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ANTIOXIDANT POTENTIAL OF ATMOSPHERIC FREEZE DRIED APPLES AS AFFECTED BY ULTRASOUND APPLICATION AND SAMPLE SURFACE

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ABSTRACT

Atmospheric Freeze Drying (AFD) yields products of a similar quality to the conventional vacuum freeze drying technique, but reduces the operating cost. However, it involves very low drying rates. The sample surface/mass ratio is one of the process variables that can be taken into account to reduce drying time. Moreover, power ultrasound (US) can also be used to intensify this process because of its effects on external and internal mass transfer resistance. However, both factors may affect not only the drying time but also the final product quality. Therefore, the aim of this study was to address the influence of both ultrasound application and the sample surface/mass ratio on the drying process and the antioxidant potential of atmospheric freeze dried apple. For that purpose, two sample geometries with different surface/mass ratio were considered: slabs (30 x 30 x 10 mm) and cylinders (diameter: 9 mm and height: 30 mm). The samples were freeze dried (-10°C) with ultrasound application (21.7 kHz) at different power levels (0, 10.3, 20.5 and 30.8 kW/m^3). The total phenolic content (TPC), antioxidant capacity (AC) and ascorbic acid content (AA) were measured in the dried apple. The drying time was significantly shorter for cylindrical samples than for slabs, probably due to their higher surface/mass ratio. The application of US increased the drying rate, this increase being greater for the slab than for the cylindrical particles used in this study. In general, AFD reduced the TPC, AC and AA, the final content being significantly greater for slabs than for cylinders. This fact can also be related to the lower surface/mass ratio in the case of slabs. US application further reduced TPC, AC and AA content, probably due to some cellular damage produced by the acoustic waves and to the oxygen transfer improvement. Nevertheless, the bigger particles (slabs) dried with ultrasound

needed a 10 % of drying time than the smaller ones (cylinders) dried without ultrasound. Moreover both kind of samples presented similar antioxidant potential.

Keywords: antioxidant capacity, ascorbic acid, ultrasonic, drying time.

INTRODUCTION

Atmospheric freeze drying (AFD) is a convective drying process that takes place at temperatures below freezing point. It is carried out using a cold gas (usually air) at atmospheric pressure with low water vapor pressure as the drying medium. In these conditions, the vapor's partial pressure gradient created between the product and the drying gas forces the frozen water of products to sublimate and to diffuse from the inner part of the materials to the surface and then to the gas [1, 2, 3]. The drying kinetics of atmospheric freeze-dried products depends on process variables, such as the drying temperature, air velocity and relative humidity, the product size (characteristic dimensions) or the initial moisture content of the products [1].

AFD allows products to be obtained with a better sensory, nutritional and functional quality than those processed by hot air drying and similar to those obtained by conventional vacuum freeze drying [2,4]. Taking place at atmospheric pressure, one advantage of AFD lies in the fact that there is no need for vacuum; moreover, the process can operate continuously, both aspects leading to a reduction in the processing cost. However, this operation involves low drying rates, which means long processing times. Therefore, there is a need to explore techniques that can contribute to an intensification of the AFD process.

Conventionally, one of the techniques used to shorten the drying time in convection drying operations involves a reduction in the particle size. Thus, increasing the surface and mass/volume of the particle facilitates moisture transport into the drying medium. However, this increase in the particle surface can also lead to degradation reactions, such as the oxidation of some interesting

compounds, like antioxidants. Therefore, it is very important to establish the optimum relationship between the surface and mass/volume particle for each product, linked not only to the drying rate but also to the quality of the obtained product.

Another alternative means of intensifying the AFD could be by considering a new technology, such as the application of power ultrasound (US). In fact, US has been applied in the hot air drying of fruits and vegetables, reducing both the drying times and the energy use [5, 6, 7]. The mechanical energy introduced into the medium by ultrasound can help to reduce both the external and the internal mass transfer resistance, with only a mild thermal effect [8]. The potential of power ultrasound to improve mass transfer phenomena during the AFD of several products (fruits, vegetables or fish), leading to shorter drying times, has also been addressed in previous studies [2, 3, 4, 9, 10, 11]. However, ultrasound application during drying can also influence the product's final quality, affecting both the physical [11, 12] and chemical [12, 13] characteristics.

Dried apples can be used as a raw material in the processing of ready-to-eat foods, such as snacks, breakfast cereals and other functional foods. Furthermore, apple constitutes one important source of antioxidant compounds with high antioxidant activity [14, 15, 16]. However, it has been observed that processing brings about a marked reduction in both the antioxidant content and the antioxidant activity [4, 15, 17]. Nowadays, there are very few studies into the effect of AFD on the antioxidant potential of dried products, and how ultrasound application can influence it.

Therefore, the main objective of this study was to address the influence of both the surface/volume ratio of the samples and ultrasound application on the antioxidant potential of dried apple during atmospheric freeze drying.

MATERIALS AND METHODS

Sample preparation

Apples (*Malus domestica* cv. Granny Smith) were purchased in a local market (Valencia, Spain). The fruits were selected to obtain a homogeneous batch, similar in size, color and ripeness (10.5-12 ºBrix). Two different apple sample geometries were obtained from the flesh of the fruit, slabs (30 x 30 x 10 mm) and cylinders (30 mm height and 9 mm diameter), with the help of a cork borer and sharp knives. The samples were pretreated, in a 1% (w/w) citric acid and 2% (w/w) ascorbic acid solution for 10 minutes, blotted, wrapped in plastic film and frozen at -18 ± 1°C until processing (at least 24 h).

Influence of ultrasound application on the antioxidant potential of raw apple

A set of experiments was designed and carried out to determine whether, regardless of the drying process, ultrasound application had an influence on the antioxidant potential of apple samples. For this purpose, slab samples were exposed to a high power ultrasonic field (Figure 1) for 5, 10 and 15 min in triplicate. An electrical power of 185 W was supplied to a generator that, with the help of an amplifier and an impedance adapter, produced an adequate signal with which to feed a sandwich type transducer. The vibration produced by the transducer is transmitted to a stepped plate (40 cm diameter) at a resonance

frequency of 25.8 kHz. At the maximum electrical power capacity, 185 W, the system is able to produce an ultrasonic field of 170 dB.

The antioxidant potential of the samples, both just after ultrasonic treatment and after 3 days of storage at 4±1 ºC, was measured in triplicate to determine the influence of the application of ultrasound on raw apple.

Drying experiments

Atmospheric freeze drying experiments were carried out in an ultrasonically-assisted drier (Figure 2), previously described [2]. The cylindrical drying chamber (internal diameter 100 mm, height 310 mm, thickness 10 mm) constitutes a vibrating element excited by an ultrasonic transducer at a frequency of 21.9 kHz. The drying air is re-circulated in the system, which can control both the temperature and the air velocity by means of two PID control algorithms. The drying experiments were carried out at a constant temperature of -10 ºC and an air velocity of 2 m/s. The relative humidity was maintained below 15 % in all cases, ensuring the vapor pressure gradient needed for the atmospheric freeze-drying. The experiments were conducted without (0 kW/m^3) and with ultrasound application. In this last case, three different power levels were tested: 10.3, 20.5 and 30.8 kW/m^3 (21.9 kHz). For each run, 4 slab samples or 12 cylinder samples, being an initial mass load of 27±3 g, were placed in a sample holder as is shown in Figure 3. The position of samples in the holder assured a uniform treatment of them for both air flowing and ultrasound application. At least four replicates were carried out for each condition tested, which means a minimum of 32 independent experiments. The drying kinetics was determined from the initial moisture content

of samples and the evolution of sample weight during drying. The experiments were stopped when the initial sample weight loss reached 73%.

