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"Stability of individual phenolic compounds and antioxidant activity during storage of a red wine powder"

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Running title: Phenolics stability in freeze dried wine

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Abstract

The addition of maltodextrin DE 10 to red wine (*Cabernet sauvignon*) followed by freeze-drying allowed to obtain a free-flowing (dealcoholized) wine powder (WP) having a phenolic concentration about 3.6 times higher than the original liquid red wine. The powder, having a water activity (a_w) of 0.053 and 0.330 was stored at 28ºC and 38ºC and the content of ten different phenolic compounds was determined by HPLC.

Caftaric acid, quercetin 3-glucoside, caffeic acid, gallic acid and resveratrol contents in the WP stored at 28 and 38 ºC, remained almost constant during 70 days of storage, while epicatechin gallate, catechin, malvidin 3-G and epicatechin showed slight losses (about 15-25%) during storage. On the contrary, epigallocatechin experienced a strong loss of concentration (around 61% loss) during storage at the same conditions.

A moderate decrease of antioxidant activity, determined by free radical scavenging capacity of the DPPH* and ferric reducing antioxidant power (FRAP) was observed along storage. This decrease was more important at higher temperature (38ºC) and higher a_w (0.33).

These results would allow the feasibility of using this wine powder as a healthy ingredient in alcohol-free powder drinks.

Key words: Wine powder - Dealcoholized - Polyphenols - Antioxidant capacity - Anthocyanins – Resveratrol

Introduction

In the past years evidence has accumulated suggesting that wine might be one of the most prominent elements contributing to the beneficial health effect of the so-called "Mediterranean diet" (Kallithraka et al., 2007; Radovanic & Radovanic, 2010). A regular and moderate wine consumption is a highly evoked fact for explaining the low incidence of cardiovascular events in France (known as "the French paradox") compared with other industrialized countries (Gorelik et al., 2008; Nikfardjam et al., 2006; Soulat et al., 2006). It has been shown that small daily intakes of wine can reduce the risk of coronary heart disease and atherosclerosis, being this benefit ascribed to the antioxidants properties of the phenolic compounds (Díaz et al., 2012; Mazza et al., 1999; Radovanovic & Radovanovic, 2010; Renaud et al., 1992) which differ from those found in grapes or must. Although the mechanisms of action are yet not fully understood, it is well known that phenolic compounds behave as radical scavengers and antioxidants. It has also been demonstrated that they can protect cholesterol in the low-density lipoprotein (LDL) from oxidation (Brouillard et al., 1997; Cioroi & Musat, 2007). The relationship between *in vitro* antioxidant capacity and the polyphenol total content of red wines has been documented (Avalos Llanos et al., 2003; Cioroi & Musat, 2007) and total polyphenol content was found to be responsible for the *in vivo* effect (Camussoni & Carnevali, 2004).

However, there are some clear drawbacks in wine consumption associated with the ingestion of alcohol: a) consumption must be moderate (i.e. 1-2 glasses per day) in order to avoid alcohol related diseases, and b) many people, either by ethnical, social or religious reasons do not consume wine (Midgley, 1971; Sanchez et al., 2011).

In a previous work Sanchez et al. (2011) reported preliminary results on the freeze drying encapsulation of red wine added with maltodextrin (MD) obtaining an alcohol free

powder. Water and almost all alcohol from wine were removed during freeze drying and the use of MD as a drying aid led to a glassy microstructure in which the wine phenolics - as well as other components of wine dry extract such as glycerol, sugars, organic acids, salts, etc - were entrapped. Sanchez et al. (2011) suggested their free flowing (dealcoholized) wine powder (WP) might be added to other powdered drinks for enrichment in red wine phenolics. Munin & Edwards-Lévy (2011) noted that although polyphenols are compounds possessing interesting properties for use in medicine, they lack of long term stability since are usually very sensitive to light and heat, and encapsulation appears to be a promising approach to gain stability. Thus, further studies including the stability of individual phenolic compounds on the WP during storage were required.

The objective of the present work was to study the stability of several red wine phenolics in a protective enclosing within a maltodextrin matrix (WP) of low moisture content during storage at selected conditions of temperature and water activity (a_w). Moreover, the change in its antioxidant activity during storage was monitored.

Materials and methods

Materials

The wine used was a commercial *Cabernet Sauvignon*, "Postales del Fin del Mundo" (*Bodega Fin del Mundo*, vintage 2010) from a cold climate wine growing region (Neuquén province, Patagonia, Argentina). Its alcohol content, pH and total polyphenols content were respectively, 13.7%, 3.8 and 2299 ± 89 mg of Gallic Acid Equivalent/L (by Folin-Ciocalteu method, subsequently described in this section). All bottles were purchased at the same time in a winery located in the city of Buenos Aires.

Maltodextrin Dextrose Equivalent 10 (MD_{10}) (Productos de Maíz S.A., Buenos Aires, Argentina) was used for encapsulation of wine and was selected on the basis of the well-

known ability of this maltodextrin to encapsulate labile biomaterials (Chranioti & Tzia, 2012, Galmarini et al; 2011; Roos, 1995).

HPLC grade reagents malvidin-3-glucoside chloride (malvidin-3-G), catechin, epicatechin, epicatechin gallate, epigallocatechin, caffeic acid, gallic acid, caftaric acid, quercetin 3-glucoside (quercetin 3-G) and resveratrol (all of them >95% purity) were purchased from *Extrasynthese*, France; the solvents acetonitrile (>99.9% purity) from Pancreac, France, formic acid (98%) from Sigma Aldrich, France and methanol (>99.5%) Chromasolv, France.

The Folin–Ciocalteau reagent was from Merck KgaA Darmstadt, Germany.

Potassium chloride buffer (pH 1) and sodium acetate buffer (pH 4.5) used for chemical anthocyanins quantification were prepared using potassium chloride, chlorhydric acid and sodium acetate purchased from Biopack, Buenos Aires, Argentina.

The *2,2-Diphenyl-1-picrylhydrazyl* free radical (DPPH*) was purchased from Merck, France. For the FRAP assay the 2,4,6-Tripyridyl.s.triazine (TPTZ) used for the was from Sigma Aldrich, France, $FeSO_4$.$7H_2O$, FeCl3.6H2O and the sodium acetate (99%) were purchased from Fluka, France.

Encapsulation procedure

MD_{10} was dissolved in wine to a 20% concentration (total weight basis) and freeze dried to encapsulate the dry extract of wine (including polyphenols) in an amorphous carbohydrate microstructure (Sanchez et al., 2011). The solution of wine and MD_{10} was poured into a stainless steel tray (round tray of 20cm diameter; depth of sample 1cm) and freeze-dried at room temperature in a FIC Ll-I-E300-CRT freeze dryer (Buenos Aires, Argentina) operated with a freezing plate and condenser at - 40ºC and a vacuum below 200µmHg. Freeze drying time was 40 hours at room temperature (22 ± 3ºC) and resulting

samples had a water activity of 0.053. Along with water, almost all of the ethanol originally present in wine was eliminated during freeze-drying, leading to a dealcoholized wine powder, since residual alcohol was below 1% (Sanchez et al., 2011). The freeze dried product - a porous cake of glassy aspect - was milled in a domestic grain coffee grinder resulting in a free-flowing powder used in the storage stability experiments.

