"Influence of storage conditions on phenolic compounds stability, antioxidant capacity and colour of freeze-dried encapsulated red wine"

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"Influence of storage conditions on phenolic compounds stability, antioxidant capacity and colour of freeze-dried encapsulated red wine"

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ARTICLE INFO

ABSTRACT

A concentration of 9% (w/w) maltodextrin (DE 10) and gum arabic was added to red wine *C. sauvignon* and freeze dried to obtain a dealcoholized wine powder having a polyphenols concentration 7.1 times higher than in liquid red wine. Malvidin 3-G and total anthocyanins were the phenolics showing greater losses during storage. Moreover, an increase of water activity from 0.11 to 0.58 greatly enhanced the losses. The decrease in malvidin 3-G content was associated with the decrease on redness (colour parameter a*) of wine powder. Gallic acid was the most stable phenolic and its content remained constant during storage at all water activity levels under investigation. Contents of epicatechine, catechine, caffeic acid and resveratrol remained constant at $a_w = 0.11$, although at $a_w = 0.33$ catechine and epicatechine suffered important losses. Results indicated that water activity was a key factor affecting phenolics stability during storage.

Antioxidant activity of the wine powder remained constant over 145 days at accelerated storage conditions.

1. Introduction

Epidemiological evidence indicates that moderate consumption of red wine reduces the incidences of coronary heart disease, atherosclerosis and platelet aggregation (Li et al., 2009; Tedesco et al., 2000). Wine is an important component in Mediterranean dietary tradition because it is very rich in antioxidant compounds. This protection is mainly attributed to the phenolic components of wines, which are particularly abundant in the red wine.

The polyphenolic contents of wine consist in two classes of components (flavonoids and non-flavonoids) and depend on a variety of factors such as the grape variety, vineyard location, climate, soil type, harvesting time, production process, etc. Although the mechanisms of action are yet to be fully understood, it is generally accepted that phenolic compounds behave as antioxidants. They can protect cholesterol in the low-density lipoprotein (LDL) from oxidation (Brouillard et al., 1997). However, there are some clear drawbacks in wine consumption associated with the ingestion of alcohol: a) consumption must be moderate (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). This was resolved by Sanchez, Baeza, Galmarini, Zamora, and Chirife (2013), who reported preliminary results on the freeze drying encapsulation of red wine with maltodextrin (20% w/w) obtaining an alcohol-free powder. Water and almost all alcohol from wine were removed during freeze drying and the use of maltodextrin as a drying aid led to an amorphous, glassy microstructure in which the wine phenolics - as well as other components of dry wine extract (glycerol, sugars, organic acids, salts, etc) were encapsulated.

Munin and Edwards-Lévy (2011) noted the lack of long term storage stability of polyphenols since are usually very sensitive to light and heat, and encapsulation appears to be a promising

approach to gain stability. Encapsulation by freeze-drying can be used in the food industry to protect the nutraceuticals by preventing the oxidation, reducing the losses of volatile substances, making handling easier, facilitating or making more difficult the premature interaction with other ingredients, and regulating food bioactive content during its industrialization processes (Deyse Gurak, Correa, & Rocha-Leão, 2013). Numerous wall materials or encapsulating agents are available for use in food. The ideal encapsulant should have film-forming properties, have emulsifying properties, be biodegradable, be resistant to the gastrointestinal tract, have low viscosity at high solids contents, exhibit low hygroscopicity and have a low cost. The hydrocolloids, such as maltodextrin and gum arabic, are among the wall materials, most commonly used in the fruit juice encapsulation process using spray drying or freeze drying techniques. These carrier agents, and also blends of maltodextrin and gum arabic also protect adequately the fruit's bioactive compounds (i.e. anthocyanins) from oxidation (Ferrari, Marconi Germer, Alvim, & de Aguirre, 2013; Tonon, Brabet, & Hubinger, 2010). Recently, Mahdavi, Jafari, Ghorbani, and Assadpoor (2014) reviewed microencapsulation of anthocyanins with different biopolymers through spray drying to develop natural colorant pigments which possess high stability, solubility, and dispersibility. They noted that biopolymer used for microencapsulation by spray drying is very important for encapsulation efficiency and microcapsule stability and indicated that typical biopolymers generally suitable for spray-drying microencapsulation of anthocyanins include gum arabic and maltodextrin, among others.

Besides of fruit juices, drying encapsulation may be also used to produce powdered pigments obtaines from winewastes products, although this requires a previous solvent extraction of polyphenols. De Souza, Thomazini, Balieiro, and Favaro-Trindade (2015) produced and evaluated powdered pigments obtained from vinification by-products of Bordo red grapes (*Vitis labrusca*). The concentrated extract obtained from the by-products was spray dried (and also freeze-dried) under different conditions of inlet air temperatures (130–170 °C) and carrier (maltodextrin) concentration (10–30%). Galmarini et al. (2013) reported storage stability of several phenolics in a red wine powder encapsulated (freeze drying) in maltodextrin (20% w/w).

The objective of the present work was to study the stability of red wine phenolics encapsulated in 9% (w/w) of maltodextrin and gum arabic mixture. The total concentration of encapsulating agents added to red wine was considerably less than previously used by Sanchez, Baeza, Galmarini, Zamora, and Chirife (2013) who added 20% (w/w) of maltodextrin alone as carrier material. A lower addition of encapsulants (while protecting the bioactive compounds) bring about benefits for the development of healthy drink powders because increasing concentration of wine polyphenols, and improved sensory profile as showed recently by Parra, Galmarini, Chirife, and Zamora (2015). Present study included the effect of water activity (a_w) on the content of various phenolics, colour and antioxidant capacity of the encapsulated red wine powder (EWP) stored at 38 °C during several months.