Drying modelling

During drying, two series resistance appear against the moisture transport, an internal resistance to the moisture transport inside the sample and an external resistance between sample surface and the surrounding drying air. The first one mainly depends on the material structure and the temperature and the second one of the turbulence created at the solid-gas interphase. At low temperatures, such as the used in this work, the slow internal diffusion of vapor supposes an important resistance of moisture transport. In this case, the external resistance to mass transfer it can be assumed as negligible when compared to the internal one [2]. For this reason, in the present work a diffusion model was considered for the purposes of quantifying the influence of the experimental variables on the drying kinetics. The model also assumed the initial moisture content to be uniform, the water diffusivity during the drying to be constant and the conditions of all samples to be isotropic and homogeneous. The last assumption, however, usually considered in hot air drying processes, is not accomplished under atmospheric freeze-drying conditions. In this case, two main layers constitute the sample, an increasing external dried layer and a retreating internal frozen core, the sample being neither isotropic nor homogeneous. This means the theoretical diffusion model becomes an empirical one when freeze drying conditions are in place. However, this model has been previously used in similar conditions to quantify and compare the influence of process variables on drying kinetics with acceptable results [2].

Thus, the analytical solutions of the diffusion model [18] obtained for the cylindrical samples is shown in Equation 1.

$$W(t)_{FC} = 8 \cdot \sum_{n=0}^{\infty} \frac{e^{-D_e \beta_n^2 t}}{(\beta_n R)^2} \cdot \sum_{n=0}^{\infty} \frac{e^{-D_e \lambda_n^2 t}}{(\lambda_n L_c)^2} \cdot (W_o - W_e) + W_e \quad \text{Eq. 1}$$

Where $W(t)_{FC}$ is the moisture content of the sample (kg water/kg dry matter; d.m.) at time t (s), W_o the moisture content of the fresh sample (kg water/kg d.m.) and W_e the equilibrium moisture content (kg water/kg d.m.), D_e is the effective diffusivity (m^2/s), R is the radius (m) and L_c the height (m) of the cylindrical samples, and λ_n and β_n are the eigenvalues calculated from:

$$\lambda_n L = (2n + 1) \cdot \frac{\pi}{2} \quad \text{Eq. 2}$$

$$J_0(\beta_n R) = 0 \quad \text{Eq. 3}$$

As regard the slab samples, the analytical solution used was:

$$W(t)_{FS} = 8 \cdot \left(\sum_{n=0}^{\infty} \frac{e^{-D_e \lambda_n^2 t}}{(\lambda_n L_1)^2} \right)^2 \cdot \left(\sum_{n=0}^{\infty} \frac{e^{-D_e \lambda_n^2 t}}{(\lambda_n L_2)^2} \right) \cdot (W_0 - W_e) + W_e \quad \text{Eq. 4}$$

Where L_1 and L_2 are the half thicknesses (m) of the sides of slab samples and λ_n are the eigenvalues calculated from Equation 2.

The equilibrium moisture (W_e) was calculated from the desorption isotherm for apple reported by Veltchev et al. [19]. This isotherm was calculated from experimental data obtained at 20, 40 and 60ºC. Therefore, and for the purposes of checking its accuracy at -10ºC, the equilibrium moisture content was also

experimentally measured by maintaining apple samples under drying conditions (-10 ºC and a relative humidity of under 15 %) until constant weight was achieved (15 ± 2 days).

The effective diffusivity (D_e) values for the different experimental runs were identified by minimizing the squared difference between the experimental and calculated (Equation 1 and Equation 4) moisture content. For that purpose, the generalized reduced gradient method, available in Solver tool from Microsoft Excel® 2007, was used. The percentage of explained variance (Equation 5) was estimated in order to quantify the goodness of the fit of the model.

$$\mathrm{VAR}\ (\%) = \left(1 - \frac{S_{xy}^2}{S_y^2}\right) \cdot 100 \qquad \text{Eq. 5}$$

Antioxidant potential

The antioxidant potential of fresh and dried samples was assessed from the measurement of the antioxidant capacity (AC), the total polyphenolic content (TPC) and the ascorbic acid content (AA) of ethanol extracts. For that purpose, approx 0.5 and 1 g of dried or fresh sample, respectively, were placed in 10 mL of ethanol (96% v/v) and were homogenized with an ultraturrax for 1 min at 13000 r.p.m. Then, the mix was filtered and stored at 4ºC, protected from light, until analysis.

Antioxidant Capacity (AC) measurement

The AC of samples was measured using the FRAP method [20]. 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 FeCl3•6H2O (Panreac, Barcelona, Spain) and 2.5 mL of 0.3 M acetate buffer (Panreac, Barcelona, Spain), pH 3.6. Readings at the maximum absorption level (595 nm) were taken using a spectrophotometer (Helios Gamma, Thermo Spectronic, Cambridge, UK). The antioxidant capacity was evaluated through a calibration curve, which was previously determined using ethanol solutions of known Trolox (Sigma-Aldrich, Madrid, Spain) concentrations and expressed as mg Trolox per g of apple dry matter.

Total phenolic content (TPC) measurement

The TPC was determined by means of the Folin-Ciocalteu method [21]. Briefly, 100 mL of sample were mixed with 200 mL of Folin-Ciocalteu's phenol reagent (Sigma-Aldrich, Madrid, Spain) and 2mL of distilled water. After 3 min at 25C, 1mL of Na_2CO_3 (Panreac, Barcelona, Spain) solution (Na_2CO_3-water 20:80, w=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 taken 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.

Ascorbic acid (AA) measurement

The ascorbic acid content (AA) was determined following the method proposed by Jagota and Dani [22]. For that purpose, 0.5 mL of sample was mixed with 0.5 mL of a trichloroacetic acid solution (7.5%). After 5 min at 4 ºC, the mix was

filtered. Then, 0.2 mL of extract, 2 mL of distilled water and 0.2 mL of diluted solution (1:10 v/v) were mixed and maintained for 10 min at room temperature. After that, absorbance was measured at 760 nm in a spectrophotometer (Helios Gamma, Thermo Spectronic, Cambridge, UK). A calibration curve was previously prepared with ethanol solutions of known ascorbic acid concentrations. The results were expressed as mg of ascorbic acid per g of apple dry matter.

All the determinations were carried out in triplicate. The retention of AC, TPC and AA at the end of drying was calculated by using Equation 6.

$$\%\text{Retention} = \frac{C_f}{C_o} \cdot 100 \qquad \text{Eq. 6}$$

where C_o is the AC, TPC and AA measurements before treatments and C_f is the AC, TPC and AA measurements obtained at the end of treatment.

Statistical analysis

A multifactor analysis of variance (ANOVA) was carried out, using the Statgraphics Centurion XVI (StatPoint Technologies, Inc), to check the significance ($p<0.05$) of the differences between dependent variables (D_e, AC, TPC or AA) of samples, cylinders or slabs, dried at different levels of ultrasonic power applied. Moreover, the Least Significant Difference (LSD) intervals were also estimated so as to determine the significance of differences between treatments.