Storage conditions

After freeze drying, samples of WP (6g) were placed in small opaque glass flasks and stored at selected (and controlled) temperatures and water activity (a_w). Wine powder was stored in constant temperature ovens kept at 28 and 38ºC in hermetically sealed flasks in order to preserve the low initial moisture conditions (a_w=0.053) and at 28ºC and 38ºC in opened flasks over a saturated solution of MgCl which provided a constant relative humidity of 33% (a value sufficiently low so as not to have adverse physical changes but also high enough to be easily maintained during storage). Samples of all systems were removed from storage and analyzed after 0, 30, 50 and 70 days.

It is well known that amorphous hygroscopic powders (such as present DWP) may suffer undesirable physical changes during storage, namely stickiness and caking. These changes are mainly controlled by the glass transition temperature (T_g) of the amorphous powder in relation to storage temperatures and relative humidity as well as storage time (Galmarini, 2010; Galmarini et al., 2011; Roos, 1995; Roos & Karel, 1971). Therefore, storage conditions at higher temperature/a_w were not selected because they might induce those adverse physical changes. The chosen temperatures represent normal and drastic ambient storage conditions.

Water activity and moisture content

Water activity (a_w) of DWP samples was determined using an electronic dew point water activity meter Aqualab series 3 (Decagon Devices, Pullman, Washington, USA). The device was calibrated and tested with saturated salt solutions in the water activity range of interest (Favetto et al., 1983). The error in a_w measurement was determined to be about ± 0.004 a_w.

Moisture content was determined gravimetrically; a thin layer of DWP was placed in a forced circulation oven at 70 ºC (± 1 ºC) until constant weight. A relatively low temperature was used to avoid the possibility of any evaporation of glycerol in the DWP, originated from the liquid red wine.

Total polyphenols

Total phenolics were measured by Folin-Ciocalteu method (Camussoni & Carnevali, 2004; Singleton & Rossi, 1965). Wine powder samples were first rehydrated with water to their original weight and they were afterwards diluted (1:10). The same dilution was used for the liquid wine. All samples were treated according to the protocol described by the International Organization of Vine and Wine (Method OIV-MA-AS2-10, 2009). As stated in the protocol, absorbance at 765 nm was measured using a spectrophotometer Shimadzu PharmaSpec UV-1700 (Shimadzu Corporation, Japan). All measurements were done in duplicate, and polyphenol concentrations were expressed as Gallic Acid Equivalent (GAE) in milligrams per Liter calculated by means of a standard curve of gallic acid.

Monomeric anthocyanin content

Monomeric anthocyanin content (Monomeric AC) of the initial wine and of WP was determined using the pH differential method (Giusti & Wrolstad, 2001; Ribereau-Gayon & Stonestreet, 1968). WP samples were rehydrated with water to their original weight before

freeze drying and diluted in buffer to achieve a proper concentration range for reading. A Shimadzu PharmaSpecUV-1700 spectrophotometer (Shimadzu Corporation, Japan) and 1cm path length disposable cells were used for spectral measurements. Absorbencies were read at 520 and 700nm. Pigment content was calculated as malvidin-3-glucoside (mg/100g wine powder) with molecular weight of 463.3gmol-1 and extinction coefficient of 25,000Lcm^{-1}mol^{-1}. All samples were prepared in duplicate and measurements were taken four times for each one.

Analysis of phenolic compounds by HPLC

Samples were prepared by weighting 60.0±0.5mg of WP which was dissolved in 1.5mL of solvent composed of a mixture of water/methanol/HCl (89/10/1) (Souquet et al., 2006). Prior to injection they were filtered through a Whatman PTFE syringe filter (13mm diameter, porosity 0.45µm). Injection volume was of 10µL. The original red wine was also analyzed by direct injection.

HPLC analysis was carried out in a 1100 series HPLC instrument (Agilent Technologies, Waldbronn, Germany) equipped with a vacuum degasser, a quaternary pump, an autosampler and a thermostated column compartment. Separation was achieved on a reverse phase Nucleosyl LC-18 column. The column's temperature was maintained at 30°C. Detection was performed thanks to a diode array detector attached to a computer (HP Chemstation).

Two solvents were used during the analysis. Solvent A composed of distilled water/ formic acid (95/5) and Solvent B consisting of acetonitrile/ water/ formic acid (80/15/5). A constant flow of 1 mL/min was applied with a linear gradient elution profile. The following proportions of solvent B were used (Salas et al., 2004): 0-7 min, 3%; 7-20 min, 13%; 20-23 min, 14%; 23-34 min, 20%; 34-38 min, 20%; 38-45 min, 24%; and 45-54 min, 35%.

Malvidin 3-G, catechin, epicatechin, epicatechin gallate, epigallocatechin, caffeic acid, gallic acid, caftaric acid, quercetin 3-glucoside (quercetin 3-G) and resveratrol were identified according to their retention time and spectral properties. Quantification was done by external standard curves of authentic standards of each compound. These chosen phenolics are representative of important polyphenol groups found in red wine, namely, flavanols, hydroxycinnamic acids, hydroxybenzoic acids, tartaric acid derivatives, flavonols, anthocyanins and stilbenes. Total anthocyanins were also analyzed by absorption at 520nm and expressed as g malvidin-3-G equivalent/100g WP. Caftaric acid was expressed as g of caffeic acid/100g WP. All samples of WP were prepared in triplicate and then analyzed.

Antioxidant capacity

Changes in the antioxidant capacity along storage of the WP was analyzed using two independent methods: free radical scavenging capacity of the DPPH* (*2,2-Diphenyl-1-picrylhydrazyl*) (Klopotek et al., 2005; Hukkanen et al., 2006; Marc et al., 2004; Stratil et al., 2006) and ferric reducing antioxidant power (FRAP) (Benzie & Strain, 1996).

For the DPPH* method, the WP was first dissolved in water (1g/100mL) and then three dilutions were done in methanol in order to obtain solutions of WP within the desired range of % inhibition. After some previous explorative dilutions, the final determinations were done using the concentrations 9.6, 6.4 and 3.2 g/L. Each storage condition and time (0, 30, 50 and 70 days) were analyzed. All the methanol solutions were centrifuged during 7 minutes at 4ºC in order to precipitate the undisolved MD_{10} before analysis.

