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Direct Formulation of a Solid Foodstuff with Phenolic-Rich Multicomponent Solutions from Grape Seed: Effects on Composition and Antioxidant Properties

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The aim of our work was to supplement a solid foodstuff with grape phenolics by osmotic treatment with an aqueous solution made of osmo-active agents (NaCl and sucrose) and a commercial grape seed extract. To investigate how the composition of the osmotic solution affected phenolic infusion, experimental conditions were set by a central composite design with two factors (the molality of NaCl and sucrose in the osmotic solution). In all experiments, the total phenolic content in the osmotic solution was kept constant (6300 ± 45 mg gallic acid equivalents/kg), and the model food (an agar−agar gel) was processed for 8 h. Throughout the response surface, the osmo-treated model food was significantly supplemented with flavan-3-ols. At the central point of the experimental design, flavan-3-ol monomers and dimers were found in concentrations of 1334 ± 126 and 486 ± 55 mg/kg, respectively. Their penetration into the model food was limited by sucrose to a different extent. The Trolox equivalent antioxidant capacity of the osmo-treated gel was higher than that of fruits with a very high free radical scavenging activity.

KEYWORDS: Antioxidant; capacity; osmotic dehydration; phenolics; polyphenols; grape flavan-3-ol; procyanidins

INTRODUCTION

The beneficial effects of grape phenolics on human health have been extensively reviewed, and their recovery from byproducts of the winemaking and grape juice industries is thus a matter of growing interest. The typical byproducts of these industries are seeds, skins, and stems, all of which are rich sources of phenolics [particularly gallic acid (GA) and the flavans catechin and epicatechin]. In addition, a wide variety of procyanidins, condensed phenolics containing the monomeric flavan-3-ol units of catechin and epicatechin, are found in grape seeds (*1, 2*).

Recently, grape seed extracts (GSE) have become a widespread nutritional supplement because of their antioxidant properties. GSEs contain heterogeneous mixtures of monomers, oligomers, and polymers made up of subunits of flavan-3-ol, which depend on both the grape variety and the extraction conditions (*3, 4*). GSEs, particularly those with high contents of oligomeric proanthocyanidins (dimer, trimer, and tetrameric), have been shown to be highly bioavailable and to provide excellent health benefits: For example, a broad spectrum of biological, pharmacological, and therapeutic activities against free radicals and oxidative stress have been reported (*2, 5*).

Osmotic treatment (OT), also called osmotic dehydration or dewatering impregnation soaking, is an operation that has a double effect: It partially dehydrates solid food material and simultaneously impregnates it with solutes. OT has been proposed as a method that will change a food formulation by (i) reducing the water content or adding water activity lowering agents and (ii) supplementing the food with compounds that modify its functional and nutritional properties (*6*).

The most common solutes in the osmotic solution are sodium chloride and sucrose, although other electrolytes and nonelectrolytes, such us polyalcohols and polysaccharides of different molecular weights (*7*), have been used to control the ratio of dehydration to solute impregnation and to optimize the end product formulation for each particular application. Generally, salts are used to treat vegetables, fish, meat, and cheese, and sugars are used to treat fruits.

The use of multicomponent solutions, basically ternary solutions of salt, sugar, and water, leads to better control of the

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water content and solute impregnation. OT with ternary solutions (NaCl/sucrose/water) has been applied to such fruits and vegetables as tomato (*8*), apple (*9, 10*), melon (*11*), carrot (*12*), and paprika (*13*). In every case, water loss was increased, and individual gain of each osmo-active solute was reduced, which improved the sensory properties of the final food product (*9, 14*).

Even though OT has been extensively used to produce intermediate moisture products, it has only been used to a limited extent to produce functional foods from fruits and vegetables. In particular, it has been used to impregnate plant foods with probiotics (*15*) and minerals (e.g., calcium and zinc) (*16*). Recently, Rózek et al. (*17*) described OT as being a suitable method for infusing solid foodstuffs with grape phenolics. In this case, an agar−agar gel was used as a model food and a concentrated red grape must as the source of the phenolics. In all of the 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 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 while the Trolox equivalent antioxidant capacity (TEAC) was three times that of fresh fruit with the highest antioxidant capacity. The concentration of the osmo-active solute, sucrose in that case, proved to control phenolic infusion and the antioxidant properties of the end product. However, the influence of the kind of osmo-active solute, used in single or binary mixtures, on the phenolic infusion pattern requires further research.

The main objective of this study is to investigate how the composition of the osmotic solution (the kind and concentration of the osmo-active agent) affected phenolic infusion and the antioxidant properties and composition of the osmo-treated solid food. A phenolic-rich commercial GSE and an agar gel are used as a source of phenolics and a model food, respectively. Both osmo-active agents, NaCl and sucrose, are studied as single osmo-active solutes or in mixtures (NaCl and sucrose) with concentrations that maintain the same water activity (a_w).

MATERIALS AND METHODS

Osmotic Solution and Model Food. A multicomponent aqueous solution made of sucrose, NaCl, and a commercial GSE (Vitisol supplied by Berkem, Gardonne, France) was used as the osmotic solution. In all experiments, the mass fraction of total phenolics was kept constant while the mass fractions of sucrose and NaCl were set by the experimental design described below.

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

Osmotic Treatment. The experimental set up 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 three shelves and guaranteed total immersion of the sample in the osmotic solution. About 50 g of agar−agar gel cubes (1 cm side) was weighed and placed in the OT basket. This basket was then submerged in 1 L of osmotic solution. The model food was processed for 8 h, and the osmotic pressure of the solution was adjusted (see below). The solution/model food ratio (w/w) was always higher than 20:1 to prevent the solution from being significantly diluted by water removal, which would lead to local reduction of the osmotic driving force during the process. The temperature was maintained at 25 ± 2 °C. Agitation was provided by a magnetic stirrer. After OT, the gel cubes were removed from the solution, gently blotted with tissue paper, and weighed. All experiments were run under atmospheric pressure.

Determination of Moisture Content. The moisture content of fresh and osmo-dehydrated food was determined with the 934.06 AOAC gravimetric method (*18*).

Determination of Salt and Sucrose Content. About 2.5 g of fine ground sample was dissolved in 50 mL of milliQ water. The chloride content of osmo-dehydrated gel was determined by Mohr's method and expressed as a sodium chloride (*18*). The sucrose content of fresh and osmo-dehydrated was determined with a kit for sugar analysis based on a chemical method (*19*) (GAB Sistemática Analítica S.L., Barcelona, Spain).

Extraction of Phenolic Compounds of the Osmo-Treated Food. To determine the extent of phenolic impregnation in the osmo-treated food, a sequential extraction was carried out. About 5 g of homogenized sample was extracted sequentially with 30 mL of methanol:water (50/50, v/v) and 30 mL of acetone:water (50/50, v/v) for 1 h in each extraction solvent at room temperature. Each solvent extraction was carried out in duplicate. Filtrated and appropriately diluted extracts were taken for the determination of individual phenolic content and TEAC. Extracts (mixtures of water/methanol/acetone) used for high-performance liquid chromatography (HPLC) analysis of individual phenolics were evaporated to dryness under reduced pressure (rotary evaporator, $T \leq 35$ °C) and kept at −26 °C until analyzed, within 2 weeks. Then, samples were resuspended in high-purity water (Milli-Q, Millipore, Bedford, MA) and filtered through 0.45 μm syringe filters (Teknokroma, Barcelona, Spain). In the case of the osmotic solution, samples were filtered through 0.45 μm syringe filters and directly analyzed by HPLC.

Determination of Total Phenolic Content. The total phenolic content of the osmotic solution was determined with Folin−Ciocalteu's method (*20*). The results were expressed as gallic acid equivalents (mg of GAE/kg of wet basis). The total phenolic content of the treated gel was determined as the sum of all phenolics as detected by HPLC analysis.

