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## Grape phenolic infusion into solid foods: studies on mass transfer and antioxidant capacity

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### Abstract

Osmotic Treatment (OT) enables to introduce controlled quantities of solution solutes into food and partially dehydrate it. The main aim of this study was to formulate intermediate-moisture solid foods with functional ingredients such as grape phenolics using OT. We evaluated how the source of phenolics and different types of binary mixtures of osmo-active solutes, affects: (i) the penetration level of grape phenolics, (ii) the intake of low-molecular-weight phenolics, and (iii) antioxidant capacity in a model food made of agar during OT. Fickian and empirical models were applied to characterise the mass transfer rate of grape phenolics during OT. Finally, the extent and rate of phenolic infusion into plant tissue (apple, banana and potato) was determined while their stability after a post-treatment such as convective air drying was evaluated. Our results confirm that OT is a suitable technology for the exploitation of solid foods into which functional ingredients can be successfully incorporated. Under the conditions that maximized the phenolic infusion, the total phenolic content of the osmo-dehydrated food was close to the values reported in some rich-in-phenolic fruits and vegetables, while the TEAC was three times that of fresh fruit with the highest antioxidant capacity. The effective diffusion coefficients of total and individual phenolics showed that the kind and the concentration of osmo-active solute in the osmotic solution, food structure and the molecular weight of grape phenolics controlled their rate of infusion in the food. OT as a pre-treatment protected against grape phenolic degradation during further convective air drying.

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### 1. Introduction

In recent years, interest has grown in developing new functional foods that have health-promoting and/or disease-preventing properties beyond the basic function of supplying nutrients. Since synthetic

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antioxidants raise toxicological concerns, grape extracts have become popular as nutritional supplements. Extracts from grapes contain a heterogeneous mixture of phenolic acids, simple flavonoids, complex flavonoids and anthocyanins. Commercial grape extracts are usually obtained from grape seeds and marc (i.e. solid residues of the wine-making process) in which the most abundant phenolics that have been isolated from grape seeds are catechin, epicatechin and procyanidins [1,2]. Numerous studies have demonstrated the benefits of the phenolic compounds in grapes, including antioxidant effects [3], antimicrobial effects [4], anticancer effects [5] and protection against cardiovascular diseases [6]. Osmotic treatment (OT) is widely used to modify the composition of solid foods (e.g. fruits, vegetables, meat and fish) by partially removing water and adding solutes. During immersion in a hypertonic (osmotic) solution, the higher osmotic pressure of the osmotic solution (OS) drives water transport from the solid food into the solution [7]. Water transport is accompanied by the simultaneous counter-diffusion of solutes from the OS into the food structure. In the food industry, OT is used to improve quality in various areas, including colour, flavour, texture, energy efficiency and packaging [8].

The aim of this study was to formulate intermediate-moisture solid foods with functional ingredients such grape phenolic using OT. To satisfy this main objective, we also evaluated how the source of phenolics, i.e. concentrated red grape juice and commercial extracts from grape seed and white grape marc, affects: (i) the penetration level of grape phenolics, (ii) the intake of low-molecular-weight phenolics, and (iii) antioxidant capacity in a model food at different operation conditions. To do so, Fickian and empirical models were applied. Besides, we determined how different types of binary mixtures of osmo-active solutes (sucrose and sodium chloride) affect the phenolic pattern and antioxidant activity of the final product. Finally the extent and rate of phenolic infusion into plant tissue (apple, banana and potato) was established and their stability after a post-treatment such as convective air drying was evaluated.

## 2. Materials and Methods

### 2.1. Fruit, vegetable and model food procedures

Fresh apples (*Malus pumila*, var. Granny Smith), bananas (*Musa acuminata*, var. Cavendish) and potatoes (*Solanum tuberosum*, var. Monalisa) were purchased from a local market. 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. Apple, banana, potato and model food samples were cut in 1 cm side cubes.