For the analysis of WP samples, 150µL of each dilution together with 3mL of DPPH* solution (2.5 mg DPPH*/100mL methanol) were placed in disposable 1cm path length plastic spectrophotometer cells. A control was prepared by adding 150µL of methanol to 3mL of DPPH* solutions and a blank was done using 3mL of methanol and 150µL of each WP

methanol dilution. All WP samples, control and blank were prepared in duplicate and were stored in the dark during 60 minutes before measuring their absorbance (Abs) at 517nm. Measurements were done by means of a spectrophotometer UV-MC2. SAFAS, Monaco. The inhibition percent (inhibition %) was obtained by using the equation 1 (eq. 1)

$$\text{Inhibition } \% = 100 - [(\text{Abs WP} - \text{Abs blank})/\text{Abs control}]*100 \quad \text{(eq. 1)}$$

Finally, for each storage time the inhibition % was plotted against concentration obtaining a linear regression (R^2 always > than 0.995) which was used to obtain the concentration of WP necessary to inhibit 50% of DPPH* (EC_{50}). This change along time for each storage condition was represented. Results are expressed as 50% inhibition, representing grams of WP needed to reduce DPPH* concentration in 50%, thus lower values mean higher antioxidant capacity.

The FRAP technique was adapted from Benzie & Stain (1996). The Frap reagent was prepared by mixing acetic buffer (300mM, pH = 3.6), 2,4,4-tripyridyl-s-triazine (TPTZ) 10mM solution in HCl (40 mM) and $FeCl_3.6H_2O$ (20mM water solution) at a ratio of 10:1:1. FRAP reagent was incubated at 37ºC, and then 1.5mL were added to 50µL of the WP solution (125mg WP /25mL of water). After 5 min of reaction the absorbance of blue coloration was measured at 595nm against a blank sample by means of a spectrophotometer UV-MC2, SAFAS, Monaco. A standard curve of $FeSO_4.7\ H_20$ expressed as Fe^{2+} µM was done in the range of 100-1200 µmol Fe^{2+} /L. Results (mean from three replicates) were expressed as micromoles of Fe^{2+} reduced per Kg of WP, based on the mentioned $FeSO_4.7H_2O$ standard.

Data analysis

Multiple factor analyses of variance (ANOVA) with subsequent Student Newman-Keuls (SNK) test were carried out to detect differences along storage times for chemical and HPLC determinations using Infostat software v.2009 (Universidad Nacional de Córdoba, Argentina).

HPLC quantification of malvidin-3-G and chemical determination of anthocyanins were correlated by means of a simple regression using Statgraphics Plus v 5.1.

For all measurements the standard deviation (S.D.) was calculated.

Results and discussion

Analysis of phenolic compounds

Table 1 shows content of the ten individual phenolic compounds investigated in *C. Sauvignon* red wine and in the WP. As a result of freeze drying and addition of MD_{10}, the concentration of phenolics in the WP (mg/100g) resulted to be in general about 3.6 times higher than in the liquid wine (also expressed in mg/100g wine) revealing that the product could be considered a concentrate of wine polyphenols. Moisture content of WP after freeze drying was determined to be 1.51 % ± 0.07 and that of the WP equilibrated to a_w 0.330 was 4.53 % ± 0.09.

As previously noted in the Materials and Methods section the phenolic compounds studied in present work are representative of important polyphenol groups found in red wine. The concentration of these selected phenolics in the used red wine (**Table 1**) falls within the range reported by several authors either for Argentinean *C. Sauvignon* wines (Kruma et al., 2010; Fanzone et al., 2012) or from other countries (Anli & Vural, 2009; Burin et al., 2011; Gambelli &Santaroni, 2004; Kruma et al., 2010). Total polyphenols content (**Table 1**) also

fits in the range reported for several red wines either from Argentina or other countries (Anli & Vural, 2009; Camussoni & Carnevali, 2004; Kruma et al., 2010).

Phenolic compounds in red wine have proved important for delivering health benefits and therefore their stability has been widely studied giving place to numerous publications in a variety of wines from different geographical regions. However, they are of limited applicability to a powder system such as the WP. In a powder food system as the WP, moisture (or a_w) is a key factor affecting chemical and physical stability since it is well known that water sorbed in an amorphous food powder acts as a plasticizer accelerating (or decreasing) chemical reactions by influencing molecular mobility (Buera et al., 2006, Li et al., 2011; Roos, 1995).

van Galde et al. (2003) used the technique of freeze drying not to obtain a wine polyphenols concentrate but to remove alcohol and water from red wine, in order to study the effects of wine polyphenols on health without the influence of alcohol. When describing the procedure used to reconstitute the freeze dried product they stated that: "distilled water was added to the dry *powder*". The meaning of "dry powder" is not clear since a powder can not be obtained after freeze drying of red wine alone. In present and previous works (Sanchez et al., 2011) we observed that after freeze-drying of *Cabernet Sauvignon* wine without the addition of MD_{10}, an amorphous rubbery mass (very difficult to handle) was obtained. However, when MD_{10} was added before freeze drying a free flowing powder (after milling) was obtained. This free flowing powder is readily dissolved in water, it is easy to handle and could be integrated as an ingredient in different food systems.

Freeze drying has proved to be a suitable method for drying thermosensitive substances, minimizing thermal degradation reaction (Munin & Edwards-Lévy, 2011). van Galde et al. (2003) also assessed the effect of freeze drying on retention of four important red

wine polyphenols (gallic acid, catechin, epicatechin and quercetin) by determining their concentration both in the original red wines and the freeze dried products. In their case, recovery amounted to 68% (average for all four phenolics in six different red wine brands). They mentioned that "apart from the intended loss of volatile substances like alcohol, about 30% of the non volatile substances is also lost " in the freeze drying process; although they acknowledged that further research was needed to analyze at which stage of their freeze drying process the reported loss of polyphenols occurred. Volatility is the tendency of a substance to vaporize (or sublimate) and it is directly related to a substance's vapor pressure. So, one would expect that gallic acid, catechin, epicatechin and quercetin would be hardly "volatilized" during freeze drying due to their very low vapour pressure. For gallic acid (25ºC) vapour pressure is $7.32E^{-11}$mmHg; for catechin (25ºC) $9.3E^{-17}$mmHg, for epicatechin (25 ºC) is $3.33\ E^{-31}$mmHg , and for quercetin (25ºC) is $4.24\ E^{-17}$mmHg. Moreover, van Galde et al. (2003) reported the retention of gallic acid and catechin after freeze drying was identical (64 %) a result which in terms of "volatility" would be difficult to explain since there is a difference of six orders of magnitude between their vapor pressures.

On the contrary, in present work the retention of gallic acid, catechin, and epicatechin after freeze drying of red wine + MD_{10} amounted nearly to 100% (average for this three phenolics). Sanchez et al. (2011) also reported that after freeze drying of red wine with MD_{10} total polyphenols retention (Folin-Ciocalteu method) amounted to 97.8%. However, it has to be stressed that these values were obtained after freeze drying of red wine with MD_{10} and not in regular red wine as done by van Galde et al. (2003). Addition of MD_{10} may had protected phenolics by generating micro-regions of reduced molecular mobility and diffusion rate, thus lowering chemical reaction rates (Buera et al., 2006 ; Li et al., 2011) which might enable polyphenols recovery.

