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Influence of drying on the retention of olive leaf polyphenols infused into dried apple

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ABSTRACT

Olive leaf extracts are rich in polyphenolic compounds. Their inclusion by impregnation in food solid matrices could improve the nutritional value and antioxidant capacity of dietary products, such as apple. Drying the food matrix is interesting not only because it speeds up the infusion, but also because of its effect on the final stabilization of impregnated food. In this work, the influence of drying method on the retention of infused olive leaf polyphenols in a solid matrix (apple) was addressed. For this purpose, apple cubes (10 mm side) were initially dehydrated by freeze drying or hot air drying at 60 °C and then impregnated with the olive leaf extract. After the polyphenolic infusion, samples were dried for the final stabilization by means of three different methods: freeze drying and hot air drying at 60 °C both with and without ultrasound application. The retention of infused polyphenols in apple samples was evaluated by determining the total phenolic content and antioxidant capacity and quantifying the main olive leaf polyphenols by HPLC-DAD/MS–MS. The drying kinetics and the loss of apple solids during impregnation were modeled by using diffusion equations and the Weibull model, respectively.

The role of fresh apple drying on the retention of infused olive leaf polyphenols was more significant than the further drying of the impregnated apple. Thus, hot air drying of fresh apple provided the highest antioxidant capacity (47.1 ± 2.6 mg Trolox/g d.m.) and oleuropein contents in the final dried apple of up to 1928 mg/100 g d.m. were found.

Keywords: dehydration, impregnation, modeling, antioxidant potential, HPLC-DAD/MS-MS.

Introduction

Due to new lifestyles, a large group of the population lacks a generous intake of basic foods (Schieber et al., 2001), such as fruit and vegetables and, therefore, of their nutritional and bioactive compounds. In consequence, the requirements of modern-day society and the demands of the market have promoted the innovation and development of new products. Nowadays, there is a growing demand for snacks that not only provide convenience and taste but also nutritional and health benefits (Jack et al., 1997; Zandstra et al., 2001). Thus, the impregnation of vegetable solid matrices with bioactive compounds has gained importance in recent years.

Apple is one of the most widely consumed fruits (fresh and dehydrated). Its tissue, composed of parenchyma cells, interspersed with air and liquid gaps (Khan & Vincent, 1990), facilitates the infusion of solutions, which is particularly noticeable if the water is previously removed, e.g. by drying. The most commonly used impregnation mediums have been water, sweet solutions or fruit juices. However, the increasing attention paid to the role played by natural active ingredients and their beneficial effects on human health, such as antioxidants (Fernandes et al., 2011), has opened up a new research topic in the field of the impregnation of food products. In this sense, although the infusion of ascorbic acid solutions (Blanda et al., 2008a) and osmotic solutions enriched with grape phenolic compounds (Rózek et al., 2010; Ferrando et al., 2011) into apples has been the subject of previous studies, none of them have addressed the infusion of pure plant extracts. Olive leaf extracts could be an interesting material with which to impregnate food products since they are rich

in phenolic compounds, such as oleuropein, verbascoside and luteolin glucoside (Ahmad-Qasem et al., 2013a and 2013b), with noticeable bioactive properties (Karakaya, 2009; Menéndez et al., 2013).

Osmotic treatments (Rózek et al., 2010; Ferrando et al., 2011) and vacuum impregnation (Blanda et al., 2008a) are the techniques which are most commonly used as a means of including compounds of interest in solid matrices. In solid-liquid treatments, mass transfer depends not only on the properties of the solution and the working pressure, but also on the structure of the solid matrix (Spiess & Behsnilian, 1998). Thus, in the rehydration operation of the previously dried matrix, the degree of rehydration is linked to the level of cellular and structural disruption (Cunningham et al., 2008). Therefore, the drying operation greatly influences the infusion rate and capacity. Moreover, once the solid matrix is impregnated, a further dehydration stage is necessary in order to improve its shelf life and reduce storage costs. To some extent, this final drying stage could also affect the infused compounds, for which reason it should be carefully designed.

On the one hand, air-force drying, using hot air, is the most commonly used drying technique due to its simplicity and the fact that it is relatively low cost. As is well known, air drying induces physical and chemical changes, such as shrinkage, porosity decrease, textural changes, loss of nutritional value and color modifications (Maskan, 2001; Lewicki & Jakubczyk, 2004). On the other hand, freeze drying provides products with the highest nutritional quality (Mujumdar & Law, 2010) and a minimal reduction of volume (Jankovié, 1993). However, the high cost of implementation and operation of freeze drying limits

its use to only high quality products. Recently, in order to provide new alternatives to conventional dehydration methods, new emerging technologies have been developed, such as power ultrasound assisted drying or low-temperature dehydration (García-Pérez et al., 2012).

The development of novel processing techniques to obtain healthier and safer food products is one of the major challenges facing the food industry in the new century (Barros, 2011). Thus, the effective incorporation of natural bioactive compounds, such as olive leaf polyphenols, into food matrices would be an interesting achievement. For that purpose, it is necessary to evaluate the different processing steps accurately. Thus, the goal of this work was to assess the influence of the drying method on the retention of olive leaf polyphenols impregnated into previously dried apple. Both the initial drying of the raw apple and the further drying of the impregnated apple with polyphenols were addressed.

Materials and methods

Raw materials

Olive leaves (*Olea europaea*, var. Serrana) were collected on a farm located in Segorbe (Castellón, Spain), packaged and stored at 4 °C until drying (less than 48 h). The initial moisture content was determined according to AOAC method nº 934.01. For that purpose, samples were kept in a vacuum chamber at 70 °C until constant weight was reached (AOAC, 1997).

The olive leaves were hot air dried at 120 °C for 12 min in a forced air laboratory drier (FD, Binder, Tuttlingen, Germany) using an initial mass load of 150 g, an air flow of 0.094 m^3/s and an air velocity of 0.683 m/s. The dehydration process was finalized when the samples lost 40 ± 1 % of the initial weight. After drying, olive leaves were packaged in plastic bags and stored at 4 °C until the extraction operation.

In order to obtain olive leaf extracts, the leaves were milled (Blixer 2, Robot Coupe USA, Inc., Jackson, MS, USA). The obtained powder was sieved (Metallic mesh size 0.05 mm, Filtra Vibración, Barcelona, Spain) to select particles with a diameter of less than 0.05 mm. The extractions were carried out in sealed containers, protected from light and immersed in a thermostatic shaking water bath (SBS40, Stuart, Staffordshire, UK). The ratio between the weight of the olive leaves and the solvent (water) volume used was 10 g/150 mL. During extraction, the mixture was stirred (170 rpm) at 22 ± 1 °C for 24 h. Afterwards, the extracts were centrifuged for 10 min at 5000 rpm (Medifriger BL-S, J.P. Selecta, Barcelona, Spain), filtered (nylon filters of 0.45 µm) and stored in opaque vials or bottles at 4 °C until used for apple impregnation.

Cubes of 10 mm side were obtained from the apple flesh (*Malus domestica* cv. Granny Smith) by using a cutting machine (CL50 Ultra, Robot Coupe USA, Inc., Jackson, MS, USA) and immediately processed. Following AOAC method nº 934.06, the initial moisture content was determined by drying in a vacuum chamber at 70 °C until reaching constant weight (AOAC, 1997).

Apple drying experiments

For the purposes of obtaining the solid matrix to be impregnated, fresh apple cubes were dehydrated by means of two different methods: freeze drying (FD) and hot air drying (HAD). Once the samples were impregnated, further dehydration was carried out by freeze drying (FD) and hot air drying with (HAD-US) or without power ultrasound (HAD) application. A scheme of the experiments carried out and the nomenclature used is shown in Figure 1.

The FD experiments were conducted at an initial temperature of -48 ± 2 °C, keeping the shelf temperature at 22 ± 2 °C and the pressure at 1.4 10^{-1} mbar (LIOALFA 6-50, Telstar, Madrid, Spain). The initial mass load used was 120 g.

