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Effect of ultrasound treatment on physicochemical parameters and drying time in nopal cladodes *Opuntia spp.*

Efecto del tratamiento ultrasónico sobre los parámetros fisicoquímicos y tiempo de deshidratado en cladodios de nopal *Opuntia spp.*

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Resumen

El nopal (*Opuntia spp.)* es una cactácea consumida como verdura que ha sido reconocido como un alimento funcional, ya que contiene compuestos bioactivos como ácidos orgánicos, fibra, mucílagos, pigmentos, minerales y antioxidantes. Las nuevas tecnologías en la industria alimentaria han demostrado que la aplicación de ultrasonido de potencia puede promover los mecanismos de transferencia de masa cuando se utiliza como pretratamiento para mejorar el secado de los alimentos. El objetivo de este trabajo fue evaluar el efecto del tratamiento ultrasónico sobre el color, la microestructura y el contenido de compuestos bioactivos para su posterior deshidratado y evaluación del tiempo de secado de los cladodios de nopal. Se realizó un diseño experimental factorial completo con punto central, considerando como factores la amplitud (20, 48 y 76%) y el tiempo de sonicación (10, 15 y 20 min). La aplicación del tratamiento con ultrasonido de potencia sobre los cladodios del nopal afecta la microestructura, deformando y distanciando las células, principalmente a 76% de amplitud por 15 min. El contenido de compuestos bioactivos como polifenoles totales y clorofila se incrementó de 11.7 hasta 18 mg EAG/g b.s. y de 30.9 hasta 93.6

mg/g b.s., respectivamente al tratar los cladodios del nopal con ultrasonido. Posterior al tratamiento con ultrasonido, el proceso de secado con aire caliente, provoca cambios de color de tonos verdes a rojo/café y disminuye entre 9-41% la concentración de polifenoles, sin embargo, el ultrasonido logró reducir el tiempo de secado hasta en un 26%.

Palabras clave: ultrasonido, secado con aire caliente, nopal, compuestos bioactivos, microestructura.

Abstract

Nopal (*Opuntia spp.*) is a cactus consumed as a vegetable that has been recognized as a functional food, since it contains bioactive compounds such as organic acids, fiber, mucilage, pigments, minerals, and antioxidants. The new technologies in the food industry have shown that the application of power ultrasound, can promote mass transfer mechanisms when used as pre-treatment to improve the food drying. The objective of this work was to evaluate the effect of ultrasonic treatment on color, microstructure and bioactive compounds content for subsequent dehydration and determination of drying time of nopal cladodes. Full factorial experimental design with a central point was performed, considering the amplitude (20, 48 and 76%) and the sonication time (10, 15 and 20 min) as factors. The application of power ultrasound treatment on nopal cladodes, affects the microstructure, deforming and distanced the cells, mainly at 76% amplitude for 15 min. The content of bioactive compounds such as total polyphenols and chlorophyll increased from 11.7 until 18 mg EGA/g d.b. and from 30.9 until 93.6 mg/g d.b., respectively by treating the nopal cladodes with ultrasound. After the ultrasound treatment, the hot-air drying process causes color changes from green to red/brown and decreases between 9-41% of the polyphenols concentration; however, ultrasound managed to reduce drying time by up to 26%.

Key Words: ultrasound, hot air-drying, nopal cladodes, bioactive compounds, microstructure.