Figure 1 shows the behavior of gallic acid, caftaric acid, quercetin 3-G, caffeic acid and resveratrol in WP equilibrated at a_w = 0.33 and stored at 28 and 38 ºC. It can be seen that phenolics concentration in WP remains practically constant, either at 28 or 38 ºC during 70 days storage. Similar results (not shown in the figure) were obtained at lower water activity (a_w = 0.053).

Some authors reported data on the stability of these phenolics in other low-moisture powders. For example, Altiok et al. (2009) studied the properties of trans-resveratrol in chitosan microspheres produced by spray drying finding that the stability of trans-resveratrol when incorporated into the microspheres remained constant. The same was observed in present work for resveratrol freeze-dried encapsulated in maltodextrin. For gallic acid, Tandale (2007) used whey protein concentrate as carrier material and stored freeze-dried microencapsulated gallic acid at 25ºC in dark or under UV light. In both conditions the retention of microencapsulated gallic acid (at a_w = 0.22 and 0.44) was above 90% after 56 days storage. The stability of caftaric acid in a freeze dried extract of a medicinal herb (*Echinacea purpurea*) was studied by Rattanadechsakul et al. (2007) who found that it remained relatively constant for at least 60 days at temperaturas of 30 and 45ºC.

The stability of some of the aforementioned phenolics "in solution" (e.g. wine) has also been studied and may be compared with present data in WP. Giovanelli & Brenna (2007) detected minor changes (degradation) in gallic and caftaric acid when red wine bottles were stored at 30ºC for up to 75 days. Zafriella et al. (2003) reported that changes in quercetin 3-G content in red wine stored at 20ºC (in the dark) were small, finding losses of 6.4% and 9.2%, after 30 and 60 days storage, respectively. Overall, these studies performed in wine might suggest that gallic acid, caftaric acid and quercetin 3-G might be relatively stable even when "in solution" (wine) during storage time (65/75 days) similar to those in present work with WP. It is to be noted, however, that studies in "liquid" wine were performed at lower

temperatures (20 and 30 ºC) than the highest temperature (38 ºC) at which the phenolics proved to be stable in WP. Jeandet et al. (1995) investigated the influence of aging on the resveratrol content of wine through the analysis of wines of different ages and reported that resveratrol appeared relatively stable in wine. Ratola et al. (2004) and Cvejic et al. (2010) also indicated a good thermal/chemical stability of resveratrol in red wines.

Behavior of epigallocatechin content in WP during storage (**Figure 2**) was different from the other catechins here studied (see next **Figure 3**) since after an initial pronounced loss of around 61 % epigallocatechin content appeared to level off during storage at studied conditions of a_w and temperature.

In **Figure 3** the data referring to epicatechin gallate, catechin and epicatechin in WP equilibrated at a_w = 0.33 and stored at 28 and 38 ºC are presented. A small loss is observed for these catechins, and this loss increased significantly ($p<0.05$) with increasing temperature from 28 to 38 ºC. Nevertheless, at the highest a_w/temperature losses ranged between 15-25% after 70 days of storage. Stability of catechins in the "dry" state has been studied in dry tea products. Friedman et al. (2009) reported changes for epicatechin and epicatechin gallate (among other catechins) during storage of eight commercial brands of tea leaves in the "dry" state (at 20ºC, in the dark). They concluded that even in the "absence of moisture" catechins content decreased with time. Unfortunately, they did not report the moisture content (or a_w) of their tea leaves neither variations of moisture content during storage. These variations were likely to happen since the tea bags were stored in the original containers and without controlling ambient relative humidity. After 60 days of storage the average loss of epicatechin and epicatechin gallate amounted to 18% and to 57%, these values being an average of the eight tea brands studied. However, inspection of their data revealed a strong dispersion of data

among different tea brands, suggesting some unknown influence on the stability of catechins. It is not known whether these differences might be due to (among other things) different moisture content of the tea bags, since moisture has an important effect on stability of reduced moisture food systems.

Li et al. (2011) studied the effect of temperature and relative humidity on the degradation kinetics of catechins (among them, epicatechin, epigallocatechin and epicatechin gallate) in spray dried green tea powder. They used a commercial brand and did not disclosure the ingredients in the product (besides from green tea extract); it may be mentioned that maltodextrin is sometimes used as carrier material for spray drying of tea extracts (Nadeem et al., 2011). Li et al. (2011) reported that catechin degradation was affected by an increase in water activity and temperature, temperature being a dominant factor. Interestingly, an inspection of their results at $a_w = 0.41$ (at 60ºC) revealed that epigallocatechin exhibited the highest rate of degradation, as compared to epicatechin and epicatechin gallate, a behaviour similar to that reported in present work (Figs. 2 and 3) for catechins but in WP at $a_w = 0.33$. From the first-order degradation kinetic curves reported by Li et al. (2011), we calculated that after 50 days storage in green tea powder (60ºC, $a_w = 0.41$) epigallocatechin experimented 40% loss while for epicatechin gallate and epicatechin the losses were 23.7% and 7.7%, respectively. No explanation was advanced for the higher instability of epigallocatechin in green tea extract powder as compared to the other catechins. It is well known that catechins can undergo degradation, oxidation, epimerization and polymerization (Ananingsih et al, 2011) and many factors could contribute to these chemical changes. Moreover, Wang et al. (2000) reported the loss of catechins in "liquid phase" of tea, (i.e. green tea extracts) during storage at 50 ºC. Their tabulated data for epigallocatechin, catechin and epicatechin content in tea extract were recalculated and plotted as % retention versus storage time (at 50 ºC) finding that the epigallocatechin evidenced the highest loss.

Changes in malvidin 3-G content in WP during storage are observed in **Figure 4**. At 28 ºC and a_w = 0.053 malvidin 3-G did not show any loss during 70 days storage. However, increasing either the a_w from 0.053 to 0.33 or the temperature from 28 to 38 ºC resulted in losses which amounted to about 26% at 70 days storage at the highest a_w/temperature. These losses were also reflected in changes in the measurements of monomeric AC. For this purpose, a correlation between malvidin 3-G content (determined by HPLC) with monomeric AC determined by differential pH method was done. An acceptable linear correlation (r^2 = 0.852; n:12) was observed suggesting that chemical determination of monomeric anthocyanins is strongly related to malvidin 3-G content, which is known to be the major anthocyanin in *C. Sauvignon* wines (Birse et al., 2004; Radovanovic & Radovanovic, 2010).