For the HAD and HAD-US experiments, apple samples were dried in an ultrasonically assisted convective drier already described in the literature (García-Pérez et al., 2010). The equipment consists of a pilot-scale convective drier with an aluminum cylindrical ultrasonic radiator working as the drying chamber and driven by a piezoelectric transducer (21.8 kHz). The drier operates completely automatically: air temperature and velocity are controlled using a PID algorithm and samples are weighed at preset times by combining two pneumatic systems and a PLC (CQM41, Omron, Japan). The HAD experiments were carried out at 60 ºC, keeping a constant air velocity of 2 ms^{-1} and using an initial mass load of 120 g. The experiments assisted by power ultrasound (HAD-US) were conducted under the same experimental conditions as the HAD experiments, but by applying an acoustic power of approximately

20 kW/m^3, which is defined as the electric power supplied to the ultrasonic transducer divided by the chamber volume.

At least three drying tests were carried out for each condition tested and they were extended until the samples lost 85 ± 1 % of the initial weight of fresh apple and 91.3 ± 0.3 % in the case of impregnated apple.

Impregnation experiments

For the infusion of olive leaf phenolic compounds into the dry apple, 4 g of dried apple cubes were immersed in 250 mL of olive leaf extract at 25 °C using a flask protected from light. The polyphenolic infusion kinetic was monitored by weighing the samples at preset times. For that purpose, apple cubes were blotted with tissue paper to remove the excess superficial extract before being weighed. It was considered that equilibrium was reached when the difference between two consecutive weights was less than 0.02 g. Experiments were conducted in triplicate.

Solids loss during apple impregnation

A new set of experiments was carried out to evaluate the loss of apple solid compounds that takes place throughout the impregnation.. For that purpose, 4 g of dry apple (HAD or FD) were rehydrated in 250 mL of distilled water at 25 °C for different times. Then samples were blotted with tissue paper to remove the excess superficially adhered water and, afterwards, the moisture content (nº 934.06; AOAC, 1997) was determined. Three replicates were made for each rehydration time. The solid content throughout the rehydration was

estimated from the difference between the rehydrated sample weight and its moisture content. The loss of solids was assumed to be the same as the one produced during the impregnation with the olive leaf extract.

The Weibull empirical model (Cunha et al., 1998) was used for the prediction of the solid content during impregnation (Eq. 1):

$$C(t) = C_e + (C_0 - C_e)\ \exp\left(-\left(\frac{t}{\beta}\right)^{\alpha}\right) \quad (1)$$

where *C(t)* (g/g of apple) represents the solid content after an impregnation time t, subscripts 0 and e represent the initial condition and equilibrium, respectively, α the dimensionless parameter assimilated to the behavior index of the product during impregnation, and β (min^{-1}) is the kinetic parameter inversely related (1/β) with the process rate. The identification of the model parameters (α, β and C_e) was carried out by minimizing the sum of the squared differences between the experimental and calculated solid content of the samples by using the Solver tool from Excel™ (Microsoft Corporation, Seattle, USA).

Sample preparation for phenolic content and antioxidant capacity determination

For the purposes of assessing the antioxidant potential, extraction experiments were carried out on the dried and dried-impregnated-dried apple samples in order to release the phenolic compounds in aqueous extracts. The extraction conditions were similar to those used for obtaining the olive leaf extracts. 10 g of milled apple sample were placed in sealed containers protected from light with 150 mL of distilled water at 170 rpm and 22 ± 1 °C for

24 h. Afterwards, the extracts were centrifuged (10 min at 5000 rpm) and filtered (nylon filters of 0.45 µm).

Total phenolic content measurement (TPC)

The phenolic content was determined by the Folin-Ciocalteu method (Singleton et al., 1999). Briefly, 100 µL of sample were mixed with 200 µL of Folin-Ciocalteu's phenol reagent (Sigma-Aldrich, Madrid, Spain) and 2 mL of distilled water. After 3 min at 25 ºC, 1 mL of Na_2CO_3 (Panreac, Barcelona, Spain) solution (Na_2CO_3-water 20:80, p/v) was added to the mixture. The reaction was kept in the dark at room temperature for 1 h. Finally, the absorbance was read at 765 nm using a spectrophotometer (Helios Gamma, Thermo Spectronic, Cambridge, UK). The measurements were carried out in triplicate. The standard curve was previously prepared using solutions of a known concentration of gallic acid hydrate (Sigma-Aldrich, Madrid, Spain) in distilled water. Results were expressed as follows: mg of gallic acid (GAE) per g of dried matter of apple (d.m.) or mg GAE per mL of olive leaf extract, for apples and olive leaf extracts, respectively.

Antioxidant capacity measurement (AC)

The antioxidant capacity of extracts was determined by using the Ferric-reducing ability power (FRAP) method, which is a simple method used to estimate the reduction of a ferric-tripyridyltriazine complex method. It was applied following the procedure described by Benzie & Strain (1996), with some modifications. Briefly, 900 µL of freshly prepared FRAP reagent were mixed

with 30 µL of distilled water and 30 µL of test sample or water as appropriate reagent blank and kept at 37 °C for 30 min. The FRAP reagent contained 2.5 mL of a 10 mM TPTZ (Fluka, Steinheim, Germany) solution in 40 mM HCl (Panreac, Barcelona, Spain) plus 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$ (Panreac, Barcelona, Spain) and 2.5 mL of 0.3 M acetate buffer (Panreac, Barcelona, Spain), pH 3.6 (Pulido et al., 2000). Readings at the maximum absorption level (595 nm) were taken using a spectrophotometer (Helios Gamma, Thermo Spectronic, Cambridge, UK). Four replicates were made for each measurement. The antioxidant capacity was evaluated through a calibration curve, which was previously determined using water solutions of known Trolox (Sigma-Aldrich, Madrid, Spain) concentrations and expressed as: mg Trolox per g of dried matter of apple (d.m.) or mg Trolox per mL of olive leaf extract, for apples and olive leaf extracts, respectively.

Identification and quantification of polyphenols by HPLC-DAD/MS-MS

In order to identify and quantify the main polyphenols present in olive leaf extracts and dried-impregnated-dried apples, these were analyzed using an HPLC instrument (Agilent LC 1100 series; Agilent Technologies, Inc., Palo Alto, CA, USA) controlled by the Chemstation software. The HPLC instrument was coupled to an Esquire 3000+ (Bruker Daltonics, GmbH, Germany) mass spectrometer equipped with an ESI source and ion-trap mass analyzer, and controlled by Esquire control and data analysis software. A Merck Lichrospher 100RP-18 (5 µm, 250 x 4 mm) column was used for analytical purposes.

Separation was carried out through a linear gradient method using 2.5 % acetic acid (A) and acetonitrile (B), starting the sequence with 10 % B and programming the gradient to obtain 20 % B at 10 min, 40 % B at 35 min, 100 % B at 40 min, 100 % B at 45 min, 10 % B at 46 min and 10 % B at 50 min. For the LC-MS pump to perform accurately, 10 % of organic solvent was pre-mixed in the water phase. The flow-rate was 1 mL/min and the chromatograms monitored at 240, 280 and 330 nm. Mass spectrometry operating conditions were optimized in order to achieve maximum sensitivity values. The ESI source was operated in negative mode to generate $[M–H]^-$ ions under the following conditions: desolvation temperature at 365 °C and vaporizer temperature at 400 °C; dry gas (nitrogen) and nebulizer were set at 12 L/min and 4.83 bar, respectively. The MS data were acquired as full scan mass spectra at 50–1100 m/z by using 200 ms for the collection of the ions in the trap.

