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Solid Foodstuff Supplemented with Phenolics from Grape: Antioxidant Properties and Correlation with Phenolic Profiles

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Osmotic dehydration was assessed as an operation for supplementing a solid foodstuff (a gel was used as the model food) with grape phenolics from a concentrated red grape must to increase its antioxidant properties. The model food was processed for up to 24 h, and the osmotic pressure was adjusted by diluting the concentrated red grape must. In all conditions tested, low molecular weight phenolics (≤610 g/mol) and, in particular, *trans*-caftaric acid, *trans*-coutaric acid, ferulic acid, coumaric acid, caffeic acid (hydroxycinnamic acids), gallic acid (hydroxybenzoic acids), quercetin, and rutin (flavonols), were quantified in the red grape must and also in the osmo-dehydrated food. Other flavonoids such as (+)-catechin and (−)epicatechin (flavan-3-ols) were detected only in the red grape must. Trolox equivalent antioxidant activity (TEAC) and ferric reducing antioxidant power (FRAP) were determined in the osmo-dehydrated food. Under the conditions that maximized phenolic infusion, the total phenolic content of the gel was close to the values reported in some rich-in-phenolic fruits and vegetables, whereas TEAC was 3 times that of fresh fruit with the highest antioxidant capacity. Regression analysis showed that the individual phenolics analyzed significantly explain the antioxidant capacity of the osmo-dehydrated food.

KEYWORDS: Antioxidant capacity; osmotic dehydration; phenolics; polyphenols; grape

INTRODUCTION

A high consumption of fruits and vegetables maintains human health and reduces the risk of disease, mainly because they contain phytochemicals that have antioxidant characteristics. Although data produced so far in relation to dietary antioxidants not only support but also challenge the antioxidant hypothesis (*1*), there is still increasing interest in producing novel foods supplemented with dietary antioxidants. Phenolics, plant secondary metabolites, are some of the most abundant antioxidants in fruits and vegetables and their recovery from the byproducts of agricultural industries is a matter of increasing interest (*2*). More specifically, grape is one of the world's largest fruit crops, and the press residues resulting from winemaking (that is, seeds, skins, and highly pressed must) are rich in phenolics and generated in huge amounts (*3*). Two phenolic-rich byproducts can be differentiated: grape pomace, which is made of the solid residues, and concentrated must, which is obtained by evaporation of the highly pressed must.

Phenolics, especially anthocyanins, have been extracted from grape pomace for decades and used as a natural food colorant. Currently, a wide range of grape pomace extracts have been put on the market. However, these extracts are usually a complex mixture of phenolic compounds, and quantitative data about their phenolic content is not available. Recent studies have reported that the antioxidant and antimicrobial properties (*4*) of grape pomace extracts depend on the extraction conditions and the varieties of the raw material (*5*−*7*).

The use of these grape pomace extracts as a source of functional compounds is still incipient, and some applications have been suggested to make confections, fruit fillings, sauces, beverages, and pasta products. However, all of those examples involve the direct addition of the grape pomace extract as an ingredient or additive. No application has been reported in which a fresh solid foodstuff is impregnated.

Osmotic dehydration (OD) is a well-known operation in food technology that enables water to be removed from the product and its functional properties to be modified by impregnation with particular solutes. OD commonly takes place by immersing the product in an aqueous solution with a greater osmotic pressure (i.e., with a relatively high concentration of dissolved substances, mainly sugars and salts). This creates two major simultaneous countercurrent mass transfer fluxes, namely, water outflow from the product to the surrounding solution and solute infusion into the product (*8*). As a result of these two main

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flows, OD has a double effect on solid foodstuffs: it partially removes the water from the food and impregnates the food with the solutes of the osmotic solution.

The main objective of this study is to investigate the possibilities to increase the antioxidant capacity of a solid foodstuff by impregnation with phenolics from grape. Because of its dewatering and impregnating effects, osmotic treatment was considered to be the process of choice, and concentrated red must was used as osmotic solution and as the source of phenolics. To assess the use of OD to supplement a model solid food with antioxidants under standard processing conditions, (i) the penetration level of grape phenolics, (ii) the intake of low molecular weight phenolics from grape must, and (iii) the impact of the increase in phenolics on the antioxidant capacity are determined. On this basis, OD is investigated as the first step in developing products with a potentially high natural antioxidant capacity.

MATERIALS AND METHODS

Osmotic Solution and Model Food. The concentrated red grape must (vars. Bobal, Garnacha, and Tintorera) was supplied by Concentrados Palleja, S.L (Riudoms, Spain). The red grape must had a mass fraction of soluble solids of 65% and a pH of 3.5 and was used as an osmotic solution. Tartaric acid (1 g/L) was added to prevent pH changes.

As a model food an agar−agar gel was prepared with 4% (w/w) agar−agar (Scharlau, Spain), 9.6% (w/w) sucrose, and distilled water. The mixture was heated to 95 °C in a microwave oven until the agar−agar was completely dissolved. Gelation was achieved by cooling at room temperature. The gel was then stored at 6 ± 2 °C prior to use.

Osmotic Dehydration. The experimental setup consisted of two parts: a basket in which the gel samples were placed, and a vessel that was filled with the osmotic solution. The basket contained five shelves and guaranteed total immersion of the sample in the osmotic solution. Agitation was provided by a magnetic stirrer. About 150 g of agar−agar gel cubes (1 cm side) was weighed and placed in the OD basket. The prepared basket was submerged in 2.7 L of osmotic solution. The model food was processed for 1, 2, 4, 8, 12, and 24 h, and the osmotic pressure was adjusted by diluting the concentrated red grape must to 40, 50, and 60% of the mass fraction of soluble solids. A 14:1 solution/gel ratio (w/w) prevented changes in the solution concentration. During the experiment, temperature was maintained at 25 ±2 °C and the setup was covered to minimize the effect of light. After osmotic treatment, the gel cubes were removed from the solution, gently blotted with tissue paper, and weighed. Each experiment was carried out in triplicate. All experiments were run under atmospheric pressure.

Determination of Moisture and Soluble Solids Content. The moisture content of fresh and osmo-dehydrated food was determined with the 934.06 AOAC gravimetric method (*9*). The concentration of soluble solids in osmotic solutions and in osmo-dehydrated food was determined by the 932.14 AOAC refractometric method (*9*).