RESULTS

Experimental drying

The average initial moisture content of the apple (96 samples) used in this study was 6.2 ± 0.4 kg water/kg d.m. and the average soluble solid content was 11.1 ± 0.8 ºBx. All drying kinetics took place in the falling rate period.

Because it can be assumed that the mass transfer at atmospheric drying conditions controls the drying rate, the experimental drying kinetics showed how the sample geometry influenced the drying rate (Figure 4), the drying process being faster for cylinders than for slabs. Thus, 37% more time was needed for slabs to reach a moisture content of 2 kg water/kg d.m. (44 ± 2 h) than for cylinders (32 ± 3 h). As showed before in materials and methods section, the mass load used in each experiment was the same and the transport characteristic dimensions of slabs and cylinder were similar (30 x 30 x 10 mm in the case of slabs and 30 mm high and 9 mm diameter for cylinders). Then, the difference in drying time could be attributed to the different surface/mass ratio, 33% higher for cylinders (0.53 m^2/kg) than for slabs (0.40 m^2/kg).This means that, for the same amount of product, the cylindrical samples presented greater surface to mass transport than the sliced samples, which made the drying process easier.

The application of ultrasound increased the drying rate: the more ultrasonic power applied, the faster the process (Figure 5). Thus, the time required to reach 2 kg water/kg d.m in the case of cylindrical samples was reduced from 32 ± 3 h to 10 ± 2 h when an ultrasonic power density of 10.30 kW/m^3 was applied, which means that the drying time was shortened by 69 %. This reduction increased to 88 % at the maximum ultrasonic power tested (30.8 kW/m^3), needing only 3.8 ± 0.3 h. Kowalski and Mierzwa [7], drying apple slices at 50 ºC and 2 m/s, observed a drying time reduction of 38 % when ultrasound (200 W) were applied (160 min

without ultrasound vs 100 min with ultrasound). At lower temperature, -10ºC, and using apple cubic samples, Santacatalina et al. [4] found higher influence of ultrasound application. These authors obtained a drying time of 10.3 h in the experiments carried with ultrasound (20.5 kW/m3) and 43.8 h in experiments carried without ultrasound that means 77% time reduction. Drying also apple cubic samples at -10 ºC, Santacatalina et al. [3] observed that the increase in the level of applied acoustic power led to a rise in the effective diffusivity.

In the case of slab samples, the application of ultrasound reduced the drying time (necessary to reach a moisture content of 2 kg water/kg d.m.) by 82% at 10.3 kW/m^3 (8.1 ± 0.7 h) and 92% at 30.8 kW/m^3 (3.7 ± 0.6 h). Therefore, the application of ultrasound seems to be more effective at reducing the drying rate in the samples that presented a lower surface/mass ratio, the slab samples.

To check the influence of ultrasound application in the total energy needs of the process, energy consumption measurements of the laboratory scale dryer were carried out. The results showed that, at the drying conditions tested (-10 ºC; 2 m/s) and not including the consumption of the refrigeration system, the total energy consumption of the system per hour increased 163 % when ultrasound were applied (30.8 kW/m3). However, the reduction of the drying time produced by ultrasound (88 % in cylinder samples and 92 % in slab samples) decreased the total energy consumption of the drying 68.8% in the case of cylinder samples and 78.8 % in the case of slabs. These results shows the energy saving potential of ultrasound application for the drying at these conditions.

Drying modeling

For the purposes of modeling the experimental drying data, it is necessary to determine the equilibrium moisture content of apple samples under the drying conditions (-10 ºC and a relative humidity under 15 %). For this reason, the equilibrium moisture content was calculated for each particular drying experiment by applying the isotherm reported by Veltchev et al. [19]. The values obtained were very similar, the average value being 0.35 ± 0.06 kg water / kg d.m for every condition tested. Moreover, as explained in the materials and methods section, the experimental equilibrium moisture content was also measured and the average value obtained, 0.4 ± 0.2 kg water/kg d.m., was not significantly different ($p<0.05$) from the one obtained from the Veltchev et al. [17] isotherm.

In general, it could be stated that the proposed model fitted the experimental data adequately, as can be observed from the percentage of explained variance obtained, higher than 97% in every drying condition tested (Table 1). Notwithstanding this, the fit of the model in both types of sample tested, cylinders and slabs, is poorer after the application of ultrasound. Thus, the lower the level of ultrasonic power applied, the higher the percentage of explained variance. In all likelihood, the effects produced by ultrasound application induced mechanisms, more intense when more power is applied, other than diffusion (internal resistance), which make the moisture transport easier. Nevertheless, because the modelling was only for the purposes of comparison, the fit was considered to be adequate and permitted the quantification of the influence of the studied process variables, ultrasound power level and sample geometry, on the AFD drying kinetics of apple.

The effective diffusivity (D_e) identified from the experiments carried out without ultrasound application (0 kW/m^3) was in the range of that obtained by other authors [23]. No significant differences ($p<0.05$) were found between the geometries tested, cylinders and slabs (Figure 6). This was expected because the moisture diffusivity is a material property and the sample geometry was considered in the model through the different characteristic dimensions of moisture transport: R and L_c for cylindrical samples and L_1 and L_2 for slabs. Even the model being highly simplified provide sound results.

In both geometries tested, the identified D_e significantly ($p<0.05$) increased when ultrasound was applied during the drying experiments (Table 1). This increase presented a linear relationship with the ultrasonic power applied (Figure 6). The analysis of variance and the estimation of the least significance difference (LSD) intervals ($p<0.05$) indicated that for the ultrasonic power levels tested, the D_e rose significantly in line with how much power was applied (Figure 6). The influence of the ultrasonic power applied on D_e has been previously observed, not only in low temperature drying processes [23] but also in the case of ones carried out at higher temperatures [6]. In fact, Ozuna et al. [6] observed a relationship between the slope of the increase of D_e with the ultrasonic power applied and some internal properties of materials, like porosity. Thus, the more porous the material, the steeper the D_e vs ultrasonic power slope and, thus, the more prone it is to ultrasonic effects on drying kinetics. As a highly porous external layer is developed in the products during the atmospheric freeze drying process, it is possible to observe that ultrasound application has a great influence on drying kinetics (Table 1). Previous measurements showed a slight increase of sample temperature during drying when ultrasound were applied (2ºC in the center of

samples at the higher ultrasonic power tested). This increase of temperature did not explain the increase of effective diffusivity obtained and it can indicate that the effects of ultrasound were mainly mechanical.

However, from the results obtained, it was observed that the influence of ultrasound on the drying kinetics was also dependent on the sample geometry. Thus, the rise in the identified D_e produced by the increase in the ultrasonic power applied was significantly ($p<0.05$) lower for cylindrical samples than for slabs (Figure 6). Thus, the identified empirical D_e value obtained for slab samples was approximately twice the value obtained for cylindrical samples at every ultrasonic power level tested, as can be observed by comparing the slope of the linear relationship between D_e and the ultrasonic power applied (Figure 6). The D_e is a parameter that includes all the resistances to mass transfer.