1. Introduction

Nopal (*Opuntia spp.*) is a cactus that has been consumed as a vegetable in the Mexican diet for thousands of years, becoming a fundamental part of some traditional meals. Nopal cladodes have been recognized as a functional food because they are an important source of bioactive compounds, including organic acids, fiber, mucilage (hydrocolloid), pigments (betalains and carotenoids), minerals (calcium and potassium), and antioxidants (polyphenols and vitamin C) [1]. Effects against diseases with benefits such as cholesterol-lowering, hypertension, diabetes, among others, have also been reported [2,3]. One of the main challenges in the food industry is to provide a good supply of nutrients without affecting the organoleptic properties of foods since they have a high water content, which favors the undesirable microbial growth responsible for degradation. Thus, the most recognized and effective method to reduce water activity and extend the shelf life of food is heat dehydration. However, within its main disadvantages are the long drying times, deterioration in the quality of the final product and the loss of heat-sensitive bioactive compounds such as vitamin C. As consumer needs for nutrition and food health increase, researchers are challenged to explore innovative methods to develop high-quality dried products, where novel food technologies as microwave, vacuum, freeze-drying, hybrid technologies or pre-treatment of non-thermal technologies such an ultrasound or pulsed electric fields are getting attention [4–6]. Ultrasound (US) pre-treatment for food drying has been a hotspot in recent years and has been reported to promote mass transfer mechanisms to improve food drying [7–9]. According to different applications, the ultrasound can be divided as low or high intensity. The first does not produce modification and is suitable for measuring the characteristics of the medium; on the other hand, high-intensity cause physical and chemical changes in the medium being applied. Other authors establish the same classification but based on frequency ranges, where >100 kHz is used for clinical diagnosis and frequencies between18 to 100 kHz, also known as high-intensity ultrasound, or power ultrasound is applied to food processing, either as a pre-treatment (usually water immersing), airborne or contacting US assisted drying [10,11]. Some authors have pointed out that the application of ultrasound technology as a pre-treatment on several fruits and vegetables affects the physicochemical parameters of food quality and reduces nutrient loss and drying times [9,12,13]. However, different results have been found without a common trend, since it's depended more on the nature of the food matrix, and no study has been reported for nopal cladodes. In addition, the effect caused by the ultrasonic treatment of raw materials before dehydration has not been reported, so it is of great interest to study step by step the changes generated in the food matrix. Therefore, the objective of this work was to evaluate the effect of ultrasonic treatment on color, microstructure and bioactive compounds content for subsequent dehydration and determination of drying time of nopal cladodes.

2. Materials and Methods

Ultrasonic treatment

Full factorial experimental design with a central point was performed to evaluate the effect of ultrasound (US) treatment in the nopal cladodes, considering the amplitude (20, 48 and 76%) and the sonication time (10, 15 and 20 min) as factors. A 750-watt ultrasonic processor (Sonics-VCX 750; CT, USA) with a frequency of 20 kHz, equipped with a 13 mm diameter titanium probe was used. The process was carried out using a plastic beaker with 750 mL of distilled water; a stainless holder supported nopal samples of 4 × 4 cm with a mesh to keep the sample immobile during the process. The sample was subjected to US treatment according to the conditions of time and amplitude of the experimental design, the total time was divided in two to give half the time to each face of the nopal sample. The amplitude range was established at extreme levels and central point, considering the recommended limits of the US processor. The high limit was left at 76% after preliminary tests, where 100% did not significantly improve the evaluated parameters, being that the recommended limit of 75% was far exceeded. The ultrasonic intensity determined was 13.6, 61.98 and 118.05 W/cm^2 for 20, 48 and 76% of amplitude, respectively. All experiments were performed in duplicate. Fresh and US treated samples were analyzed to determine changes in color, microstructure, chlorophyll, total polyphenols, and flavonoids.

Drying kinetics

To evaluate the reduction of drying time, five drying kinetics were performed in duplicate with samples of nopal cladodes whether or not treated with US follow by a convective drying process. The experimental conditions of US pretreatment were amplitude: 20 and 76% and sonication time:10 and 20 min. It was used a laboratory convection oven (Electrodux Modelo 5958-13801) at 60 ± 5 °C with air velocity of 2.3 ± 0.3 m/s, monitoring the weight loss in samples of nopal cladodes every 10, 20 and 30 min. The moisture content of the dried samples was determined by the vacuum oven method at 70 °C with a gauge pressure of 43 KPa. Approximately 5 g were placed in aluminum dishes in a vacuum oven kept for 48 h and then weighed using a balance (Wim Systems WMB-50G-3) with a sensitivity of 0.001 g. The results were expressed as mass fraction of moisture on a dry basis $X/X_{0.}$ All experiments were performed in duplicate. The color and total polyphenols were determined in the dehydrated nopal cladodes and it was reported as the % of loss.