Extraction of Phenolic Compounds from the Osmo-dehydrated Food. To determine the extent of phenolic impregnation in the gel after osmotic dehydration, a sequential extraction was carried out. A sample of crushed gel (2.5 g) was extracted sequentially with 15 mL of methanol/water (50:50, v/v) and 15 mL of acetone/water (50:50, v/v) solutions, for 1 h in each extraction solvent and at room temperature. Each extraction was carried out in triplicate.

Determination of Total Phenolic Content. The total phenolic content of red grape must and gel extracts was determined with Folin−Ciocalteu's method (*10*). The test sample (1 mL) was mixed with 50 mL of distilled water, 5 mL of Folin−Ciocalteu's reagent, and 20 mL of 20% sodium carbonate solution. After 30 min, the absorbance at 750 nm was recorded. The results were expressed as gallic acid equivalents (mg of GAE/kg on wet basis).

Determination of Individual Phenolics by HPLC. Phenolics were identified and quantified by HPLC (Hewlett-Packard (HP)/Agilent). An automatic injector, HP 1000, was used for the injection. A Supelcosil LC-18 column (25 cm × 4.6 mm), with a particle size of 5 μm and an injection volume of 100 μL was kept at 40 °C. A constant flow rate of 1.5 mL/min was used with two solvents: solvent A, acetic acid in water at pH 2.60; solvent B, 20% solvent A mixed with 80% acetonitrile. Peaks were monitored by an HPLC system equipped with a diode array detector and were identified by their retention times and spectra in comparison with external standards. A diode array UV−vis detector (DAD) was used to choose the maximum absorbance for each group of compounds, to control peak purity, and to identify the spectra of some phenolics (*11*). The concentrations of the phenolic compounds identified were measured using external standard curves. Those hydroxycinnamic acids for which standards were not available were identified using their spectra and retention time, as described by other authors (*11*).

Calibration curves (standard area in absorbance versus concentration in mg/L) were performed over the range of concentration observed, except for caftaric acid (*cis*- and *trans*-) and coutaric acid (*cis*- and *trans*-), which were calibrated using a caffeic acid and coumaric acid standard, respectively.

Gallic acid, *trans*-caftaric acid, *trans*-coutaric acid, caffeic acid, coumaric acid, ferulic acid, rutin, quercetin, (+)-catechin, and (−)-epicatechin were purchased from Sigma-Aldrich (Steinheim, Germany). Results were expressed as milligrams of phenol per kilogram on wet basis.

Trolox Equivalent Antioxidant Activity Assay (TEAC). The 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) method is a decolorization assay applicable to both lipophilic and hydrophilic antioxidants (*12*). The method is based on the ability of antioxidant molecules to quench the long-lived $ABTS^{\bullet+}$, a blue-green chromophore with characteristic absorption at 734 nm, compared with 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), a water-soluble vitamin E analogue. The addition of antioxidants to the preformed radical cation reduces it to ABTS and leads to decolorization.

Trolox (Acros Organics, Geel, Belgium) was used as the antioxidant standard. Trolox was prepared in phosphate-buffered saline (PBS), pH 7.4 (Sigma-Aldrich, Steinheim, Germany), for use as the stock standard. ABTS was obtained from Sigma-Aldrich (Steinheim, Germany) and potassium persulfate (potassium peroxodisulfate) from J. T. Baker (Deventer, The Netherlands).

ABTS was dissolved in water to a concentration of 7 mM. An ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12−16 h before use. To study the antioxidant capacity of the extracts, the $ABTS^{\bullet+}$ solution was diluted with PBS, pH 7.4, to an absorbance of 0.70 (±0.02) at 734 nm and equilibrated at 30 °C. The absorbance was verified by a diode array spectrophotometer (Hewlett-Packard 8452A).

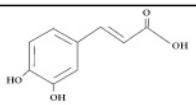
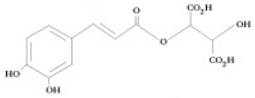
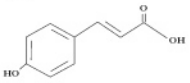
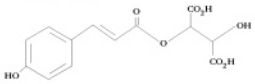
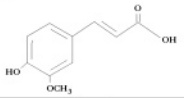
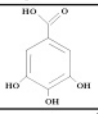
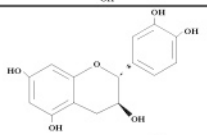
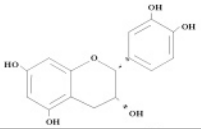
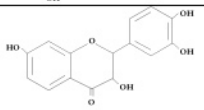
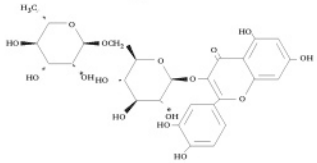
After 20 μL of extract or PBS had been added to 2 mL of diluted $ABTS^{\bullet+}$ solution ($A_{734nm} = 0.70 \pm 0.02$), the absorbance reading was taken at 30 °C exactly 5 min after initial mixing. Appropriate solvent blanks were run in each assay. All determinations were carried out in duplicate. The percentage inhibition was calculated using eq 1

$$\Delta A_{(sample)} = \frac{A_{t=0min(sample)} - A_{t=5min(sample)}}{A_{t=0min(sample)}} - \frac{A_{t=0min(PBS)} - A_{t=5min(PBS)}}{A_{t=0min(PBS)}} \quad (1)$$

where $A_{t=0min}$ and $A_{t=5min}$ are the initial absorbance reading and that after 5 min, respectively. Percent inhibition values were obtained by multiplying $\Delta A_{(sample)}$ values by 100. The percentage inhibition was compared with the standard calibration curve for Trolox ($R^2 = 0.999$), and the results were expressed as the Trolox equivalent in millimoles per kilogram on wet basis.

Ferric Reducing Antioxidant Power Assay (FRAP). The method is based on reducing the Fe^{3+}−2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) complex to the ferrous form at low pH (*13*). This reduction is monitored by measuring the absorption change at 593 nm. Electron-donating substances for which the half-reaction has a lower redox potential than Fe^{3+}/Fe^{2+}−TPTZ drive the reaction and the formation of the blue complex forward.