2. Materials and methods

2.1. Materials

Wine used was a commercial Cabernet Sauvignon, "Postales del Fin del Mundo" from Neuquén province, Argentina. Its alcohol content was 13.7% (v/v) and pH 3.6. Total polyphenol content was 2230 ± 160 mg GAE/L as determined Folin– Ciocalteau method (see below). Carbohydrates used for encapsulation were a mixture of Maltodextrin (Dextrose Equivalent 10 (MD_{10}) from Productos de

Maíz, S.A., Argentina) and gum arabic (from Gelfix, Buenos Aires, Argentina).

Salts (reagent grade) used for relative humidity (RH %) control were: Potassium acetate (CH_3COOK), magnesium chloride ($MgCl_2$), potassium carbonate (K_2CO_3), and sodium bromide (NaBr); they were purchased from Biopack, Argentina.

The Folin–Ciocalteau reagent was obtained from Merck KgaA Darmstadt, Germany. The 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH*), β-Carotene, Linolenic acid ≥ 99%, TWEEN® 20 (Polyethylene glycol sorbitan monolaurate) and chloroform used for antioxidant quantification were purchased from Sigma Aldrich, St. Louis, USA. HPLC-grade reagents malvidin-3-glucoside chloride (malvidin 3-G), catechine, epicatechine, caffeic acid, gallic acid, and resveratrol (all of them > 95% purity) were purchased from Sigma Aldrich, St. Louis, USA; Solvents used were: acetonitrile (ACN), formic acid (FA), methanol (MOH), and Hydrochloric acid (HCL). All were obtained from J.T. Baker, USA, Cicarelli, Argentina and Carlo Herba, Spain. Double-distilled water (HPLC grade) was elaborated at a facility of the University.

2.2. Encapsulation procedure

A blend (65:35) of MD_{10} and gum arabic was dissolved in red wine at a ratio of 9% concentration (total weight basis). The mixture was then freeze-dried to encapsulate the dry extract of wine (containing its polyphenols). The wine with carrier agents was poured into an aluminium tray (depth of sample, 1 cm) and frozen at −20 °C during 24 h. The freeze-dried process was performed using a laboratory-scale FIC-LI-I-E300-CRT freeze dryer (Rificor, Buenos Aires, Argentina) operated with a freezing plate and condenser at −40 °C and a vacuum of 100 μm Hg during 40 h and at room temperature. Freeze dried samples had a water activity (a_w) of 0.11; it was measured using a dew point hygrometer "Aqualab" (Decagon Devices USA). Ethanol from wine as well as water were eliminated during freeze-drying, leading to a dealcoholized EWP. The freeze-dried product - a porous cake of glassy aspect -was milled in a domestic grain coffee grinder leading to a free-flowing powder which was stored in hermetic, dark glass flasks for further analysis.

2.3. Storage conditions

EWP in small opaque glass flasks was stored in a constant temperature oven kept at 38 °C in one or the other of the following conditions, a) in hermetically sealed flasks in order to preserve its initial moisture condition ($a_w = 0.11$); b) in open flasks placed over a saturated solution of $MgCl$ which provided a constant relative humidity of 33%, and c) in open flasks placed over a saturated solution of NaBr which provided a constant relative humidity of 58%. Temperature 38 °C is representative of accelerated shelf life studies; thus storing at 38 °C and water activity 0.33 are called hereinafter "accelerated storage conditions".

Samples of all systems were periodically removed from storage and analyzed during 145 days.

2.4. Adsorption isotherm

Equilibrium moisture contents of wine powder were determined using the well known static gravimetric method (Iglesias & Chirife, 1982). Samples of EWP were placed in desiccators containing four different saturated salt solutions which provided the following relative humidities), CH_3COOK (22%RH), $MgCl_2$ (33%RH)), K_2CO_3 (RH 43%) and NaBr (58% RH). Desiccators were placed in an oven at 38 °C and after an equilibrium time of three weeks moisture content of samples was determined gravimetrically using 2 g
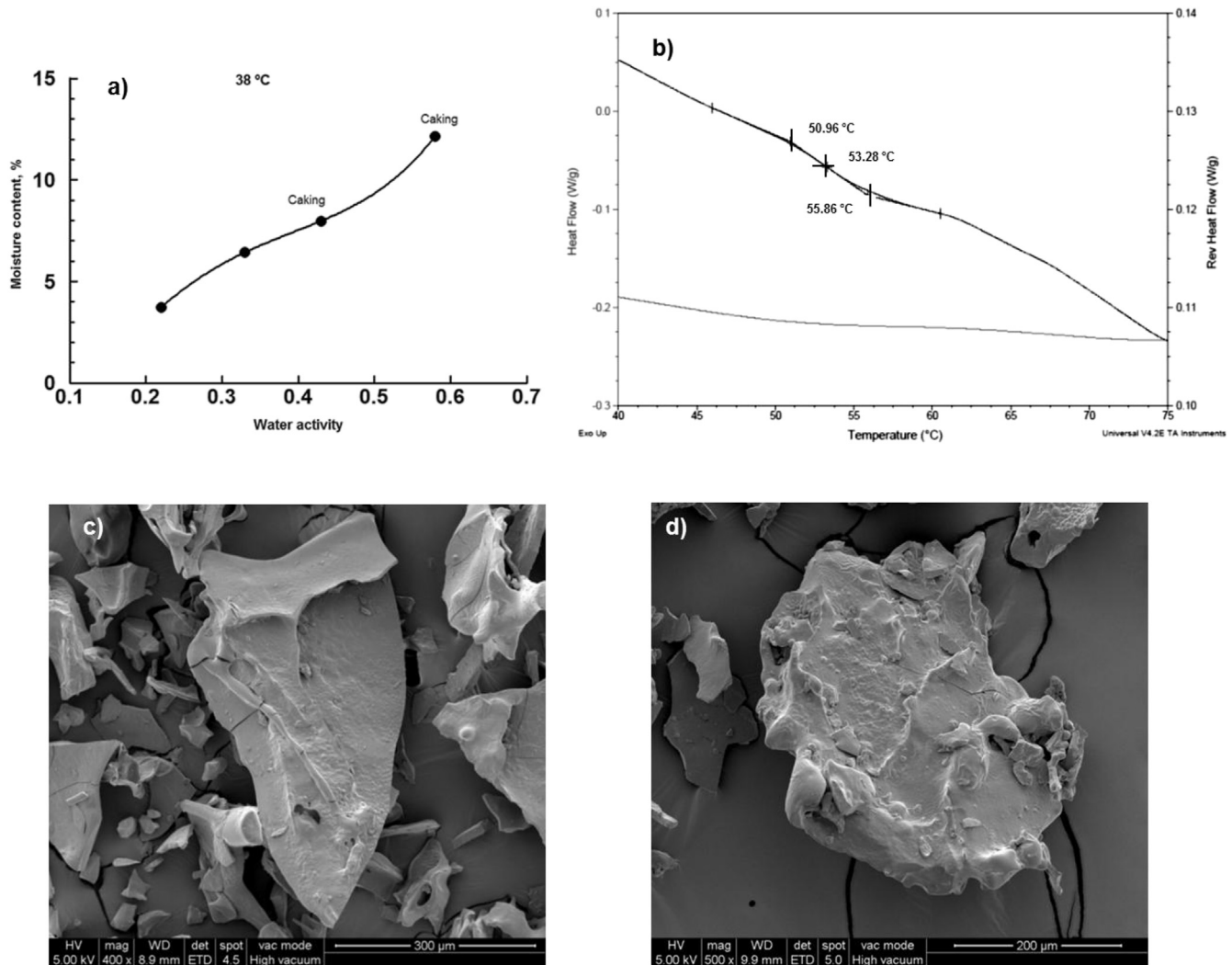
Fig. 1. Physical characteristics of EWP; a) adsorption isotherm (38 °C); b) DSC thermogram (at 0.33 a_w); SEM Micrographs before (c) and after (d) 145 days at accelerated storage conditions.