Determination of Individual Phenolics by HPLC. Phenolics were identified and quantified by HPLC (Hewlett-Packard (HP)/Agilent, Wardborn, Germany). 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, glacial acetic acid in water to adjust the pH to 2.60, and solvent B, 20% solvent A with 80% acetonitrile. The composition of the mobile phase during the analysis was set according to the elution program explained by Betés-Saura et al. (*21*). Peaks were monitored by an HPLC system equipped with a diode array and were identified by their retention times and spectra 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 (*21*). The concentrations of the phenolic compounds identified were measured using external standard curves. Calibration curves (standard area in absorbance vs concentration in mg/L) were performed over the range of concentration observed.

Gallic acid (GA), protocatechuic acid (PA), (+)-catechin (CT), (−)-epicatechin (ECT), (−)-epicatechin 3-*O*-gallate (ECG), (−)-epigallocatechin 3-*O*-gallate (EGCG), (−)-epigallocatechin (EGC), procyanidin B1 (PAB1), and procyanidin B2 (PAB2) purchased from Sigma-Aldrich (Steinheim, Germany) were used as standards for the identification and quantification of individual phenolics. Results were expressed as milligrams of phenol per kilogram on a wet basis.

Trolox Equivalent Antioxidant Capacity (TEAC). The antioxidant capacity of the osmo-treated material extracts obtained as described above was determined as Trolox equivalents. The method compares the ability of antioxidant molecules to quench the long-lived $ABTS^{+}$, a blue-green chromophore with characteristic absorption at 734 nm, with that of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), a water-soluble vitamin E analogue (*22*).

All determinations were carried out in duplicate. 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 a wet basis.

Experimental Design. A second-order central composite rotatable design (CCRD) with two factors (NaCl and sucrose molality of the osmotic solution) was used to determine their effect on the response pattern. The experimental design consisted of three sets of points: the 2^k vertices (± 1, ± 1) of a k-dimensional "cube", the 2^k vertices ($\pm\alpha$, 0; 0, $\pm\alpha$) of an axial or "star" at distance α from the center of the design, and a number, n_0, of "center points" for two factors ($k = 2$). Five levels of each actual variable were considered following the rotatability criterion (the values required were $\alpha = 1.414$), and the central point was replicated six times according to Khuri and Cornell (*23*). The actual factor values and the corresponding coded values are given in **Table 1**.

The molalities of the two osmo-active solutes (sucrose and NaCl) were the actual factors selected to adjust the osmotic strength and, therefore, the a_w of the osmotic solution. In OT, the solid foodstuff loses water while the a_w difference between the solid food and the osmotic solution is significant. The maximum values of the actual factors were those leading to a water activity of 0.935, while the minimum value considered for them both was zero. Previous results showed that phenolic impregnation was significant and water loss considerable in OT with a concentrated grape juice of 0.935 a_w (*17*).

Statistical Analysis. Variance analysis, calculation of regression coefficients, and tridimensional graphics were performed using SigmaPlot 9.0 and SPSS 13.0 programs.

Calculation Procedures. The mass transfer of the model food during OT was evaluated by calculating the water loss ($-\Delta M^w$), total solid gain (ΔM^{SS}), sucrose gain (ΔM^{SUC}), NaCl gain (ΔM^{NaCl}), and phenolic gain (ΔM^{TPH}). These parameters were calculated as:

$$\Delta M^w = \frac{M_t x_t^w - M_0 x_0^w}{M_0} \quad (1)$$

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

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

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

$$\Delta M^{TPH} = \frac{M_t x_t^{TPH} - M_0 x_0^{TPH}}{M_0} \quad (5)$$

where M and $x$are the mass of the gel and the mass fraction of each component in the gel, respectively, the subindexes 0 and t indicate initial conditions and conditions at time t of treatment, and superindexes w, SS, SUC, NaCl, and TPH are water, total solids, sucrose, sodium chloride, and total phenolics, respectively. The total phenolic content of the treated gel was determined as the sum of all phenolics as detected by the HPLC analysis. From this point on, the mass fraction of each component in the gel will be expressed as kg/kg on a wet basis.

To establish how the kind of osmo-active solute affected the response variables (gain and mass fraction of sucrose, sodium chloride, phenolics, TEAC, etc. in the osmo-treated food), we calculated the response values from the second-order model for those combinations of actual factors that led to an osmotic solution of 0.935 a_w. By applying the Norrish (*24*) and Pitzer (*25*) equations for predicting the a_w of binary solutions of sucrose and NaCl, respectively, and the Ross equation for ternary solutions (*26*), we calculated the concentrations of sucrose and NaCl required to obtain several aqueous ternary solutions with a common a_w value of 0.935.

RESULTS AND DISCUSSION

Phenolic Profile of the Osmotic Solution. A commercial GSE was used as a source of phenolics, and all experiments were performed with a total phenolic concentration of 6300 ± 45 mg GAE/kg in the osmotic solution. **Table 2** shows the total and individual phenolics quantified by Folin−Ciocalteu's method and HPLC, respectively, their molecular weight and structure, and classification. Phenolics of low molecular weight were identified and quantified (<600 g/mol) since they were found to be the ones that infused most in solid food during OT (*17*). Of the individual phenolics, flavan-3-ol monomers were the major group quantified followed by flavan-3-ol dimers and hydroxybenzoic acids, which represented 69, 28, and 3%, respectively, of all of the individual phenolics identified by HPLC. In addition, the individual phenolics of low molecular weight quantified were almost 59% of the total phenolics determined by Folin−Ciocalteu's method.

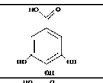
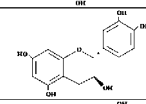
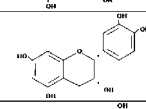
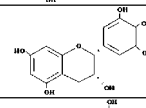
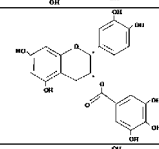
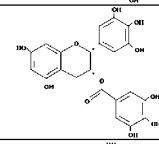
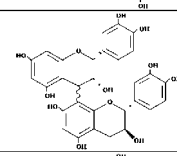
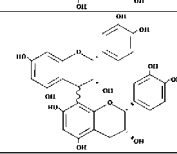
Of the flavan-3-ol monomers, EGC was found at the highest level (1153.4 ± 2.7 mg/kg) followed by ECT (741.0 ± 0.2 mg/

Table 1. Experimental Design and Water Loss ($-\Delta M^W$), Total Solid (ΔM^{SS}), Sucrose (ΔM^{SUC}), and NaCl Gain (ΔM^{NaCl}), Sucrose and NaCl Content, Water Activity, Total Phenolics Determined by HPLC, and TEAC of the Osmo-Treated Gel at the Different Factor Levels[a]

	osmotic solution		osmo-treated food								
	sucrose (molality)	NaCl (molality)									
no.	X_1[b]	X_2[b]	ΔM^W	ΔM^{SS}	ΔM^{SUC}	ΔM^{NaCl}	sucrose (kg/kg)	NaCl × 10^3 (kg/kg)	a_w	TPH_{HPLC} (mg/kg)	TEAC (mmolTrolox/kg)
1	2.49 (1)	1.62 (1)	−0.234	0.340	0.334	0.049	0.394 ± 0.004	44.3 ± 0.2	0.941	1598.8 ± 39.0	42.1 ± 1.2
2	0.43 (−1)	1.62 (1)	−0.033	0.072	0.044	0.065	0.141 ± 0.002	62.5 ± 0.2	0.931	2177.2 ± 93.4	68.4 ± 5.2
3	2.49 (1)	0.28 (−1)	−0.206	0.300	0.336	0.009	0.400 ± 0.004	8.5 ± 0.1	0.936	1660.6 ± 177.6	41.4 ± 0.9
4	0.43 (−1)	0.28 (−1)	−0.011	0.012	0.040	0.012	0.142 ± 0.001	11.9 ± 0.2	0.935	2427.6 ± 49.1	64.1 ± 4.6
5	1.46 (0)	0.95 (0)	−0.143	0.228	0.231	0.035	0.307 ± 0.003	32.4 ± 0.4	0.938	1852.1 ± 74.0	48.2 ± 0.3
6	1.46 (0)	0.95 (0)	−0.144	0.229	0.237	0.035	0.313 ± 0.003	32.4 ± 0.8	0.938	1848.5 ± 66.1	45.5 ± 5.1
7	1.46 (0)	0.95 (0)	−0.143	0.229	0.229	0.036	0.305 ± 0.002	32.8 ± 0.2	0.942	2208.1 ± 34.1	47.5 ± 1.1
8	0 (−1.414)	0.95 (0)	0.049	−0.067	−0.087	0.042	0.016 ± 0.000	42.7 ± 0.2	0.935	2997.2 ± 139.3	65.7 ± 6.7
9	1.46 (0)	0 (−1.414)	−0.102	0.186	0.241	0.000	0.316 ± 0.002	0	0.955	1958.8 ± 100.3	45.0 ± 2.6
10	2.92 (1.414)	0.95 (0)	−0.271	0.323	0.329	0.027	0.410 ± 0.003	25.9 ± 0.1	0.932	1893.9 ± 80.4	37.7 ± 0.6
11	1.46 (0)	1.9 (1.414)	−0.143	0.237	0.210	0.067	0.285 ± 0.001	61.7 ± 0.1	0.912	2112.5 ± 158.3	47.7 ± 2.1
12	1.46 (0)	0.95 (0)	−0.132	0.211	0.214	0.035	0.293 ± 0.001	32.2 ± 0.1	0.942	1881.9 ± 80.6	44.4 ± 0.6
13	1.46 (0)	0.95 (0)	−0.149	0.211	0.212	0.034	0.296 ± 0.001	32.1 ± 0.2	0.938	1816.5 ± 117.2	46.3 ± 0.9
14	1.46 (0)	0.95 (0)	−0.134	0.215	0.203	0.035	0.282 ± 0.001	31.9 ± 0.1	0.940	1772.7 ± 91.5	45.1 ± 0.9