The low temperatures used in AFD implies that the drying is mainly controlled by the internal mass transfer resistance. Santacatalina et al. [24] did not found significance difference in the drying rate of eggplant (-10 ºC) dried at different air velocities (1, 2, 4 and 6 m/s). This fact indicates that, if the main factor that can reduce external resistance that is the air turbulence, did not significant affect the drying kinetics, then the external resistance is negligible compared with the internal one. Additionally, the slab geometry used in the present study presented a lower surface/mass ratio (0.40 m^2/kg) than the cylindrical one (0.53 m^2/kg). This means that slab particles presented a smaller drying surface; therefore, for these samples, the internal resistance had a greater influence on the drying than in the case of cylinders. The fact that the influence of ultrasound application on the slab samples is more marked can indicate that ultrasound has a proportionally greater

influence on the reduction of internal mass transport resistance than external. Then, the increase in drying kinetics produced by ultrasound was higher in the samples that presented greater internal resistance to mass transfer. The porous external dried layer developed during AFD is highly prone to the ultrasonic effects. Therefore, under AFD conditions, the effects of ultrasound on internal mass transport resistance may be both more marked and more significant than the effects on external resistance.

This result is of great interest because it indicates that ultrasound could be used to accelerate the AFD of samples with a low surface/mass ratio. In fact, in conventional AFD operations, small particle sizes are usually used to achieve a higher surface/mass ratio, which shortens the drying time. The increase in the drying rate produced by ultrasound can provide the possibility of using bigger particles and obtaining new products.

Influence of ultrasound application on the antioxidant potential of raw apple

For the purposes of differentiating the influence of ultrasound application on the antioxidant potential of raw apple from the influence of ultrasound application during apple atmospheric freeze drying, a set of experiments was carried out, as pointed out in the materials and methods section. In this sense, the treatment times chosen for these experiments were short enough to avoid significant moisture changes in samples. Moreover, the ultrasonic power applied (170 dB) was higher that the maximum applied in drying experiments (155 dB) to test the influence of a more intense ultrasonic treatment.

The TPC, AA and AC contents of the fresh apple used in this study was 4.4±0.6 mg GAE/g d.m., 2.6±0.1 mg ascorbic acid/g d.m. and 17.72±1.99 mg Trolox/g d.m., respectively. Thus, the TPC obtained is in the range of that reported by Vega-Gálvez et al. [24] (1.6 ±0.01 mg GAE/g d.m.) or Heras-Ramírez et al. [16] (11.9±1.0 mg GAE/g d.m.). As regards AC, the value obtained was higher than that reported by Santacatalina et al. [4], probably due to the natural variability of the product. The experimental results showed that the pre-treatment applied in order to prevent Maillard browning did not affect the antioxidant potential of samples. In fact, no significant ($p<0.05$) differences were found between the TPC, AA and AC of fresh and pre-treated apple samples.

In non-drying experiments, the application of ultrasound affected neither the AA nor the AC content of apple (Table 2). On the contrary, the observed post-sonication TPC value was lower than in raw apple. Although these differences were only significant for the treatments carried out for 5 and 15 min, they could indicate a negative effect of ultrasound on these compounds. In this sense, Wiktor et al. [25] applied ultrasound to apple (var. Ligol) disks using a stainless steel mesh mounted in a ring sonotrode (24 kHz). They observed an increase in the phenolic content of 77, 29 and 34%, compared to the fresh samples, for treatments of 5, 10 and 20 min, respectively. It is likely that the ultrasonic treatment allowed for a better extraction of the phenolic compounds. However, after 30 min of treatment, they obtained a 30 % reduction. This fact can be linked both to the oxidative degradation produced by the oxygen included in the tissue pores and to the intensification of the enzymatic activity, which reduced the phenolic compounds.

After 3 days of storage at 4 ºC, the TPC of non-ultrasonically treated fresh samples was reduced by 51.4 %. On the contrary, the ultrasonically treated samples exhibited a smaller reduction in TPC; the longer the treatment time, the smaller the reduction (47.8, 38.6 and 30.2% for treatments of 5, 10 and 15 min of, respectively). This can be attributed to the formation of new phenolic compounds during storage due to the response to the abiotic stress produced by ultrasound application. Abiotic stress affects the phytochemical content due to the alteration of the activity of key enzymes that generate other secondary bioactive compounds [26]. In this sense, Cisneros-Zevallos [27] stated that the controlled abiotic stress treatments can be used as tools for the purposes of improving the nutritional quality of products.

As regards the AA and AC content of apple samples after 3 days of refrigerated storage, it was not significantly different from that obtained for fresh apple, regardless of the ultrasonic treatment applied.

Influence of sample geometry and ultrasound application on the antioxidant potential of dried apple

The influence of both the sample geometry and the application of ultrasound during drying on the antioxidant potential of dried apple was also addressed. For this purpose, the TPC, the AA and the AC of apple samples dried both without ultrasound application (AIR) and at the maximum ultrasonic power tested, 30.8 kW/m^3 (AIR+US), were measured.

Total Phenolic Content

In general, drying reduced the TPC of apple samples. Stawczyk et al. [17] also found reductions in the phenolic compound content of apple of 28.6% and 23.9% during atmospheric freeze drying at -8 and -12°C, respectively. The TPC reduction was affected by the variables considered: sample geometry and ultrasound application. Thus, in the case of AIR dried samples, the percentage of TPC retention was significantly lower for slabs (61%) than for cylinders (83%) (Figure 7A). This degradation of the phenolic compounds may be related with the cellular damage produced by the prior freezing of the sample that, among other things, contributed to the release of the oxidative enzymes. Therefore, the longer drying time needed for the slab samples afforded the oxidative enzymes a longer time in which to act, and so a more marked phenolic compound degradation during processing.

In the case of cylindrical particles, the application of ultrasound reduced the retention of the TPC content (Figure 7A), this retention being significantly ($p<0.05$) lower in AIR+US samples (57%) than in AIR ones (83%). On the contrary, the TPC retention of AIR+US slab samples (75%) was significantly higher than that obtained for AIR ones (61%). On the one hand, the mechanical stress produced by acoustic waves can produce some structural effects that may increase the cell damage induced by freezing [23]. On the other hand, as the results shown in the section before seemed to indicate, the application of ultrasound can activate a response mechanism of the tissue that induces the formation of new phenolic compounds, not only through the combination of existing compounds but also via the activation of secondary metabolic pathways. Moreover, the fact that the ultrasonic treatment produced a possible inactivation of oxidative enzymes must also be considered [28]. The predominance of one of

these effects can be related with the surface mass ratio of the sample that, as stated before and in the case of the samples used in this study, was higher in cylinders than in slabs. Thus, the structural damages produced by ultrasound will be favored by high surface/mass ratio values, while the ultrasonic activation of response reactions will be favored by low values.

Antioxidant Capacity

In general, drying leads to a reduction in the AC of samples (Figure 7B). However, there were differences between the AC of the geometries tested. Thus, while the cylindrical samples exhibited a significant ($p<0.05$) AC decrease, no significant difference between the AC of fresh apple and that of the dried slab samples was observed. The difference between slabs and cylinders can be related to the higher surface/mass ratio of the cylinders. Thus, for the same mass, the cylindrical samples presented a greater surface of exchange than slabs, which makes it easier for some oxidation reactions to take place.

The application of ultrasound produced a reduction in AC, similar for both geometries tested. The mechanical stress on the cellular structure and the enhancement of the oxygen transport into the damaged structure induced by ultrasound effects can be linked to this reduction. It must be highlighted that, even though the ultrasound induced a reduction, the AC of AIR+US slab samples was in the range of that of the AIR cylinder samples (Figure 7B).