sample dried in an air-circulating oven at 90 °C during 15 h.

2.5. Differential scanning calorimetry (DSC)

The glass transition temperature (Tg) of the EWP was determined using a differential scanning calorimeter (model DSC 2010, TA Instruments, New Castle, DE, USA). Approximately 4–5 mg of powder equilibrated with $MgCl_2$ saturated solution (33% RH) was placed into aluminium pans (20 μl). The equipment was calibrated with sapphire (600 °C). The samples were scanned from −30 to 100 °C at a rate of 10 °C/min. An empty pan was used as a reference. The onset values for glass transition temperature of the samples were calculated using the software Universal Analysis (TA instruments).

2.6. Scanning electron microscopy (SEM)

Scanning Electron Microscopy, morphological analysis was performed by SEM using a FEI, Quanta 200 microscope (Netherlands). The samples were placed in a carbon support and coated with a layer of gold (40–50 nm) and examined using an acceleration voltage of 5 kV. Three samples of EWP were observed: powder before and after 145 days at accelerated storage conditions

and powder after storage at 38 °C and 0.58 a_w.

2.7. Colour

Colour measurements was analyzed using a Minolta Spectrophotometer CM-600d (Konica Minolta Observer), with D65 illuminant and an observer angle of 2°. The colour measurement was obtained by placing samples of EWP in plastic white containers. CIELab parameters (CIE 1976, L* a* b*) were L* for lightness, a* for redness and b* for yellowness.

2.8. HPLC analysis of polyphenols

Samples were prepared by weighting 60.0 ± 0.5 mg of EWP which was dissolved in 1.5 ml of solvent composed of a mixture of H_2O/MOH/HCl (89/10/1) (Souquet, Veran, Mané, & Cheynier, 2006). Prior to injection they were filtered through a Whatman PTFE syringe filter (diameter = 13 mm, porosity = 0.45 μm). The injection volume was 20 μl.

HPLC analysis was carried out in a 1200 series HPLC instrument (Agilent Technologies, Waldbronn, Germany) equipped with a vacuum degasser, a quaternary pump, an autosampler and a thermostated column compartment.
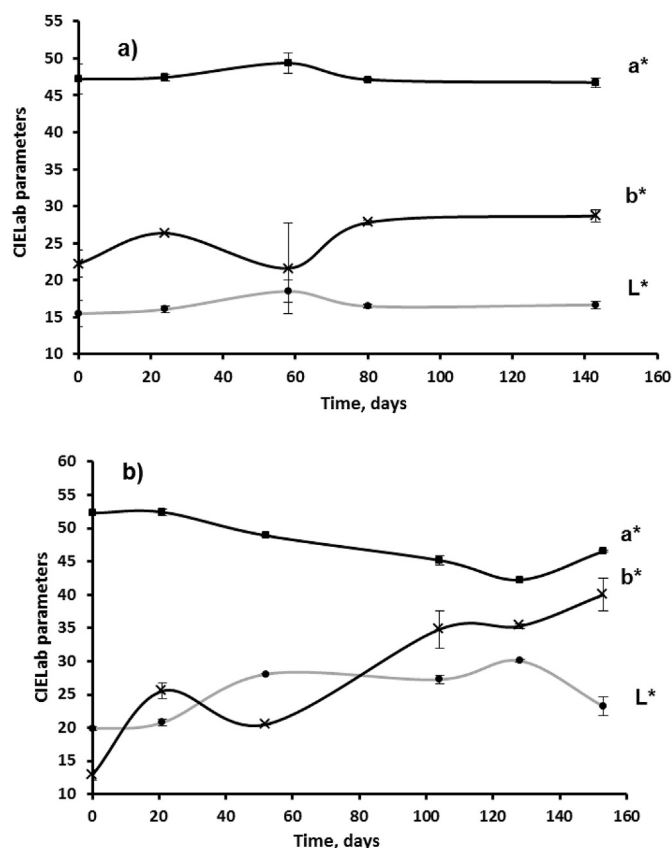
Fig. 2. Evolution of colour parameters (a*, b*, L*) for EWP stored at 38 °C up to 145 days: a) 0.11 a_w; b) 0.33 a_w.- In many cases SD bars overlap with data points.

