Use of novel drying technologies to improve the retention of infused olive leaf polyphenols

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ABSTRACT

The infusion of phenolic extracts in dried fruits constitutes an interesting means of improving their nutritional content. However, drying can affect the further process of impregnation. In this work, different drying treatments (air temperature and ultrasound application) were applied to apple samples and impregnated with olive leaf extract. The application of ultrasound during drying did not significantly ($p<0.05$) affect the infusion capacity of samples but the ultrasonically assisted dried samples showed a greater antioxidant capacity than those conventionally dried. The highest content of oleuropein and verbascoside was found in samples dried at low temperature using ultrasound.

Keywords: drying; infusion; extract; antioxidant potential; HPLC-DAD/MS-MS

INTRODUCTION

Apple is one of the most widely-consumed fruits, not only raw but also in the form of juice or as a dried product included in snack preparations or whole grain breakfast cereals (Biedrzycka and Amarowicz, 2008)[1]. Apple is also characterized by a high concentration of phenolic compounds, with an important portion of free phenolics compared with other fruits (Boyer and Liu, 2004)[2]. The Granny Smith variety is one of the apple cultivars that is richest in polyphenols (66.2-211.9 mg/100 g fresh weight). Processing could provoke changes in the apple, affecting not only the matrix structure but also the bioactive components (Tiwari and Cummins, 2013)[3].

Nowadays, consumers demand high quality products with an extended shelf life, which not only preserve the fresh-like characteristics of flavor, texture or color well but also enjoy an improved nutritional content (Rodríguez et al., 2014)[4]. Thus, the infusion of interesting compounds into vegetable solid matrices, compounds such as antioxidants (Fernandes et al, 2011)[5], has gained importance in recent years. The internal structure of apple is composed of parenchyma cells interspersed with air spaces (Khan and Vincent, 1990)[6] that makes the infusion of solutions easier than in more closed and compact structures. The process of infusion is made particularly easy if the water content has previously been reduced, e.g. by drying. In this sense, apple has been used as a matrix for the infusion of ascorbic acid solutions (Blanda et al., 2008)[7] and grape phenolic compounds (Rózek et al., 2010; Ferrando et al., 2011)[8, 9]. Olive leaf extracts could be an interesting alternative means of impregnating food products, since they are rich in phenolic compounds, such as oleuropein, verbascoside and luteolin glucoside (Ahmad-Qasem et al., 2013a and 2013b)[10, 11] with proven bioactive properties (Karakaya, 2009)[12]. The infusion of olive leaf polyphenols in the dried apple matrix could greatly improve their bioactive content and, therefore, their benefits for human health.

Infusion can be addressed as a particular rehydration-impregnation operation. The structural damage caused by removing the water during the drying of the fresh product could greatly affect not only the infusion capacity and rate (Cunningham et al., 2008)[13] but also the interaction force between the infused compounds and the solid matrix. Due to its simplicity and its relatively low cost, one of the most frequently used dehydration methods in the food industry is that of conventional hot air drying. The high temperature used can help to inactivate some enzymatic reactions (Sanjuan et al., 2001)[14], some of which can degrade antioxidant compounds. However, it can produce changes in the nutritional value, physical properties and microstructure of the products.

Recently, the feasibility of employing new drying technologies to improve drying has been evaluated. In this sense, the use of low temperature drying can represent an interesting alternative with which to reduce the changes produced by drying (García-Pérez et al., 2012) [15]. On the other hand, the application of power ultrasound has been proven to be an interesting means of increasing the drying rate, not only in conventional high-temperature drying (Cárcel et al., 2011)[16] but also in low-temperature drying processes (García-Pérez et al., 2012)[15].

All these different drying methods can affect the samples' structure and composition in different ways, thus influencing the further infusion of the antioxidant compounds. Therefore, the main objective of this work was to evaluate how the drying method used on the fresh apple affects the further infusion of the olive leaf extract. The retention of the polyphenols in the apple matrix and the antioxidant capacity of the obtained samples will also be addressed.

MATERIAL AND METHODS

To achieve this main goal, porous matrixes of apple were obtained by drying fresh samples by means of different methods. Then the dried samples were infused with olive leaf extract and, afterwards, dried again to obtain a final, stable product. The antioxidant capacity and phenolic content of the final product was assessed to determine the influence of the first drying process on the obtained product. Subsequently, a more detailed description of the different parts of the working plan is shown.

Obtaining of olive leaf extracts

Olive leaves (*Olea europaea*, var. Serrana) were collected on a farm located in Segorbe (Castellón, Spain), packaged and stored at 4°C (for less than 48 h). The initial moisture content was determined following AOAC method nº 934.06 [17]. The olive leaves were separated in different sets and dried at 120°C (1±0.1% relative humidity) for 12 min in a forced air laboratory drier (FD, Binder, Tuttlingen, Germany) using an air flow of 0.094 m^3/s and an air velocity of 0.683 m/s following the experimental procedure reported by Ahmad-Qasem et al. [10]. For each set, an initial mass load of 150 g was used. The dehydration process was extended until the samples lost 40±1% of the initial weight. After drying, the olive leaves of the different sets were mixed and packaged in plastic bags and stored at 4°C until the extraction operation.

The dried leaves were milled (Blixer 2, Robot Coupe USA, Inc., Jackson, MS, USA) and the obtained powder was sieved (Metallic mesh size 0.05 mm, Filtra Vibración, Barcelona, Spain) selecting particles with a diameter of under 0.05 mm.