Color

The color determination of fresh and treated samples was measured with a colorimeter (Konica Minolta, CR-20) CIELab color parameter. were obtained for each measurement. In this space $L*$ represents lightness in a range of 0 to 100, the parameters of $a*$ (+red, -green) and $b*$ (-blue, +yellow). The colorimeter was calibrated with a standard calibration plate of a white surface according to the manufacturer's instructions. Each sample was measure three times. The total color difference (ΔE) between control and treated samples was calculated with Eq. (1).

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$$

(Eq. 1)

where the subscript zero indicates the color measurement in the fresh nopal samples.

Microstructure analysis

An optical microscope (Biological Microscope, YJ-2016T-LCD) was used to cladodes microstructure analysis. The fresh and treated nopal samples were cutting with 1.5 mm thick. In order to evaluate microstructural changes in cladodes due to ultrasound treatment at least 150 images were analyzed for each treatment condition. RGB images of each sample were captured with 10x magnification, in JPG format (836 x 3264 px), and these images were converted to grayscale. The entropy is a measure of the texture image and was evaluated using he graylevel co-occurrence matrix (GLCM) algorithm by means of Image J 1.43u software (The National Institutes of Health, Bethesda, MD, USA). GLCM is a second-order statistic algorithm that compares two neighboring pixels at a time and compiles the frequency at which different gray levels can be found within a restricted area [14].

Determination of total chlorophyll

The content of total chlorophyll was determined by spectrophotometry of the Bruinsma method cited by Aguilar-Becerril *et al.* (2006) [15]. with some modifications. This technique consisted of preparing an acetone solution 80% (v/v), 0.5 g of the sample was macerated with 5 mL of acetone 80 (v/v) and finally centrifuge at 7000 rpm for 15 min at 4 °C. The total chlorophyll was calculated from chlorophyll α (measured at 663 nm) and chlorophyll β (measured at 645nm). The results were expressed as mg/g of nopal cladodes in dry basis.

Determination of total polyphenols

The total polyphenols content was quantified by Folin-Ciocalteau technique [16]. For the standardization of the method, a calibration curve of gallic acid at concentrations of 20, 50, 100, 150 and 200 µg/mL was used. The results were expressed as mg equivalent of GA / g of nopal cladodes in dry basis.

Determination of flavonoids

The flavonoids content was determined according to the method of Tounsi *et al.* (2011) [17]. For the quantitative analysis, a calibration curve was constructed with quercetin at concentrations of 125-1000 mg/mL. The results were expressed as mg equivalent of quercetin/g of nopal cladodes in dry basis.

Statistical Analysis

Statistical analysis was executed by ANOVA and Tukey's multiple comparison test procedure using an Addinsoft (2020) XLSTAT statistical and data analysis solution (New York, USA). The multiple regression method was used to fit the data (95% confidence) and evaluate the main effect of factors on each response variable. Eq. (2) represents the quadratic model used, where y represents each response variable, A is the amplitude level (20, 48 and 76%) and t is the time (10, 15 and 20 min). From β_0 to β_5, are the regression coefficients of the model.

$$y = \beta_0 + \beta_1 A + \beta_2 t + \beta_3 A^2 + \beta_4 t^2 + \beta_5 A \times t \quad \textbf{(Eq. 2)}$$

3. Results and Discussion

Changes in physical properties

The effect of the ultrasound treatment on the nopal cladodes color was evaluated by exploring the values $L*$, $a*$ and $b*$. Specifically, in the $L*$ and $b*$ values, it was observed that the ultrasound treatment amplitude and time did not affect the color. It is feasible to consider that the ultrasound treatments were not severe enough to cause changes in $L*$ and $b*$ parameters range. Another hand, the green color intensity is one of the most important quality characteristics of green vegetables. For this reason, the value $a*$ has been used as a physical parameter that represents this characteristic or their loss. Due to the above, the effect of US treatment on $a*$ value of the samples treated was evaluated, and it was found that there is not a statistical difference of this variable that indicates a loss of the green color ($p<0.05$). ΔE_{US} values are in the range of 0.98 to 2.54; however, that data does not show the statistical difference (see Table 1).

Table 1. Results of coordinates CIELab evaluated in nopal cladodes after US treatment.