Table 1. Phenolic Composition of the Concentrated Red Must with a Soluble Solids Mass Fraction of 64.5% (Mean ± Standard Deviation of Determinations Performed in Triplicate)

	Concentration [mg/kg]	Molecular weight [g/mol]	Molecular structure	Phenolic classification
caffeic acid	11.8±1.8	180.20		Non-flavonoids: Hydroxycinnamic acids/ Hydroxycinnamates
trans-caftaric acid	147.2±5.8	312.23		
coumaric acid	29.8±1.4	164.16		
trans- coutaric acid	104.0±6.4	295.00		
ferulic acid	35.5±2.9	194.18		
gallic acid	318.7±13.2	170.12		Non-flavonoids: Hydroxybenzoic acids
(+)-catechin	66.7±2.4	290.28		Flavonoids: Flavan-3-ols
(-)-epicatechin	59.6±1.6	290.27		
quercetin	28.9±1.2	338.27		Flavonoids: Flavonol
rutin	77.5±1.6	610.53		
Total phenolics$_{FC}$[a]	13152±276	-	-	-
Total phenolics$_{HPLC}$[b]	879.7	-	-	-
Total hydroxycinnamics*	328.2	-	-	-
Total flavonoids*	232.8	-	-	-
Total benzoic acids*	318.7	-	-	-

[a,b] Total phenolics determined using the Folin–Ciocalteu method and HPLC, respectively. Total phenolics$_{FC}$ is expressed as mg of GAE/kg. *Calculated from HPLC results.

The FRAP reagent was prepared as a mixture of 0.1 M acetate buffer (pH 3.6), 10 mM TPTZ (Sigma-Aldrich, Steinheim, Germany) in 40 mM hydrochloric acid at 50 °C, and 20 mM ferric chloride (10:1:1, v/v/v). Every day 3 mL of working FRAP reagent was prepared and mixed with 100 μL of sample. The absorbance at 593 nm was recorded after 5 min of incubation at 30 °C on a diode array spectrophotometer (Hewlett-Packard 8452A).

FRAP values were obtained by comparing the absorption changes in the test mixture with those when Trolox was used as a standard. Results were expressed as the Trolox equivalent in millimoles per kilogram on wet basis. This procedure was used to analyze all solid gel extracts in duplicate.

Calculation Procedures. The osmotic dehydration kinetics of the model food was evaluated by calculating the water loss ($-\Delta M^{w}$), soluble solid gain (ΔM^{SS}), and phenolic gain (ΔM^{TPH}). These parameters were calculated as

$$\Delta M^{w} = \frac{M_t x_t^{w} - M_0 x_0^{w}}{M_0} \quad (2)$$

$$\Delta M^{SS} = \frac{M_t x_t^{SS} - M_0 x_0^{SS}}{M_0} \quad (3)$$

$$\Delta M^{TPH} = \frac{M_t x_t^{TPH} - M_0 x_0^{TPH}}{M_0} \quad (4)$$

where M and x are the mass of the gel and the mass fraction of each component in the gel, respectively, the subindices 0 and t indicate initial conditions and conditions at time t of treatment, and the superindices

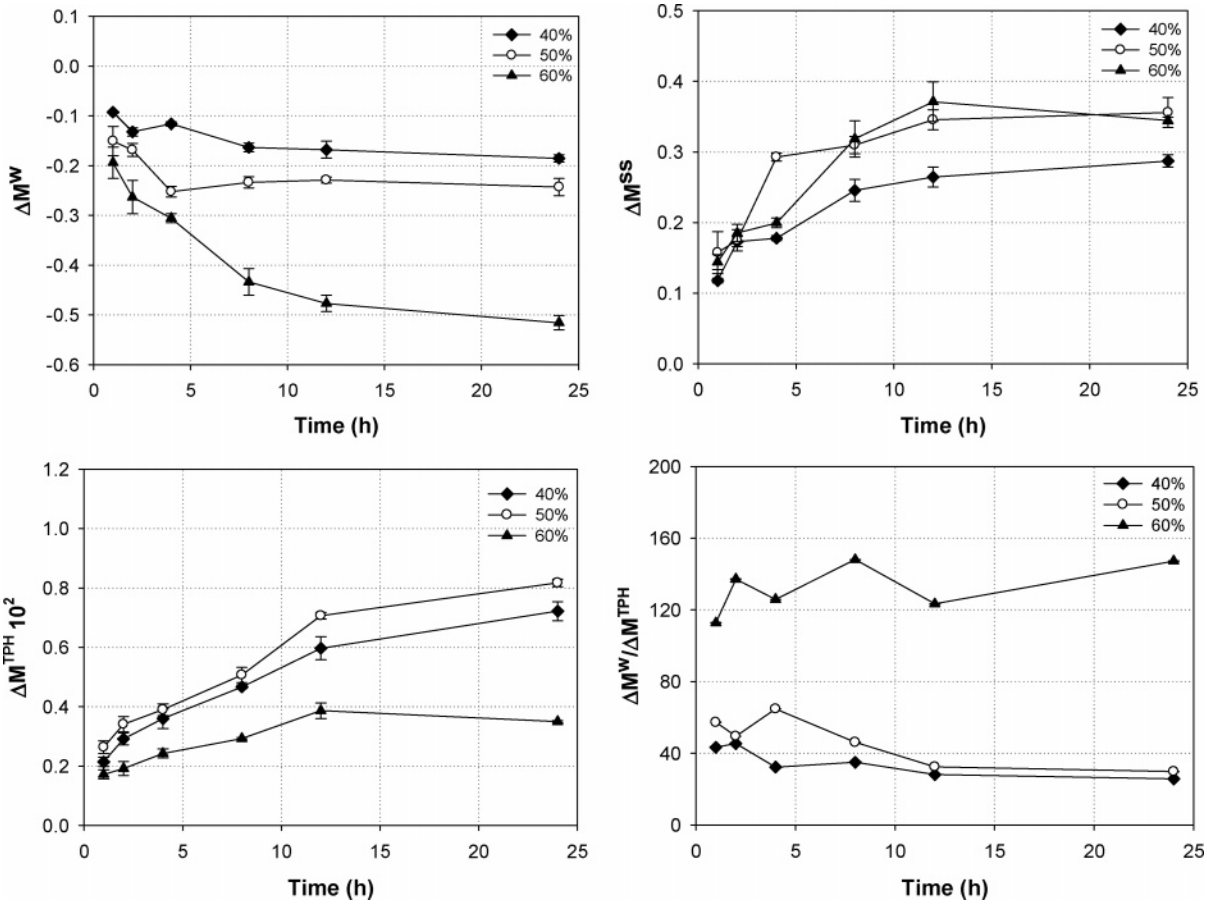


Figure 1. Mass changes of water (ΔM^{w}), gain in soluble solids (ΔM^{SS}) and total phenolics (ΔM^{TPH}), and ratio of water mass changes to gain in total phenolics ($\Delta M^{w}/\Delta M^{TPH}$) during OD with red grape must (mean ± standard deviation of experiments performed in triplicate). Mass fraction of soluble solids in the red must was adjusted to 40, 50, and 60%.