The extraction experiments were carried out in sealed containers, protected from light and immersed in a thermostatic (22±1°C) shaking (170 rpm) water bath (SBS40, Stuart, Staffordshire, UK) for 24 h. The ratio between olive leaf powder and solvent (water) was 10 g/150 mL. 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 Tm), characterized (phenolic content and antioxidant capacity) and stored in opaque vials at 4°C until their use for apple infusion.

Apple drying experiments

Cubes of 10 mm side were obtained from apples (*Malus domestica* cv. Granny Smith) by using a cutting machine (CL50 Ultra, Robot Coupe USA, Inc., Jackson, MS, USA) and immediately processed. The initial moisture content was measured by placing the samples at 70ºC and 200 mmHg until constant weight was reached, following AOAC method nº 934.06 (AOAC, 1997)[17].

Drying experiments were carried out with and without ultrasound application at 60 ºC, a commonly high temperature used in the dying of fruits and vegetables, and at -1ºC, a low temperature that can contribute to preserve the natural components of apple. Therefore, four different methods were used to dry the apple cubes: hot air drying at 60ºC (relative humidity of 8±1%), without (HAD) and with ultrasound (USHAD) application and low temperature drying at -1ºC (relative humidity of 15±2%), without (LTD) and with ultrasound (USLTD) application. The drying experiments at 60ºC and -1ºC were carried out in convective driers showed in Figure 1A and Figure 1B respectively, already described in detail in previous studies (Riera et al., 2011 and García-Pérez et al, 2012) [18, 15]. The ultrasonically assisted experiments (USHAD and USLTD) were conducted using an acoustic power of 20.5

kW/m^3, which is defined as the electric power supplied to the ultrasonic transducer divided by the volume of the drying chamber. Ultrasound was applied in continuous way during drying. For each run, 110 apple cubic samples, that mean an initial mass load of 80±3 g, were placed in a sample holder such as the showed in Figure 2. The position of samples in the 9 trays of the holder assured a uniform treatment of them for both air flowing and ultrasound application [15]. Experiments were carried out at least in triplicate, using an air velocity of 2 m/s and extended until the samples lost 83±1% of the initial weight.

The dried samples were infused with the olive leaf extract and further dried for the final stabilization. Ahmad-Qasem et al. (2014)[19] found that the influence the final drying step had on the antioxidant capacity and phenolic content of infused apples was negligible. For this reason, every sample was dried at 60ºC and 2 m/s using an initial mass load of 14±1 g until the samples achieved a constant weight.

Drying kinetics modeling

A diffusion model was used to describe the drying kinetics (HAD, USHAD, LTD and USLTD) of fresh apple cubes. The differential equation of diffusion was obtained combining Fick's first law and the microscopic mass balance. For cubic geometry, the diffusion model considering constant the effective moisture diffusivity and isotropic solid is shown in equation (1).

$$\frac{\partial W_p(x,y,z,t)}{\partial t} = D_e\left(\frac{\partial^2 W_p(x,y,z,t)}{\partial x^2} + \frac{\partial^2 W_p(x,y,z,t)}{\partial y^2} + \frac{\partial^2 W_p(x,y,z,t)}{\partial z^2}\right) \quad (1)$$

where W_p is the local moisture (kg water/kg dry matter, d.m.), t is the time (s), D_e is the effective moisture diffusivity (m^2/s) and x, y and z represent the characteristic coordinates in cubic geometry (m).

In order to solve equation (1), the following assumptions were considered: solid symmetry, uniform initial moisture content and temperature, constant shape during drying and a negligible external resistance to moisture transport. Taking these assumptions into account, the analytical solution of the diffusion equation, expressed in terms of the average moisture content, is shown in equation (2) (Crank, 1975)[20].

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

where W is the average moisture content (kg water/kg d.m.), L the half-length of the cube side (m) and subscripts 0 and e represent the initial and equilibrium state, respectively.

The diffusion model was fitted to the experimental drying kinetics in order to identify the effective moisture diffusivity. The identification was carried out by minimizing the sum of the squared differences between the experimental and the calculated average moisture content. For that purpose, the Generalized Reduced Gradient (GRG) optimization method, available in Microsoft Excel™ spreadsheet (Microsoft Corporation, Seattle, WA, USA) was used. The goodness of the fit was determined by calculating the percentage of explained variance (%VAR, equation (3)).

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

where S_{xy} and S_y are the standard deviation of the estimation and the sample, respectively.

Infusion experiments

The infusion of the olive leaf extract into the dried apple samples was carried out in flasks protected from the light at 25ºC. In each experiment, 4 g of dried apple cubes were immersed in 250 mL of olive leaf extract. The infusion kinetics were determined by weighing the samples at preset times. For that purpose, apple cubes were extracted from the solution, blotted with tissue paper to remove the excess of superficial extract and jointly weighed. It was considered that the equilibrium state was reached when the difference between two consecutive sample weights (at least, 1200 s of delay) was less than 0.02 g. The experiments were conducted in triplicate for each drying condition tested (HAD, USHAD, LTD and USLTD).

The infusion capacity (IC) was calculated from equation (4).

$$IC = \frac{(M_t - M_0)}{M_0} \qquad (4)$$

where M_t is the weight (g) of the infused samples at time t and M_0 the initial weight of the dried samples (before the infusion).