Amp %	**Time *min***	**Coordinates CIELab/ after US treatment**			
		L*	**a***	**b***	ΔE_{US}
0	0	42.9^a	-9.6^a	23.7^a	-
20	10	44.7^a	-9.6^a	24.7^a	1.65^a
48	10	46.8^a	-10^a	27.9^a	2.54^a
76	10	43.7^a	-9.4^a	22.7^a	2.49^a
20	15	45.4^a	-9.7^a	25.1^a	2.17^a
48	15	43.2^a	-9.4^a	22.9^a	1.71^a
76	15	41^a	-9.0^a	19.8^a	1.63^a
20	20	46.7^a	-10^a	26.1^a	0.98^a
48	20	42.8^a	-9.0^a	21.9^a	1.38^a
76	20	44.6^a	-9.5^a	23.6^a	1.72^a

Different letters in the same column indicate significant differences ($p<0.05$).

The changes in microstructure of nopal cladodes were evaluated by image analysis. Figure 1b. shows the fresh nopal structure, where the closed occlusive cells are distinguished, and their pair of attached cells, and the general cell structure is visible. Figure 1a. shows the optical microscopy images gallery for different conditions of amplitude and time of US, it can be observed especially in treatment at 76% amplitude and 15 min some rounded structures with a dark tone known as calcium oxalate; it helps the plant to reduce the toxicity of oxalic acid by precipitating it and acting as a store of nutrients. The effect of cavitation on the nopal microstructure was noted in deformed and distanced cells of different sizes.

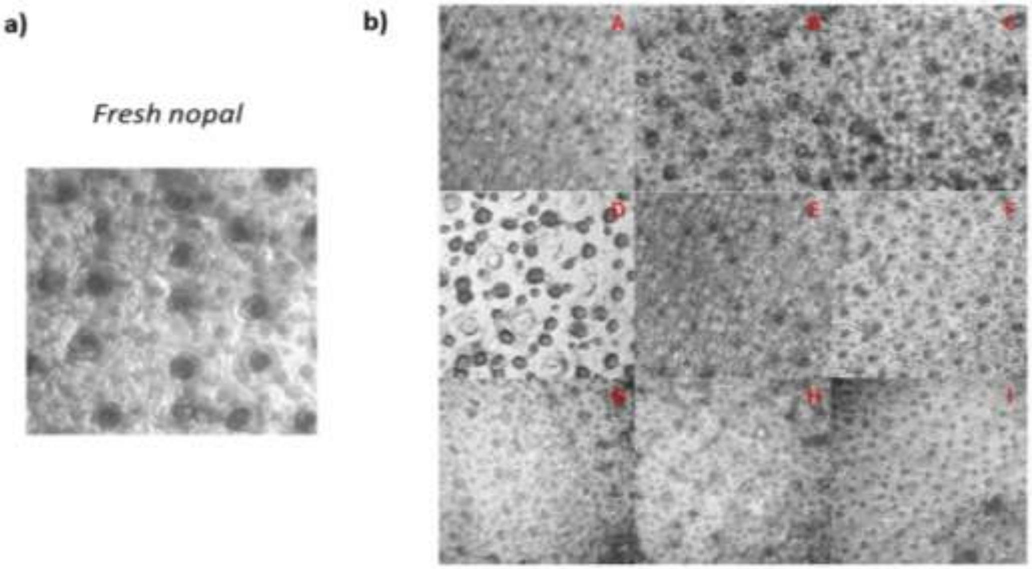

Figure 1. Optical microscope images at 10x, longitudinal cut of nopal cladodes a) fresh nopal cladode, b) pretreated with US at different conditions A) 20% - 10 min, B) 48% - 20 min C) 76% - 10 min, D) 76% - 15 min, E) 48% - 10 min, F) 20% - 20 min, G) 20% 15 min 7, H) 76% - 20 min y I) 48% - 15 min.

To quantitatively evaluates the microstructural changes due to ultrasound treatment, a texture analysis of images was performed using entropy as a measurement parameter. Entropy measures the disorder of images and it is an indication of the complexity within an image, so the more complex images, the higher entropy values [18]. Fresh nopal image presents a high entropy value due to the presence of all intact structures cells of the nopal microstructure; while a low entropy value is related to more significant microstructure damage due to the rupture of cells by cavitation, as observed in the treatment at 76% for 15 min.