Separation was achieved on a reverse phase using a size 150 × 4.60 mm column size, Phenomenex Gemini 5 μ C18, 110 ˚A. The column's temperature was maintained at 30 °C. Detection was performed using 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 ACN/H$_2$O/FA (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 following Galmarini et al. (2013) method, with some modifications: 0–3 min, 3%; 3–5 min, 7%; 5–10 min, 10%; 10–12 min, 14%; 12–20 min, 15%; 20–23 min, 20%; 23–32 min, 25%; 32–34 min, 40%; 34–39 min, 40%; 39–41 min, 20%; and 41–45 min, 3%. Malvidin 3-G, catechine, epicatechine, caffeic acid, gallic acid and resveratrol were identified according to their retention time and spectral properties. Absorption wavelengths (λ) at which each analyte was measured, were: 280 nm (catechine, epicatechine and gallic acid); 320 nm (caffeic acid and resveratrol) and 520 nm (malvidin 3-G). Quantification was done by external standard curves of authentic standards of each compound. The compounds were expressed as mg compound/100 g powder. Total anthocyanins were also analyzed by absorption at 520 nm and expressed as mg malvidin3-G equivalent/100 g powder.

2.9. Total polyphenols

Total polyphenols of red wine and EWP were determined by the Folin− Ciocalteau method (Camussoni & Carnevali, 2004). Concentrations were expressed as milligrams gallic acid equivalent/100 g powder. EWP was dissolved in water (1 g of powder in 8 g of water); then absorbance at 765 nm was measured and polyphenol concentrations of samples derived from a standard curve of gallic acid.

2.10. Antioxidant capacity

Changes in the antioxidant capacity along storage of the EWP was analyzed using two independent methods: free radical scavenging capacity of the DPPH* (2,2-diphenyl-1-picryl- hydrazyl) (Stratil, Klejdus, & Kuban, 2006) and B-Carotene/linoleic system (Lu & Yeap Foo, 2000). For both methods 1 g of freeze dried powder was dissolved in 8 ml of distilled water. Five dilutions were done in water in order to obtain solutions of EWP within the desired range of linearity of both methods.

2.10.1. DPPH*

An aliquot of 100 μl of EWP dissolved was mixed with 3.9 ml of DPPH* ethanol solution (25 mg DPPH*/l). Absorbance was determined at 517 nm after 60 min in darkness. Antioxidant activity was expressed as mM of gallic acid equivalents necessary to inhibit 50% of DPPH* (EC50).

2.10.2. β-Carotene/linoleic acid assay

One millilitre of a solution of β-carotene in chloroform (3.34 mg/ml) was pipetted into a flask containing 40 mg linoleic acid and 400 mg Tween 20. The chloroform was removed by rotary evaporation at 40 °C for 5 min and, to the residue, 100 ml of distilled water was added slowly with vigorous agitation, to form an emulsion. A 5 ml aliquot of the emulsion was added to a tube containing 0.2 ml of EWP dissolved and the absorbance was measured at 470 nm, immediately, against a blank, consisting of the emulsion without b-carotene. The tubes were placed in a water bath at 40 °C and the absorbance measurements were made again at 120 min.

The result was expressed as β-carotene inhibition of oxidation Index (AI %).

2.11. Data analysis

All experiments were conducted in triplicate and the results were analyzed by one-way analysis of variance (ANOVA) test using Infostat v.2013 (Universidad Nacional de Córdoba, Argentina). Means comparisons among storage time were carried out by Tukey test at $P < 0.05$.

3. Results and discussion

The EWP obtained after freeze-drying was a free-flowing powder having $a_w = 0.11$. Total polyphenol content was 1583 ± 98 mg GAE/100 g powder which is 7.1 times higher than in liquid wine.