[a] OT was performed for 8 h. [b] In parentheses, the coded values of the two factors.

Table 2. Phenolic Composition of the Osmotic Solution (Mean ± Standard Deviation of Determinations Performed in Triplicate)

	Concentration [mg/kg]	Molecular weight [g/mol]	Molecular structure	Phenolic classification
Gallic acid (GA)	106.0± 0.4	170.12		Non-flavonoids: Hydroxybenzoic acids
Protocatechuic acid (PA)	7.4±0.1	154.12		
(+)-Catechin (CT)	563.0±0.5	290.27		Flavonoids: Flavan-3-ol Monomers
(-)-Epicatechin (ECT)	741.0±0.2	290.27		
(-)-Epigallocatechin (EGC)	1153.4±2.7	306.27		
(-)-Epicatechin 3-O-gallate (ECG)	56.5±4.1	442.37		
(-)-Epigallocatechin 3-O-gallate (EGCG)	42.4±0.3	458.37		
Procyanidin B1 (PAB1)	611.4±5.8	578.5		Flavonoids: Flavan-3-ol Dimers
Procyanidin B2 (PAB2)	432.1±3.56	578.5		
Total phenolics$_{FC}$[a]	6300±45.0			
Total phenolics$_{HPLC}$[b]	3713.3±17.6			
Hydroxybenzoic acids	113.5 ±0.4			
Monomers	2556.3±7.8			
Dimers	1043.5±9.3			

kg) and CT (563.0 ± 0.5 mg/kg). The gallate esters, ECG and EGCG, were present in lower concentrations (56.5 ± 4.1 and 42.4 ± 0.3 mg/kg, respectively) than the corresponding ECT and EGC. PAB1 was the flavan-3-ol dimer detected in the highest concentration (611.4 ± 5.8 mg/kg) followed by PAB2 (432.1 ± 5.55 mg/kg), while GA and PA were the hydroxybenzoic acids found at average levels of 106.0 ± 0.4 and 7.4 ± 0.1 mg/kg, respectively.

The GSEs of different grape cultivars have been widely characterized (*27, 28*), and their profile of low molecular weight phenolics was similar to the one determined in the osmotic solution. CT and ECT are usually the major catechins, and PAB1 and PAB2 are the most abundant procyanidin dimers. However, grape variety, maturation stage, cultural practices, and extraction conditions may cause significant differences in the particular phenolic profile of each GSE (*29*).

Response Surface Analysis of OT Performed with Multicomponent Osmotic Solutions with Two Osmo-Active Solutes (NaCl/Sucrose). Several responses were analyzed as follows: Those related to mass transfer during OT (ΔM of water, total solids, sucrose, NaCl, and phenolics), the composition of the osmo-treated food (mass fraction of sucrose, NaCl, and individual phenolics), and its antiradical scavenging capacity (TEAC). **Tables 1** and **3** show the results of the experimental design for all of the responses analyzed. **Tables 4** and **5** show the linear, quadratic, and interaction effects of the second order polynomial model (coefficient value and significance level). Standard errors (SE) and the t and P values of the regression coefficients are also shown.

The response surfaces computed using only the significant effects at the $P < 0.05$ level are shown in **Figures 1** and **2**. ΔM^{TPH} was significant throughout the response surface (from 0.17 ± 0.05 to 0.27 ± 0.03%) corresponding to ΔM^{NaCl} and ΔM^{SUC} values between 0 and 2.3 ± 0.1 and −7.4 ± 2.6 and 36.0 ± 3.0%, respectively. Negative values of ΔM^{SUC} indicate that low sucrose concentrations in the osmotic solution wash out the sucrose initially present in the model food (8.6%, w/w). Throughout the range studied, the significant gains in phenolics corresponded with a different extent of water loss: $-\Delta M^{w}$ was between 0 and 27 ± 3%. Overall, the ratio of water loss to phenolic gain, $-\Delta M^{w}/\Delta M^{TPH}$, was mainly controlled by the sucrose concentration of the osmotic solution and reached a

Table 3. Experimental Design and Content of Individual Phenolics Determined by HPLC in the Osmo-Treated Gel at the Different Factor Levels[a]

	osmotic solution		osmo-treated food								
	sucrose (molality)	NaCl (molality)	mg/kg								
no.	X_1[b]	X_2[b]	GA	PA	CT	ECT	EGC	ECG	EGCG	PAB1	PAB2
1	2.49 (1)	1.62 (1)	54.7 ± 0.1	6.6 ± 0.0	272.5 ± 3.3	354.1 ± 4.3	448.6 ± 25.5	30.8 ± 0.5	27.0 ± 0.0	265.3 ± 0.6	139.1 ± 4.8
2	0.43 (−1)	1.62 (1)	68.7 ± 0.1	6.5 ± 0.1	357.6 ± 4.9	467.2 ± 7.6	575.2 ± 45.6	51.0 ± 1.7	30.3 ± 0.6	415.3 ± 5.8	205.4 ± 26.9
3	2.49 (1)	0.28 (−1)	63.9 ± 0.1	5.8 ± 0.4	298.9 ± 15.1	387.4 ± 20.3	458.1 ± 90.8	33.3 ± 1.4	27.5 ± 0.5	244.9 ± 45.7	140.9 ± 3.3
4	0.43 (−1)	0.28 (−1)	75.1 ± 0.8	8.1 ± 1.7	390.9 ± 1.8	513.0 ± 5.6	704.1 ± 37.5	51.8 ± 0.5	31.1 ± 0.0	445.2 ± 0.9	208.2 ± 0.2
5	1.46 (0)	0.95 (0)	65.4 ± 0.2	6.6 ± 0.6	321.6 ± 5.3	415.6 ± 9.4	517.0 ± 44.7	38.1 ± 0.6	27.6 ± 0.1	309.6 ± 6.5	150.5 ± 6.5
6	1.46 (0)	0.95 (0)	62.0 ± 0.0	7.1 ± 1.4	311.6 ± 4.3	406.0 ± 5.4	513.9 ± 36.8	36.8 ± 0.3	27.8 ± 0.2	317.2 ± 7.7	166.1 ± 10.0
7	1.46 (0)	0.95 (0)	74.6 ± 0.3	6.9 ± 0.3	371.6 ± 1.8	482.3 ± 2.3	625.6 ± 22.5	43.2 ± 0.4	29.6 ± 0.1	385.2 ± 2.4	189.3 ± 4.1
8	0 (−1.414)	0.95 (0)	86.9 ± 0.1	6.9 ± 0.1	457.0 ± 11.9	617.8 ± 16.5	836.4 ± 77.0	71.3 ± 4.0	34.7 ± 0.5	598.9 ± 18.1	287.3 ± 11.2
9	1.46 (0)	0 (−1.414)	62.4 ± 0.7	5.0 ± 0.1	345.1 ± 5.7	448.1 ± 8.0	553.3 ± 42.0	41.2 ± 0.5	29.0 ± 0.2	317.5 ± 31.4	157.2 ± 11.9
10	2.92 (1.414)	0.95 (0)	65.5 ± 0.1	5.8 ± 0.1	319.6 ± 3.4	417.0 ± 6.1	528.5 ± 39.2	35.0 ± 0.1	28.6 ± 0.2	313.7 ± 12.3	180.1 ± 18.9
11	1.46 (0)	1.9 (1.414)	72.6 ± 0.6	6.3 ± 0.2	361.9 ± 12.6	474.3 ± 18.1	575.5 ± 74.1	42.6 ± 0.9	28.5 ± 0.4	365.6 ± 40.7	185.2 ± 10.6
12	1.46 (0)	0.95 (0)	64.4 ± 0.3	5.3 ± 0.4	320.4 ± 4.2	417.0 ± 7.1	519.8 ± 39.3	37.1 ± 0.1	28.3 ± 0.0	328.6 ± 19.6	161.0 ± 9.4
13	1.46 (0)	0.95 (0)	65.1 ± 0.1	5.7 ± 0.2	316.5 ± 8.8	412.1 ± 12.6	499.0 ± 56.7	36.1 ± 1.1	27.8 ± 0.0	316.1 ± 17.4	138.2 ± 20.2
14	1.46 (0)	0.95 (0)	62.5 ± 0.4	5.7 ± 0.3	306.4 ± 6.8	398.6 ± 8.6	482.3 ± 40.3	35.8 ± 0.2	28.2 ± 0.1	308.1 ± 21.3	145.3 ± 13.6