Phenolic content and antioxidant capacity

The total phenolic content and the antioxidant capacity of both the olive leaf extracts and of the dried, infused and re-dried apple samples was assessed. The measurements were carried out directly in the olive leaf extract but the apple samples had to be pre-conditioned in order to extract the polyphenols. To that end, 10 g of the apple sample were placed in sealed containers protected from the light with 150 mL of distilled water at 22±1°C and agitated at 170 rpm for 24 h. Afterwards, the extracts were centrifuged (10 min at 5000 rpm) and filtered (nylon filters of 0.45 µm); the phenolic content and antioxidant capacity in the permeate solution were analyzed as is subsequently described (Ahmad-Qasem et al., 2013a)[10].

Total phenolic content measurement (TPC)

The phenolic content was determined by means of the Folin-Ciocalteu method (Singleton et al, 1999)[21]. 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 25ºC, 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 mg of gallic acid (GAE) per g of dried matter (d.m.) of apple samples or mg GAE per mL of olive leaf extract.

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 and Strain (1996)[22] 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)[23]. Readings were taken at the maximum absorption level (595 nm) 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 (d.m.) of apple sample or mg Trolox per mL of olive leaf extract.

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

In order to identify and quantify the main polyphenols present in the olive leaf extracts and apple samples, 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 (d.m.) of apple sample or mg polyphenol per mL of olive leaf extract.

RESULTS AND DISCUSSION

Characterization of olive leaf extract

The antioxidant potential of the olive leaf extracts was assessed from the determination of TPC and AC. As can be observed in Table 1, the average TPC and AC values were 1.7±0.3 mg GAE/mL and 5.1±0.7 mg Trolox/mL, respectively.

These figures are slightly lower than others published in previous studies (Ahmad-Qasem et al, 2013a and 2013b)[10,11], which can be ascribed to the use of a different solvent, water in this study, while Ahmad-Qasem et al (2013a and 2013b)[10,11] used an ethanol-water solution at 80:20 (v/v). As regards the profile of the identified phenolic compounds, it was similar to the ones previously found by Ahmad-Qasem et al, (2013a and 2013b)[10, 11], the main polyphenols identified being oleuropein, verbascoside and luteolin and apigenin derivates.

Apple drying

Four different methods were used to dry the fresh apple cubes: HAD, USHAD, LTD and USLTD. The experimental drying kinetics are shown in Figure 3A for HAD and USHAD and in Figure 3B for LTD and USLTD. LTD was the longest drying process; under these conditions, apple cubes needed an average of 76 h to lose 83% of the initial weight. The application of US (USLTD) shortened the drying to 28 h, which implies a 63% reduction of the drying time. This kinetic improvement was similar to the ones reported for the ultrasonically assisted low temperature drying of different vegetables or fruits. Thus, when US was applied to the drying of eggplant, carrot and apple at -14ºC, García-Pérez et al (2012)[15] found that, on average, the drying time was between 65 and 70% shorter. Santacatalina et al (2014)[24] applied US during the drying of apple cubes at 0ºC and obtained a drying time reduction of around 60%.

The experiments carried out at 60ºC (HAD and USHAD) were much faster than those conducted at low temperature (-1ºC, LTD and USLTD); the difference in drying time between HAD and LTD was greater than one order of magnitude (approximately 2 hours as opposed to 80 hours, Figures 3A and 3B). The application of power ultrasound (USHAD) under these conditions also shortened the drying time (by 15%), but to a lower extent than in USLTD experiments. During high temperature drying, ultrasound application has been observed to exert only a mild influence. Rodriguez et al. (2014)[4] found a drying time reduction of 17.4% when US was applied (30.8 kW/m^3) during the drying of apple at 70ºC. Ultrasound provides additional energy to the thermal energy available in the drying air. When low temperatures are used, there is only a little energy available in the drying medium, which greatly increases the importance of the energy introduced by ultrasound. At high temperatures, the amount of energy in the medium is high and the acoustic energy provided by ultrasound is less relevant to the drying rate. This issue explains why the influence which power ultrasound exerts on drying performance is more marked at low temperatures than at high (Garcia-Perez et al., 2006)[25].

The drying kinetics of fresh apples cubes were modeled in order to identify the effective moisture diffusivity (D_e) and to assess the differences between the drying techniques tested (Table 2). The model fitted the experimental drying kinetics of LTD and USLTD adequately, as suggested by the %VAR figures obtained, over 98%. This fact shows that, at low temperatures, the drying kinetics can be explained by considering a controlling diffusional mechanism; the assumptions considered should be close to the actual drying conditions. In the case of HAD and USHAD, the %VAR obtained drastically dropped to under 91%. The poor fit of the diffusion model in HAD can also be observed in Figure 3A, where the model deviated from the experimental curves, indicating that it is not only diffusion that acts on the mass transfer control, but other factors as well. Garcia-Perez et al. (2006) [25] found similar results when applying this model to experimental drying kinetics of carrot drying obtained at 1m/s and temperatures ranging between 30 and 70ºC. They also used other model including external resistance that described better the experimental data providing percentages of explained variance above 99.9%. The high air temperature used in HAD and USHAD experiments reduced the internal resistance compared to the one found in the LTD and USLTD experiments, while the same air velocity makes that the external resistance remains similar. Therefore, the external resistance to water transfer plays a major role in controlling the drying rate, which could explain the poorer fit of the diffusion model proposed, that neglects the external resistance, in HAD and USHAD.

