez, J., & Saura-Calixto, F. (2007). Metodología para la evaluación de capacidad antioxidante en frutas y hortalizas. In *Proceedings of the 5th Congreso Iberoamericano de Tecnología Postcosecha y Agroexportaciones*. Cartagena, Murcia, España.

Peynaud, E. (1981). *Le goût du vin*. Paris: Dunod Ed.

Polydera, A. C., Stoforos, N. G., & Taoukis, P. S. (2003). Comparative shelf life study and vitamin C loss kinetics in pasteurized and high pressure processed reconstituted orange juice. *Journal of Food Engineering*, *60*(1), 21-29. http://dx.doi.org/10.1016/S0260-8774(03)00006-2.

Rapisarda, P., Carollo, G., Fallico, B., Tomaselli, F., & Maccarone, E. (1998). Hydroxycinamic acids as markers of Italian blood orange juice. *Journal of Agricultural and Food Chemistry*, *46*(2), 464-470. http://dx.doi.org/10.1021/jf9603700. PMid:10554264.

Ravani, A., & Joshi, D. (2011). Standardization of processing parameters for the production of Ready-To-Serve unripe Mango beverage (Pana). *Journal of Dairying, Foods & Home Sciences*, *30*(2), 94-98.

Reddy, L. V. A., & Reddy, O. V. S. (2011). Effect of fermentation conditions on yeast growth and volatile composition of wine produced from mango (Mangifera indica L.) fruit juice. *Food and Bioproducts Processing, 89*(4), 487-491. http://dx.doi.org/10.1016/j.fbp.2010.11.007.

Sánchez-Moreno, C. (2002). Review: methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Science & Technology International*, *8*(3), 121-137. http://dx.doi.org/10.1177/1082013202008003770.

Stahl, W., & Sies, H. (2005). Bioactivity and protective effects of natural carotenoids. *Biochemical and Biophysical Acts*, *1740*(2), 101-107. http://dx.doi.org/10.1016/j.bbadis.2004.12.006. PMid:15949675.

Sun, J., Chu, Y. F., Wu, X., & Liu, R. H. (2002). Antioxidant and antiproliferative activities of common fruits. *Journal of Agricultural and Food Chemistry*, *50*(25), 7449-7454. http://dx.doi.org/10.1021/jf0207530. PMid:12452674.

Tomás-Barberán, F. A. (2003). Los polifenoles de los alimentos y la salud. *Alimentación Nutrición y Salud*, *10*(2), 41-53.

Torija, M. J., Rozès, N., Poblet, M., Guillamón, J. M. N., & Mas, A. (2002). Effects of fermentation temperature on the strain population of Saccharomyces cerevisiae. *International Journal of Food Microbiology*, *80*(1), 47-53. http://dx.doi.org/10.1016/S0168-1605(02)00144-7.

Torrens, J. (2000). El análisis del aroma en el control de calidad de los vinos. *ACE: Revista de Enología*, (*5*). Retrieved from http://www.acenologia.com/ciencia53_2.htm

Varnam, A. H., & Sutherland, J. P. (1997). *Bebidas, tecnología, química y microbiología*. Zaragoza: Editorial Acribia.

Ward, O. P. (1991). *Biotecnología de la Fermentación: principios, procesos y productos*. Zaragoza: Editorial Acribia.

Yaping, Z., Suping, Q., Wenli, Y., Zheng, X., Hong, S., Side, Y., & Dapu, W. (2002). Antioxidant activity of lycopene extracted from tomato paste towards trichloromethyl peroxyl radical CCl3O2. *Food Chemistry*, *77*(2), 209-212. http://dx.doi.org/10.1016/S0308-8146(01)00339-9.

Yen, G. C., Duh, P. D., & Tsai, H. L. (2002). Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. *Food Chemistry*, *79*(3), 307-313. http://dx.doi.org/10.1016/S0308-8146(02)00145-0.

Yoo, K. M., Lee, K. W., Park, J. B., Lee, H. J., & Hwang, I. K. (2004). Variation in major antioxidants and total antioxidant activity of Yuzu (Citrus junos Sieb ex Tanaka) during maturation and between cultivars. *Journal of Agricultural and Food Chemistry*, *52*(19), 5907-5913. http://dx.doi.org/10.1021/jf0498158. PMid:15366841.