Zafrilla et al. (2003) determined changes in phenolic compounds of bottle-stored red wine in the dark at 20ºC. Their data for malvidin 3-G and total anthocyanins were calculated as % retention and compared to present data for malvidin 3-G and total anthocyanins retention in WP stored at 28 ºC and a_w = 0.33. **Figure 5** shows that malvidin 3-G and total anthocyanins content is much more stable in the dry matrix of WP (28 ºC) than in regular red wine stored at 20ºC. Giovanelli & Brenna (2007) also reported that in red wine a sharp decrease in total anthocyanins occurred during storage at 30 ºC for up to 75 days.

The higher stability of anthocyanins in WP, as compared to regular red wine, may be attributed to the limited molecular mobility and diffusion rates associated with the low moisture content of WP (Buera et al., 2006). Li et al. (2011) reported similar results when comparing the degradation kinetics of catechins in green tea extract solution and green tea powder (at various a_w) and found that powder products were much more stable than solutions.

The storage stability of spray-dried or freeze-dried encapsulated fruit anthocyanins has been studied in many works. Stupiñan et al. (2011) investigated the stability of anthocyanin freeze-dried powders from Andes berry during storage and concluded that addition of

maltodextrin DE_{20} improved the color and stability of antioxidants present suggesting a protective enclosing of anthocyanin within a maltodextrin matrix. Laine et al. (2008) reported that a polyphenol-rich raspberry extract was stabilized by freeze-drying, with maltodextrins (DE_{5-8} and $DE_{18.5}$) as material coating and found the freeze-dried particles were stable over long periods and provided to polyphenols an effective protection against the oxidation phenomenon during their storage, whereas antioxidant activity remained identical. Iham et al. (2010) and Osorio et al. (2010) also reported that the stability of anthocyanins was enhanced by spray drying encapsulation in maltodextrin and/or arabic gum matrixes. Tonon et al. (2010) studied anthocyanin stability of spray-dried açai (*Euterpe oleracea Mart.*) juice produced with different carriers and found that temperature influenced anthocyanin stability in a negative way and the increase of water activity also resulted in higher degradation. This was attributed to the higher molecular mobility, which allows easier oxygen diffusion, thus accelerating the oxidation reactions.

Antioxidant activity

One of the important characteristics of polyphenolic compounds is their antiradical property. Different methods are used for the determination of antioxidant capacity, such as the chromogen free radical DPPH* assay (Kruma et al., 2010) and FRAP (Ou et al., 2002; Stratil, et al., 2008). **Figure 6** shows changes in antioxidant activity of WP (determined with chromogen radical DPPH*) during storage at all studied conditions. A moderate decrease of antioxidant activity along storage time was observed, this decrease being enhanced by higher temperature and water activity. Further on, **Figure 7** shows the change in ferric reducing capacity (FRAP method) of WP during storage at different conditions of water activity and temperature which is in good agreement with results obtained by DPPH* method (**Figure 6**).

The antioxidant capacity as determined by FRAP method declined slightly during storage, the greatest loss being of only 14% at 38ºC and a_w= 0.33.

Several authors tried to correlate (with more or less success) the antioxidant activity and the polyphenolic contents of red wine. For example, a linear correlation between antioxidant capacity and the contents of total polyphenols have been reported by many authors (Avalos-Llanos et al., 2003; Cioroi & Musat, 2007; Anli et al., 2008; Kruma et al., 2010). However, Zafrilla et al. (2003) reported a pronounced fall in the concentration of total phenols in red wine stored at 20 ºC, but the antioxidant activity remained almost constant. These authors also failed to correlate the anti-radical activity with some individual phenolic compounds in red wine. These behaviour may be attributed to two main facts, a) polyphenols usually identified by different authors represented only a small proportion of the several hundreds of phenolic compounds existing in red wine (Zafrilla et al., 2003) and b) many factors which influence hydrolysis, oxidation and condensation reactions may take place during wine storage (Kallithraka et al., 2009).

Without attempting to correlate changes in antioxidant capacity (either by DPPH* or FRAP method) with changes of phenolics in WP, it may be mentioned that the observed loss of antioxidant capacity after 70 days storage (at a_w = 0.33 and 38 ºC) was 18% for DPPH* and 14% for FRAP. These values were aligned with similar losses found in catechine (19.1 %), epicatechine (25.7 %) epicatechin gallate (13.6 %) and malvidin 3-G (26.1 %) for identical storage conditions.

Very recently, Munin & Edwards-Lévy (2011) presented a review on encapsulation of natural polyphenolic compounds for cosmetics, nutrition and health, and concluded that encapsulation is an interesting means to potentialize their activity (i.e. via spray drying or

freeze-drying). They concluded that encapsulation provided a significant protection for polyphenols against drastic conditions such as oxidation and thermal degradation.

Present results on the stability of several red wine phenolics in a protective enclosing within a maltodextrin matrix (WP) of low moisture content, appears to be on line with above suggestion.

Conclusions

A free-flowing dealcoholized wine powder having a phenolic concentration about 3.6 times higher than the original liquid red wine (compared on the same weight basis) was obtained. Most phenolics remained stable in the maltodextrin matrix during the 70 days storage time at the a_w (0.053 and 0.33) and temperatures (28 and 38 ºC) considered. The WP remained free flowing at all conditions studied; undesirable physical changes (such as caking) were not visually observed.

Epigallocatechin was the only polyphenol which experienced an important decrease during storage (61%). Even though no correlation was attempted, a moderate decline of the antioxidant activity (either measured by DPPH* or FRAP methods) was also observed along storage time. This was enhanced by higher temperature and water activity (38ºC and a_w=0.33).

This freeze-dried, highly stable wine product, with a polyphenol composition similar to that of red wine could be used as a healthy ingredient in alcohol-free powder drinks since encapsulation provided protection against conditions such as oxidation and thermal degradation. Future work goes in this direction.

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Legends for figures

Figure 1 – Stability of caftaric acid, quercetin 3-G, caffeic acid and resveratrol in WP equilibrated at a_w = 0.33 and stored at 28ºC and 38 ºC. Open symbols: 28 ºC; closed symbols: 38 ºC. (In most cases data at 28 ºC overlap with those at 38 ºC; S.D. bars mostly overlap with data symbols).

Figure 2 – Changes in epigallocatechin content in WP during storage at selected storage conditions. (In some cases S.D. bars overlap with data symbols).

Figure 3 - Stability of epicatechin gallate, catechin, and epicatechin in WP equilibrated at a_w = 0.33 and stored at 28 ºC and 38 ºC. (In most cases S.D. bars overlap with data symbols).

Figure 4 – Changes in malvidin 3-G content in WP during storage at 28 and 38 ºC.

Figure 5 – Comparison of malvidin 3-G retention in WP equilibrated at a_w = 0.33 and stored at 38 ºC with malvidin 3-G retention in regular red wine (calculated with data reported by Zafrilla et al.; 2003) stored at 20 ºC in the dark.

Figure 6 – Evolution of antioxidant activity (DPPH*) of WP during storage at different conditions of water activity and temperature. Values in ordinate are grams of WP needed to reduce DPPH concentration in 50%, thus lower values mean higher antioxidant capacity.