Other authors also observed a lower AC in atmospheric dried apple samples. Thus, Santacatalina et al. [4] found a 50% reduction for apple dried at -10ºC while

Stawczyk et al. [17] reported a reduction of 7.8% when the drying was carried out at -12ºC.

Ascorbic acid content

As in the case of AC, atmospheric freeze drying produced a significant reduction in the AA content of apple. In this sense, Duan et al. [29] found a 56.4% AA retention in apple (var. Fuji) samples dried at -10ºC and Reyes et al. [15] reported a 30% retention in the case of apples dried at -5ºC.

The cylindrical samples retained less AA (80%) than the slabs (90%) (Figure 7C). This can also be explained by the higher surface/mass ratio of the cylindrical particles used in this study compared with the slabs; this facilitates oxidation reactions, which are particularly important in the degradation of ascorbic acid.

The application of ultrasound during drying significantly reduced ($p<0.05$) the retention of AA. The influence was similar for the two geometries tested. Thus, for both geometries, the retention in AIR+US samples fell by 20% compared with what takes place in AIR experiments. This indicated that the effects of US were not related with the solid-gas interface because the surface/mass ratio of samples did not seem to affect the observed AA retention. Therefore, the US effects on the antioxidant potential could mainly be located inside the samples. The contractions and expansions produced by US can cause some cellular damage that promotes oxidation reactions. Moreover, US can make it easier for the external oxygen to diffuse into the samples.

In general, from the antioxidant potential results obtained, it must be highlighted that the TPC, AC and AA retention of AIR dried cylindrical particles was not

significantly different from that obtained for AIR+US dried slabs. As is shown in Table 1, the drying time for AIR+US slabs at 30.8 kW/m^3 was 10% of that needed for the AIR cylinders without ultrasound application. This means that the application of ultrasound permitted an important reduction in the drying time without affecting the quality parameters. This could be translated into savings, both of money and energy, and permit the drying of bigger particles in a reasonable processing time.

CONCLUSIONS

The application of ultrasound increases the atmospheric freeze drying rate of apple. This increase was greater in the slabs than in the cylindrical particles used in this study. This can be related with the lower surface/mass ratio of the slabs compared with the cylinders. The application of ultrasound to raw apple samples did not affect the antioxidant capacity or the ascorbic acid content. However, after three days of storage, the total phenolic content of ultrasonically treated samples compared with non-treated ones can indicate the activation of a response mechanism which may induce the formation of new phenolic compounds. The application of ultrasound during drying produced a decrease in the antioxidant potential, which was related to the cell disruption under acoustic stress and oxygen transfer. However, the significant reduction in the drying time produced by ultrasound can compensate for a slight drop in the antioxidant potential, this being of particular importance in those samples with a lower surface/mass ratio.

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REFERENCES

1. Claussen, I. C.; Ustad, T.S.; Strømmen, I.; Walde, P. M. Atmospheric freeze drying - A review. Drying Technology 2007, 25(6), 947-957.
2. García-Pérez, J.V.; Cárcel, J.A.; Riera, E.; Rosselló, C.; Mulet, A. Intensification of Low-Temperature Drying by Using Ultrasound. Drying Technology 2012, 30(11-12), 1199-1208.
3. Santacatalina, J.V.; Fissore, D.; Cárcel, J.A.; Mulet, A.; García-Pérez, J.V. Model-based investigation into atmospheric freeze drying assisted by power ultrasound. Journal of Food Engineering 2015, 151, 7-15.
4. Santacatalina, J.V.; Rodríguez, O.; Simal, S.; Cárcel, J.A.; Mulet, A.; García-Pérez, J.V. Ultrasonically enhanced low-temperature drying of apple: Influence on drying kinetics and antioxidant potential. Journal of Food Engineering 2014, 138, 35-44.
5. Sabarez, H.T.; Gallego-Juárez, J.A.; Riera, E. Ultrasonic-assisted convective drying of apple slices. Drying Technology 2012, 30, 989-997.
6. Ozuna, C.; Gómez, T.; Riera, E.; Cárcel, J.A.; Garcia-Perez, J.V. Influence of material structure on air-borne ultrasonic application in drying. Ultrasonics Sonochemistry 2014, 21, 1235-1243.
7. Kowalski, S.J.; Mierzwa, D. US-assisted convective drying of biological materials. Drying Technology 2015, 33, 1601-16013.
8. Awad, T.S.; Moharram, H.A.; Shaltout, O.E.; Asker, D.; Youssef, M.M. Applications of ultrasound in analysis, processing and quality control of food: A review. Food Research International 2012, 48(2), 410-427.
9. Bantle, M.; Eikevik, T.M. Parametric study of high-intensity ultrasound in the atmospheric freeze drying of peas. Drying Technology 2011, 29, 1230-1239.

10. Bantle M.; Hanssler, J. Ultrasonic convective dring kinetics of clipfish during the initial drying period. Drying Technology 2013, 31, 1307-1316.
11. Ozuna, C.; Cárcel, J.A.; Walde, P.M.; García-Pérez, J.V. Low-temperature drying of salted cod (Gadus morhua) assisted by high power ultrasound: Kinetics and physical properties. Innovative Food Science & Emerging Technologies 2014, 23(0), 146-155.
12. Soria, A.C.; Corzo-Martínez, N.; Montilla, A.; Riera, E.; Gamboa-Santos, J.; Villamiel, M. Chemical and physicochemical quality parameters in carrots dehydrated by power ultrasound. Journal of Agriculture and Food Chemistry 2010, 58, 7715-7722.
13. Rodríguez, O.; Santacatalina, J.V.; Simal, S.; Garcia-Perez, J.V.; Femenia, A.; Rosselló, C. Influence of power ultrasound application on drying kinetics of apple and its antioxidant and microstructural properties. Journal of Food Engineering 2014, 129, 21-29.
14. Vrhovsek, U.; Rigo, A.; Tonon, D.; Mattivi, F. Quantitation of polyphenols in different apple varieties. Journal of Agricultural and Food Chemistry 2004, 52(21), 6532-6538.
15. Reyes, A.; Mahn, A.; Huenulaf, P. Drying of apple slices in atmospheric and vacuum freeze dryer. Drying Technology 2011, 29(9), 1076-1089.
16. Heras-Ramírez, M. E.; Quintero-Ramos, A.; Camacho-Dávila, A. A.; Barnard, J.; Talamás-Abbud, R.; Torres-Muñoz, J. V.; Salas-Muñoz, E. Effect of Blanching and Drying Temperature on Polyphenolic Compound Stability and Antioxidant Capacity of Apple Pomace. Food and Bioprocess Technology 2012, 5(6), 2201-2210.