[a] OT was performed for 8 h. [b] In parentheses, the coded values of the two factors.

Table 4. Regression Coefficients and Analysis of Variance of the Second Order Polynomial Model for Water Loss, Total Solids, NaCl, Sucrose, and Total Phenolic Gains and Their Corresponding Mass Fractions as Well as TEAC in the Osmo-Treated Food[a]

coefficient	ΔM^W	ΔM^{SS}	ΔM^{NaCl}	ΔM^{SUC}	$\Delta M^{TPH_{HPLC}}$	x^{NaCl}	x^{SUC}	monomers[d]	dimers[d]	TEAC
b_0	−0.1409[c]	0.2204[c]	0.0349[c]	0.2209[c]	0.002[c]	0.0323[c]	0.2993[c]	1338.9[c]	485.84[c]	46.2[c]
					linear					
b_1	−0.1061[c]	0.1384[c]	−0.0049[c]	0.1467[c]	−0.0003[c]	−0.0057[c]	0.1336[c]	−225.9[c]	−129.9[c]	−11.1[c]
b_2	−0.0135[b]	0.0215[c]	0.0235[c]	NS	NS	0.0217[c]	NS	NS	NS	NS
					quadratic					
b_{11}	0.0139[c]	−0.0433[c]	NS	−0.046[c]	NS	0.0008[c]	−0.040[c]	NS	80.8[c]	4.03[c]
b_{22}	0.0082[b]	NS	−0.0007[b]	NS	NS	−0.0009[c]	NS	NS	NS	NS
					interaction					
b_{12}	NS	NS	−0.0033[c]	NS	NS	−0.0037[c]	NS	NS	NS	NS
R^2	0.9930	0.9956	0.9993	0.9915	0.7252	0.9994	0.9913	0.7833	0.8708	0.9396
SE	0.0091	0.0097	0.0007	0.0142	0.0002	0.0006	0.013	134.8	58.8	3.00
F	226.8	364.0	2204.5	187.2	4.2227	2700.3	181.4	5.8	10.8	24.9
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0355	<0.0001	<0.0001	<0.015	<0.0021	0.0001

[a] Standard errors (SE). t and P values of the regression coefficients are included. [b] Effect significant at the $P < 0.05$ confidence level. [c] Effect significant at the $P < 0.01$ confidence level. [d] Mass fraction expressed in (mg/kg).

Table 5. Regression Coefficients and Analysis of Variance of the Second Order Polynomial Model for Contents of Individual Phenolics Determined by HPLC in the Osmo-Treated Food (mg/kg)[a]

coefficient	GA	PA	CT	ECT	EGC	ECG	EGCG	PAB1	PAB2	TPH_{HPLC}
b_0	65.66[c]	6.22[c]	324.69[c]	421.92[c]	526.25[c]	37.87[c]	28.21[c]	327.47[c]	158.4[c]	1896.67[c]
					linear					
b_1	−6.92[b]	NS	−46.44[c]	−65.34[c]	−100.99[c]	−11.25[c]	−1.95[c]	−94.21[c]	−35.67[c]	−363.23[c]
b_2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
					quadratic					
b_{11}	NS	NS	NS	33.02[b]	58.88[b]	6.20[c]	1.41[c]	50.38[c]	30.4[c]	205.8[b]
b_{22}	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
					interaction					
b_{12}	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
R^2	0.6215	0.4441	0.7478	0.7592	0.7961	0.9102	0.8870	0.8869	0.8256	0.8141
SE	6.13	0.79	29.69	41.06	59.96	3.40	0.853	38.22	21.33	198.43
F	2.63	1.28	4.74	5.04	6.25	16.22	12.57	12.55	7.57	7.01
P	0.1081	0.36	0.0261	0.022	0.0119	0.0005	0.0013	0.0013	0.0067	0.0085

[a] Standard errors (SE). t and P values of the regression coefficients are included. [b] Effect significant at the $P < 0.05$ confidence level. [c] Effect significant at the $P < 0.01$ confidence level.

maximum value of about 149 when the sucrose concentration was the highest.

Figure 2shows the model food composition after 8 h of OT vs the concentration of NaCl and sucrose in the osmotic solution. Under these conditions, an intermediate moisture product was obtained with a_w values between 0.912 and 0.955 (**Table 1**). Even though the osmo-treated food presents quite a narrow range of a_w, its composition depended heavily on the concentration of NaCl and sucrose in the osmotic solution. When the composition of the osmotic solution was varied, the NaCl content in the osmo-treated food ranged between 0 and 0.079 ± 0.002 kg/kg and sucrose levels between 0.03 ± 0.01 and 0.427 ± 0.024 kg/kg. In addition, the osmo-treated model food was significantly supplemented with phenolics,