Figure 7 - Changes in ferric reducing capacity (FRAP method) of WP during storage at different conditions of water activity and temperature. Bars represent S.D.

Table 1 - Phenolic compounds in dealcoholized red wine powder (WP; a_w = 0.053; moisture content 1.5%) following freeze drying (time 0 days) and in the liquid red wine used ("Postales del Fin del Mundo", *Cabernet Sauvignon*).

Phenolic compound	**Concentration in WP** ***mg/100 g*** [a]	**Concentration in liquid wine, *mg/100 g*** [a]
Epigallocatechin	176.5 ± 3	46.7 ± 2.8
Malvidin 3-G	59.3 ± 5	13.7 ± 1.3
Epicatechin gallate	44.0 ± 0.5	13.5 ± 0.34
Catechin	31.5 ± 1	8.7 ± 0.2
Gallic acid	21.5 ± 1.5	6.0 ± 0.22
Caftaric acid	18.5 ± 0.5	5.4 ± 0.1
Epicatechin	17.5 ± 0.5	4.7 ± 0.1
Quercitin 3-G	7.0 ± 0.5	2.1 ± 0.1
Caffeic acid	1.5 ± 0.1	0.4 ± 0.02
Resveratrol	0.50 ± 0.02	0.01 ± 0.008
Total phenolic content [b]	845.5 ± 10.4 mg GAE/100 g	2,299 ± 89 mg GAE/L

[a] mean value ± S.D.

[b] by Folin-Ciocalteu method.

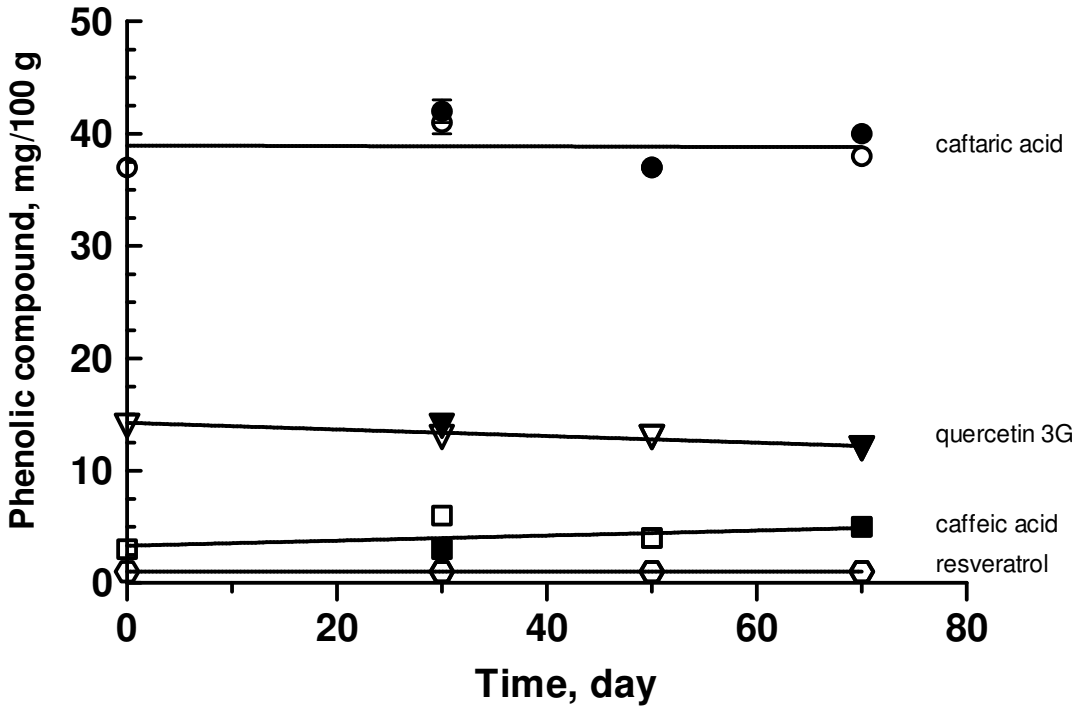
Phenolic compound, mg/100 g
50
40
30
20
10
0
0
20
40
60
80
Time, day
caftaric acid
quercetin 3G
caffeic acid
resveratrol

Figure 1.

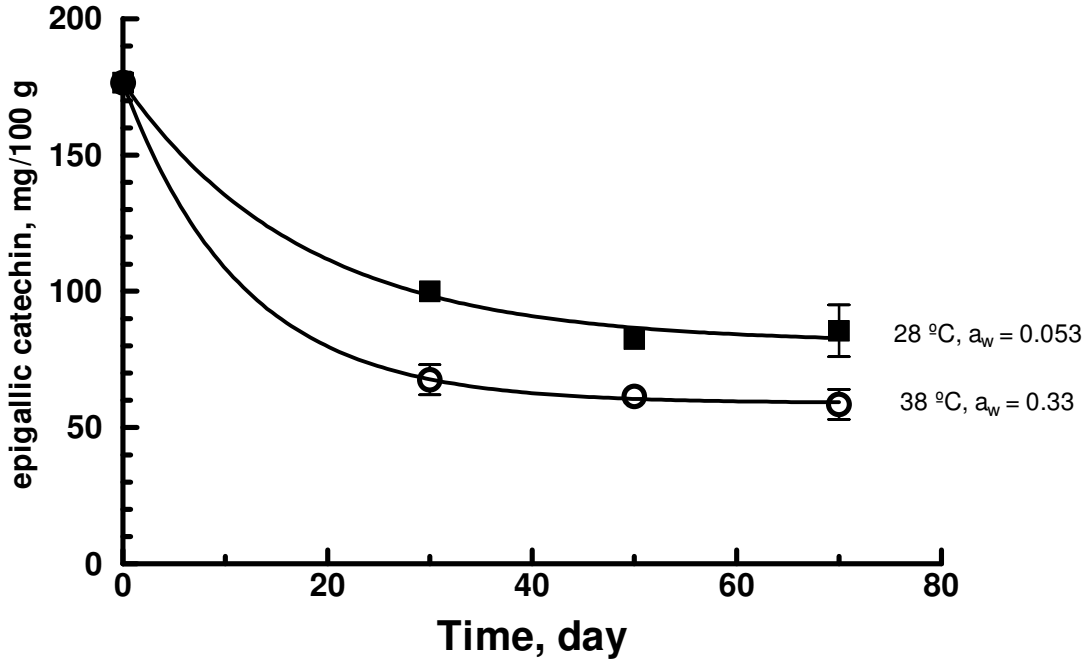
200
150
100
50
0
epigallic catechin, mg/100 g
0
20
40
60
80
Time, day
28 ºC, a_w = 0.053
38 ºC, a_w = 0.33

Figure 2.