More mechanistic approaches for the drying modelling have been proposed in the literature including heat and mass transfer coupling, variable diffusivity or shrinkage of samples (Perré and May, 2001, Mihoubi et al., 2004, Perré and May, 2007, Garcia-Perez et al 2011)[26,27,28,29] fitting better drying kinetics than the model used in this work. However, the effective diffusivity identified in this case allowed evaluating the influence of the different drying methods tested on drying rate. On the one hand, the D_e values identified (Table 2) for USLTD experiments were significantly ($p<0.05$) higher (107%) than for LTD. At 60ºC, the influence of ultrasound on the D_e identified was lower compared to experiments carried out at -1ºC (26% higher in USHAD than in HAD). From preliminary tests, it was observed that the ultrasonically dried samples showed an increase of temperature at the end of drying lower than 3ºC. Similar increase of temperature has been observed by Kowalski [30] drying apple slices at 30ºC and an ultrasonic power of 50 W. This fact can indicate that the effect of ultrasound in drying kinetics was not only associated to the thermal effect. Thus, Garcia-Perez et al. (2006) [25], for drying carrots, found D_e values higher at 50ºC with ultrasound application than at 60ºC without ultrasound application. According to the literature, the improvement of D_e brought about by ultrasonic application can be mainly linked to the mechanical effects provoked in the material (García-Perez et al, 2009)[31]. The alternating expansions and contractions produced by acoustic waves when travelling through a medium (Gallego-Juárez et al, 1999)[32] generate a mechanical stress that facilitates the movement of water through the product. In any case, it will be interesting to carry out a deep study to differentiate thermal and mechanical effects of ultrasound during drying."

Infusion of the olive leaf extract into the dried apple

Apple cubes dried by means of the four different techniques were impregnated with the olive leaf extract and the infusion kinetics were experimentally determined by weighing the samples at preset times. The results showed that the method employed to dry fresh apples had a significant ($p<0.05$) influence on the final infusion capacity (IC) (Figure 4). Thus, the IC after 3.5 h of HAD (3.26±0.03) and USHAD (3.17±0.15) samples infusion was significantly ($p<0.05$) greater than that observed in LTD (2.90±0.05) and USLTD (2.75±0.15) samples. These differences could be linked to the fact that LTD experiments were carried out at a temperature (-1ºC) close to the freezing point of the apple. Previously, it has been reported that freezing could introduce changes in the rehydration pattern of vegetables (Eshtiaghi et al., 1994)[33]. The application of power ultrasound did not lead to significant ($p<0.05$) differences in the IC. Therefore, it could be stated that ultrasonic assisted drying at low or high temperatures did not affect the solvent gain during the impregnation of the olive leaf extract into the dried apple. It is known that the mechanical stress produced by ultrasound can affect the internal structure of materials (Puig et al. 2012)[34] and, therefore, the later infusion capacity. But this influence depends of process variables (temperature, ultrasonic power applied and product) and the final structure of ultrasonically assisted dried product can be less degraded than conventionally dried one (Puig et al. 2012)[34]. For the process studied in this work, it seems that the effects of ultrasound were enough to improve drying but not so high to significantly affect the infusion capacity.

Influence of drying method on antioxidant potential

The apple cubes impregnated with the olive leaf extract were further stabilized by a final drying operation. According to the results reported by Ahmad-Qasem et al (2013c)[19], the influence of which final drying method was used on the apples that had been impregnated with the olive leaf extract was negligible compared to the influence of the method employed to dry the fresh apple. For this reason, the same drying method was used to dry the impregnated samples (hot air dried at 60ºC and 2 m/s). Therefore, in the following sections, it is reported how the drying method used on the fresh apple affects the TPC, AC and the main polyphenols infused into the dried apple.

Total phenolic content (TPC)

The TPC value obtained for fresh apple was 0.40±0.05 mg GAE/g d.m. This value is lower than the reported for Fu et al. [35] for different apple varieties. After infusion, the lowest value of TPC was obtained in LTD (14.0±355 0.8 mg GAE/g d.m.) samples, while the highest one was found in HAD (30.2±1.6 mg GAE/g d.m.) (Figure 5). That means that, in all cases, the infusion of olive leaf extracts significantly increased the phenolic content of fresh apple. The difference between LTD and HAD samples could be due to the high temperatures which can induce the formation of some phenolic compounds and inactivate enzymatic reactions of phenolic compounds degradation (Ahmad-Qasem et al, 2013a)[10].

The samples dried by means of US application presented intermediate values of TPC, with no significant differences ($p<0.05$) found between samples dried at low (-1ºC; USLTD; 17.1±1.0 mg GAE/g d.m) and high temperatures (60ºC; USHAD; 18.6±0.8 mg GAE/g d.m). Therefore, the application of ultrasound during the drying of fresh apple led to a negligible influence of the drying temperature on the TPC. Thereby, the difference observed between the TPC of HAD and LTD was not found for in the case of USHAD and USLTD. High temperature drying could induce the formation of some phenolic compounds (Ahmad-Qasem et al., 2013d)[36]. To a certain extent, this could be different when US is applied due to its widely recognized capacity to form free radicals, which could reduce the amount of available polyphenols (Paniwynk et al., 2001)[37]. Otherwise, the kinetic intensification caused by US application at low temperatures involved a great shortening (48 hours) of the exposure time to the air flow and so, could reduce the degree of oxidation of the phenolic compounds. In addition to the aforementioned effects, the inactivation of oxidative enzymes by ultrasound waves should also be considered (Islam et al., 2014)[38], something which is almost negligible at high temperatures, but that could be meaningful at low temperature drying where the enzymes are well preserved.