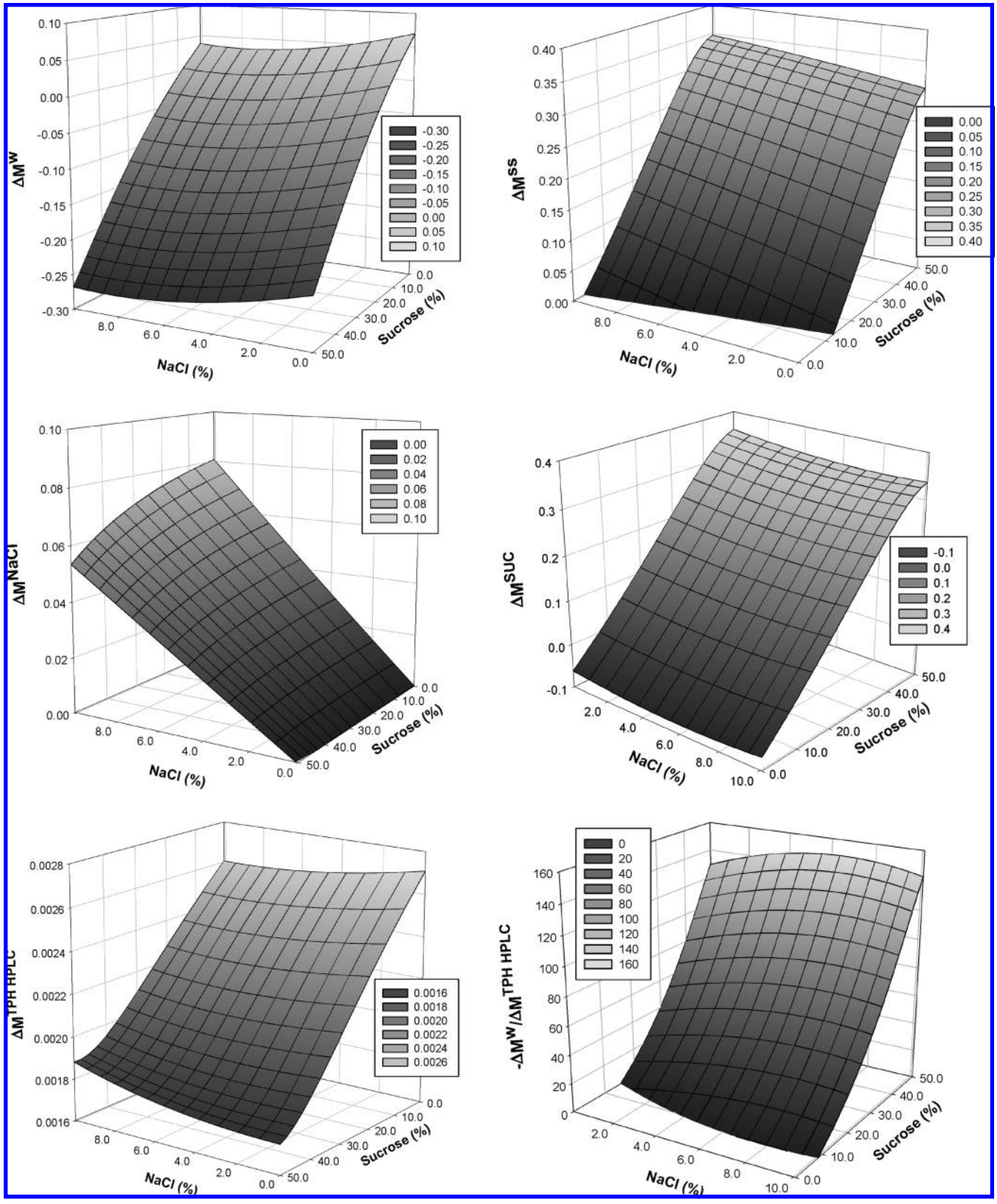


Figure 1. Surface responses of water mass changes (ΔM^{w}), gain in total solids (ΔM^{ss}), NaCl (ΔM^{NaCl}), sucrose (ΔM^{SUC}), total phenolics (ΔM^{TPH}), and ratio of water loss to gain in total phenolics ($\Delta M^{w}/\Delta M^{TPH}$) vs sucrose and NaCl mass fraction in the osmotic solution.

in particular with flavan-3-ols. The monomers of this phenolic group were found in concentrations between 1231 ± 126 and 2020 ± 246 mg/kg and the dimers between 386 ± 107 and 832 ± 214 mg/kg. In comparison with the values reported for commonly consumed fresh and processed foods, then, the content of osmo-treated food was similar to that of proanthocyanidin-rich food products (see **Table 6**).

The coefficients of the second order model show that linear and quadratic terms significantly determined the extent of ΔM^{w} (**Table 4**), while the interaction between NaCl and sucrose did

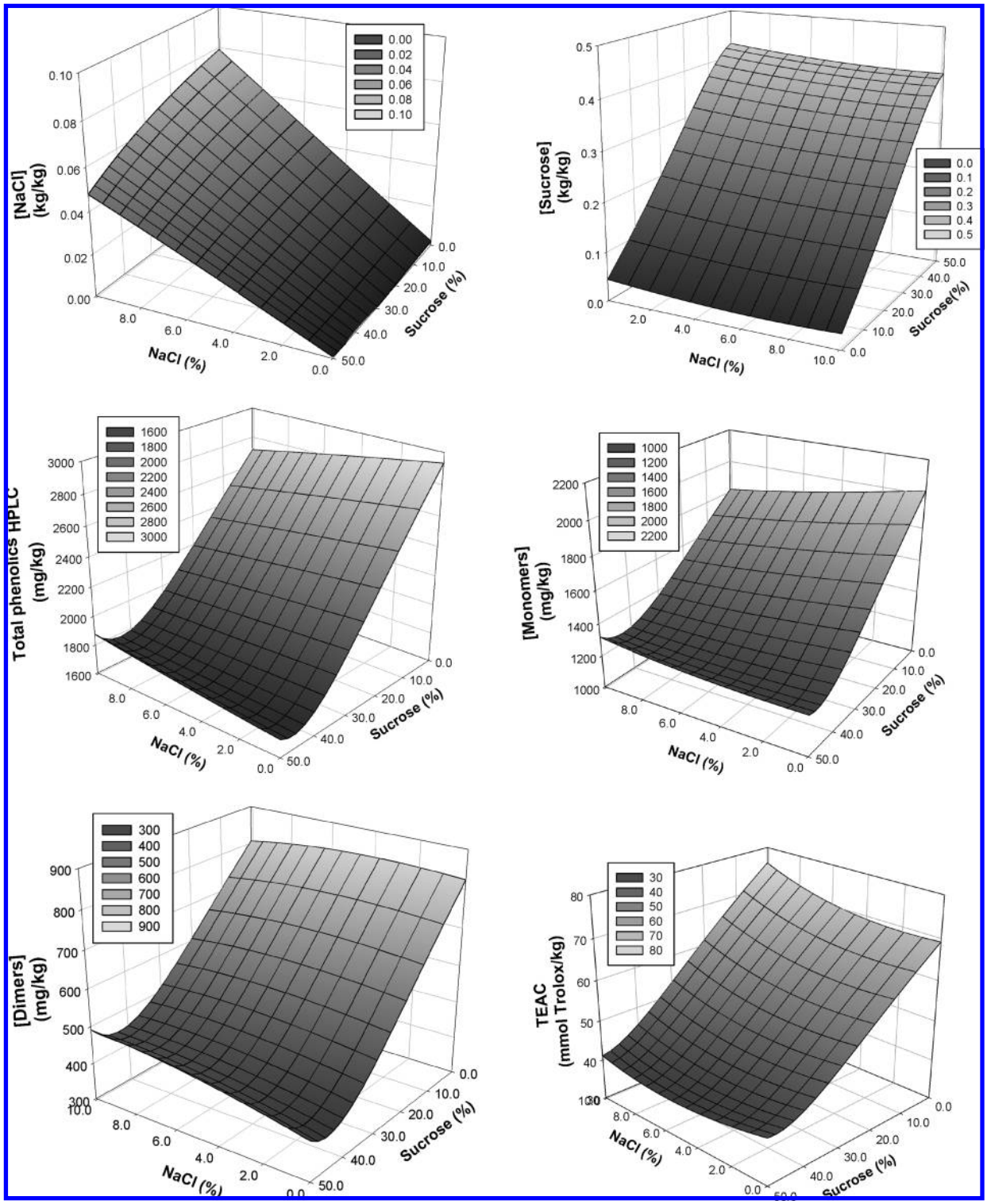


Figure 2. Surface responses of NaCl, sucrose, total phenolic, flavan-3-ol monomer and dimer contents, and TEAC of the osmo-treated food after 8 h of OT.

not have a major influence. Similarly, ΔM^{SS} is mainly controlled by linear and quadratic effects, whereas the interaction term is not significant. The gain in individual solutes presented some differences: Although linear, quadratic and interaction terms are significant on ΔM^{NaCl}, only linear and quadratic effects of sucrose molality determine ΔM^{SUC} and ΔM^{TPH}. In addition, ΔM^{TPH} and $-\Delta M^{w}$ were more sensitive to variation in the sucrose molality than the NaCl molality of the osmotic solution.