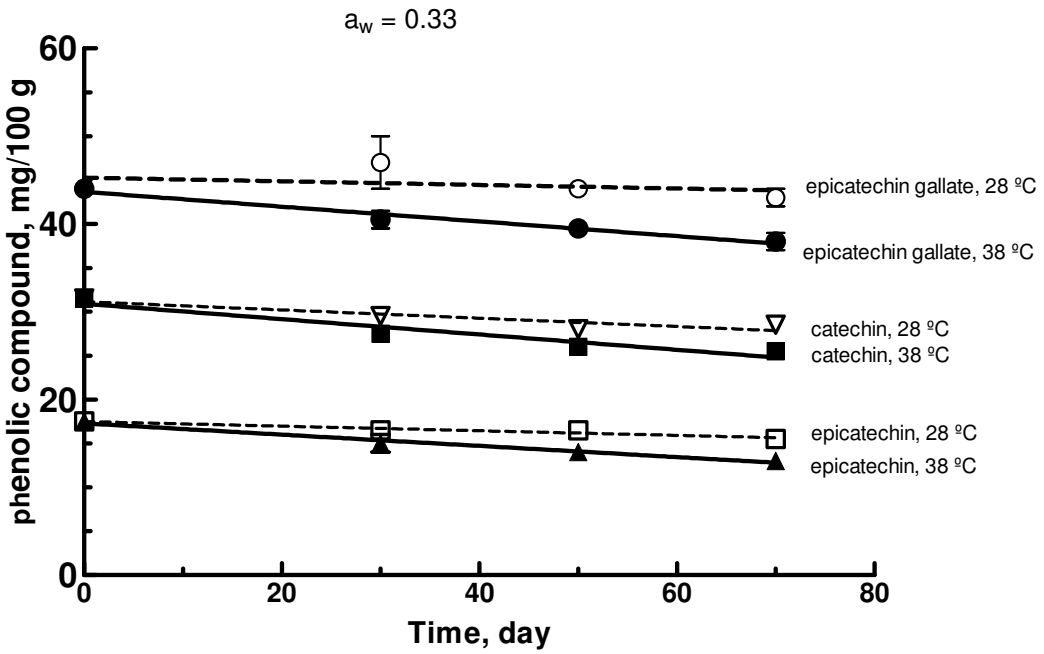
a_w = 0.33
phenolic compound, mg/100 g
Time, day
0
20
40
60
80
epicatechin gallate, 28 ºC
epicatechin gallate, 38 ºC
catechin, 28 ºC
catechin, 38 ºC
epicatechin, 28 ºC
epicatechin, 38 ºC

Figure 3.

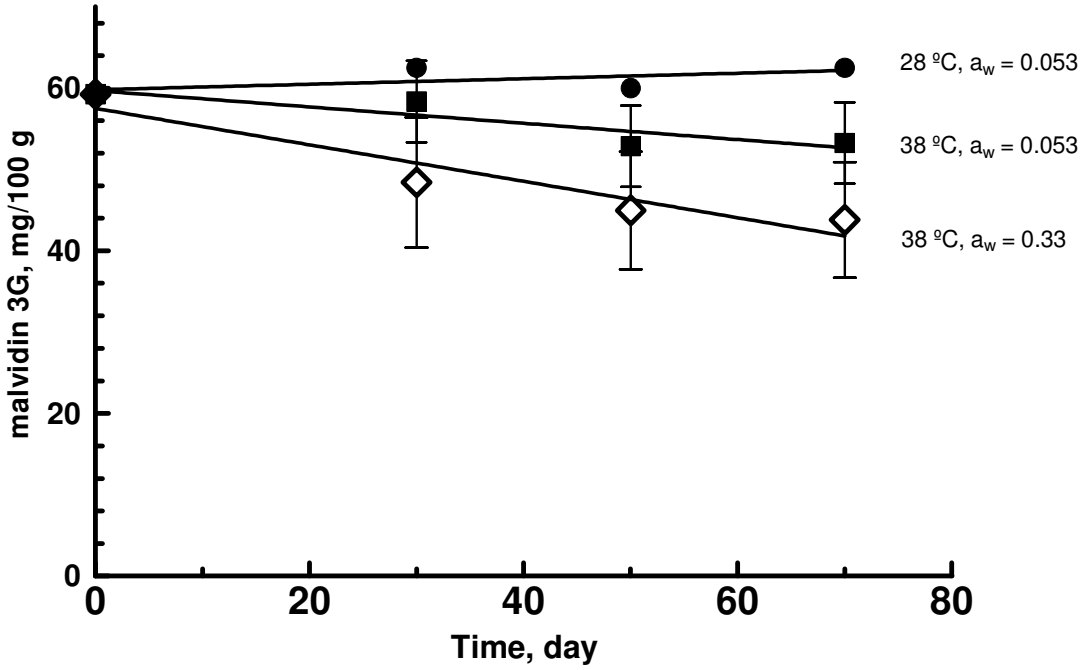
malvidin 3G, mg/100 g
Time, day
0
20
40
60
80
28 ºC, a_w = 0.053
38 ºC, a_w = 0.053
38 ºC, a_w = 0.33

Figure 4

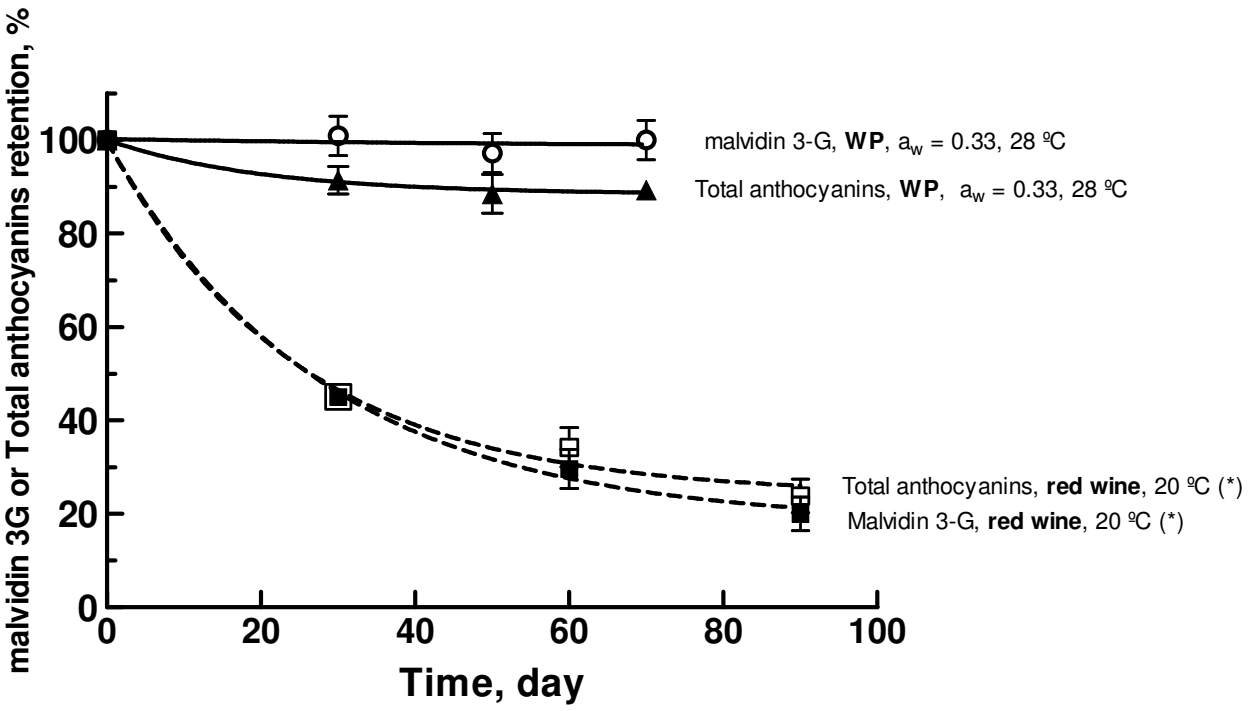
malvidin 3G or Total anthocyanins retention, %
100
80
60
40
20
0
0
20
40
60
80
100
Time, day
malvidin 3-G, **WP**, a_w = 0.33, 28 ºC
Total anthocyanins, **WP**, a_w = 0.33, 28 ºC
Total anthocyanins, **red wine**, 20 ºC (*)
Malvidin 3-G, **red wine**, 20 ºC (*)