Fig. 1 shows **(a)** adsorption isotherm of EWP at 38 °C for a selected range of water activity (0.22−0.58). After an equilibrium time of three weeks, powder caking was observed at $a_w = 0.43$ and 0.58, but not at $a_w = 0,33$ and below. This behaviour may be explained considering that physical changes in an amorphous matrix are time dependent being a function of (T-T$_g$), where T is the storage temperature and T$_g$ is the glass transition temperature (Roos & Karel, 1991). Fig. 1. (b) shows the DSC thermogram at water activity of 0.33; a glass transition seems to be apparent in the thermogram. Furthermore, the onset, midpoint and end point glass transition temperature are indicated (Roos, 1995). The onset glass transition temperature (50.96 °C) was taken as representative of the glass transition temperature of EWP at $a_w = 0.33$. Since this value is higher than storage temperature (38 °C), the absence of
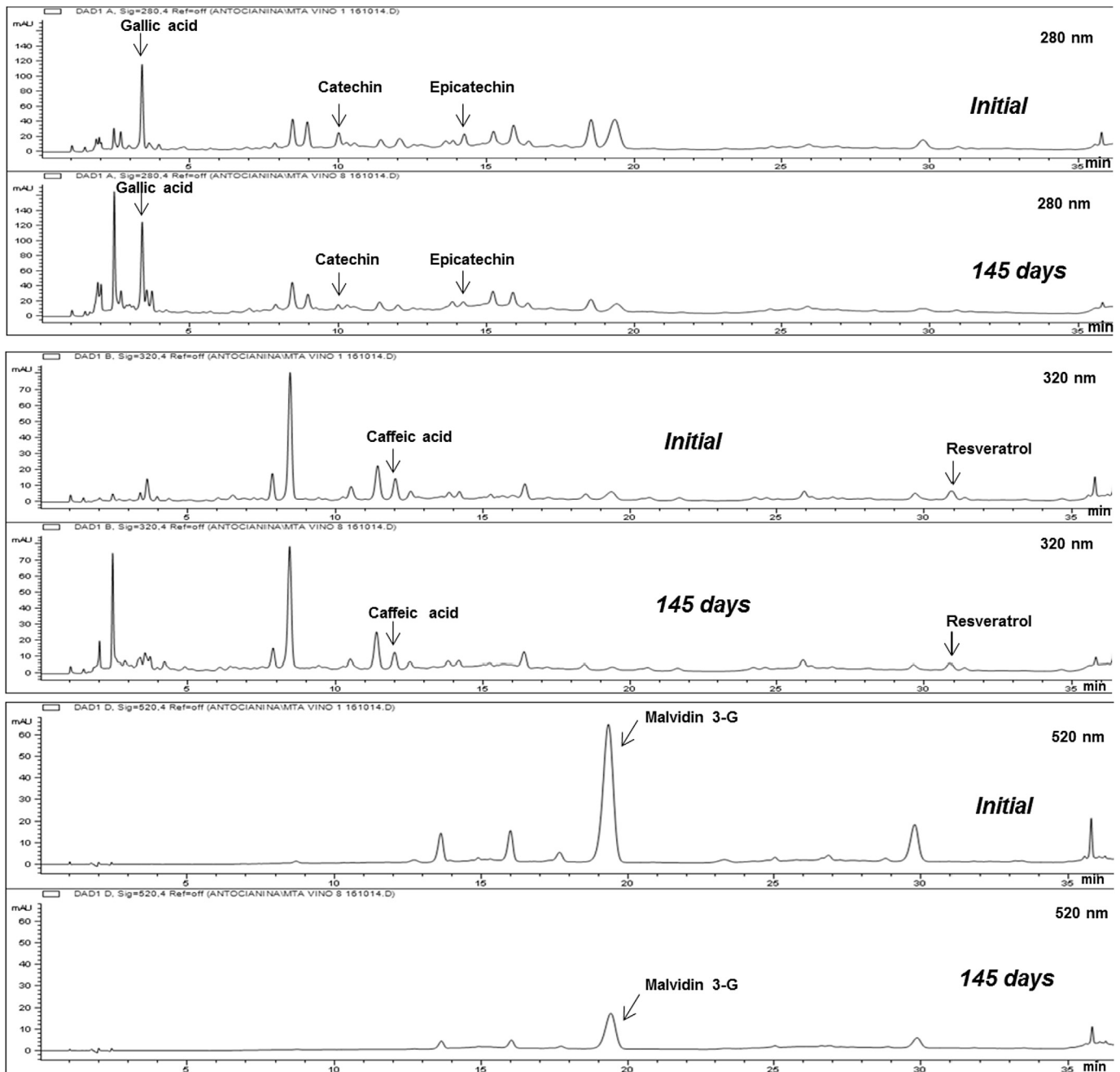
Fig. 3. Comparison of HPLC chromatogram of EWP before and after 145 days at accelerated storage conditions − Selected phenolics peaks are indicated.

caking at $a_w = 0,33$ and below (as observed in the isotherm) may be attributed to the presence of a glassy state (Roos & Karel, 1991). Fig. 1 (c,d) shows micrographs (scanning electron microscope) of EWP before and after storage at 38 °C and $a_w = 0.33$. The powder **(c)** shows irregular plate shaped particles of different sizes because they were ground after freeze drying. Similar results were reported by Deyse Gurak et al. (2013) for grape juice freeze dried encapsulated with maltodextrin and gum arabic. After 4.5 months of storage at 38 °C **(d)** some shrivelling of the surfaces plate shaped particles is noted.

Fig. 2 shows the changes in all colour parameters: a* (redness), b* (yellowness), and L* (lightness), values for EWP stored at 38 °C and two different water activities (0.11 and 0.33) over 145 days

storage. Overall, it can be observed that at $a_w = 0.11$ all colour parameters remained approximately constant and this was confirmed by an ANOVA test performed over the data ($p \leq 0.05$). At water activity 0.33 some decrease is observed in parameter a* (redness), with an unexpected increase by the end of storage. An increase in b* (yellowness) and L* (lightness) were also noted along the 145 day storage period; and this was again confirmed by an ANOVA test. Mazza and Francis (1995) reported that during 42 °C storage of red wine, redness decreased but yellowness increased due to the higher concentration of chalcone; this observation is in agreement with measured increase of parameter b* during accelerated storage of EWP.