The effect of NaCl and the sucrose concentration on mass transfer during OT with ternary aqueous solutions has been extensively investigated for several food commodities, and quite a widespread conclusion is that NaCl enhances water loss while sucrose seems to reduce NaCl gain (*9, 13*). However, to investigate the effect of the osmo-active agent on mass transfer, we have to take into account that the gradient of a_w (directly correlated to osmotic pressure) between the solid food and the osmotic solution that drives water transport during OT. For water/NaCl/sucrose solutions, the contribution of each individual solute to mass transfer should be established from the results obtained with solutions of different compositions but constant

Table 6. Proanthocyanidin Content (mg/kg) of Different Food Commodities

commodity	mg/kg momomer	mg/kg dimer	refs
chocolate products	797.9 ± 248.6 3633.3 ± 632.9	590.5 ± 140.7 3066.7 ± 514.0	*30,31*
nuts			
hazelnuts	98.3 ± 15.7	125.1 ± 38.4,	*31*
pecans	172.2 ± 25.5	421.3 ± 54.2	
pistachio	109 ± 43.5	132.64 ± 18.0	
fruits			
plums	108.8 ± 29.1	385.4 ± 107.2	*31,32*
nectarines	106.4 ± 46.9	119.3 ± 82.8	
cranberries	72.6 ± 15.1	259.3 ± 61.2	*31,33*
raspberries	39.1 ± 27.4	86.4 ± 83.6	
strawberries	37.1 ± 8.0	52.6 ± 18.9	

values of a_w. When the experimentation does not fulfill this condition, NaCl has the greatest dehydration effect because of the important differences in molecular weight between NaCl and sucrose: A small increase in the NaCl mass fraction significantly changes a_w and leads to major water loss. Thus, the experimental design considered osmotic solutions within quite a narrow range of a_w (from 0.887 to 0.976). From this point on, every response variable was calculated from its corresponding response surface but only for osmotic solutions (water/NaCl/sucrose/phenolics) with a constant a_w. Assuming that phenolics did not contribute to a_w in the osmotic solution (*17*), we calculated several combinations of sucrose and NaCl concentrations that led to a 0.935 a_w within the range stated for each of them in the experimental design (sucrose, 0−2.9 molal; NaCl, 0−1.9 molal). Chirife et al. (*34, 35*) found that the models applied (Norrish, Pitzer, and Ross) predict the a_w of aqueous binary and ternary NaCl and sucrose solutions with deviations that can be even less than the error of the typical instrumentation available for measuring it.

Figures 3 and **4** plot variable responses after 8 h of OT with osmotic solutions of different NaCl/sucrose compositions but with a constant a_w of 0.935. Variable responses are plotted against y_{OS}^{SUC}, which was calculated as:

$$y_{OS}^{SUC} = \frac{n_{OS}^{SUC}}{n_{OS}^{SUC} + n_{OS}^{NaCl}} \quad (6)$$

where n_{OS} is the number of moles of each osmo-active solute in the osmotic solution.

Figure 3 shows that water loss ($-\Delta M^w$) increased with y_{OS}^{SUC} so sucrose has a higher dewatering effect than NaCl when agar gel is used as the model food. As far as solute impregnation was concerned, the ΔM^{SS} and sucrose mass fraction in the osmo-treated food also increased with y_{OS}^{SUC} while the NaCl mass fraction decreased. The maximum contents of sucrose and NaCl were 0.43 ± 0.04 and 0.080 ± 0.002 kg/kg, respectively, which were obtained with y_{OS}^{SUC} values of 1 and 0.

These impregnation results can be explained by considering the ratio of sucrose to NaCl moles in the osmo-treated food, n_F^{NaCl}/n_F^{SUC}. **Figure 3** shows the exponential decrease in this ratio vs y_{OS}^{SUC}, which indicates that sucrose limits NaCl gain, particularly when y_{OS}^{SUC} is below 0.5. When y_{OS}^{SUC} increases slightly to 0.25, the ratio of NaCl to sucrose moles in the osmo-treated food drastically decreases to values close to 2. For values of y_{OS}^{SUC} above 0.5, the ratio of NaCl to sucrose moles in the osmo-treated food is lower than 1 (**Figure 3**). When y_{OS}^{SUC} increases to 0.75, the ratio of NaCl to sucrose moles in the osmo-treated food reaches values close to 0.333. These results are similar to those reported during the OT of carrot with water/NaCl/sucrose solutions of 0.91 a_w (*12*) in which case the ratio of NaCl to sucrose moles in the osmo-treated carrot was 0.29 for osmotic solutions with 0.76 y_{OS}^{SUC} and above 1 for $y_{OS}^{SUC} < 0.5$. These results show that when $y_{OS}^{SUC} > 0.5$, the penetration of sucrose moles predominates and results in a higher mass fraction of sucrose than of NaCl and an increase in total solute gain (ΔM^{SS}). Regarding flavan-3-ol monomers and dimers, their penetration into the model food is limited by sucrose to a different extent. By increasing y_{OS}^{SUC}, the monomer content decreases from 1816 ± 461 to 1245 ± 457 mg/kg, while the dimer content decreases from 806 ± 200 to 406 ± 199 mg/kg. When $y_{OS}^{SUC} < 0.75$, the monomer and dimer contents reach minimum values of 1232 ± 206 and 405 ± 136 mg/kg, respectively.

Considering all of this, a range of different osmo-treated model foods can be formulated with a very high content of flavan-3-ols (monomers and dimers), similar a_w but very different contents of NaCl and sucrose. By using more than one osmo-active solute and adjusting the composition of the osmotic solution, OT can control not only the phenolics content but also the sensory properties of the end product. For instance, with $y_{OS}^{SUC} = 0.75$ (3.7% of NaCl and 41.3% sucrose in the osmotic solution), the osmo-treated food presents the lowest monomer and dimer impregnation but a significant water loss ($-\Delta M^w = 0.19 \pm 0.01$), relatively low NaCl (0.0220 ± 0.0005 kg/kg), and high sucrose (0.37 ± 0.01 kg/kg) content. Even though sensory properties can be negatively affected by such a high gain in total solutes, NaCl impregnation may reduce the sensory effect caused by very high sucrose levels (*36*).

Influence of the Osmo-Active Agent on the Phenolic Profile of the Osmo-Treated Food. The regression coefficients and analysis of variance of the fitted second order model for individual phenolics (**Table 5**) show that only linear and quadratic effects of sucrose molality have a significant influence on the mass fraction of flavan-3-ols quantified in the osmo-treated food. In the case of hydroxybenzoic acids, a lack of fit was detected with PA content, while in the GA mass fraction, only the linear term of sucrose molality had an significant effect. The results obtained for the PA surface response are not considered in the following discussion of results. For each response variable, we calculated the confidence limits (95%) of the predicted value. In case of individual phenolics, we found that the mean relative error of these estimations was about 15%, what was related to R^2 values between 0.62 and 0.91. As expected, the error distribution throughout the surface response was much higher at its lower and upper limits than around the central point. In spite of it, the surface response showed a clear behavior vs the composition of the osmotic solution, which is thoroughly discussed next. From this point on, to improve clarity, error bars are not plotted in the graphs.The effect of the osmo-active solute on the impregnation of each individual phenolic was analyzed from their response surfaces. Only those osmotic solutions with a constant value of 0.935 a_w were considered (**Figure 4**). Although the trend is similar for all phenolics, the extent of the decrease in content vs y_{OS}^{SUC} depends on the kind of phenolic. The content of all individual phenolics, except EGC and PAB1, is minimum for y_{OS}^{SUC} values between 0.80 and 0.85. EGC and PAB1 contents decrease throughout the y_{OS}^{SUC} range and are minimum for osmotic solutes that contain only sucrose as the osmo-active agent.