w, SS, and TPH are water, soluble solids, and total phenolics, respectively. From this point on, the mass fraction of each component in the gel will be expressed as kilogram per kilogram on wet basis.

Statistical Analysis. For regression analysis, SPSS 13.0 was used.

RESULTS AND DISCUSSION

Phenolic Profile of the Concentrated Red Must (Osmotic Solution). A concentrated red must with a soluble solid mass fraction of 64.5%, from a mixture of three grape varieties, was used as a source of phenolics in the osmotic solution. **Table 1** shows the content of soluble solids, total phenolics, and the individual phenolics identified and quantified in the concentrated red must. The soluble solids concentration of the concentrated red must was 3 times higher than that of a fresh grape must. The identification and quantification of grape phenolics was focused on those with a low molecular weight because molecular weight has been reported to strongly limit solute infusion during OD (*8*). On this basis, hydroxycinnamic acids and their corresponding hydroxycinnamate and hydroxybenzoic acids were quantified from the group of non-flavonoids, whereas two flavan-3-ols and two flavonols were identified from the group of flavonoids. Although some anthocyanins might be extracted from grape skins during the production of the concentrated red must, they are not analyzed here as the work has focused on the intake of low molecular weight phenolics. **Table 1** lists the concentration of the phenolics identified and quantified in the concentrated red must, together with their molecular weight and structure.

From the hydroxycinammates, *trans*-caftaric acid was found at highest level (147.2 ± 1.7 mg/kg) followed by *trans*-coutaric acid (104.0 ± 1.7 mg/kg). Fertaric acid was not detected. Ferulic acid was the free cinnamic acid detected in the highest concentration in the must (35.5 ± 0.9 mg/kg), probably because of an extended hydrolysis of the tartrate ester, fertaric acid, which might explain why this ester was not detected. Coumaric acid and caffeic acid were the cinnamic acids found in the lowest concentrations (29.8 ± 0.9 and 11.8 ± 1.8 mg/kg, respectively).

Gallic acid, the only benzoic acid detected, was present in an extremely high concentration (318.9 ± 4.8 mg/kg), which is several orders of magnitude higher than the usual concentration in grape must (*11*). The hydrolysis of the ester forms of some flavonols produces free gallic acid. Therefore, the operating conditions during the production of the concentrated grape must could lead to this high gallic acid concentration.

Flavonoids were present in lower concentrations than hydroxycinnamates and gallic acid; about 26% of the total phenols quantified by HPLC were flavonoids. Because the flavonoids quantified (flavan-3-nols and flavonols) are typically found in the grape seed and/or skin, they may be in the must as a result of the extraction method used to obtain the grape juice. Must was richer in (+)-catechin (66.7 ± 0.3 mg/kg) than in (−)-epicatechin (59.6 ± 0.5 mg/kg). Quercetin and rutin were the flavonols detected at average levels of 28.9 ± 0.5 and 77.5 ± 0.6 mg/kg, respectively.

Effect of the Operating Conditions on Water Loss and Soluble Solid and Phenolic Gain. Figure 1 plots the effect of the soluble solids concentration of the osmotic solution on water loss and soluble solid and total phenolic gain during OD. As expected, water loss ($-\Delta M^{w}$) increased with time and also with the soluble solids concentration of the osmotic solution. The