### 2.2. Osmotic solutions

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. In order to evaluate how the source of phenolics affect the phenolic infusion into model food two kinds of phenolic extracts were used: grape seed extract (GSE) and white grape marc extract (WGME) (Seppic, Paris, France). The osmo-active solute was sucrose (refined, 99.9%), and the osmotic pressure of the OS was adjusted by a 50% (w/w) sucrose solution. The total phenolic content of the various OSs used in the OT experiments was adjusted using GSE and WGME to  $15.0 \pm 0.4$ ,  $7.70 \pm 0.5$  and  $3.5 \pm 0.2$ g GAE/L.

In order to evaluate how binary mixtures of osmo-active solutes affect the phenolic pattern and antioxidant capacity of the final product, a multi-component aqueous solution made of sucrose, NaCl and a commercial GSE (Vitolol® supplied by Berkem, Gardonne, France) was used as the osmotic solution. In all experiments the mass fraction of total phenolics was kept constant ( $6300 \pm 45$  mg GAE/kg) while the mass fraction of sucrose and NaCl was set by a second-order Central Composite Rotatable Design (CCRD) with two factors (NaCl and sucrose molality of the osmotic solution). The molality of sucrose

and NaCl ranged from 0 to 2.92 and 1.9, respectively, therefore, the  $a_w$  of the osmotic solution was from 0 to 0.935[9].

In the experiments of OT followed by air-drying, a commercial grape seed extract (Vitisol® supplied by Berkem, Gardonne, France) was used as a source of phenolic compounds. In all experiments, the mass fraction of the total phenolics in the osmotic solution was set to  $6300 \pm 45$  mg GAE/kg. Solutions with 50 % (w/w) sucrose (refined, 99.9% sucrose) were used during the OT of apple and banana, while solutions with 10 % (w/w) sodium chloride (J.T. Baker, Germany) were used to treat potato. At these concentrations, all osmotic solutions presented a similar water activity of  $0.935 \pm 0.010$ .

### 2.3. Osmotic treatment

The experimental set-up consisted of two parts: a basket to allocate the samples and a vessel to be filled with osmotic solution. The sample cubes were placed in a basket and then immersed into the osmotic solution vessel. Then the vessel was placed in a magnetic stirrer. The agitation level was chosen in order to make the surface mass transfer negligible. The solution/food ratio (w/w) was always higher than 20:1, preventing significant alteration of the solution concentration during the OT. The samples were processed up to 24 hours. The temperature was maintained at  $25 \pm 2$  °C. All experiments were run under atmospheric pressure. Each experiment was performed in duplicate.

### 2.4. Air drying

The osmotically pre-treated apple, banana, potato and agar gel samples were placed in a laboratory scale dryer. This basically consisted of a through flow chamber with controlled temperature and air flow velocity (Mulet et al., 2000). Experiments were conducted at 55 °C with an air rate of 4 m/s, which ensured that mass transfer was controlled by the internal resistance. The initial load of osmo-treated samples to be dried was approximately 100 g. In order to characterize drying kinetics, the weight of the samples was monitored during drying, and the final moisture content was determined at least in triplicate. The samples were dried until a constant weight was reached. Each experiment was carried out in duplicate. Dried samples were stored at 5 °C for 1 week until the phenolic extraction process.

### 2.5. Analytical methods

The moisture content of fresh, osmo-treated and osmo-air dried samples was determined gravimetrically following the official AOAC method 920.151 [10]. A hygrometer was used to measure the water activity of the osmotic solution, and the apple, banana, potato and agar gel (Novasina, IC-500, AW-LAB).

The total sucrose content was determined by the Rebelein method [11] using a GAB kit for sugar analysis (GAB Sistemática Analítica S.L., Barcelona, Spain). The sodium chloride content was quantified according to Mohr's method [12].