The main compounds were identified by HPLC-DAD analysis, comparing the retention time, UV spectra and MS/MS data of the peaks in the samples with those of authentic standards or data reported in the literature. Only the main olive leaf polyphenols were quantified using commercial standards: oleuropein (Extrasynthese, Genay Cedex, France), luteolin-7-O-glucoside (Phytolab, Vestenbergsgreuth, Germany) and apigenin (Nutrafur, Murcia, Spain). A purified extract (96.85 %) provided by Universidad Miguel Hernández (Elche, Spain) was used to quantify verbascoside. The quantitative evaluation of the compounds was performed with a calibration curve for each polyphenol, using ethanol (oleuropein), methanol (verbascoside and luteolin) or dimethyl sulfoxide (apigenin) solutions of known concentration. The polyphenol

concentrations were expressed as mg polyphenol per g of dried matter of apple (d.m.) or mg polyphenol per mL of olive leaf extract, for apples and olive leaf extracts, respectively.

Drying kinetics modeling

A diffusion model (Eq. 2) was used to mathematically describe the drying kinetics of impregnated samples (Simal et al., 2005).

$$W(t) = W_e + (W_e - W_0) \cdot \left[\sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} e^{\left(-\frac{D_w (2n+1)^2 \pi^2 t}{4L^2} \right)} \right]^3 \quad (2)$$

where W is the average moisture content (kg w/kg d.w.), L the half-length of the cube side (m), t is the time (s), D_w is the effective moisture diffusivity (m^2/s) and subscripts 0 and e represent the initial and equilibrium state, respectively.

D_w was identified by fitting a diffusion model to experimental kinetics. Thus, the Generalized Reduced Gradient (GRG) optimization method, available in Microsoft Excel™ spreadsheet (Microsoft Corporation, Seattle, WA, USA) was used, defining the objective function to be minimized as the sum of the squared difference between experimental and calculated average moisture content. The percentage of explained variance (%VAR, Eq. 3) was calculated in order to determine the goodness of the fit to the experimental data.

$$\%VAR = \left[1 - \frac{S_{xy}^2}{S_y^2} \right] \cdot 100 \quad (3)$$

where S^2_{xy} is the variance of the estimation and S^2_y the variance of the sample.

Results and discussion

Characterization of dried apple samples and olive leaf extract

In order to obtain different solid matrices for impregnation, apple cubes were dried by means of two different methods, hot air (HAD) and freeze drying (FD). The moisture of fresh apple (85.3 ± 0.9 g w/100 g) was reduced to a final moisture content of 3.1 ± 0.2 g w/100 g, which represents a reduction of 96 % in the initial water mass. Thereby, stable dehydrated products with water activity of under 0.31 ± 0.03 were obtained.

The antioxidant potential of the dried apple was estimated from the determination of TPC and AC, as described in the Materials and Methods section. HAD apples showed a TPC (1.16 ± 0.03 mg GAE/g d.m.) and AC (3.87 ± 0.08 mg Trolox/g d.m.) that were significantly ($p<0.05$) higher than the one measured in FD (TPC of 0.45 ± 0.09 mg GAE/g d.m. and AC of 1.07 ± 0.15 mg Trolox/g d.m.). Previous works have reported different results for apple. Thus, Vega-Gálvez et al. (2012) suggested that total phenolics decreased as the drying temperature rose (40-80 °C), while Joshi et al. (2011) did not find any meaningful differences between the drying methods tested (freeze-, air-, oven- and vacuum drying).

As regards the olive leaf extracts, the average TPC and AC values were 2.0 ± 0.6 mg GAE/mL and 5.9 ± 0.5 mg Trolox/mL, respectively, as can be observed in Table 1. These figures are slightly lower than the ones published in previous works (Ahmad-Qasem et al., 2013a and 2013b), which could probably be ascribed to the different solvent used in this work (water instead of hydro-

alcoholic solutions) and the harvest period of the olive leaves. However, the profile of bioactive compounds identified was similar to the ones previously found (Ahmad-Qasem et al., 2013a and 2013b), oleuropein, verbascoside and luteolin and apigenin derivates being the main polyphenols.

Impregnation of dried apple with the olive leaf extract

FD and HAD apples were impregnated with the olive leaf extract. During this process, two opposite mass transfer processes took place. On the one hand, the infusion of the extract compounds into the solid matrix and, on the other hand, the lixiviation of some solid compounds of the matrix to the liquid medium. The latter was observed from the increase in the soluble solid content in the olive leaf extracts (from 1.6 ± 0.2 to 2.6 ± 0.3 ºBrix). As a consequence, the quantification of total solids loss in the apples during impregnation was studied, and the kinetics of solids loss in water was determined (Figure 2) and assumed to be roughly the same as in the olive leaf extract. Once the dry apple was soaked in water, it underwent a sudden rehydration, which caused a meaningful decrease in the solid content. Thus, in FD samples, the solid content dropped from 0.97 to 0.25 g/g apple in less than 10 s, while in HAD, the solid content decreased to 0.50 g/g apple in approximately 60 s. The release of the solids from the apple matrix is coupled to the water gain, but it is only noticeable once the sample is almost fully rehydrated. This latter stage was accurately described using the Weibull model (Figure 2), which provided explained variances of over 0.97 for both FD and HAD. The major differences between FD and HAD were found in the rate of solids loss, since it was much

faster in FD (Figure 2). However, both FD and HAD reached a similar solid content in the equilibrium (0.059 ± 0.005 g/g of apple). It is important to highlight that the impregnated apple could be considered as practically a sugar-free product.

Figure 3 depicts the global mass change (ΔM) for apples during impregnation. As observed, the drying method of fresh apples had a significant ($p<0.05$) influence on the further impregnation rate (Figure 3). Thus, the infusion of olive leaf extract in FD was faster than in HAD. Thus, after 50 min of treatment, FD samples achieved practically a constant ΔM, whereas HAD required 2 h 30 min. The faster infusion of olive leaf extract (Figure 3), as well as the solids loss (Figure 2), into the FD apple could be related to the cellular disruption suffered by the vegetable material as a result of freezing (Van Buggenhout et al., 2006) and the formation of a high porosity matrix. These facts are also evidenced in the final mass gain, which was slightly larger in FD samples.

The evolution of AC in the apple during impregnation may be estimated (Figure 4) from the global mass change (Figure 3), the solids loss kinetics (Figure 2) and by considering the AC of the extract entering the particle. Fresh apple drying did not significantly ($p<0.05$) affect the final estimated AC (Figure 4). Thus, an average AC of 84.7 ± 0.2 mg Trolox/g d.m. was found for both FD and HAD apples. Notwithstanding, in order to reach the same AC, HAD needed almost twice as long as FD. Therefore, the technique of freeze-drying could be considered a reliable means of speeding up the impregnation of dried apple with the olive leaf extract.

The impregnated apple is an unstable matrix due to its high water content (close to 94 %). As a consequence, further dehydration is necessary in order to reduce the storage costs and increase shelf life. How the further drying affects not only the dehydration rate but also the antioxidant potential are relevant aspects to be considered and are addressed in the following sections.

Drying kinetics of impregnated apple

Impregnated apples (FD+I and HAD+I) were dehydrated by freeze drying (FD) or hot air drying with or without power ultrasound (HAD-US and HAD) application. The drying kinetics were determined (Figure 5) due to the water removal of the impregnated apple constitutes an additional cost, both in terms of energy and time consumption. The kinetic study could not be completed in FD samples due to the fact that the freeze-drier operates in batch (24 h).

The explained variances reached with the proposed diffusion model were low, ranging from 88 to 91 % (Table 2). This fact suggests that diffusion was not the only controlling mass transport mechanism, probably because of the high rate of the impregnated water moving freely through the solid to the surface, lending a significant role to convection. Even the differences in drying kinetics were not marked (Figure 5); a significantly ($p<0.05$) higher effective moisture diffusivity was found in FD+I+HAD ($12.9 \pm 0.7 \times 10^{-10}$ m^2/s) than in HAD+I+HAD ($11.7 \pm 0.5 \times 10^{-10}$ m^2/s) (Table 2). This fact was probably due to the more porous matrix promoted by freeze drying, which aids the further removal of the impregnated water.