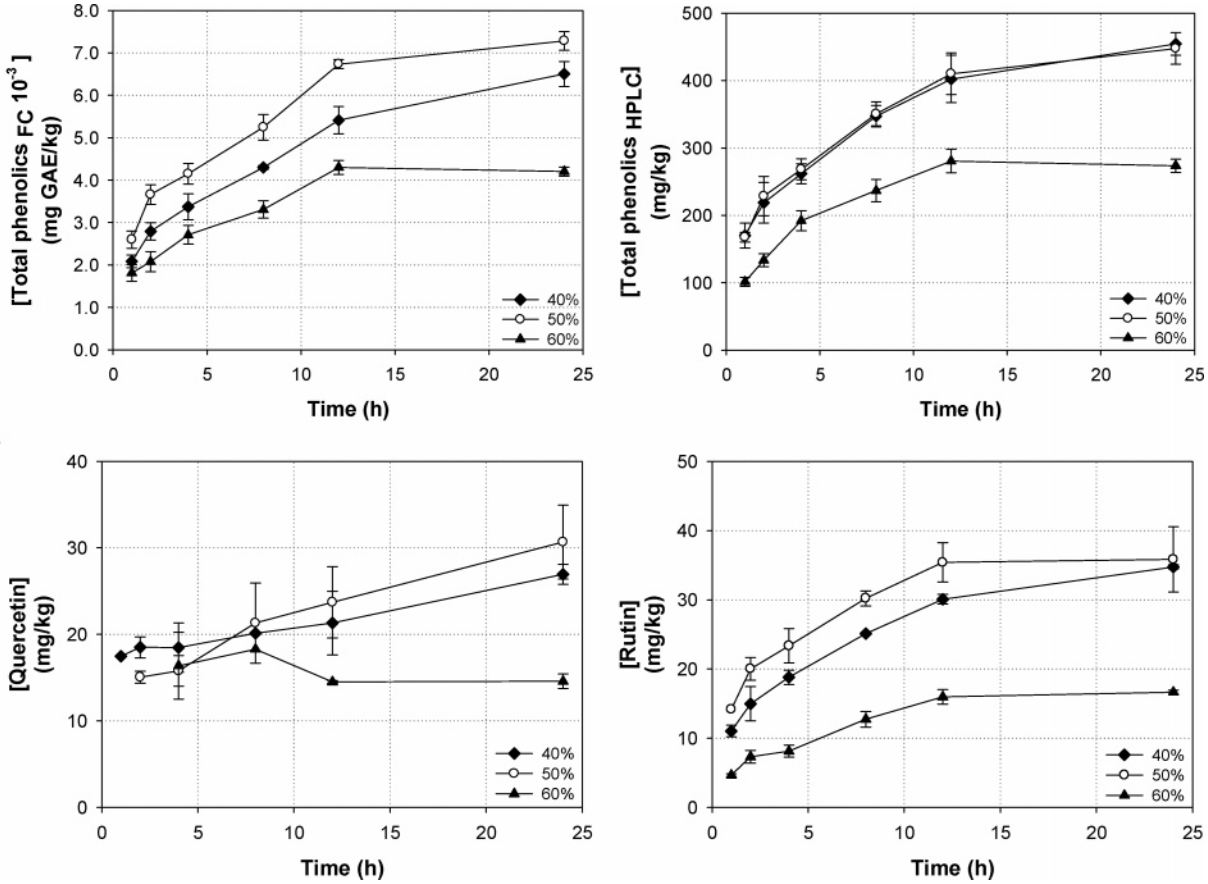


Figure 2. Total phenolic content, determined by Folin–Ciocalteu's method and HPLC, and content of the flavonoids (quercetin and rutin) identified in the osmo-dehydrated food during OD with red grape must (mean ± standard deviation of experiments performed in triplicate). Mass fraction of soluble solids in the red must was adjusted to 40, 50, and 60%.

highest water loss was 52% (with a 60% soluble solids mass fraction in the osmotic solution) followed by 24.3 and 18.5% (with 50 and 40% soluble solids mass fraction in the osmotic solution, respectively) for 24 h of OD. The gain in soluble solids (ΔM^{SS}) and total phenolics (ΔM^{TPH}) depends on the soluble solids concentration of the osmotic solution. Whereas soluble solids increased with the soluble solids concentration of the osmotic solution, the total phenolic gain was highest (0.82%) with a 50% mass fraction of soluble solids in the osmotic solution. Gains of 0.72 and 0.35% were obtained, respectively, with 40 and 60% mass fractions of soluble solids in the osmotic solution. OD with 50 and 60% mass fractions of soluble solids in the osmotic solution led to a 35% gain in soluble solids, which decreased to 29% when the mass fraction of soluble solids in the osmotic solution was reduced to 40%.

Some authors (*14*, *15*) have reported that highly concentrated sugar solutions (mass fraction of soluble solids >55%) hinder the penetration of the solute (solid gain) either because of a surface layer of solids, formed by the high counter current flows of water and solids, or because of the high viscosity of the solution. The slight differences between gains in soluble solids with 50 and 60% mass fractions of soluble solids in the osmotic solution support this. However, the impregnation with total phenolics (total phenolics gain) can be explained considering that total phenolics are minor components of the osmotic solution (11.13 ± 0.28, 9.84 ± 0.11, and 9.78 ± 0.16 g of GAE/kg in the 60, 50, and 40% soluble solids concentration of the osmotic solution, respectively) and contribute little to the osmotic pressure or a_w. On this basis, the impregnation of total phenolics during OD with an osmotic solution of 60% mass fraction of soluble solids might be limited either by the concentrated surface layer of soluble solids or by the high viscosity of the osmotic solution, which not only prevents any further increase in soluble solids but also the penetration of other minor compounds in the osmotic solution (phenolics, for example).

Figure 1 depicts the progress of the ratio of water loss to phenolic gain, $-\Delta M^{w}/\Delta M^{TPH}$. At a high concentration of soluble solids in the osmotic solution (60%), the water loss was up to 150 times greater than the phenolic gain, which indicates that dewatering is the prevailing effect with regard to impregnation. Although at lower concentrations of soluble solids in the osmotic solution (40 and 50%) dewatering was still much higher than phenolic gain—water loss was between 25 and 65 times higher than phenolic gain, the ratio of water loss to phenolic gain decreased significantly.

Phenolic Profiles in the Osmo-dehydrated Food. Figure 2 shows the total phenolic content determined according to the Folin−Ciocalteu method and the phenolic content determined by HPLC. The latter was calculated from the content of the individual phenolics identified and quantified in the osmo-dehydrated food and plotted in **Figures 2** and **3**. Hereinafter, this will be referred to as the HPLC phenolic content. The total phenolic content in the osmo-dehydrated food increased with processing time (**Figure 2**). OD for 24 h with a 50% mass fraction of soluble solids in the osmotic solution led to the highest total phenolic content in the osmo-dehydrated food (up to 7284 ± 219 mg of GAE/kg), followed by 6504 ± 294 and 4203 ± 106 mg of GAE/kg obtained after 24 h of OD with 40 and 60% soluble solids in the osmotic solution, respectively.