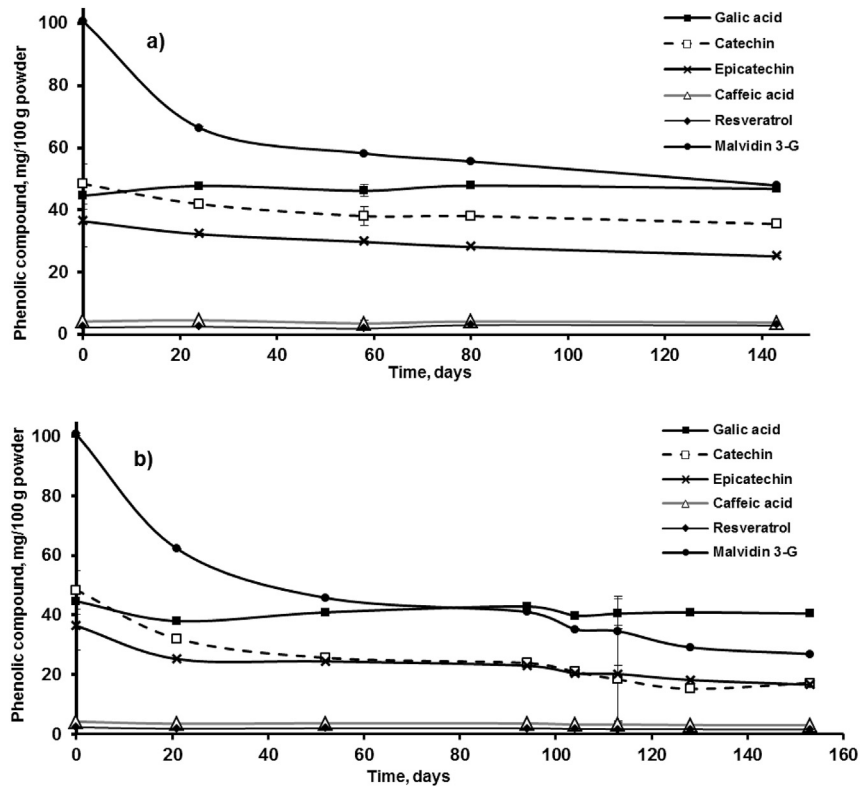In a low moisture system (EWP), a_w (or moisture content) is a

Fig. 4. Evolution of selected phenolics in EWP stored at 38 °C and different water activities. a) 0.11 a_w; b) 0.33 a_w. − In many cases SD bars overlap with data points.
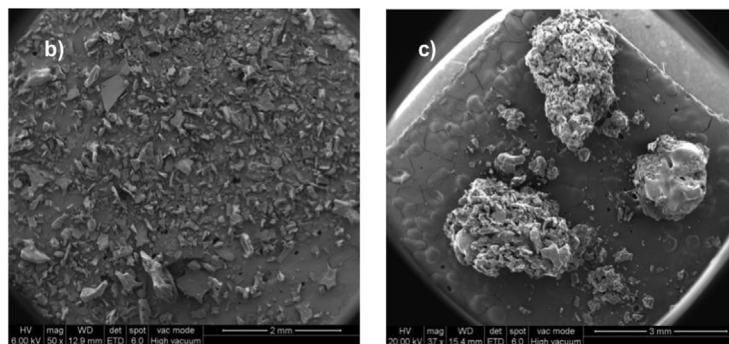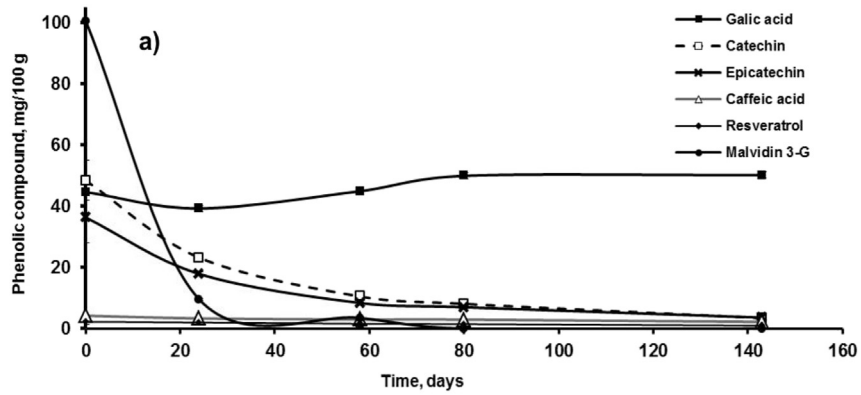

Fig. 5. a) Evolution of selected phenolics during storage of EWP at $a_w = 0.58$ (In many cases SD bars overlap with data points). b) SEM micrograph of EWP before storage c) SEM micrograph of caked wine powder after storage at 38 °C and 0.58 a_w.
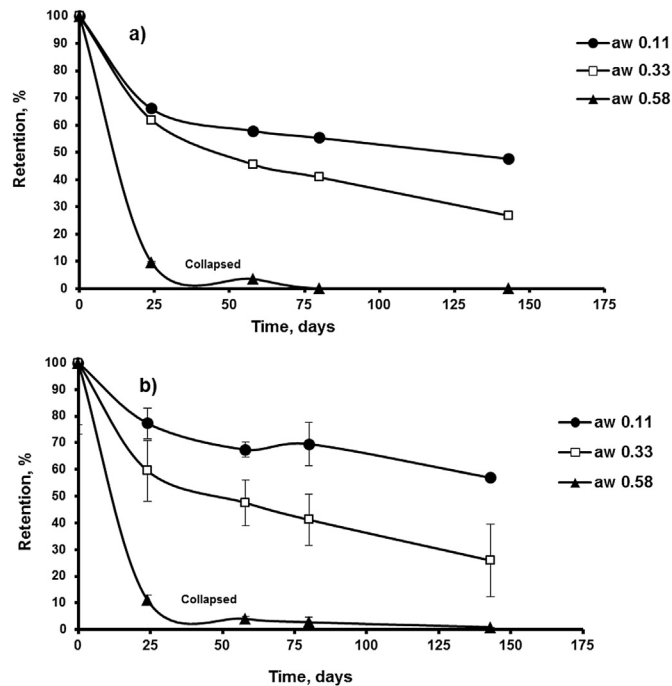
Fig. 6. Effect of water activity on malvidin 3-G (a) and Total anthocyanins (b) during storage at 38 °C of EWP. − In many cases SD bars overlap with data points.

key factor affecting chemical (and physical) stability. Since water acts as a plasticizer accelerating (or decreasing) chemical reactions by influencing molecular mobility (Buera, Welti-Chanes, Lillford, & Corti, 2006; Roos, 1995).

Fig. 3 shows the HPLC chromatogram of EWP before and after 145 days at accelerated storage conditions; the peaks of selected phenolic compounds are indicated with arrows on the figure. A qualitative overall view of these peaks anticipates that malvidin 3-G and catechin are the less stable of the selected phenolics. It is to be noted that at 320 nm and 280 nm some unidentified peaks appeared in the chromatogram and this will be discussed later in the manuscript.