As to ultrasound application during drying, the effective diffusivity identified for HAD+I+HAD-US was only 5.1 % higher than that obtained for HAD+I+HAD (Table 2). In the case of FD+I samples, the increase in D_e when ultrasound was applied was of 14.7 %. In both cases, the improvement was less significant than that reported for the ultrasonic drying of fresh vegetables and fruits (García-Pérez et al., 2012; Ozuna et al., 2014). Therefore, the use of ultrasound for the purposes of improving the drying of impregnated apples seems not to be very promising as a means of increasing productivity and reducing energy consumption.

Effect of drying of the impregnated apple on antioxidant potential

Once the impregnated apple was dried, the obtained final product had much higher antioxidant potential values than those found in the dehydrated raw apples (Figures 6 and 7). However, the type of drying operation had a noticeable effect on the final antioxidant potential achieved, as observed in Figures 6 and 7.

Firstly, the drying of the fresh apple greatly affected the antioxidant potential of dried-impregnated-dried apple (Figures 6 and 7). Thereby, FD samples achieved significantly ($p<0.05$) lower TPC (Figure 6a) and AC (Figure 7a) than HAD (Figures 6b and 7b). The average final TPC and AC for HAD apples (HAD+I+HAD, HAD+I+HAD-US and HAD+I+FD) was 2-3 times higher than for FD (FD+I+HAD, FD+I+HAD-US and FD+I+FD). As far as we are aware, these results have not previously been reported and could be explained by considering, among other facts, the residual enzyme activity present in the

unfrozen rubbery-state water fraction of frozen samples, as well as how freezing affects the solid matrix. Thus, in impregnated FD apples, both enzymatic and hydrolytic reactions could take place (Blanda et al., 2008b), reducing the antioxidant potential achieved with the olive leaf extract infusion. As regards the influence of freezing on the solid matrix, the injury to the cell integrity caused would facilitate the release of intra-cellular components, thus polyphenols, polyphenol oxidase and oxygen may be placed in contact (Ferreira et al., 2002) during impregnation favoring the abovementioned residual enzymatic activity. In addition, the growth of ice crystals pushes, compresses and breaks cells , greatly degrading the native structure (Voda et al., 2012) and creating an open, weak structure (Sham et al., 2001). This suggests that polyphenols are more exposed to dehydration conditions, due to their weak interaction with the poorly consolidated solid matrix of FD samples.

Although the influence of the further drying of the impregnated apple was much less noticeable on the retention of infused polyphenols (Figures 6 and 7) than the drying of the fresh apple, some facts could be highlighted. Thus, the TPC of HAD+I+HAD (Figure 6) was 115 and 67 % higher than HAD+I+HAD-US and HAD+I+FD, respectively. However, HAD+I+HAD showed a similar AC to HAD+I+HAD-US and HAD+I+FD (Figure 7a), which suggests that the method used to dry the impregnated apples did have an effect, but to a lesser extent. The ultrasound assisted drying of FD impregnated samples (FD+I+HAD-US) slightly increased ($p<0.05$) the TPC as compared to those dried using other techniques (Figure 6a), but no positive effects were observed in AC (Figure 7a).

Therefore, once the impregnated apples were dried, the products obtained presented a much higher antioxidant potential than that found in the dehydrated raw apple (Figures 6 and 7). As a consequence, the method proposed in this work, combining drying-impregnation-drying steps, could be considered a convenient apple-processing alternative in order to obtain a stable product, low in sugar and enriched with olive leaf bioactive polyphenols with high antioxidant activity.

Finally, additional experiments were conducted for the purposes of investigating how the further drying affects the antioxidant potential of the apple itself. Thus, FD and HAD samples were again subjected to FD, HAD and HAD-US experiments. Obviously, it cannot be considered a dehydration step due to the fact that the initial water content was only 0.032 kg w/kg d.m. The experimental results (Figures 6 and 7) showed that the additional HAD step (both with and without ultrasound application) significantly ($p<0.05$) increased the TPC and AC for both FD and HAD samples. Thus, HAD+HAD apples showed significantly ($p<0.05$) higher TPC and AC (109 and 74 %, respectively) than HAD ones. This fact could be linked to the formation of Maillard-derived melanoidins, responsible for color changes during HAD, since these molecules have already been linked to the potential antioxidant enhancement of dried products as a result of the formation of novel compounds with antioxidant activity (Manzocco et al., 2001). However, the additional FD step did not imply any increase in the TPC and AC for either HAD and FD apples, as may be observed if FD+FD and HAD+FD are compared to FD and HAD (Figures 6 and 7), respectively.

Effect of drying of the impregnated apple on phenolic composition

In order to gain insight into the influence of the different drying techniques on the retention of infused polyphenols, the phenolic compounds were identified and quantified by HPLC-DAD/MS-MS.

The main polyphenols identified and quantified in the olive leaf extract (Table 1) were also found in the dried-impregnated-dried apple samples (Table 3). In agreement with the antioxidant potential results, the polyphenol retention was mostly affected by how the fresh apple was dehydrated. Dried HAD+I apples had a significantly ($p<0.05$) higher content of the main polyphenols than the FD+I ones (Table 3). These differences were particularly noticeable in the case of the oleuropein, its HAD+I content being up to 3 orders of magnitude higher than in FD+I. Oleuropein was not even detected in FD+I+HAD-US apples.

As far as the drying method applied to the dehydration of impregnated samples was concerned, no meaningful effect was found. Indeed, no significant ($p<0.05$) differences were found between FD+I+HAD, FD+I+HAD-US and FD+I+FD. In the case of HAD+I, the drying method had a significant ($p<0.05$) influence on the concentration of some compounds, such as oleuropein, oleuropein glucoside and luteolin glucoside. Thus, HAD+I+FD apples showed the highest concentrations of the main compounds: oleuropein (1928 ± 111 mg/100 g d.m.) and oleuropein glucoside (338 ± 17 mg/100 g d.m.). Therefore, FD seemed to be a convenient method with which to dehydrate the HAD+I samples, which appears contradictory if

compared with the already mentioned negative effect on the drying of fresh apple. This fact could be explained by considering different aspects. On the one hand, the freezing step did not favor the release of oxidative enzymes due to they were previously inactivated by HAD (drying of fresh apple). On the other hand, the low temperature applied during FD caused less degradation of the bioactive compounds in HAD+I+FD apples than in HAD+I+HAD and HAD+I+HAD-US.

Conclusions

The method proposed in this work, combining drying-impregnation-drying steps, could be considered as a convenient apple processing alternative as a means of obtaining a stable product of high antioxidant potential and low-sugar content enriched with olive leaf polyphenols. However, the retention of infused polyphenols was greatly dependent on how the drying steps were performed. In this regard, the fresh apple drying process influenced the retention of infused olive leaf polyphenols more than the further drying process of the impregnated apple. Firstly, the infusion rate was improved by freezing prior to drying; thus, freeze-dried apples impregnated faster than hot air dried ones. Secondly, hot air dried apples were found to retain a greater quantity of the olive leave polyphenols than those that were freeze-dried. An oleuropein content of up to 1928 mg/100 g d.m. was achieved in the dried-impregnated-dried apple. Finally, further research should be carried out in order to elucidate the biochemical mechanisms involved.

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Figure captions

Figure 1. Sequence of different treatments undergone by apple samples.

Figure 2. Solid content in FD and HAD apples during soaking in water.