Phenolic compounds were sequentially extracted with solution of methanol/water (50/50, v/v) from fresh, osmo-treated and osmo-air-dried food in order to determine the total and individual phenolics. The total phenolic content (TPH) of the osmotic solution and osmo- and osmo-air-dried food extracts was determined spectrophotometrically (CECIL, CE2021) by Folin-Ciocalteu's colorimetric method [13]. TPH was expressed as the gallic acid equivalent using the standard curve prepared at different concentrations of gallic acid. Data presented are the average of two measurements for each extract. Antioxidant capacity of the prepared extracts was assessed according to the ABTS decolorization assay [14], which is based on to what extent antioxidants inhibit the absorbance of the radical cation of 2,2'-azinobis(3-ethylbenzothiazoline 6-sulfonate) (ABTS). Individual phenolics were identified and quantified using liquid chromatography HPLC (Hewlett-Packard, HP/Agilent, Wardborn, Germany) equipped with

ChemStation software. The concentrations of the phenolic compounds identified were measured using external standard curves. Calibration curves (standard area in absorbance versus concentration in mg/L) were performed over the range of concentration observed [15, 16, 19].

2.6. Calculation procedures

Mass transfer parameters during OT of apple, banana, potato and agar gel cubes were analyzed by calculating the following parameters: water loss ( $-\Delta M^w$ ), soluble solid gain ( $\Delta M^{ss}$ ), gain in moles of osmo-active solute ( $\Delta N^{ss}$ ) and total phenolic gain ( $\Delta M^{TPH}$ ) according to the equations reported by Rózek et al. (2007) [15]. The effective diffusion coefficients ( $D_e$ ) were calculated assuming that the samples behave like an isotropic structure and considering the external mass transfer negligible compared to the internal resistance, the solution of Fick’s second law for cubical geometry was used to model the mass transfer of water and soluble solids during OT [16]. The model proposed by Peleg [17] and redefined by Palou et al. [18] was employed to fit the progress of total and individual phenolic content during OT.

3. Results and Discussion

The results obtained during OT with concentrated red grape must showed that the concentration of osmo-active solute (sugars) in the osmotic solution (OS) significantly affected the penetration level of grape phenolics. Increasing the concentration of soluble solids of red grape must to 50% (w/w) significantly decreased the penetration of total and individual phenolics in the model food. Figure 1 shows the total phenolic content determined according to the Folin-Ciocalteu’s method and the phenolic content determined by HPLC. The total phenolic content in the osmo-dehydrated food increased with processing time. OT for 24 hours 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  $7284 \pm 219$  mg of GAE/kg).

The effective diffusion coefficients of total and individual phenolics showed that not only the concentration of soluble solids in the osmotic solution but also the molecular weight of grape phenolics controlled their rate of infusion in the model food. The penetration of phenolics with a molecular weight of over 612 g/mol was low and made a poor contribution to total phenolic impregnation. The  $D_e$  of total phenolics for all conditions tested was influenced by the soluble solids concentration in the osmotic solution.  $D_e$  increased from  $2.9 \cdot 10^{-11}$  to  $4.9 \cdot 10^{-11}$  m<sup>2</sup>/s during OT with 40 and 50% of soluble solids and significantly decreased to  $0.9 \cdot 10^{-11}$  m<sup>2</sup>/s during OT with 60% of soluble solids in OT.

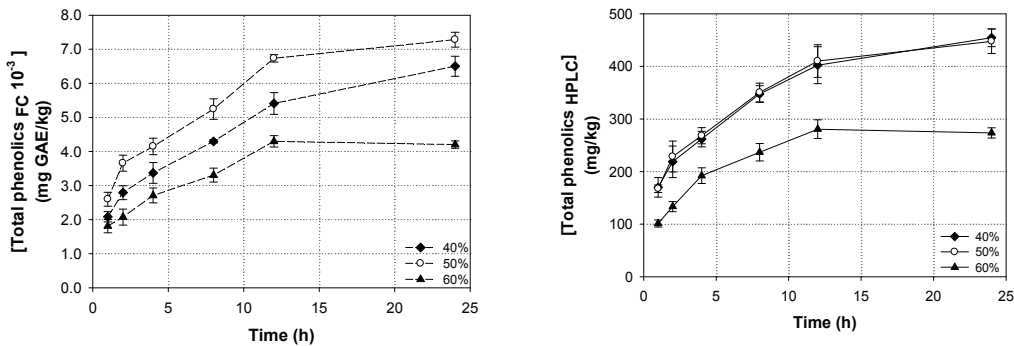


Fig. 1. Total phenolic content, determined by Folin-Ciocalteu’s method and HPLC identified in the osmo-dehydrated food during OT with concentrated red grape juice (mean ± standard deviation of experiments performed in triplicate).