Fig. 4 (a,b) shows the changes in phenolic contents in EWP stored at 38 °C and two different water activities (0.11 and 0.33) over 145 days storage. As indicated by an ANOVA test performed over the data (p ≤ 0.05). Gallic acid, catechine, epicatechine, caffeic acid and resveratrol remained approximately constant throughout storage period at a_w 0.11. On the contrary, malvidin 3-G showed an important initial decrease followed by a slower one. At a higher a_w = 0,33 only gallic acid remained constant. Catechine and epicatechine exhibited an initial decrease but then remained constant up to the end of storage (145 days); their final losses were 65 and 54% respectively. For the same storage conditions, losses of caffeic acid and resveratrol were 29 and 31% respectively. Malvidin 3-G showed the largest decrease amounting to about 70% of its initial value. It is interesting to note that the decrease in malvidin 3-G content is associated with the decrease in redness (colour parameter a*) previously noted in Fig. 2. This behaviour was confirmed by Pearson positive correlation 0.80 (p ≤ 0.05). Sanchez, Baeza, and Chirife (2015) also reported that loss of monomeric anthocyanin was associated with a decrease in redness colour (parameter a*) in stored encapsulated cherry juice.

Overall, the stability behaviour of selected phenolics is similar to that reported by Galmarini et al. (2013) for same phenolics in red wine but encapsulated with 20% of maltodextrin instead of 9% of maltodextrin + gum arabic, as used in the present work. They

reported stability data for similar values of water activity and temperature, but during a shorter storage period (70 days). Other authors also reported data on stability of phenolics in low-moisture food powders. For example, Tandale (2007) used whey protein concentrate as carrier material and stored freeze-dried encapsulated gallic acid at 25 °C. It was found to have very good retention (above 90%) at aw = 0.22 and 0.44 after 56 days storage. Tonon et al. (2010) studied anthocyanin stability of spray-dried açai juice produced with different carriers and found that temperature negatively influenced anthocyanin stability 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.

Fig. 5(a) shows the evolution of selected phenolics during storage of EWP at a_w = 0.58 and 38 °C. As mentioned before, at this combination of water activity and temperature the amorphous structure collapsed and caking was observed (see Fig. 5 b,c). This was reflected in a dramatic loss of malvidin 3-G as well as losses of the other phenolics with the exception of gallic acid which remained constant.

Fig. 6 compiles the effect of water activity on the % retention of malvidin 3-G and total anthocyanins in EWP stored at 38 °C. Increasing the water activity from 0.11 to 0.58 strongly affected the retention of these phenolics and stress the importance of water activity as a control parameter for anthocyanins stability during storage. It is to be noted that the behaviour of malvidin 3-G and total anthocyanins is similar because malvidin 3-G is the main phenolic compound of total anthocyanins in red wine (see Fig. 3.).

The **"antioxidant power"** of a food is an expression of its capability both to defend the human organism from the action of the free radicals and to prevent degenerative disorders deriving from persistent oxidative stress (Di Majo, La Guardia, Giammanco, La Neve, & Giammanco, 2008). Thus, one of the important characteristics of polyphenolic compounds is their antiradical property.

Fig. 7 shows the evolution of Total Polyphenols **(a)**, Antioxidant capacity determined with chromogen radical DPPH* **(b)** and Antioxidant capacity determined by the β -Carotene/Linoleic acid assay **(c)**, for EWP at accelerated storage conditions Total polyphenols, antioxidant capacity determined with chromogen radical DPPH* and antioxidant capacity determined by β-Carotene/Linoleic acid assay remained approximately constant during storage, as determined by ANOVA test performed on the data shown in Fig. 7 a, b,c.

A good correlation between the antioxidant activities (determined by several methods) and total phenol content (Folin Method) has been observed for red wines (Büyüktuncel, Porgalı, & Çolak, 2014; Stratil, Kubáň, & Fojtová, 2008). However, the relationship between antioxidant capacity and specific phenolic compounds was unclear. Di Majo et al. (2008) indicated that the wine's antioxidant properties of red wines from Sicilia are influenced differently by each polyphenolic molecule. Van Leeuw, Kevers, Pincemail, Defraigne, and Dommes (2014) studied several different wines having large variability in the levels of individual phenolic compounds as well as in antioxidant capacity. Comparisons of the different wines based on their individual phenolic profile and antioxidant capacities (ORAC, DPPH, hemolysis, ESR, and total phenols) showed limited differences.