According to the entropy response surface (Figure 2), entropy was mainly affected by time and amplitude. Entropy rapidly increased as time increased; this may be due to the severity of the treatment. Thus, the time and the interaction $A\times t$ are the parameters with higher effect ($p<0.05$) in the microstructure of nopal cladodes, whose corresponding model equation is presented below (Eq. 3) with an $R^2=0.72$.

$$Entropy(S) = 8.01 + 0.084A + 0.115t - 0.074A^2 + 0.331t^2 + 0.147A\times t \quad \textbf{(Eq. 3)}$$

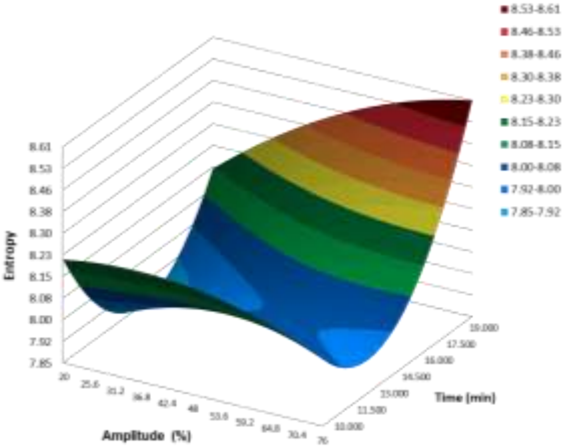

Figure 2. Response surface of images entropy of nopal cladodes treated at different amplitudes and times of US.

Changes in bioactive compounds

One of the most interesting issues in using emerging technologies is maintaining or even improving the availability of bioactive compounds in food. Table 3 shows the concentration of bioactive compounds found in nopal cladodes after US treatment. All the samples treated with US showed a higher concentration of chlorophyll and total polyphenols concerning the fresh untreated nopal, but not for flavonoids content. The above is explained by the more significant release of compounds present in the vacuole (polyphenols) and chloroplasts (chlorophyll) due to the structural damage in the cells caused by the US treatment. Regarding the effect of the US parameters (amplitude and sonication time), no statistically significant trend or effect explained by the proposed model was found; however, the ANOVA shows a significant effect of the amplitude and the interaction $A \times t$ on the chlorophyll content. Chlorophyll is a characteristic green pigment involved in the photosynthesis process of plants, of which biological properties have been related, such as antioxidant, antimutagenic, anti-inflammatory, antimicrobial, among others [19]. The concentration of chlorophyll are mainly affected by the sonication amplitude in interaction with time, where low intensity of sonication for a longer time promotes the delivery of chlorophyll, but a high amplitude and long times implies sonication intensity so high that it could degrade some of the released chlorophyll into other compounds as chlorophyllides, pheoforbides or pheophytins [19].

Table 2. The bioactive compounds present in nopal cladodes samples under different conditions of US treatment.

Amplitude *%*	**Time** *Min*	**Chlorophyll** *mg/g d.b.*	**Polyphenols** *mg EGA/g d.b.*	**Flavonoids** *mg EQ/g d.b.*
0	0	30.87^{d}	11.70^{a}	8.62^{a}
20	10	62.01^{abcd}	13.21^{b}	5.46^{a}
48	10	84.39^{a}	18.08^{b}	5.76^{a}
76	10	63.05^{abcd}	15.95^{b}	5.64^{a}
20	15	79.68^{ab}	14.73^{b}	5.15^{a}
48	15	42.40^{cd}	13.57^{b}	9.55^{a}
76	15	64.31^{abcd}	13.57^{b}	6.92^{a}
20	20	93.65^{a}	14.30^{b}	5.86^{a}
48	20	70.00^{abc}	14.59^{b}	5.32^{a}
76	20	46.94^{bcd}	14.64^{b}	10.19^{a}

EGA: Equivalent of Gallic Acid; **EQ**: Equivalent quercetin; **d.b.**: dry basis. Different letters in the same column indicate significant differences ($p<0.05$).