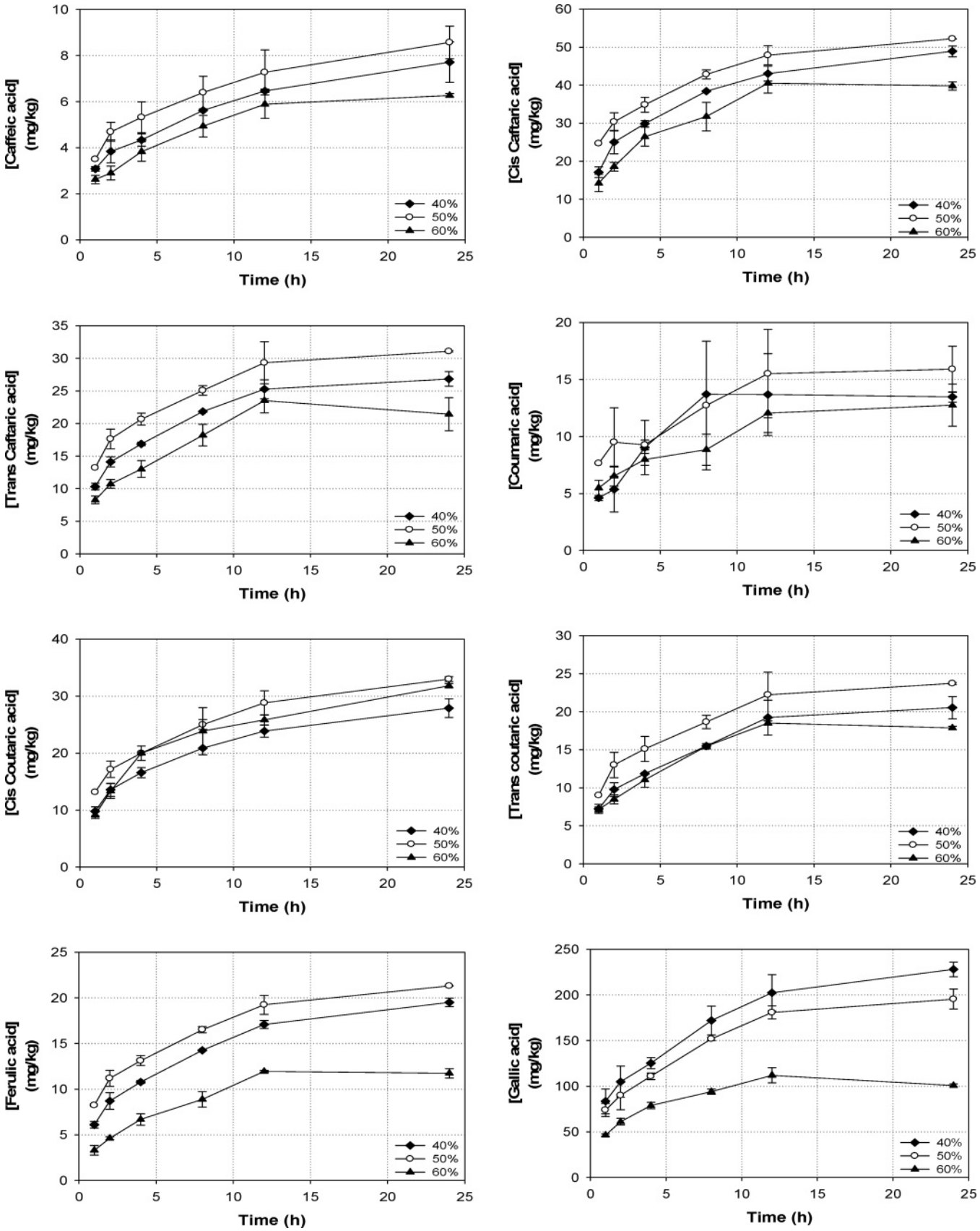


Figure 3. Content of the non-flavonoids (hydroycinnamic acids and gallic acid) identified in the osmo-dehydrated food during OD with red grape must (mean ± standard deviation of eperiments performed in triplicate). Mass fraction of soluble solids in the red must was adjusted to 40, 50, and 60%.

Comparing these total phenolic contents with those reported in the literature for commonly consumed fresh fruits and vegetables (*16*−*18*), we can easily verify that the osmo-dehydrated model food obtained in these OD conditions had a similar or higher content of total phenolics than the richest fruits and vegetables. Fruits such as black- and blueberries, plums, strawberries, and grapefruits have a total phenolic content that may be around 267−9610, 1740−3686, 1600−2250, and 1617 mg of GAE/kg

of FW, respectively, depending on the variety. Broccoli, cabbage, or spinach, some of the vegetables that are richest in total phenolics, have a total phenolic concentration between 250 and 2900 mg of GAE/kg of FW, between 450 and 925 mg of GAE/kg of FW, and between 325 and 1000 mg of GAE/kg of FW, respectively.

Much shorter processing times, as short as 1 or 2 h of OD, would be long enough to provide osmo-dehydrated food with a total phenolic content close to that of the richest fruits and vegetables. The high concentration of total phenolics in the concentrated red must (13154 mg of GAE/kg) may explain the extent of phenolic gain. Mass transfer is usually assumed to occur between the food liquid phase (i.e., food containing water and soluble components) and the osmotic solution. On this basis, the equilibrium criterion considered is that the food liquid phase and the osmotic solution are compositionally equal (*19*). After 24 h of OD with a 50% mass fraction of soluble solids in the osmotic solution, the phenolic content in the osmo-dehydrated food (7466 mg of GAE/kg of food liquid phase equivalent to 7284 ± 219 mg of GAE/kg) was still below the total phenolic content of the osmotic solution (9840 ± 108 mg of GAE/kg), which shows that equilibrium had still not been reached from the point of view of phenolic mass transfer.

During OD with a mass fraction of 40, 50, and 60% of soluble solids in the osmotic solution, the concentration of individual phenolics in the osmo-dehydrated food was monitored (**Figures 2** and **3**). The hydroxycinnamics and gallic acid quantified in the concentrated red grape must were also detected in the osmo-dehydrated food and were the major phenols present in the osmo-dehydrated food in all of the conditions tested. However, the *cis* isomers of caftaric and coutaric acids, which were not detected in the concentrated red must, were identified in the osmo-dehydrated food. These *cis* forms are reported to be the result of a *cis*/*trans* isomerization under exposure to UV light (*11*). In addition, the effect of UV radiation seems to affect hydroxycinnamates differently: whereas in white free run juices, *trans*-coutaric was reported to be more sensitive to this isomerization than *trans*-caftaric, in the osmo-dehydrated food the opposite effect was observed. The ratio of *cis*-coutaric to *trans*-coutaric concentration was slightly lower than that of *cis*-caftaric to *trans*-caftaric concentration in all cases analyzed (**Figure 3**).

Of the phenolics identified and quantified in the concentrated red must (**Table 1**), the only flavonoids that were detected in the osmo-dehydrated food in all of the operating conditions considered were quercetin and rutin. Other flavonoids such as (+)-catechin and (−)-epicatechin were not detected in any of the samples of the osmo-dehydrated food analyzed. As the molecular weight of these compounds is in the same range as the others detected in the osmo-dehydrated food, (+)-catechin and (−)-epicatechin might be absent because they were oxidized during OD or further extraction steps. The high antioxidant potential of both compounds has been extensively reported (*20*), and although the pH of the grape must was maintained at 3.4 to prevent the phenolics from oxidizing, agitation during OD and contact with air during the further extraction might oxidize them both.