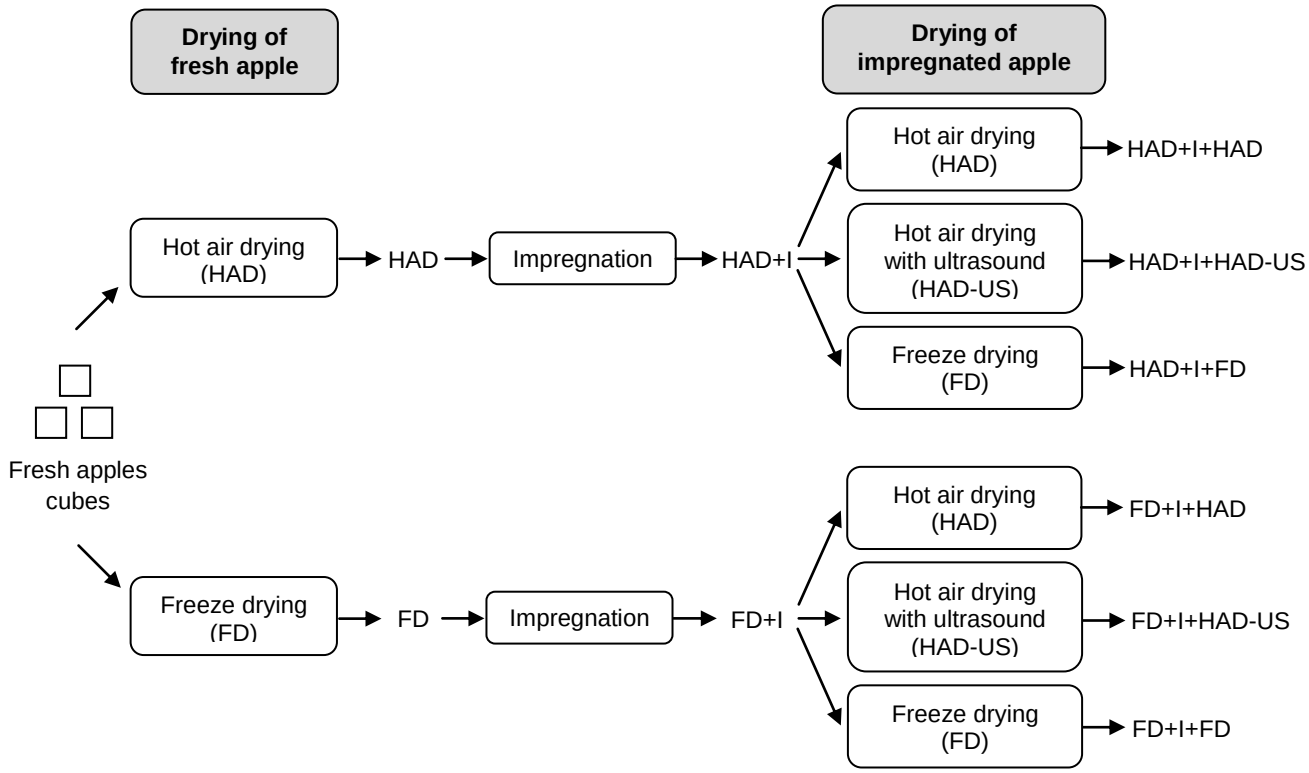
Figure 3. Global mass change ratio (ΔM) of FD and HAD samples during impregnation with olive leaf extract.

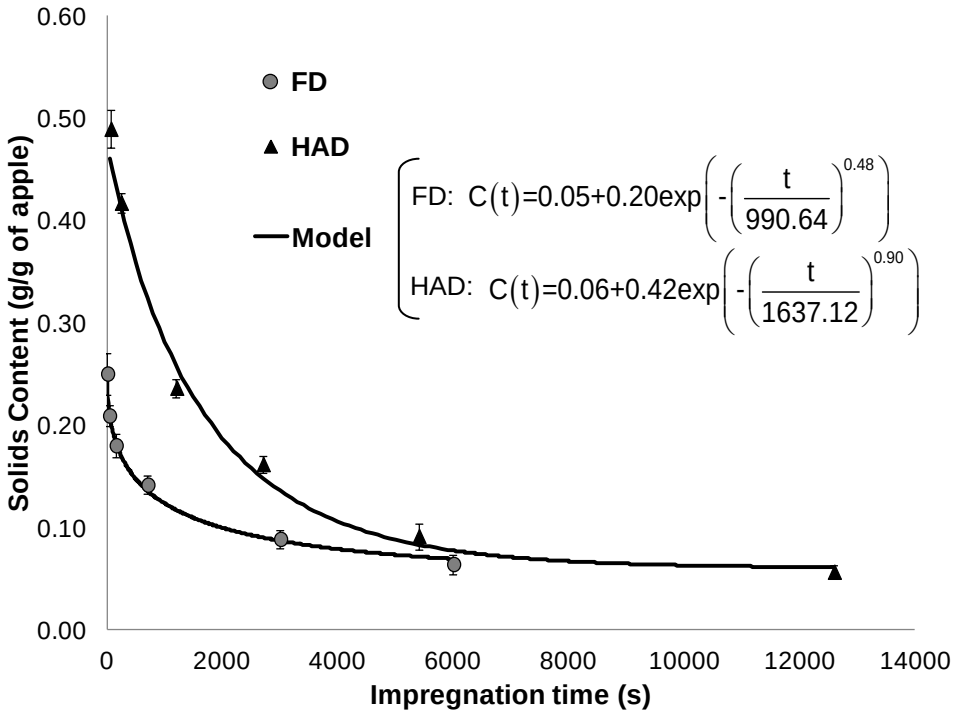
Figure 4. Kinetics of polyphenolic infusion into freeze (FD) and hot air dried (HAD) apples. Means ± Standard Deviation of antioxidant capacity (AC) are plotted.

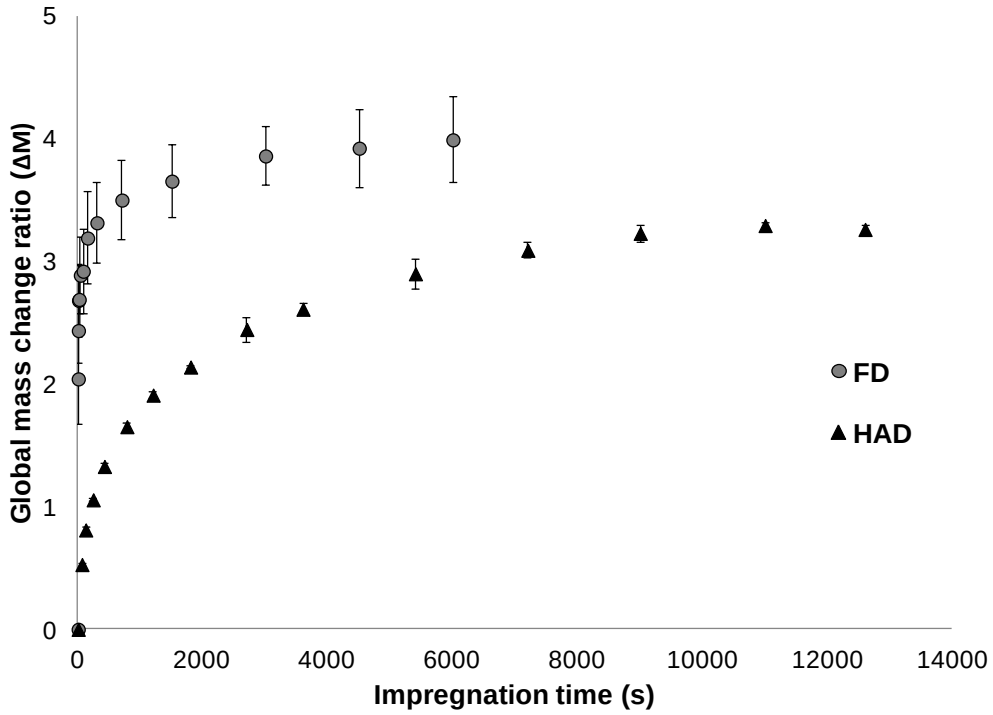
Figure 5. Hot air drying kinetics with (HAD-US) or without ultrasound assistance (HAD) of apples impregnated with olive leaf extract (a: FD+I; b: HAD+I). Means ± Standard Deviation of moisture (kg w/kg d.m.) are plotted.