As shown before contents of malvidin 3-G, catechin and epicatechin in EWP had an important decrease after 145 days at accelerated storage conditions; however these losses were not reflected in a change of antioxidant capacity. This lack of correlation between loss of some phenolics and antioxidant capacity has been also reported by others authors in different food systems. Kotseridis, Kallithraka, Kyraleou, Proxenina, and Makris (2013) studied the effect of storage on antioxidant capacity of wine and noted that oxidised phenolics may produce the formation of novel
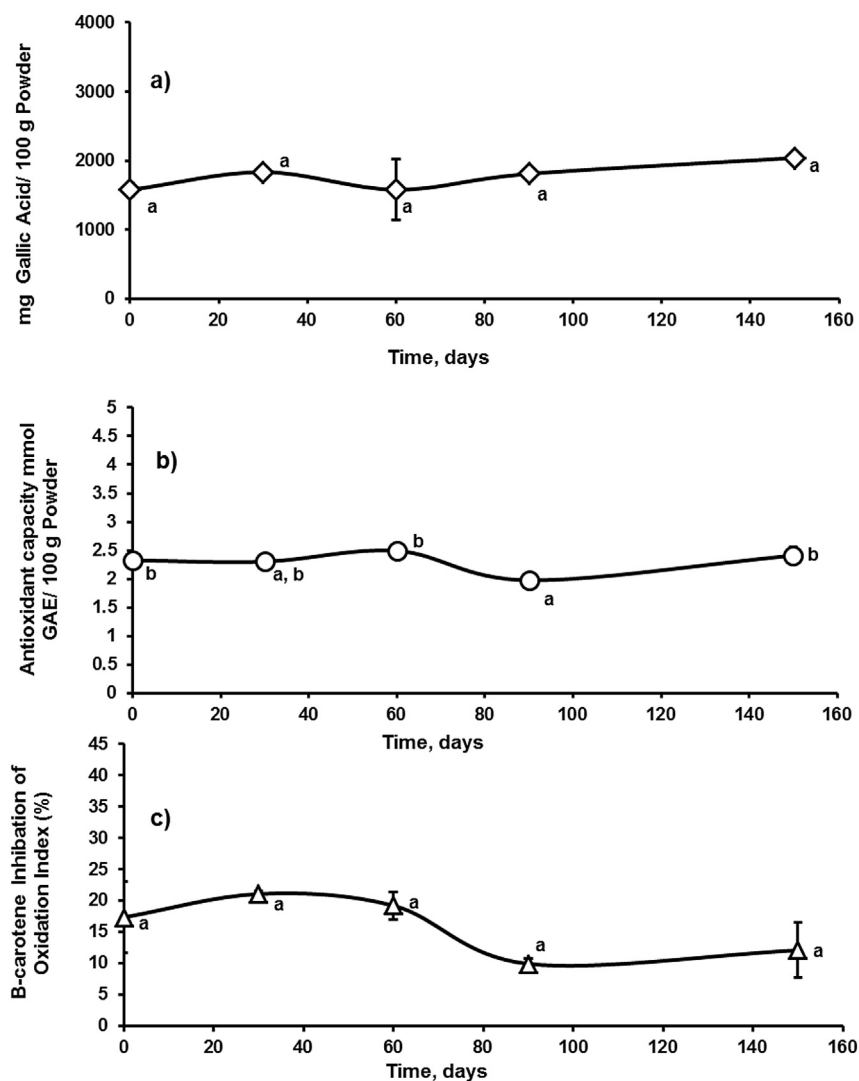
Fig. 7. Evolution of Total Polyphenols (a), Antioxidant capacity (b) DPPH* (c) B-Carotene/Linoleic acid, for EWP at accelerated storage conditions. − In many cases SD bars overlap with data points. Different letters denote statistically significant difference at $p < 0.05$.

antioxidants, and an increase in the wine antioxidant status may be observed. Kallithraka, Salacha, and Tzourou (2009) measured antioxidant activity and phenolics content during storage (9 months at 15 °C) in bottled white wine and measured the concentrations of several phenolics. They found that content of most phenolics diminished with time, but the antioxidant activity increased with storage and stated that although one would expect oxidation of antioxidants to yield a lower antioxidant capacity, reactions between oxidised phenolics may produce formation of new antioxidants. Brownmiller, Howard, and Prior (2008) evaluated the effects of processing and 6 months of storage on total monomeric anthocyanins, percent polymeric colour, and antioxidant capacity of blueberries. Storage at 25 °C resulted in dramatic losses in total anthocyanins, ranging from 62% in berries to 85% in clarified juices. This coincided with marked increases in percent polymeric colour values of these products. However, the antioxidant capacity (ORAC) showed little change during storage, indicating that the formation of polymers compensated for the loss of antioxidant capacity due to anthocyanin degradation.

The observed stability of antioxidant capacity of EWP during storage may be explained by reactions between oxidised phenolic compounds which bring about formation of new antioxidants. As

noted before (Fig. 4.) at 280 nm and 320 nm some unidentified peaks appeared in the chromatograms which were not present in initial EWP sample, but appeared after accelerated storage. Galmarini et al. (2013) also found that antioxidant capacity of red wine encapsulated with maltodextrin alone remained almost constant after 70 days storage on spite of 33% loss of malvidin 3-G and also some losses in catechin and epicatechin.

4. Conclusions

The addition of a 9% mixture (65:35) of the encapsulating agents maltodextrin (DE 10) and gum arabic to red wine C. sauvignon followed by freeze-drying, allowed to obtain a dealcoholized wine powder having a phenolic concentration 7.1 times higher than the original liquid red wine. The glass transition temperature (T_g) permitted the wine powder to remain free flowing avoiding adverse physical changes (i.e. caking) during accelerated storage conditions.

Gallic acid, catechin, epicatechin, caffeic acid and resveratrol remained approximately constant throughout storage period (38 °C) at a_w 0.11; on the contrary malvidin 3-G showed an important initial decrease followed by a slower one.

At a higher a_w = 0,33 only gallic acid remained constant. Catechin and epicatechin exhibited an initial decrease but then remained constant up to the end of accelerated storage (145 days); final losses were 65 and 54% respectively. Losses of caffeic acid and resveratrol were 29 and 31% respectively. Malvidin 3-G showed an important decrease amounting to about 70% of the initial value.

Malvidin 3-G and total anthocyanins were the phenolics that showed greater losses during storage. Increase of water activity from 0.11 to 0.58 enhanced the loss of these phenolics indicating that water activity (or moisture content) is a key factor affecting the stability of these compounds during storage.

Antioxidant activity of the wine powder exhibited a good stability over 145 days at accelerated storage conditions. In spite of some phenolic losses the antioxidant capacity of EWP remained constant and this may be explained by reactions between oxidised phenolic compounds which may bring about formation of new antioxidants.

Due to its high polyphenols content (7.1 times the original wine) the wine powder may be used for polyphenol enrichment of healthy powder drinks; also its encapsulation technique may provide protection to phenolics against conditions such as oxidation.

Acknowledgements

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