The influence of the mass fraction of soluble solids of the osmotic solution on the gain in individual phenolics was the same as on the gain in total phenolics. The phenolic concentration in the osmo-dehydrated food was highest in all cases for the 24 h of OD with a 50% mass fraction of soluble solids in the osmotic solution. In these conditions, gallic acid was the phenolic found in the highest concentration (227.9 mg/kg) (**Figure 3**). Of the hydroxycinnamics (**Figure 3**), *cis*-caftaric was found at the highest level (52.2 mg/kg), followed by *cis*-coutaric acid (33.0 mg/kg). Both *trans* isomers of caftaric and coutaric acids were detected in lower concentrations (31.1 and 23.7 mg/kg, respectively). Ferulic acid (21.3 mg/kg) was the free cinnamic acid found at the highest concentration, followed by coumaric (15.9 mg/kg) and caffeic acids (8.6 mg/kg).

In the case of flavonoids, the concentrations of quercetin and rutin in the osmo-dehydrated food after 24 h of OD with an osmotic solution of 50% mass fraction of soluble solids were 30.7 and 35.8 mg/kg, respectively.

According to these results, OD proved to be an efficient operation for supplementing a solid foodstuff with grape phenolics when a concentrated red grape must was used. Adjusting the operating conditions (basically the soluble solids content in the osmotic solution) makes it possible to maximize phenolic impregnation. However, other operating conditions such as the total phenolic content of the grape must and agitation/aeration should be optimized. In particular, the effect of agitation/aeration on the oxidation of such phenolics as (+)-catechin and (−)-epicatechin should be further investigated. The application of OD to supplement real foods requires additional research to determine the influence of the food structure and composition on the impregnation pattern of phenolics.

Antioxidant Capacity of the Osmo-dehydrated Food. A one-assay protocol cannot evaluate the effectiveness of antioxidants in complex heterogeneous foods because antioxidant protection involves several mechanisms. Two in vitro antioxidant capacity assays, TEAC and FRAP, were chosen to measure the free radical scavenging activity and the total reducing power, respectively, in the osmo-dehydrated food during OD. Although these assays are nonspecific and provide little information about the mechanisms controlling the antioxidant action, they are both widely used to determine antioxidant capacity in foods, and they have provided a great deal of antioxidant data (*21*).

Figure 4 shows the increase in TEAC and FRAP during OD with a 40, 50, and 60% mass fraction of soluble solids in the osmotic solution. As observed with total and individual phenolics, the antioxidant capacity measured by both methods was highest with a soluble solid mass fraction of 50% in the osmotic solution. In these conditions and after 24 h of OD, TEAC and FRAP values were 66.3 and 89.9 mmol of Trolox/kg, respectively. In general, berries are the edible fruits that have the greatest antioxidant capacity. Reported TEAC values of blackberry, raspberry, and strawberry are 20.24, 16.79, and 10.94 mmol of Trolox/kg of FW, respectively (*22*). Vegetables usually have lower antioxidant capacity than fruits, and spinach and peppers have been found to have the highest (TEAC values of 8.49 and 8.40 mmol of Trolox/kg of FW, respectively). Therefore, with OD in the conditions that led to the highest phenolic gain (50% mass fraction of soluble solids in the osmotic solution and 24 h) it was possible to obtain an end product with a TEAC value that was 3 times higher than that observed in the fruits with highest antioxidant capacity. At shorter processing times, between 2 and 4 h, and with any of the three osmotic solutions used (40, 50, and 60% mass fraction of soluble solids), TEAC values ranged from 20 to 40 mmol of Trolox/kg: that is, between 1 and 2 times the antioxidant capacity of the berries.

Comparing these results with the total gain in phenolics, we observed that under the same operating conditions (50% mass fraction of soluble solids in the osmotic solution and 24 h), the total phenolic content was at the same level as the fruit that was richest in polyphenols, whereas the antioxidant capacity,

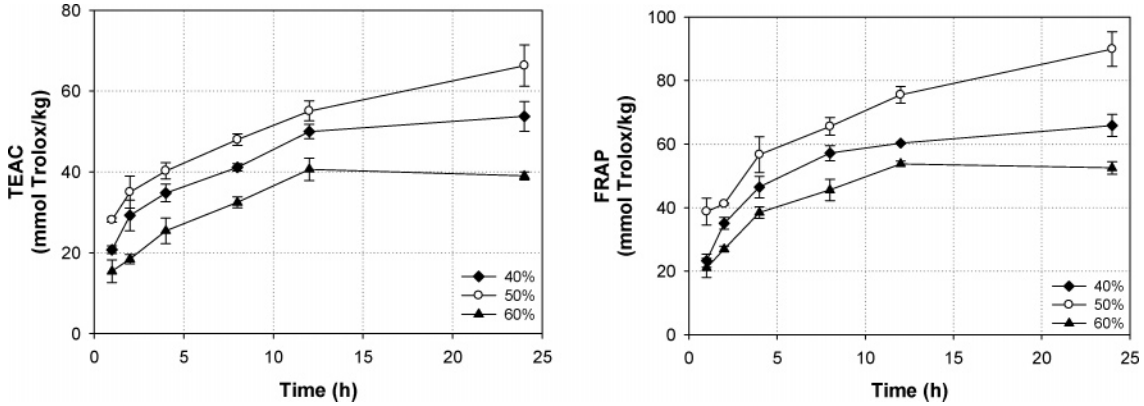


Figure 4. Antioxidant activity of the osmo-dehydrated food measured as TEAC and FRAP during OD with red grape must.