Antioxidant capacity (AC)

The drying method applied to fresh apple also significantly ($p<0.05$) affected the AC (Figure 5), the AC of the samples dried at low temperatures (LTD) being significantly ($p<0.05$) lower than that of HAD ones, which was consistent with the results reported for TPC. As regards the ultrasound application, on the one hand, USLTD showed not only higher TPC, as already reported, but also higher AC than LTD. On the other hand, USHAD also showed a higher AC than HAD and USLTD which, in this case, is not consistent with the behavior found in the TPC. The high figure found for the AC of USHAD samples could be linked to several facts. Firstly, the synergetic effect of the combined high temperature-ultrasound treatment could favor the inactivation of oxidative enzymes, thus preserving the antioxidant capacity of the available polyphenols. Secondly, the new compounds resulting from the binding of the polyphenols with the free radicals promoted by ultrasound could be highly reactive, increasing the antioxidant capacity. Finally, further work focusing on clarifying the biochemical principles should be carried out to elucidate these hypotheses.

Quantification of the main characteristic polyphenols

In order to characterize the infusion process, the main polyphenols of the olive leaf extracts were analyzed in the impregnated apples with the aim of quantifying their retention in the solid matrix after the final drying. The four main polyphenols identified in the olive leaf extract (Table 1) were also found in the impregnated apple samples (Figures 6 and 7). However, the method employed to dry the fresh apple influenced the content of these compounds.

In the case of oleuropein (Figure 6), no significant ($p<0.05$) differences were found between LTD and HAD samples, showing that the drying temperature did not affect this compound. Ultrasound application greatly increased the oleuropein content, which was especially remarkable at low temperatures (USLTD). As far as we know, this result has not been previously reported. As regards verbascoside

(Figure 6), ultrasound application was found to produce the same effect, since it promoted an increase in both LTD and HAD. In this case, it should be emphasized that the verbascoside content of HAD was significantly ($p<0.05$) lower than that of LTD.

For the minority compounds, luteolin glucoside and apigenin-6,8-glucoside, the influence of the drying method used on the fresh apple was less marked. No significant differences were found in the case of the apigenin-6,8-diglucoside content (Figure 7), while only the USLTD samples showed a significantly ($p<0.05$) different luteolin-glucoside content.

Therefore, the drying method applied before infusing the apple with olive leaf extract had a significant influence on the subsequent conservation of the added polyphenols. A probably explanation for this fact is the different sensitivity of the original enzymes of fresh apple to the inactivation caused by the different drying methods applied. Thus, a remarkable influence of the drying method is observed in some infused components, such as oleuropein, but other compounds, like apigenin-6,8-diglucoside, seem to be quite stable. A biochemical study must be carrying out to confirm this fact. The USLTD samples were the ones that showed the highest concentrations of the main compounds: oleuropein (2416±159 mg/100 g d.m.), verbascoside (141±11 mg/100 g d.m.) and luteolin glucoside (172±8 mg/100 g d.m.).

CONCLUSIONS

The method used to dry fresh apple not only affected the drying kinetic but also the further infusion of olive leaf extract. As regards the drying kinetics, the influence of ultrasound application was more important at the lowest temperature, -1ºC. The application of ultrasound during drying did not significantly ($p<0.05$) affect the infusion capacity of the samples. However, the ultrasonically assisted dried samples showed a greater antioxidant capacity than those conventionally dried at the same temperature. The highest content of polyphenols added with olive leaf extracts (oleuropein and verbascoside) was found in samples that had been submitted to ultrasound assisted low temperature drying. Further research is needed to elucidate the actual mechanisms of influence of the drying method on the polyphenol content.

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Figure captions

Figure 1. Scheme of ultrasonically assisted convective driers.

A; high temperature drier: 1, fan; 2, heating unit; 3, anemometer; 4, three-way valve; 5, thermo-couple; 6, sample loading chamber; 7, coupling material; 8, pneumatic moving arms; 9, ultrasonic transducer; 10, vibrating cylinder; 11, sample holder; 12, balance; 13, impedance matching unit; 14, wattmeter; 15, high-power ultrasonic generator; 16, PC.

B; low temperature drier: 1, fan; 2, Pt-100; 3, temperature and relative humidity sensor; 4, anemometer; 5, ultrasonic transducer; 6, vibrating cylinder; 7, sample load device; 8, retreating pipe; 9, slide actuator; 10, weighing module; 11, heat exchanger; 12, heating elements; 13, desiccant tray chamber; 14, sample holder.