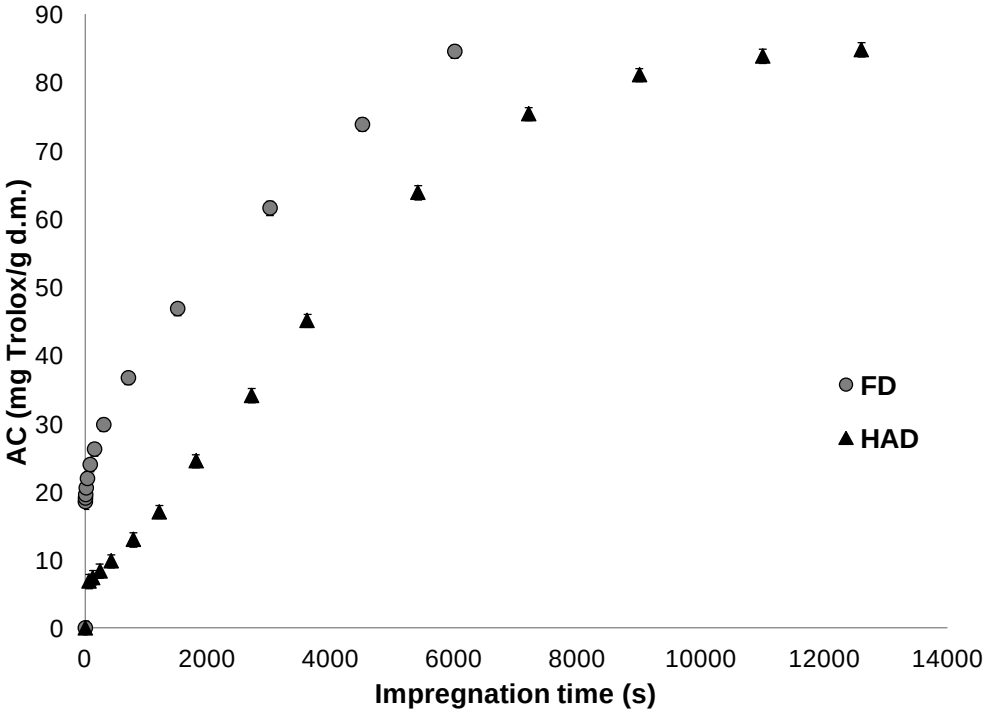
Figure 6. Influence of the different treatments on the total phenolic content (TPC) of freeze dried (a) or hot air dried (b) apples. Means ± Standard Deviation are plotted. Superscript letters show homogeneous groups established from LSD (Least Significance Difference) intervals ($p<0.05$). I: impregnated, HAD (hot air dried), HAD-US (ultrasound assisted hot air dried), FD (freeze dried).

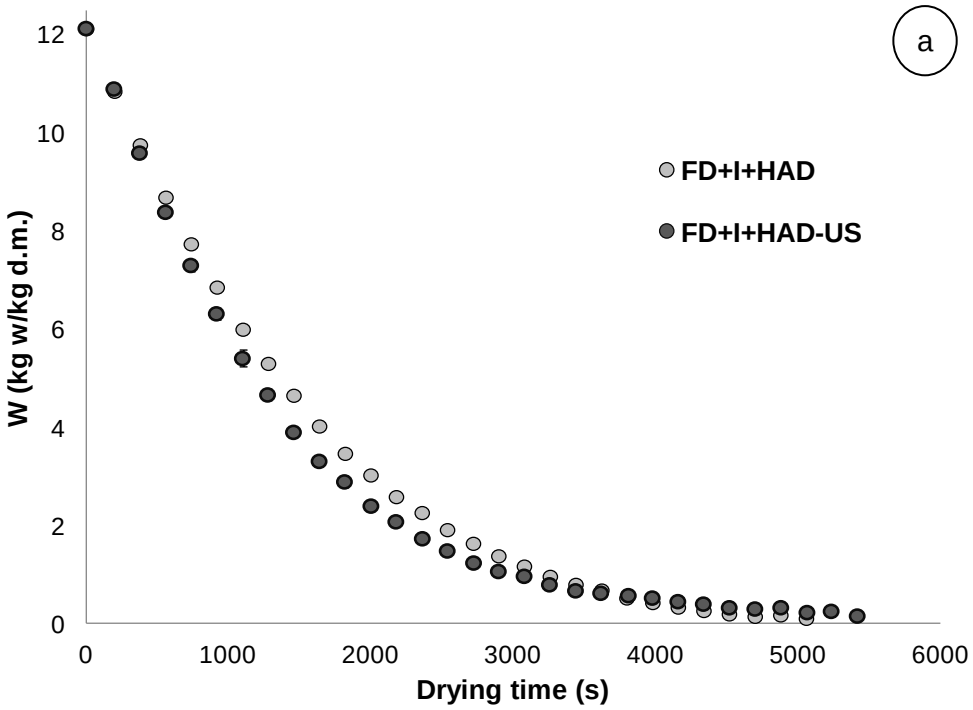
Figure 7. Influence of the different treatments on the antioxidant capacity (AC) of freeze dried (a) or hot air dried (b) apples. Means ± Standard Deviation are plotted. Superscript letters show homogeneous groups established from LSD (Least Significance Difference) intervals ($p<0.05$). I: impregnated, HAD (hot air dried), HAD-US (ultrasound assisted hot air dried), FD (freeze dried).

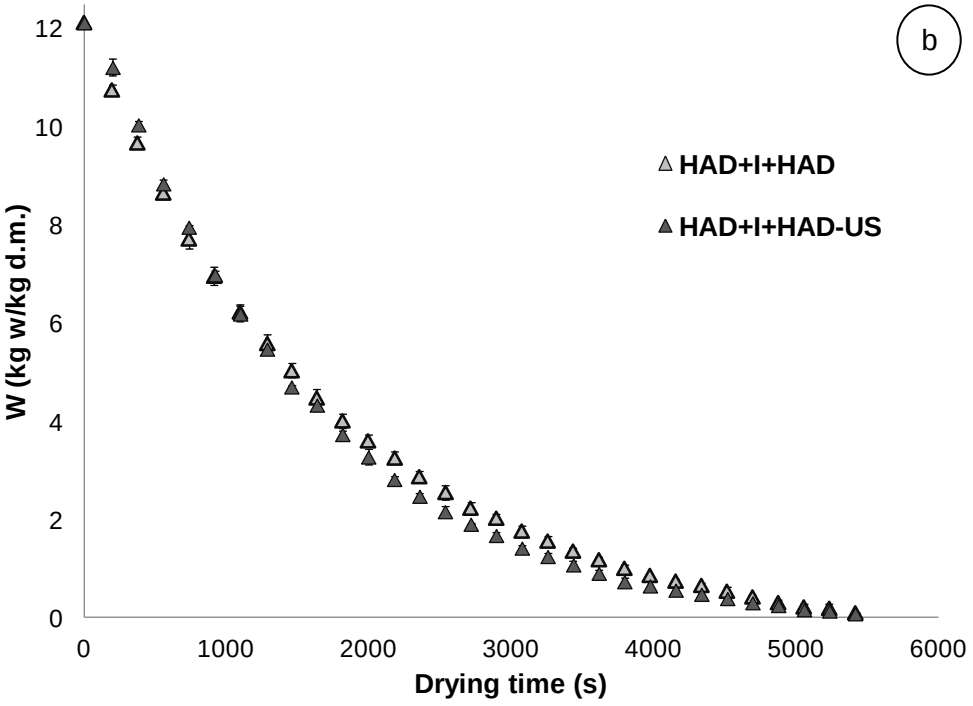

Drying of fresh apple
Drying of impregnated apple
Hot air drying (HAD)
HAD
Impregnation
HAD+I
Hot air drying (HAD)
HAD+I+HAD
Hot air drying with ultrasound (HAD-US)
HAD+I+HAD-US
Freeze drying (FD)
HAD+I+FD
Fresh apples cubes
Freeze drying (FD)
FD
Impregnation
FD+I
Hot air drying (HAD)
FD+I+HAD
Hot air drying with ultrasound (HAD-US)
FD+I+HAD-US
Freeze drying (FD)
FD+I+FD