17. Stawczyk, J.; Li, S.; Witrowa-Rajchert, D.; Fabisiak, A. Kinetics of atmospheric freeze-drying of apple. Transport in Porous Media 2007, 66(1-2), 159-172.
18. Crank, J. The mathematics of diffusion; Clarendon Press: Oxford, 1975.
19. Veltchev, Z. N.; Menkov, N. D. Desorption isotherms of apples at several temperatures. Drying technology 2000, 18 (4-5), 1127-1137.
20. Pulido, R.; Bravo, L.; Saura-Calixto, F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. Journal of Agricultural and Food Chemistry 2000, 48(8), 3396-3402.
21. Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology 1999, 299, 152-178.
22. Jagota, S. K.; Dani, H. M. A new colorimetric technique for the estimation of vitamin C using Folin phenol reagent. Analytical Biochemistry 1982, 127(1), 178-182.
23. Santacatalina, J.V.; Contreras, M.; Simal, S.; Cárcel, J.A.; García-Pérez, J.V. Impact of applied ultrasonic power on the low temperature drying of apple. Ultrasonics Sonochemistry 2016, 28, 100-109.
24. Vega-Gálvez, A.; Ah-Hen, K.; Chacana, M.; Vergara, J.; Martínez-Monzó, J.; García-Segovia, P.; Lemus-Mondaca, R.; Di Scala, K. Effect of temperature and air velocity on drying kinetics, antioxidant capacity, total phenolic content, colour, texture and microstructure of apple (var. Granny Smith) slices. Food Chemistry 2012, 132(1), 51-59.

25. Wiktor, A.; Sledz, M.; Nowacka, M.; Rybak, K.; Witrowa-Rajchert, D. The influence of immersion and contact ultrasound treatment on selected properties of the apple tissue. Applied Acoustics 2016. 103, 135-142.
26. Pirovani, M. E.; Piagentini, A. M.; Van de Velde, F.. Bioactive compounds in fresh-cut fruits: Occurrence and impact of processing and cold storage. Biotechnology of Bioactive Compounds: Sources and Applications; Wiley Blackwel, 2015.
27. Cisneros-Zevallos, L. The use of controlled postharvest abiotic stresses as a tool for enhancing the nutraceutical content and adding-value of fresh fruits and vegetables. Journal of Food Science 2003, 68(5), 1560-1564.
28. Islam, M.N.; Zhang, M.; Adhikari, B. The Inactivation of Enzymes by Ultrasound-A Review of Potential Mechanisms. Food Reviews International 2014, 30(1), 1-21.
29. Duan, X.; Ding, L.; Ren, G.Y.; Liu, L.L.; Kong, Q.Z. The drying strategy of atmospheric freeze drying apple cubes based on glass transition. Food and Bioproducts Processing 2013, 91(4), 534-538.
30. Santacatalina, J.V.; Soriano, J.R.; Carcel J.A.; Garcia-Perez, J.V. Influence of air velocity and temperature on ultrasonically assisted low temperature drying of eggplant. Food and Bioproducts Processing. In press (DOI: 10.1016/j.fbp.2016.07.010)

FIGURE CAPTIONS

Figure 1. Scheme of the set-up used for the application of power ultrasound to raw apple samples..

Figure 2. Diagram of the ultrasonically assisted low temperature dryer: A, fan; B, Pt-100; C, temperature and relative humidity sensor; D, anemometer; E, ultrasonic transducer; F, vibrating cylinder; G, sample load device; H, retreating pipe; I, slide actuator; J, weighing module; K, heat exchanger; L, heating elements; M, desiccant tray chamber; N, computer; O, amplifier; P, resonance dynamic controller.

Figure 3. Positioning of slabs and cylinders in the sample holder

Figure 4. Experimental drying kinetics of cylinder and slab samples of apple dried (-10 ºC; 2 m/s) without ultrasound application.

Figure 5. Experimental drying kinetics (-10ºC and 2 m/s) of apple cylinders (A) and slabs (B) without and with the application of ultrasound (21.9 kHz) at different power levels.

Figure 6. Identified effective diffusivity and LSD intervals ($p<0.05$) for the atmospheric freeze-drying of apple (-10 ºC; 2 m/s) with ultrasound application at different power levels.

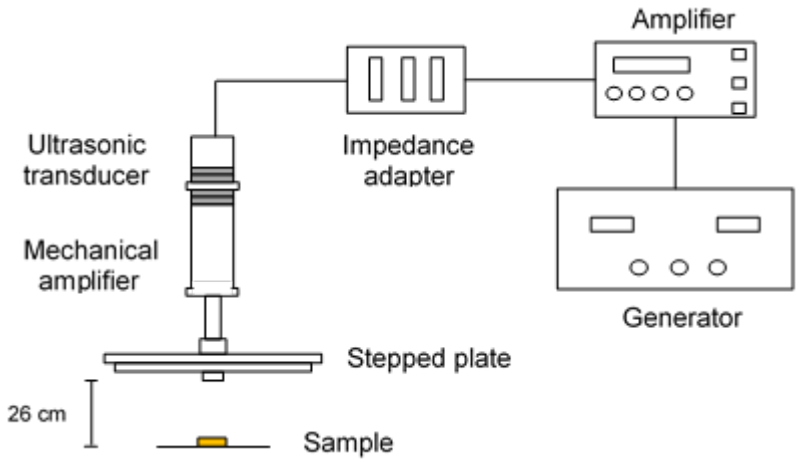
Figure 7. Retention of Total Phenolic Content (TPC), antioxidant capacity (AC) and ascorbic acid (AA) in cylinders and slabs of apple after atmospheric freeze drying (-10 ºC, 2 m/s) without (AIR) and with (AIR+US)

ultrasound application (21.9 kHz; 30.8 kW/m^3). Different letters show significant differences according to LSD intervals ($p < 0.05$).

TABLE CAPTIONS

Table 1. Effective diffusivity (D_e) identified from modelling the atmospheric freeze drying kinetics of cylinder and slab apple samples without and with ultrasound application at different ultrasonic power levels. ΔD_e is the increase in effective diffusivity produced by ultrasound application, VAR is the percentage of the explained variance, t is the drying time needed to reach an 80% initial weight loss and ΔTR is the reduction in the drying time produced by ultrasound application.

Table 2. Antioxidant potential of apple slabs treated with ultrasound, before and after storage at 4ºC.



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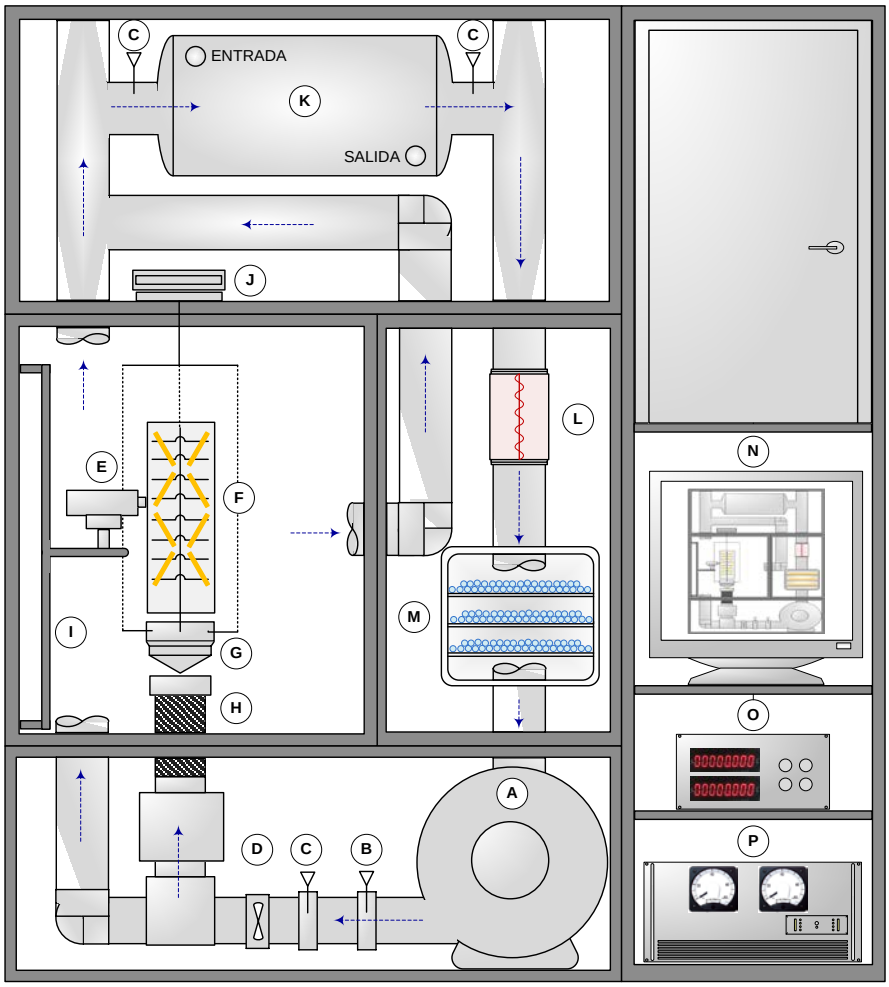