Figure 2. Scheme of distribution of apple cubes in the simple holder

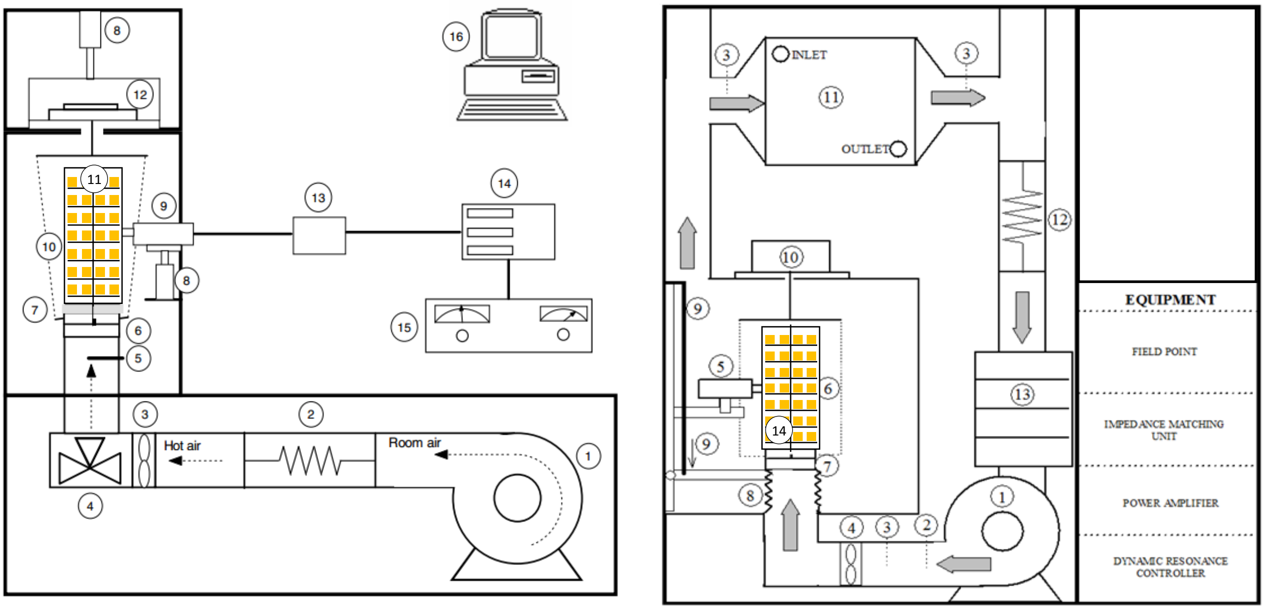
Figure 3. Experimental drying kinetics of fresh apple cubes (side 10 mm) and diffusion model. **A**: hot air drying without (HAD, 60ºC, 2m/s) and with power ultrasound application (USHAD, 60ºC, 2m/s, 20.5 kW/m^3) and **B**: low temperature drying without (LTD, -1ºC, 2m/s) and with power ultrasound application (USLTD, -1ºC, 2m/s, 20.5 kW/m^3).

Figure 4. Infusion kinetics of olive leaf extract into LTD, USLTD, HAD and USHAD dried apple cubes (side 10 mm).

Figure 5. Influence of the drying method used on the fresh apple (LTD, USLTD, HAD and USHAD) on the total phenolic content (TPC) and antioxidant capacity (AC) of samples impregnated with the olive leaf extract. Means±LSD intervals are plotted. Superscript letters (a, b, c) and (x, y, z) show homogeneous groups established from LSD (Least Significance Difference) intervals ($p<0.05$) for TPC and AC, respectively.

Figure 6. Influence of the drying method used on the fresh apple (LTD, USLTD, HAD and USHAD) on the content of oleuropein and verbascoside of samples impregnated with the olive leaf extract. Means±LSD intervals are plotted. Superscript letters (a, b, c) and (x, y, z) show homogeneous groups established from LSD (Least Significance Difference) intervals ($p<0.05$) for the content of oleuropein and verbascoside, respectively.

Figure 7. Influence of the drying method used on the fresh apple (LTD, USLTD, HAD and USHAD) on the content of luteolin glucoside and apigenin-6,8-diglucoside of samples impregnated with the olive leaf extract. Means±LSD intervals are plotted. Superscript letters (a, b) and (x) show homogeneous groups established from LSD (Least Significance Difference) intervals ($p<0.05$) for the content of luteolin glucoside and apigenin-6,8-diglucoside, respectively.