Total polyphenols are a large group of organic compounds found in plants and certain foods, and they can exist in different groups, depending on the number of phenolic rings they contain. Much evidence for their role in preventing degenerative diseases such as cancer and cardiovascular diseases is emerging [20]. Among the polyphenols, there are flavonoids, which provide many health benefits, acting mainly against free radicals, which makes them perfect antioxidants [21]. The total content of polyphenols in fresh cladodes was 11.7 mg EGA/g d.b. while for treated samples increase in a range from 13.21 to 18.08 mg EGA/g d.b. The flavonoids content ranges from 5.15 to 10.19 EQ/g d.b. considering fresh and US-treated nopal cladodes, whose low concentrations are due to the flavonoids are considered part of the group of polyphenols. For nopal cladodes, the total polyphenols content ranging from 2.18 to 19.9 mg EGA/g are reported in the literature [22,23], which are within the same range of the values of this work. However, many differences can be found that depends on the solvents used and the extraction methods used. Different authors have used solvents of different polarities, either alone or in mixtures, to optimize the extraction of bioactives, but the extraction efficiency of these, strongly depends on the matrix. Thus, for nopal cladodes, water, methanol, ethanol, acetone, acidified water, or acidified methanol have been used [22–24]. Special attention is drawn to the work of Ammar *et al*. (2015) [25], who obtains differences of up to 5 times more concentration of polyphenols extracting with methanol compared to water in cactus *(O. ficus-indica*) flowers. In this sense, to better visualize the changes due to different US parameters, greater extraction values of polyphenols and flavonoids could help, so, to explore different solvents could be recommended. Or it would also be important to identify the target bioactive compounds so that they can be monitored during all processing steps.

Changes due to drying process

The fresh nopal presented a humidity of 91.7 ± 3.7%, with a water activity of 0.95 ± 0.01, which was cut into squares of 4 × 4 cm; later, it was dehydrated with hot air, obtaining a total drying time of 360 min (6h) without any treatment. Figure 3, are shows typical hot air-drying curves of the nopal cladodes with or without US treatment. As can be seen, the effect of US treatment was more pronounced in lowering the moisture content using 76% of amplitude. The drying time at $X/X_0 = 0.1$, varies from 168 to 240 min depending on US conditions used, so the experiment at 76% amplitude for 10 min achieved the maximum reduction of drying time from 228 to 168 min (2.8 h), corresponding to a 26% of reduction time. The above is due to the microstructure damage presented in nopal cladodes by the rupture of cells by cavitation phenomenon (mentioned in Figure 1.); this damage facilitates the release of water, reducing drying time. Very different drying times have been reported in the literature nopal cladodes ranging from 1.25 to 18h, whose difference lies in the temperature, the air velocity and the selected geometry, for example: in nopal cladodes sectioned in small cylinders of 2.5 cm in diameter, reported drying times that ranged between 2.5 and 5.5h [26] and values between 1.25 and 1.68 for dehydration at 60 °C and different air velocity between 1 and 2 m/s, but with nopal cladodes cut into strips [27]. In this work, a square geometry (16 cm^2) was selected, high temperature (60 °C) and air velocity (2.3 m/s) and the reduction of drying times is attributed to US treatment before mentioned. Other authors have also reported a decrease in drying times caused by US treatment in different fruits and vegetables [8,12,13], which range from 4.5% (banana) to 50% (chili pepper) of reduction.