Although sucrose reduced the phenolic infusion in all cases, the extent of the reduction was determined by the phenolic

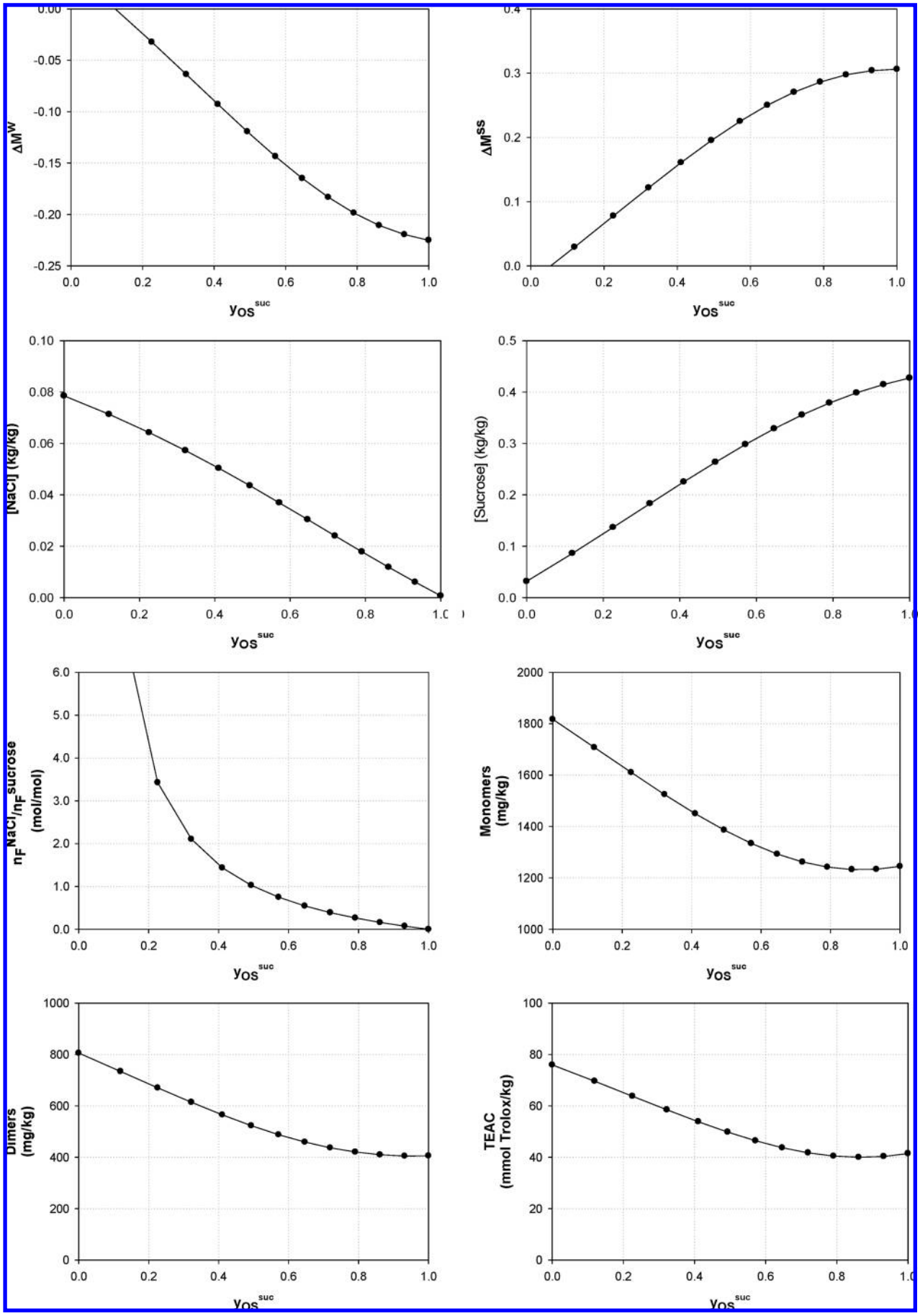


Figure 3. Mass changes of water (ΔM^w), gain in total solids (ΔM^{ss}), NaCl, sucrose, flavan-3-ol monomer and dimer contents, the ratio of sucrose to NaCl moles in the osmo-treated food, (n_F^{NaCl}/n_F^{SUC}), and TEAC vs y_{OS}^{SUC}. Data were obtained from the surface response for osmotic solutions of 0.935 a_w.

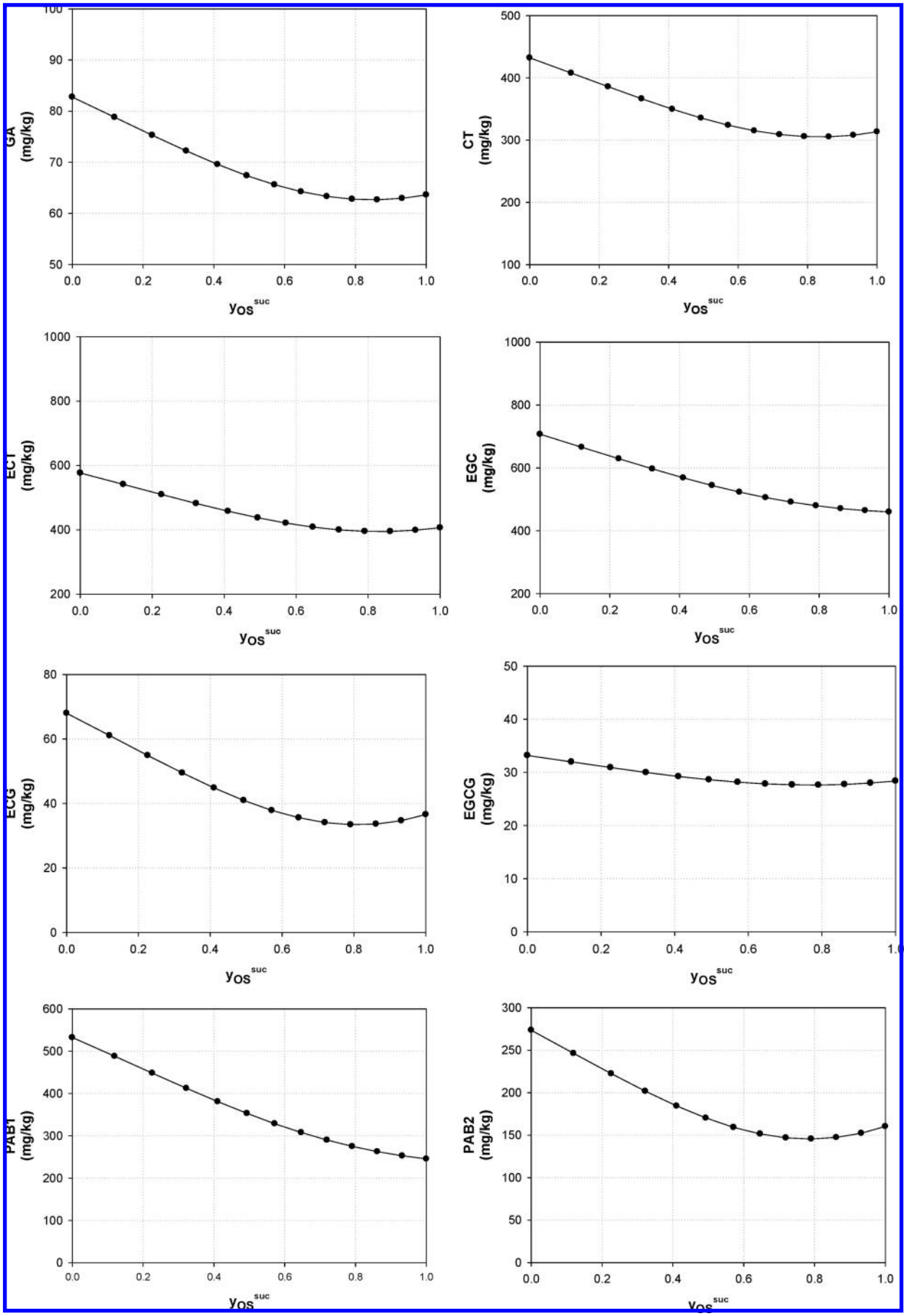


Figure 4. Content of GA, CT, ECT, EGC, ECG, EGCG, PAB1, and PAB2 vs y_{OS}^{SUC}. Data were obtained from the surface response for osmotic solutions of 0.935 a_w.