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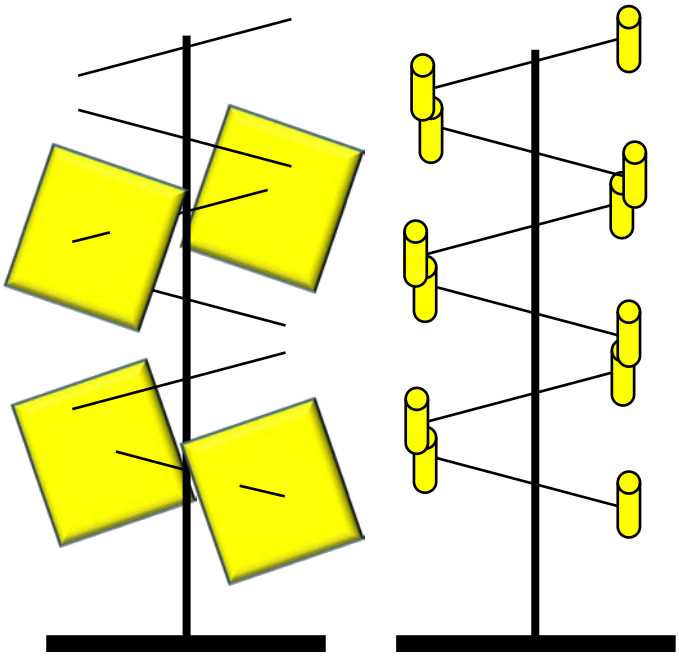


Figure 3. Positioning of slabs and cylinders in the sample holder

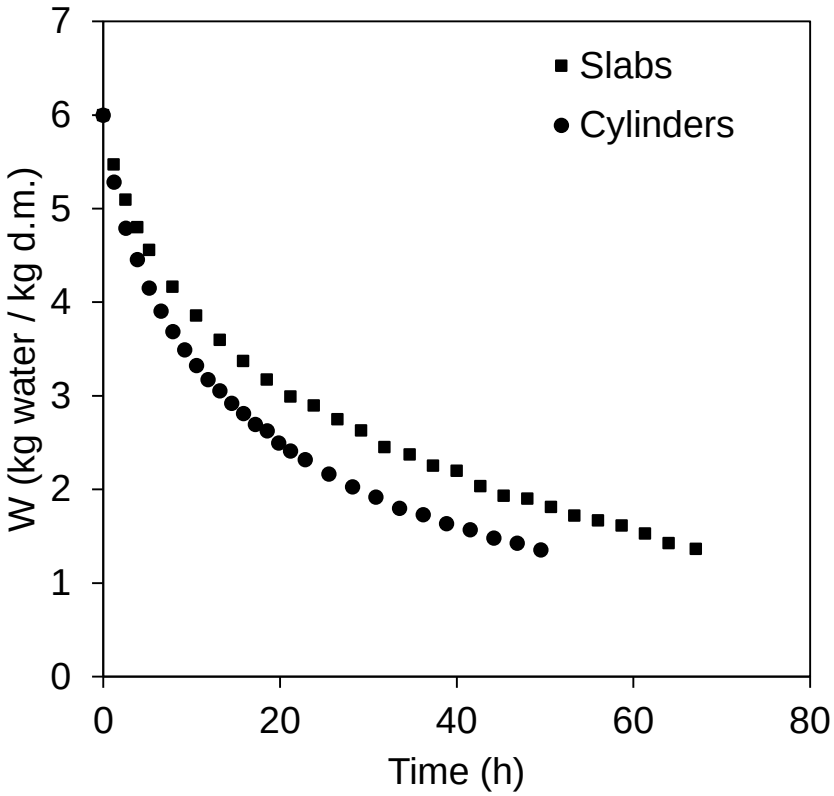


Figure 4. Experimental drying kinetics of cylinder and slab samples of apple dried (-10 ºC; 2 m/s) without ultrasound application.