Table 2. Linear Regression Analysis of Antioxidant Capacity, TEAC, and FRAP versus Individual and Total Phenolics Content

	TEAC				FRAP			
phenolic	a^a	b^a	r^2	P^b	a^a	b^a	r^2	P^b
caffeic acid	7.72	–2.55	0.956	2.61×10^{-12}	10.14	–2.78	0.928	1.42×10^{-10}
cis-caftaric acid	1.18	–2.30	0.951	7.18×10^{-12}	1.56	–2.80	0.935	6.63×10^{-11}
trans-caftaric acid	1.98	–0.77	0.967	2.72×10^{-13}	2.61	–0.74	0.949	8.58×10^{-12}
coumaric acid	3.53	1.36	0.856	3.88×10^{-8}	4.74	1.22	0.871	1.61×10^{-8}
cis-coutaric acid	1.61	4.03	0.735	5.48×10^{-6}	2.20	4.05	0.773	1.54×10^{-6}
trans-coutaric acid	2.49	0.86	0.912	7.38×10^{-10}	3.31	1.04	0.909	9.46×10^{-10}
ferulic acid	2.55	7.21	0.979	8.95×10^{-15}	3.30	10.55	0.925	2.07×10^{-10}
gallic acid	0.23	8.93	0.809	3.81×10^{-7}	0.29	13.89	0.719	8.71×10^{-6}
quercetin	2.05	0.77	0.617	5.20×10^{-4}	2.68	1.50	0.558	1.37×10^{-3}
rutin	1.30	11.48	0.909	9.57×10^{-10}	1.67	16.41	0.843	7.98×10^{-8}
$TPH_{FC}{}^c$	8.13 10^{-03}	4.65	0.971	1.12×10^{-13}	1.06 10^{-2}	6.81	0.936	5.91×10^{-11}
$hydroxycinnamics_{HPLC}$	0.33	–0.72	0.946	1.50×10^{-11}	0.438	-0.955	0.939	3.91×10^{-11}
$flavonols_{HPLC}$	0.73	11.02	0.879	9.85×10^{-9}	0.929	16.02	0.805	4.56×10^{-7}
$TPH_{HPLC}{}^d$	0.124	3.42	0.936	5.69×10^{-11}	0.159	5.94	0.873	1.46×10^{-8}

[a] *a* (mmol of Trolox/mg of phenol) and *b* (mmol of Trolox/kg) are the slope and the intercept, respectively. [b] *P* values of the regression coefficients. [c,d] Total phenolics determined using the Folin–Ciocalteu method and HPLC, respectively.

in terms of TEAC, was 3 times the antioxidant capacity of the most antioxidant fresh fruits.

Data from TEAC and FRAP correlated well: the effect of OD conditions on both parameters was comparable. On average, FRAP values were 31% higher than the TEAC values (FRAP $= 1.31 \times$ TEAC $+ 0.69$, $r^2 = 0.966$). These differences, previously reported by other authors (*23*, *24*), have been related to the fundamental characteristics of both methods. TEAC shows the ability of an antioxidant to scavenge the artificial $ABTS^{\bullet+}$ radical, wheres FRAP measures its reducing capacity. However, neither reflects the antioxidant capacity due to other effective mechanisms. As the main goal of this study is to assess whether OD with a concentrated red must, in the operating conditions considered, can increase the antioxidant capacity of a model food (gel in this case) to the same or higher levels than those of the most antioxidant fresh fruits, both FRAP and TEAC values are used to complement the phenolic profile.

Correlations between Phenolic Content and Antioxidant Capacity. Correlations between the antioxidant capacity and phenolic profile were determined to detect the extent to which the phenolics identified by HPLC describe the antioxidant capacity of the osmo-dehydrated food.

Regression analysis (**Table 2**) showed that the HPLC phenolic content was significantly correlated with TEAC ($r^2 = 0.936$, $P < 0.001$) and FRAP ($r^2 = 0.873$, $P < 0.001$), but the correlation between the antioxidant capacity and total phenolic content, determined with the Folin−Ciocalteu method, was slightly higher: TEAC ($r^2 = 0.971$, $P < 0.001$) and FRAP ($r^2 = 0.936$, $P < 0.001$). Considering that the total phenolic content determined using the Folin−Ciocalteu method in the osmo-dehydrated food was approximately 1 order of magnitude higher than that determined by HPLC (**Figure 2**), the regression analysis suggests that other phenolics that are present in the osmo-dehydrated food have a rather low impact on the free radical scavenging activity and the total reducing power.

When TEAC and FRAP were correlated with the total contents of the various phenolic groups, statistically significant dependencies could be stated. The total content of hydroxycinnamics, hydroxybenzoic acids, and flavonols identified by HPLC was highly correlated with both TEAC and FRAP. The highest correlation was shown by hydroxycinnamics (TEAC, $r^2 = 0.946$, $P < 0.001$; and FRAP, $r^2 = 0.939$, $P < 0.001$), followed by flavonols (TEAC, $r^2 = 0.879$; $P < 0.001$; and FRAP, $r^2 = 0.805$, $P < 0.001$) and gallic acid (TEAC, $r^2 = 0.809$, $P < 0.001$; and FRAP, $r^2 = 0.719$, $P < 0.001$).

In the hydroxycinnamics group, all of the phenolics analyzed exhibited a high and significant correlation with TEAC and FRAP. Ferulic acid and caffeic acid (and both isomers of its tartrate ester, *cis*-caftaric and *trans*-caftaric acids), however, showed the highest correlation, with r^2 values above 0.95 and 0.92 for TEAC and FRAP, respectively. Coumaric acid and its tartrate esters, *cis*-couratic and *trans*-coutaric acids, exhibited the weakest correlations in the hydroxycinnamics group, although they were still significant (r^2 values between 0.735 and 0.909). Gallic acid, the only hydroxybenzoic acid analyzed, was significantly correlated with TEAC and FRAP, but its correlation was low compared to its high contents in the osmo-dehydrated food (up to 4.5 times that of the major hydroxy-

cinnamics). The correlations of the individual flavonols analyzed with TEAC and FRAP were significant ($P < 0.001$) and higher for rutin than for quercetin. This can be explained only by taking into account the high dispersion obtained in the quercetin determinations.

These results showed that the individual phenolics analyzed significantly explain the antioxidant capacity of the osmodehydrated food in terms of free radical scavenging activity and total reducing power. However, the statistical analysis used, a simple linear regression, did not seem able to properly evaluate the contribution of each individual phenolic to the antioxidant capacity. What can be inferred from the above correlations may, in some cases, not fit with what has been reported (*20*). A further statistical analysis should be designed so that the possible synergic effects that might mask the present results can be evaluated.

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