Table 7. Maximum and Minimum Mass Fractions of Individual Phenolics, Their Penetration Ratios, and z Values (Content of Each Phenolic in the Osmo-Treated Food Expressed as mg/kg Food Liquid Phase) Obtained with Two Osmotic Solutions: $y_{OS}^{SUC} = 1$ (Only Sucrose as the Osmo-Active Agent) and $y_{OS}^{SUC} = 0$ (Only NaCl as the Osmo-Active Solute)[a]

		mg/kg			z (mg/kg fluid food phase)	
phenolics	molecular weight (g/mol)	$(phj)_{max}$	$(phj)_{min}$	PR^{phj} (%)	$y_{OS}^{SUC} = 0$	$y_{OS}^{SUC} = 1$
GA	170.12	83 ± 21	63 ± 7	24.3	86.2	66.1
CT	290.27	432 ± 102	306 ± 32	29.3	449.8	325.4
ECT	290.27	577 ± 140	395 ± 45	31.5	600.2	422.2
EGC	306.27	707 ± 205	480 ± 65	34.9	736.3	477.7
ECG	442.37	68 ± 14	33 ± 4	50.8	70.8	38.0
EGCG	458.37	33 ± 3	28 ± 1	16.7	34.6	29.5
PAB1	578.50	532 ± 130	275 ± 41	53.9	554.1	254.9
PAB2	578.80	274 ± 73	146 ± 23	46.8	284.6	166.5

[a] Data obtained from the surface responses for osmotic solutions of 0.935 a_w.

molecular weight. The penetration ratio of each phenolic, PR^{phj}, was calculated as:

$$PR^{phj} = \frac{(x^{phj})_{max} - (x^{phj})_{min}}{(x^{phj})_{max}} \quad (7)$$

where x^{phj} is the mass fraction of an individual phenolic in the osmo-treated gel and the subindexes max and min indicate maximum and minimum values, respectively. **Table 7** shows the maximum and minimum contents of individual phenolics, and it can be seen that PR^{phj} increased with the phenolic molecular weight. For instance, the PR of GA (170.12 g/mol) was 24%, while for the flavan-3-ol dimers PAB1 and PAB2 (578.5 g/mol), it was 54 and 47%, respectively. Only EGCG had a PR that was low (17%) for its molecular weight (458.37 g/mol).

From the point of view of phenolic mass transfer, it is interesting to evaluate if 8 h of OT leads to equilibrium. 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 (*37*). **Table 7** shows values of z (that is, the content of each individual phenolic in the osmo-treated food) expressed as mg/kg food liquid phase and obtained with two osmotic solutions: $y_{OS}^{SUC} = 1$ (only sucrose as the osmo-active agent) and $y_{OS}^{SUC} = 0$ (only NaCl as the osmo-active solute). In experiments performed with $y_{OS}^{SUC} = 1$, the z values for all of the phenolics are lower than for those in the osmotic solution, and the ratio of the content in the osmo-treated gel to that in the osmotic solution is above 50% for all phenolics except for EGC and PAB1. When $y_{OS}^{SUC} = 0$, z values were higher and the ratio of the content in the osmo-treated gel to that in the osmotic solution was 78% on average. For ECG, z values were higher than their content in the osmotic solution. Besides the errors of the predicted values calculated from the surface response, this result can also be explained if we consider that GSE (the actual source of the phenolics used in the osmotic solution) is a complex mixture of flavan-3-ols with different degrees of polymerization. Such a high content of ECG in the food liquid phase may be because the polyphenolics with a high number of flavan-3-ol units hydrolyzed (*38*). The low pH (3.4) of the osmotic solution together with agitation during OT may promote this reaction pathway. Taking all of this into account, we can conclude that the rate of phenolic mass transfer was higher when NaCl and not sucrose was the osmo-active solute. In addition, when sucrose is present in the osmotic solution, it seems to hinder the penetration of flavan-3-ols, in particular, of those with higher molecular weights (e.g., PAB1 and PAB2).

Antioxidant Properties and Their Correlation with the Phenolic Profile. The antioxidant properties of the osmo-treated food (in particular, its antiradical scavenging capacity) were determined in vitro by TEAC. Although the correlation between the TEAC and the real antioxidant capacity in vivo is still controversial, the considerable amount of TEAC data now available on many fruits and vegetables means that TEAC is now extensively used to compare the antioxidant scavenging capacities of many different food commodities.

The analysis of the regression coefficients of the second order model showed that only the linear and quadratic coefficients of the sucrose molality are significant, as was observed with the individual phenolics analyzed (**Table 4**). These results indicate that sucrose is the osmo-active solute that mainly limits phenolic penetration and, then, the antioxidant scavenging capacity of the osmo-treated gel.

As with the individual phenolics, to discuss the effect of the osmo-active solute on TEAC, we took into consideration the results obtained from the TEAC response surface for osmotic solutions with a constant a_w of 0.935. **Figure 3** shows that TEAC values decreased with y_{OS}^{SUC} from 76.0 ± 10.2 to 40.0 ± 4.6 mmol Trolox/kg. Comparing these results with average TEAC values for some fruits, we can establish that the TEAC of the osmo-treated gel was higher than that of fruits with a very high free radical scavenging activity. Blackberry and raspberry, for instance, have reported TEAC values of 20.24 and 16.79 mmol Trolox/kg of FW, respectively (*39*). Previous results (*17*) obtained with an osmotic solution of concentrated grape juice with a mass fraction of soluble solids of 50% (0.935 a_w) showed TEAC values of 66.3 mmol Trolox/kg in an osmo-treated gel. However, the total phenolic content of the concentrated grape juice was 13152 ± 276 mg GAE/kg, which is twice that of the total phenolic content in the osmotic solution (6300 ± 45 mg GAE/kg) used here.

To determine the extent to which the individual phenolics identified describe TEAC in the osmo-treated food, correlations between the TEAC and the phenolic profile were determined. Linear regression analysis (**Table 8**) showed that the total phenolics identified by HPLC have a significant effect on TEAC ($R^2 = 0.6182$, $P < 0.001$). In particular, flavan-3-ol dimers ($R^2 = 0.6618$, $P < 0.001$) seem to make a slightly higher contribution to the radical scavenging capacity than the flavan-3-ol monomers ($R^2 = 0.5844$, $P < 0.005$), while the hydroxybenzoic acid, GA, made a significant but lower contribution ($R^2 = 0.4672$, $P = 0.007$). All flavan-3-ol monomers made a significant contribution, particularly ECG followed by EGCG, CT, ECT, and EGC. Of the flavan-3-ol dimers, both PAB1 and PAB2 had very similar correlations with TEAC. Similarly, in a study of the correlations between TEAC and phenolic content of GSEs

Table 8. Linear Regression Analysis of Antioxidant Capacity, TEAC, vs Individual and Total Phenolic Content

phenolics	a^b	b^b	R^2	F	P^c
GA	0.839	−7.32	0.4672	10.52	0.0070
CT	0.157	−3.97	0.5752	16.25	0.0017
ECT	0.111	0.19	0.5728	16.09	0.0017
EGC	0.069	10.52	0.5647	15.57	0.0019
ECG	0.786	16.40	0.7379	33.79	0.0001
EGCG	3.836	−61.97	0.6346	20.84	0.0006
PAB1	0.089	17.82	0.6880	26.46	0.0002
PAB2	0.182	17.41	0.5759	16.29	0.0016
total phenolics$_{HPLC}$[a]	0.021	7.17	0.6182	19.42	0.0009
flavan-3-ol monomers	0.032	3.65	0.5844	16.87	0.0015
flavan-3-ol dimers	0.061	17.16	0.6618	23.48	0.0004

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

obtained from several grape varieties (*28*), flavan-3-ol dimers made a higher contribution than monomers.

The extent of phenolic infusion significantly increased the antiradical scavenging capacity of the osmo-treated gel, while all of the flavan-3-ols detected and the hydroxybenzoic acid, GA, also made significant contributions. Further applications of OT to infuse solid foodstuffs with grape phenolics should take into consideration other sources of grape phenolics so that the extent of infusion in different operating conditions can be determined. In addition, the results obtained with GSE and grape juice as a source of phenolics should be validated when real structured foods are used. The stability of phenolics during further processing steps and the sensory properties of the end product require further investigation.

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