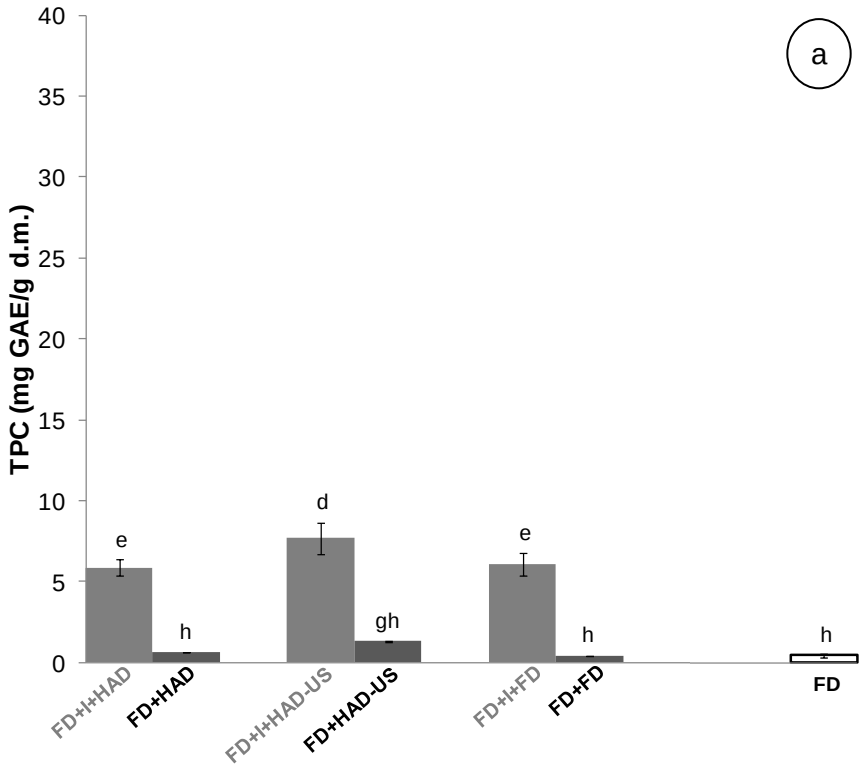

0.60
0.50
0.40
0.30
0.20
0.10
0.00
Solids Content (g/g of apple)
0
2000
4000
6000
8000
10000
12000
14000
Impregnation time (s)
FD
HAD
Model
FD: C(t)=0.05+0.20exp(-(t/990.64)^0.48)
HAD: C(t)=0.06+0.42exp(-(t/1637.12)^0.90)

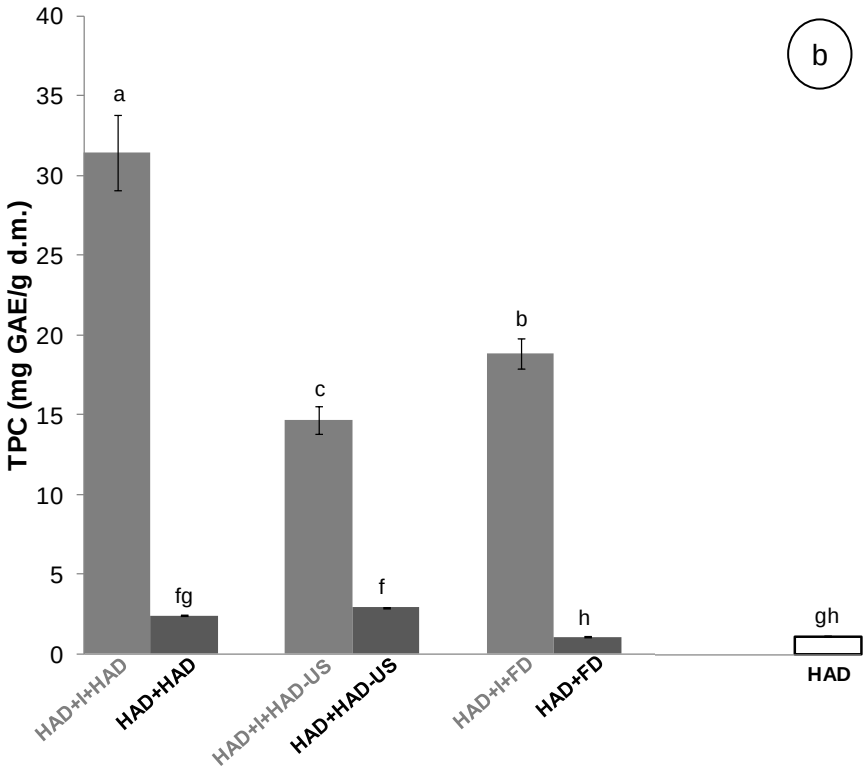

Global mass change ratio (ΔM)
Impregnation time (s)
0
1
2
3
4
5
0
2000
4000
6000
8000
10000
12000
14000
FD
HAD

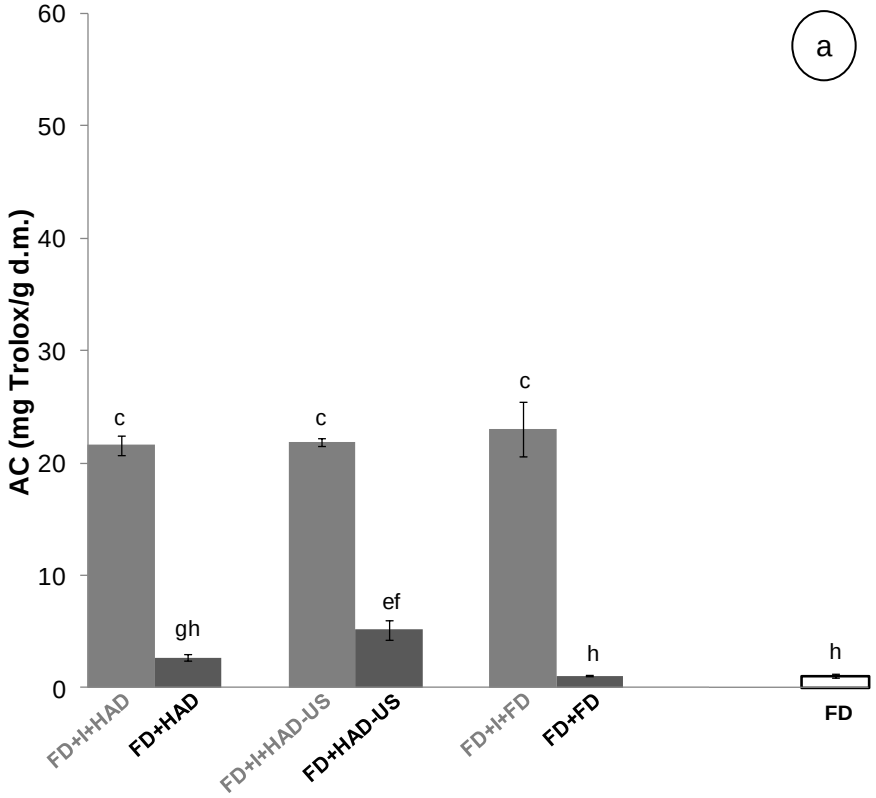

AC (mg Trolox/g d.m.)
Impregnation time (s)
FD
HAD
0
10
20
30
40
50
60
70
80
90
0
2000
4000
6000
8000
10000
12000
14000


a
12
10
8
6
4
2
0
W (kg w/kg d.m.)
0
1000
2000
3000
4000
5000
6000
Drying time (s)
FD+I+HAD
FD+I+HAD-US


b
12
10
8
6
4
2
0
W (kg w/kg d.m.)
0
1000
2000
3000
4000
5000
6000
Drying time (s)
HAD+I+HAD
HAD+I+HAD-US


a
TPC (mg GAE/g d.m.)
40
35
30
25
20
15
10
5
0
e
h
d
gh
e
h
h
FD+I+HAD
FD+HAD
FD+I+HAD-US
FD+HAD-US
FD+I+FD
FD+FD
FD


b
TPC (mg GAE/g d.m.)
40
35
30
25
20
15
10
5
0
a
fg
c
f
b
h
gh
HAD+I+HAD
HAD+HAD
HAD+I+HAD-US
HAD+HAD-US
HAD+I+FD
HAD+FD
HAD


a
AC (mg Trolox/g d.m.)
60
50
40
30
20
10
0
c
c
c
gh
ef
h
h
FD+I+HAD
FD+HAD
FD+I+HAD-US
FD+HAD-US
FD+I+FD
FD+FD
FD