Using concentrated red grape must as osmotic solution enabled us to infuse low molecular phenolic compounds such as *trans*-caftaric acid, *trans*-couteric acid, caffeic acid, coumaric acid, ferulic acid, gallic acid and flavonols such as rutin and quercetin. Regression analysis showed that the individual phenolics analyzed significantly explained the antioxidant capacity (measured by the TEAC methods) of the osmo-dehydrated food. Under the conditions that maximized the phenolic infusion, the total phenolic content of the osmo-dehydrated model food was close to the values reported in some rich-in-phenolic fruits and vegetables, while the TEAC was three times that of fresh fruit with the highest antioxidant capacity (up to 66.3 mmol of Trolox/kg) [15].

Using two commercial grape extracts from seed and white grape marc, it was also studied how the phenolic concentration and profile, as well as the presence of osmo-active solute in OS, affected the rate of phenolic mass transfer during OT. When sucrose was not present in the OS, the total phenolic content in the osmo-treated food was almost twice that obtained with 50% (w/w) sucrose in OS. This means that phenolic infusion was affected more by the sucrose content in OS than by the source of the extract. The phenolic profile of the osmo-treated food was directly linked to the chemical composition of the grape extract used. For all conditions tested, the hydroxybenzoic acid: gallic acid (GA), the flavan-3-ol monomers: (+)-catechin (CAT), (-)-epicatechin (ECT), (-)-epicatechin 3-*O*-gallate (ECG), (-)-epigallocatechin, (EGC), (-)-epigallocatechin 3-*O*-gallate (EGCG), and the flavan-3-ol dimers: procyanidin B1 (PAB1) and procyanidin B2 (PAB2) quantified in OS were determined in the osmo-treated food. When compared the phenolic gain obtained with both grape extracts in the same conditions (total phenolic content and osmotic pressure of the OS), it was found that the phenolic content in the osmo-treated model food was highest when grape seed extract (GSE) was used as the source of phenolics. These results may be explained by the differences in the individual phenolic composition between GSE and white grape marc extract (WGME). WGME is richer in flavan-3-ol dimers, whereas GSE contains more flavan-3-ol monomers, which, because of their lower molecular weight, penetrate the model food more during OT, causing differences in the profiles of individual phenolics in the osmo-treated model food depending on the source of phenolics used. Accordingly, CT, ECT and EGCT were found in greater concentrations in the model food osmo-treated with GSE, while the contents of the flavan-3-ol dimers, PAB1 and PAB2 were higher in the model food after OT with WGME. The mass transfer of solutes present in the multicomponent solution during OT was described using the solution to Fick's second law for cubical configuration and Peleg's model. The diffusional model, however, did not describe as precisely as Peleg's model the progress of total and individual phenolic content during OT [19]. Only the progress of flavan-3-ol dimers (PAB1 and PAB2) was not predicted or was poorly predicted by the models studied. Overall, the diffusion coefficients of individual phenolics were significantly higher during OT with non-osmo-active solute.

It was also studied 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. OT was performed using aqueous solutions made of osmo-active agents (NaCl and sucrose) and a commercial grape seed extract with a constant phenolic concentration. Experimental conditions were set by a central composite design with two factors (the molality of NaCl and sucrose in osmotic solution). In all experiments, the total phenolic content in the osmotic solution was kept constant (6300±45 mg GAE/kg) and the model food (an agar-agar gel) was processed for 8 hours. Throughout the response surface, the osmo-treated model food was significantly supplemented with flavan-3-ols. A range of osmo-treated model foods can be formulated with a very high content of flavan-3-ols (monomers and dimers) and a similar  $a_w$  but with 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. The penetration of grape phenolics into the model food was limited by sucrose to different extents. The TEAC of the osmo-treated gel was higher than that of fruits with very high free radical scavenging activities. The extent of phenolic infusion

significantly increased the antiradical scavenging capacity of the osmo-treated gel, while all the flavan-3-ols detected and the hydroxybenzoic acid, GA, also made significant contributions [9].