Hot air
Room air
INLET
OUTLET
EQUIPMENT
FIELD POINT
IMPEDANCE MATCHING UNIT
POWER AMPLIFIER
DYNAMIC RESONANCE CONTROLLER

A B

Figure 1

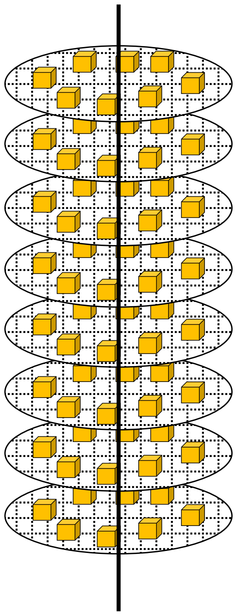


Figure 2

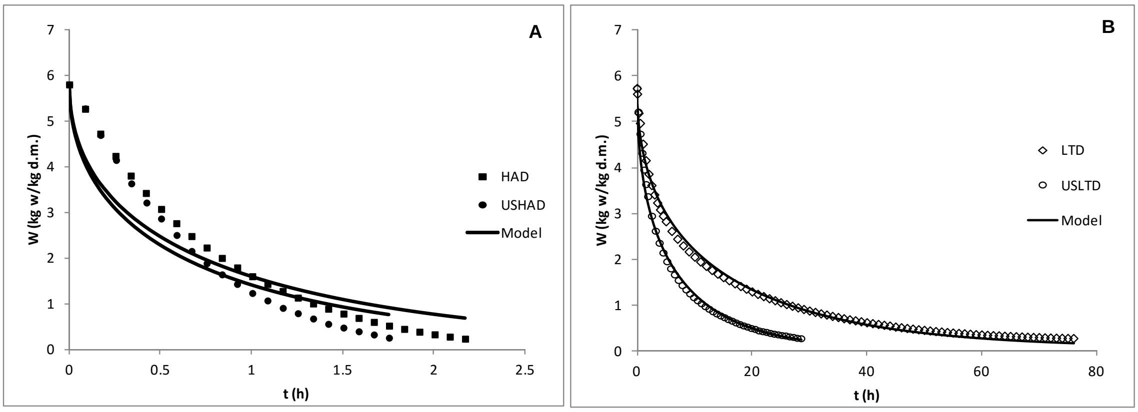
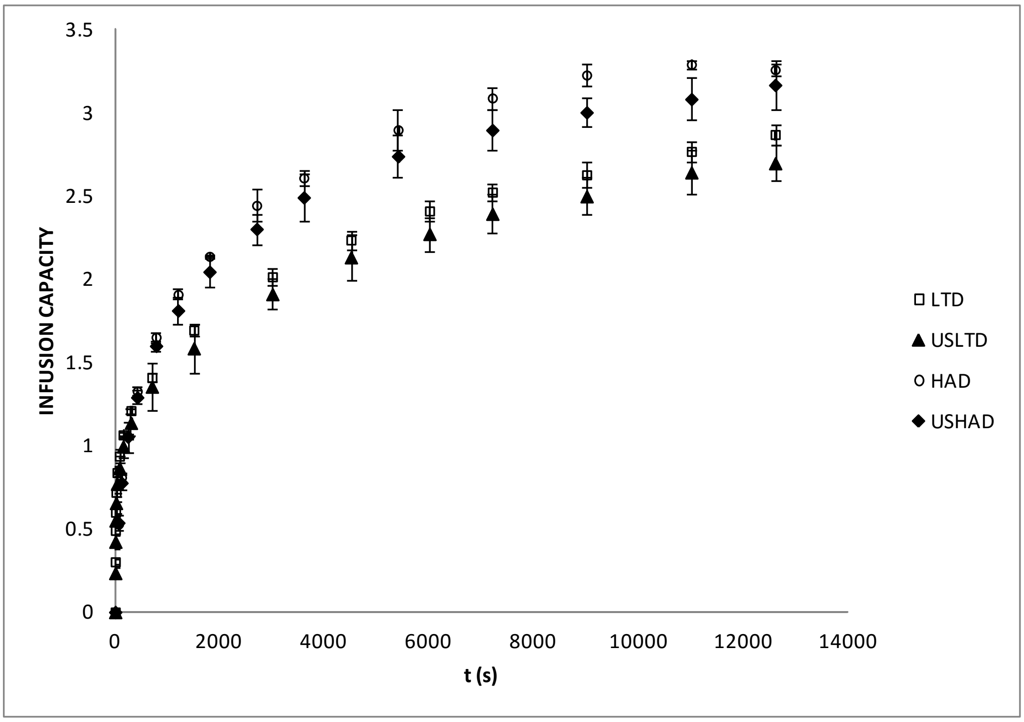


Figure 3



INFUSION CAPACITY
3.5
3
2.5
2
1.5
1
0.5
0
0
2000
4000
6000
8000
10000
12000
14000
t (s)
LTD
USLTD
HAD
USHAD

Figure 4

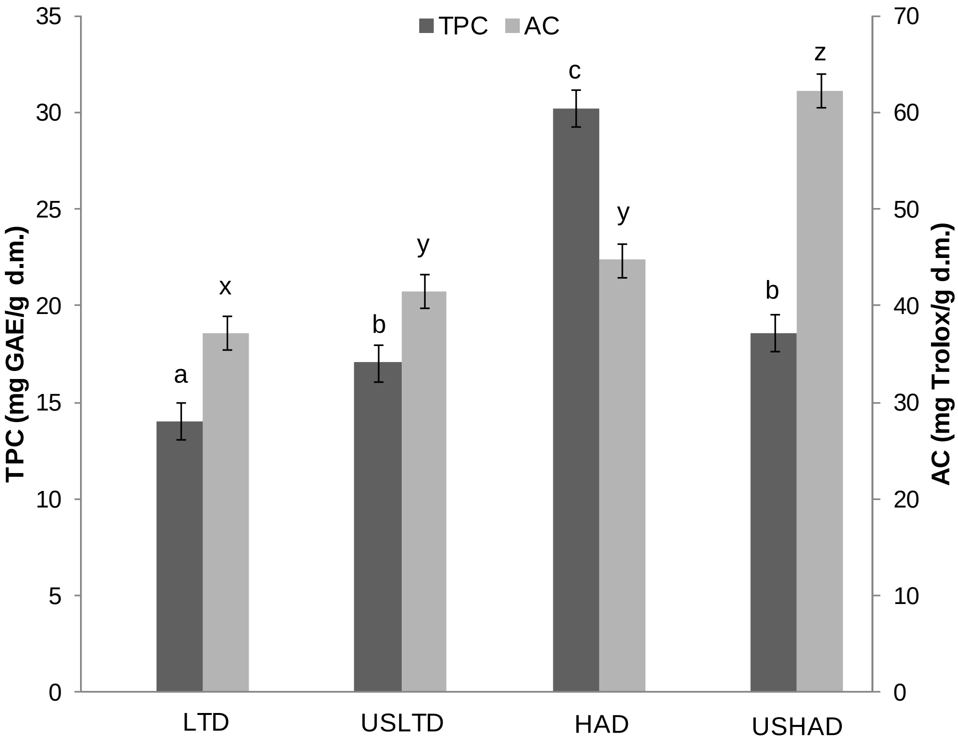

TPC
AC
TPC (mg GAE/g d.m.)
AC (mg Trolox/g d.m.)
0
5
10
15
20
25
30
35
0
10
20
30
40
50
60
70
a
x
b
y
c
y
b
z
LTD
USLTD
HAD
USHAD