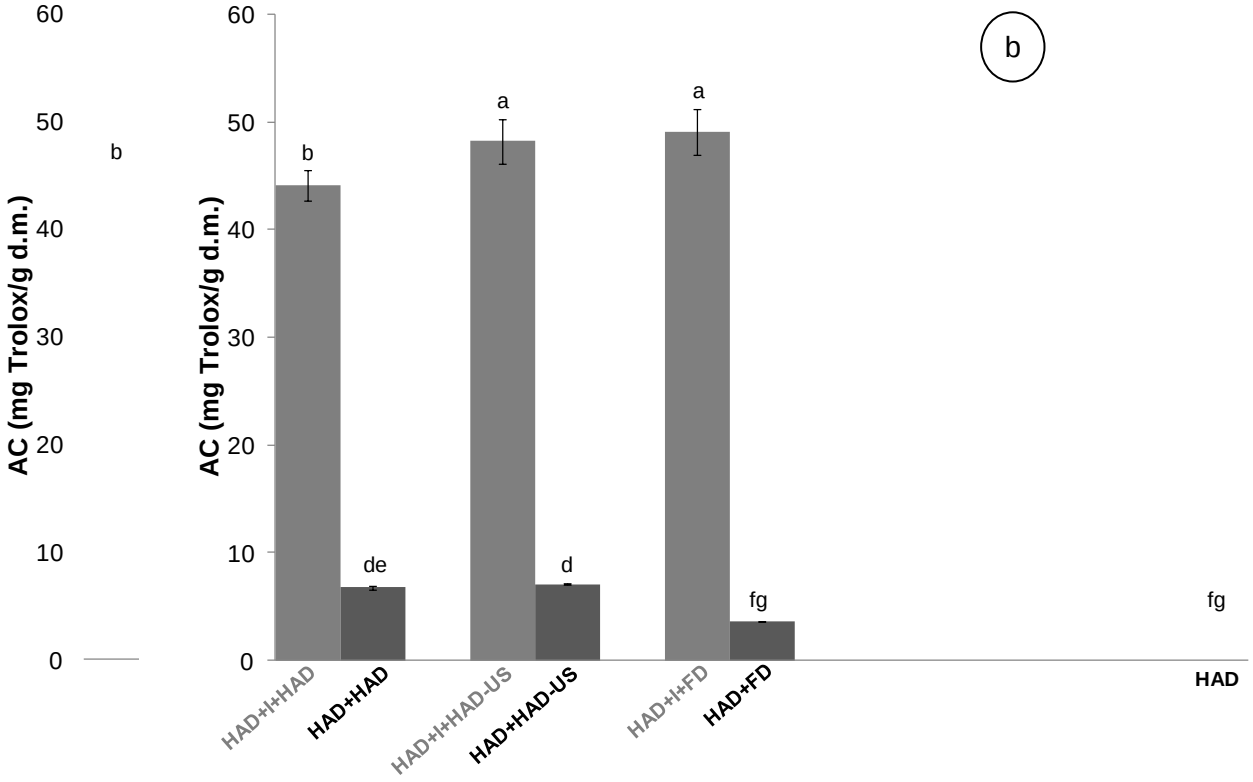

b
AC (mg Trolox/g d.m.)
60
50
40
30
20
10
0
b
b
a
a
de
d
fg
fg
HAD+I+HAD
HAD+HAD
HAD+I+HAD-US
HAD+HAD-US
HAD+I+FD
HAD+FD
HAD

Table 1. Olive leaf extracts characterization: antioxidant potential and phenolic composition.

Olive leaf extract characterization	
TPC (mg GAE/mL)	2.0 ± 0.6
AC (mg Trolox/mL)	5.9 ± 0.5
Oleuropein (mg/mL)	3.8 ± 0.3
Oleuropein glucoside *	0.060 ± 0.007
Verbascoside (mg/mL)	0.25 ± 0.02
Luteolin glucoside (mg/mL)	0.44 ± 0.03
Luteolin diglucoside **	0.037 ± 0.012
Luteolin-7-O-rutinoside **	0.07 ± 0.03
Apigenin-6,8-diglucoside ***	0.023 ± 0.002
Apigenin-7- rutinoside ***	0.036 ± 0.004

* Content expressed as equivalents of oleuropein (mg/mL)
** Content expressed as equivalents of luteolin-7-O-glucoside (mg/mL)
*** Content expressed as equivalents of apigenin (mg/mL)

Table 2. Effective moisture diffusivity (m^2/s) and percentage of explained variance (VAR) identified from the modeling of the drying of impregnated apples.

	D_e ($x10^{-10}$ m^2/s)	VAR (%)
FD+I+HAD	12.9 ± 0.7 [b]	88.0
FD+I+HAD-US	14.8 ± 0.3 [a]	89.8
HAD+I+HAD	11.7 ± 0.5 [d]	90.5
HAD+I+HAD-US	12.3 ± 0.2 [c]	88.2

[a–d] Show homogeneous groups in the same row established from LSD (Least Significance Difference) intervals ($p<0.05$)

Table 3. Main polyphenols retained in the apple matrix after impregnation (I) with olive leaf extract and different drying treatments: FD (freeze drying), HAD (hot air drying), HAD-US (hot air drying assisted by power ultrasound).

	FD+I+HAD	FD+I+HAD-US	FD+I+FD	HAD+I+HAD	HAD+I+HAD-US	HAD+I+FD
Oleuropein (mg/100 g d.m.)	11 ± 4 ^{d}	nd	6.7 ± 0.5 ^{d}	1152 ± 82 ^{c}	1710 ± 225 ^{b}	1928 ± 111 ^{a}
Oleuropein glucoside *	197 ± 10 ^{b}	304 ± 11 ^{a}	232 ± 20 ^{b}	238 ± 74 ^{b}	285 ± 84 ^{a}	338 ± 17 ^{a}
Verbascoside (mg/100 g d.m.)	nd	nd	nd	11 ± 2 ^{b}	26 ± 4 ^{a}	25 ± 2 ^{a}
Luteolin glucoside (mg/100 g d.m.)	52 ± 9 ^{b}	56 ± 12 ^{b}	52 ± 13 ^{b}	80 ± 25 ^{ab}	109 ± 38 ^{a}	108 ± 15 ^{a}
Luteolin diglucoside **	nd	nd	nd	nd	15 ± 5 ^{a}	7 ± 2 ^{b}
Luteolin-7-O-rutinoside **	nd	nd	nd	nd	nd	nd
Apigenin-6,8-diglucoside ***	8.9 ± 1.4 ^{b}	7.4 ± 0.6 ^{b}	8.1 ± 0.4 ^{b}	10.2 ± 0.4 ^{b}	14 ± 3 ^{a}	14 ± 3 ^{a}
Apigenin-7- rutinoside ***	5.0 ± 0.8 ^{c}	10 ± 2 ^{b}	5.7 ± 0.03 ^{c}	9.8 ± 1.4 ^{b}	17 ± 3 ^{a}	11.4 ± 0.6 ^{b}

* Content expressed as equivalents of oleuropein (mg/100 g d.m.)
** Content expressed as equivalents of luteolin-7-O-glucoside (mg/100 g d.m.).
*** Content expressed as equivalents of apigenin (mg/100 g d.m.)
$^{a–d}$ Show homogeneous groups in the same row established from LSD (Least Significance Difference) intervals ($p<0.05$)