Mass transfer of grape phenolics was studied during OT of plant tissue with a commercial grape seed extract in the osmotic solution. In addition, the stability of grape phenolics infused in the osmo-treated food after convective air drying was evaluated. The total phenolic content and antiradical scavenging capacity of plant foods such apple, banana, and potato were significantly increased by OT. Plant tissue showed a lower grape phenolic infusion than that of the model food. Total and individual phenolic content and TEAC were determined before and after air drying (AD) to investigate how AD affects the stability of grape phenolics infused into apple, banana, potato and the model food by a previous OT. During AD (Figure 2), the model food osmo-treated with the control OS (i.e. with non osmo-active solute) showed the highest reduction in total phenolic, followed by osmo-treated banana and apple.

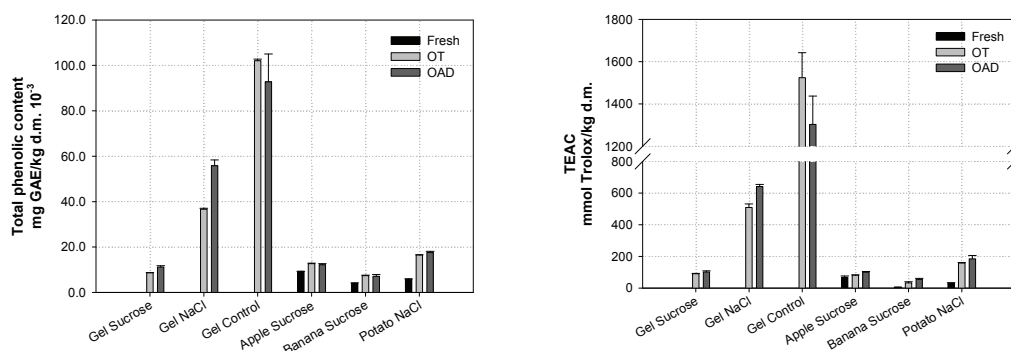


Fig. 2. Total phenolic content (a), and TEAC (b) of fresh, osmo-treated (OT) and osmo-air-dried (OAD) food. X-axis shows the type of food and the osmo-active solute used, in each case, during OT.

Contrary, the total phenolic content increased during AD of the model food osmo-treated with sucrose and sodium chloride OSs. To a lesser extent, this increase was also observed in osmo-treated potato. TEAC in the model food followed a similar trend as that described for total phenolic reduction (Figure 2). There was high TEAC reduction in samples osmo-treated with the control OS and an increase of TEAC in samples osmo-treated with sucrose and sodium chloride OSs. However, the total phenolics increased about twice as much as TEAC in the model food osmo-treated with sucrose and sodium chloride OSs. Unlike the total phenolic reduction observed in apple and banana, TEAC showed an important increase. In potato, TEAC increase was about two times greater than that of total phenolics. It was concluded that OT as a pre-treatment protected against grape phenolic degradation during further convective air drying, though the mechanisms controlling the chemical changes undergone by grape phenolics require further research [20].

#### 4. Conclusions

The presented results confirm that OT is a suitable technology for the exploitation of jelly foods, fruits and vegetables as matrices into which functional ingredients can be successfully incorporated to provide novel functional products of intermediate moisture. Concentrated red grape must and commercial grape seed and white grape marc extracts were successfully used as nutritional supplements. The type and

concentration of the osmo-active solute controlled the infusion rate of grape phenolics in the model food. In addition the molecular weight limited the penetration of grape phenolics during OT of a gel model food: those with a molecular weight over 612 g/mol made a poor contribution to total phenolic impregnation. By using mixtures of osmo- active solutes, e.g. NaCl and sucrose, the extent and rate of phenolic impregnation could be better controlled.

Although apple, banana and potato tissues showed a higher resistance to grape phenolic mass transfer than that of the model food during OT, the total phenolic content and antiradical scavenging capacity of these plant foods were significantly increased by OT with a grape seed extract as a source of phenolics. Finally, OT with sucrose or sodium chloride, as a pre-step, seemed to protect against grape phenolic degradation during further air-drying.

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