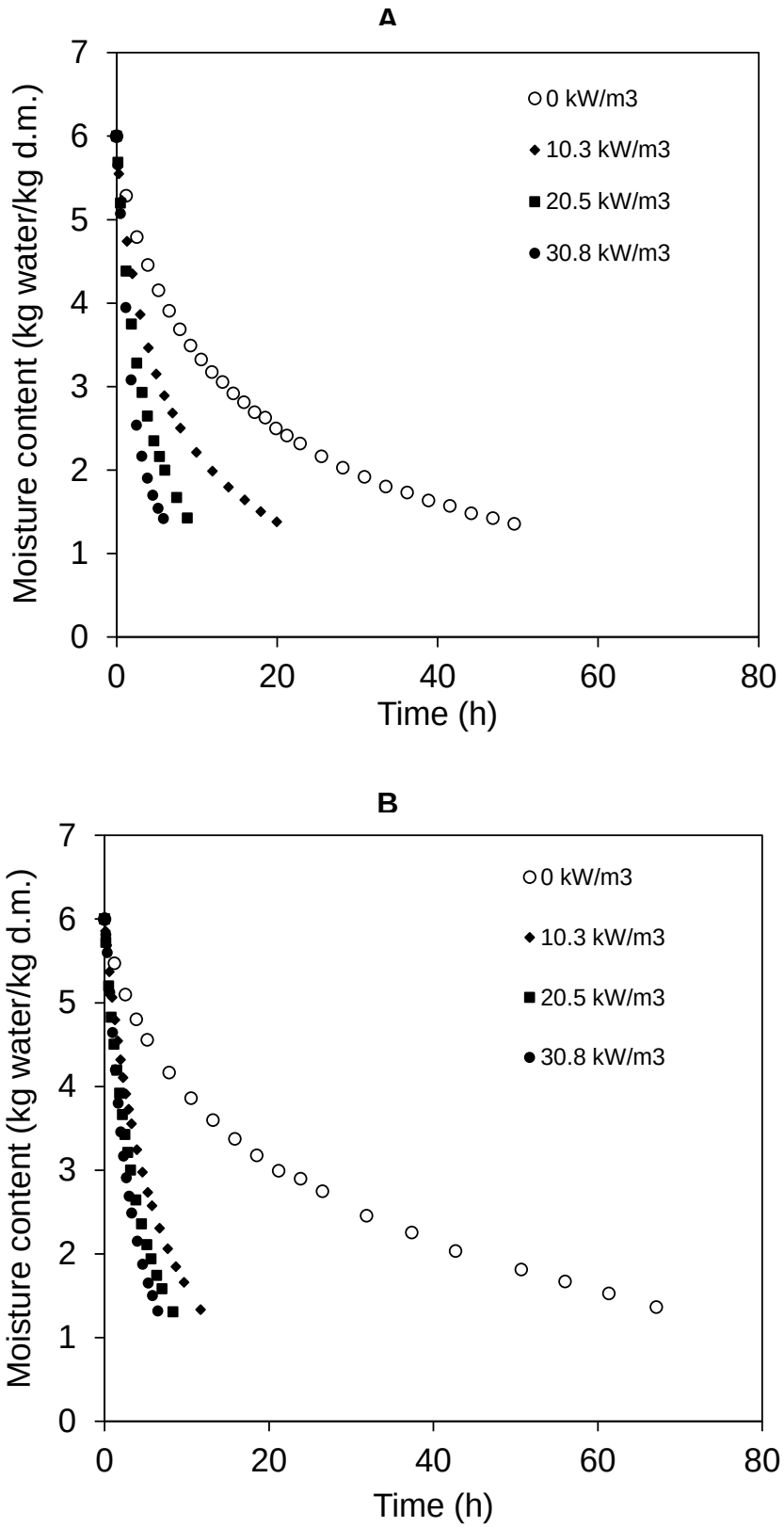


Figure 5. Experimental drying kinetics (-10ºC and 2 m/s) of apple cylinders (A) and slabs (B) without and with the application of ultrasound (21.9 kHz) at different power levels.

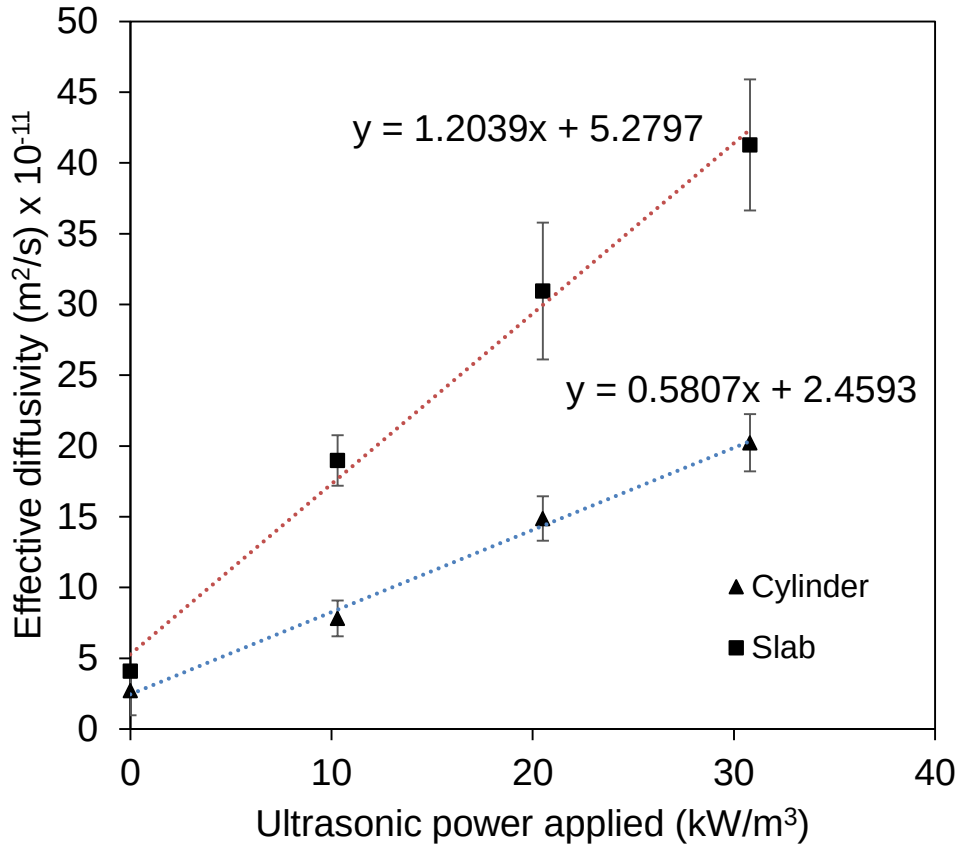
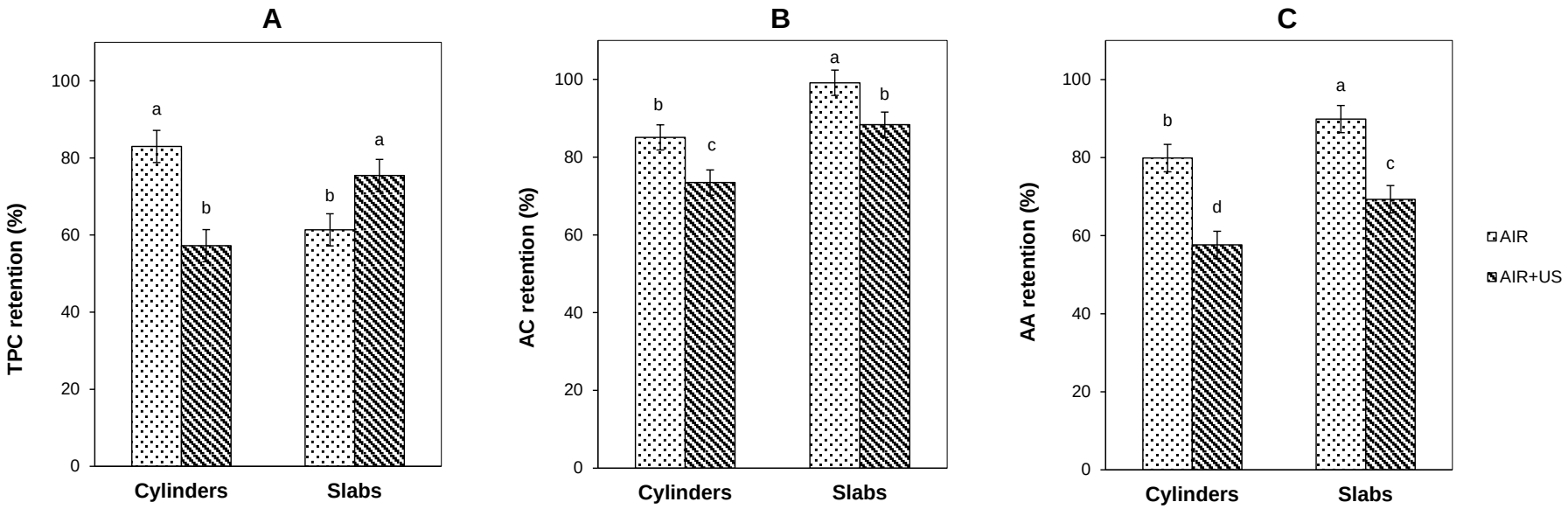


Figure 6. Identified effective diffusivity and LSD intervals ($p<0.05$) for the atmospheric freeze-drying of apple (-10 ºC; 2 m/s) with ultrasound application at different power levels



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