Figure 5

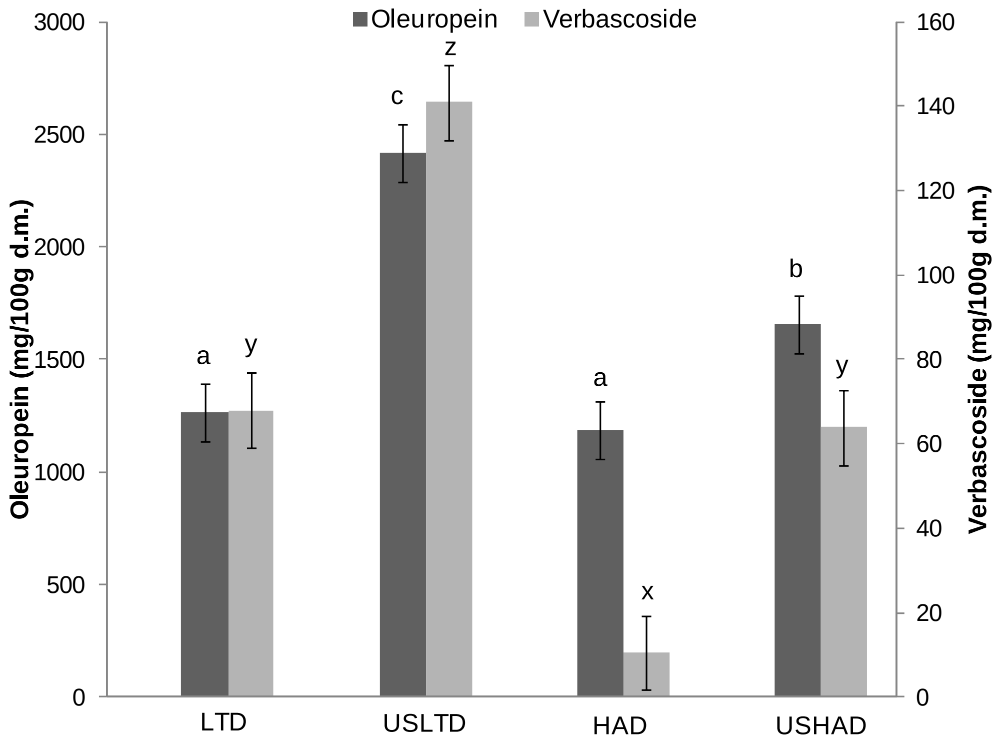

Oleuropein
Verbascoside
Oleuropein (mg/100g d.m.)
Verbascoside (mg/100g d.m.)
3000
2500
2000
1500
1000
500
0
160
140
120
100
80
60
40
20
0
a
y
c
z
a
x
b
y
LTD
USLTD
HAD
USHAD

Figure 6

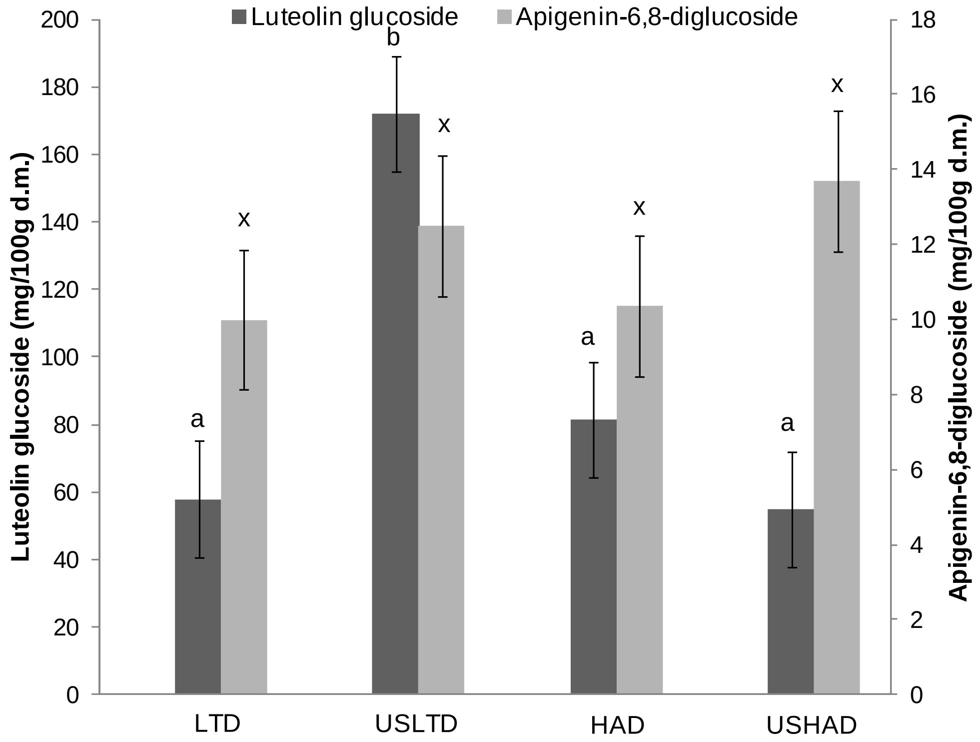

Luteolin glucoside
Apigenin-6,8-diglucoside
Luteolin glucoside (mg/100g d.m.)
Apigenin-6,8-diglucoside (mg/100g d.m.)
200
180
160
140
120
100
80
60
40
20
0
18
16
14
12
10
8
6
4
2
0
a
x
b
x
a
x
a
x
LTD
USLTD
HAD
USHAD

Figure 7