Figure 5

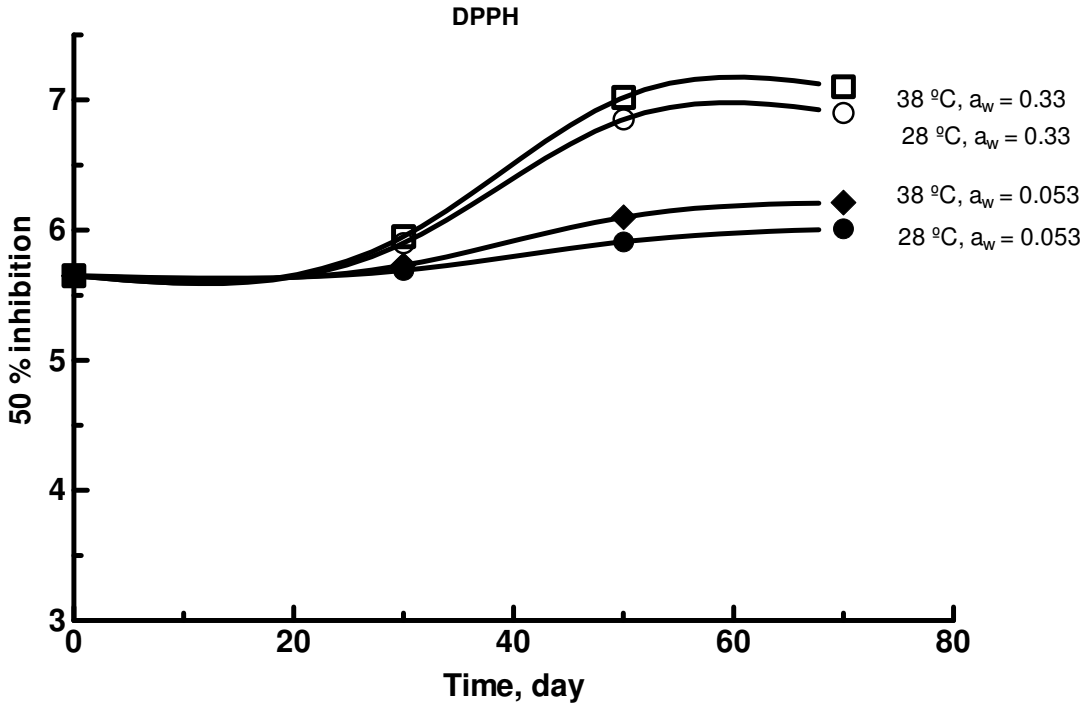
DPPH
50 % inhibition
Time, day
38 ºC, a_w = 0.33
28 ºC, a_w = 0.33
38 ºC, a_w = 0.053
28 ºC, a_w = 0.053
3
4
5
6
7
0
20
40
60
80

Figure 6.

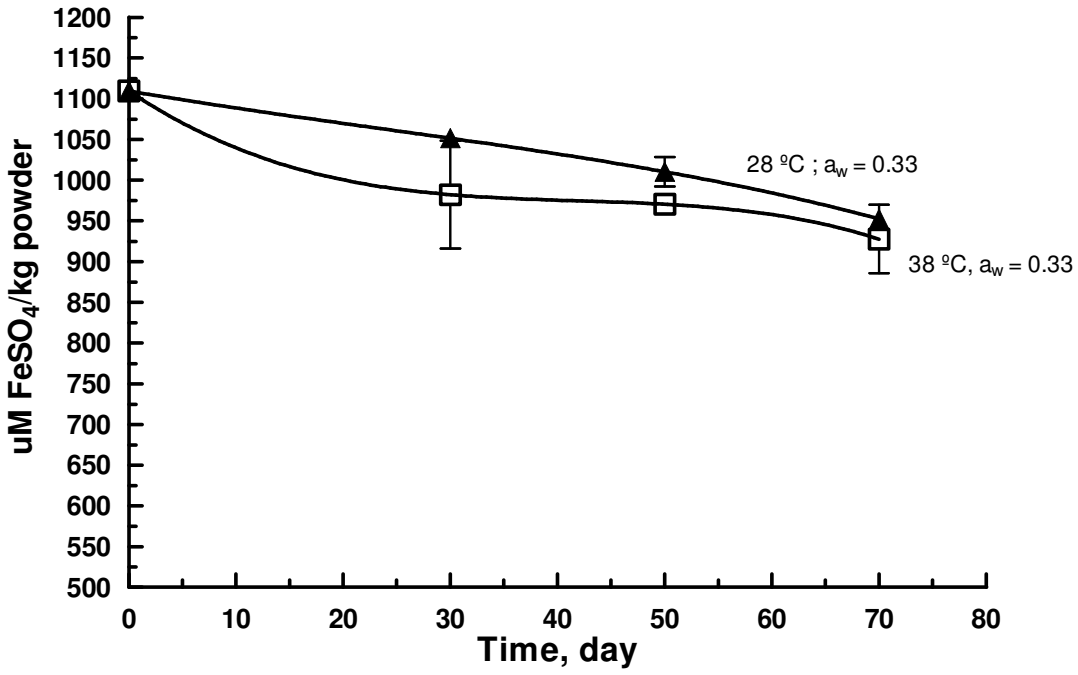
1200
1150
1100
1050
1000
950
900
850
800
750
700
650
600
550
500
uM FeSO4/kg powder
0
10
20
30
40
50
60
70
80
Time, day
28 ºC ; aw = 0.33
38 ºC, aw = 0.33

Figure 7.