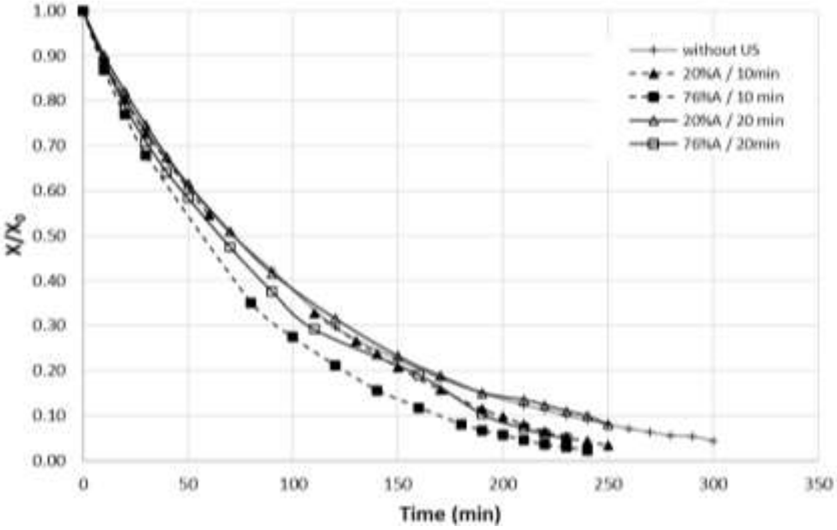

Figure 3. Drying curves for nopal cladodes under convective drying conditions of 60 °C and 2.3 m/s, with or without US treatment at different times and amplitudes.

The color changes in nopal cladodes due to drying process was evaluated with coordinates CIELab and are showed in Table 3. ΔE_D showed a statistical difference between the drying conditions evaluated, whose values are in a range of 9.6 to 14.9. These values are higher than those found in nopal samples treated only with ultrasound, which shows that heat treatment for long periods of time, such as drying, causes greater color changes. The main differences can be observed in the *a** parameter; according to values in Table 3 the samples show green color degradation with a tendency towards red/brown color. The reason for these changes may be that some enzymes like peroxidase or polyphenol oxidase (mainly related to enzymatic browning of food), or maybe due to the loss of chlorophyll, due to its conversion to pheophytins, caused by the increase in temperature during the drying process [28].

Table 3. Results of coordinates CIELab evaluated and polyphenols retention in nopal cladodes after hot-air drying.

Amp %	**Time *min***	**Coordinates CIElab/ after drying**				**Polyphenols *% of loss***
		L*	***a****	***b****	**ΔE_D**	
0	0	43.74^{a}	-1.17^{bc}	18.05^{a}	10.27^{a}	-
20	10	38.90^{a}	0.12^{a}	13.70^{a}	14.86^{a}	41.24
76	10	44.12^{a}	-0.17^{bc}	24.91^{a}	9.60^{a}	20.79
20	20	40.85^{a}	-1.70^{c}	20.99^{a}	11.41^{a}	8.93
76	20	43.75^{a}	0.64^{a}	20.49^{a}	11.83^{a}	12.68

Different letters in the same column indicate significant differences ($p<0.05$).

Regarding the retention of bioactive compounds, the loss of these compounds due to the hot-drying processes, even though it depends on the food matrix, has been documented to depend strongly on temperature and time conditions [24,29,30]. Thus, a higher temperature for a longer exposure time decreases the content of bioactive compounds and antioxidant activity. In an attempt to visualize the retention of compounds, in this work, the % of polyphenols loss concerning the value of fresh nopal was quantified, whose values are shown in Table 3. The greatest loss of polyphenols was obtained in the experiment with US pre-treatment with 20% amplitude for 10 min (41.2%), while the lowest loss was obtained with 20% amplitude for 20 min (8.9%). This coincides with the conditions that favor the release of chlorophyll, where a low intensity of sonication for long periods promotes the release of compounds without degrading them. The above was assuming that if there is a higher concentration of bioactive compounds available after US pre-treatment, higher concentrations will also be obtained after dehydration, considering a certain percentage of loss by hot-air drying. However, a more detailed study is required, focused on evaluating the effect of drying considering the ultrasonic pre-treatment to assert the best conditions, and confirm this assumption.

4. Conclusions

The application of power ultrasound treatment on nopal cladodes, does not induce significant color changes, but it does affect the microstructure at 76% amplitude for 15 min. The content of bioactive compounds such as polyphenols and chlorophyll were increased by treating the nopal cladodes with ultrasound. The hot-air drying process causes color degradation from green to red/brown color, decreases the concentration of polyphenols, and a prior US treatment with 76% amplitude reduces drying time by up to 26%.

Amplitude seems to be the most important parameter since low sonication intensities favor the release of bioactive compounds without causing extreme damage to cells. In contrast, high sonication intensities (76% amplitude) cause greater damage to the nopal microstructure, decreasing the drying time, but it does degrade part of the released bioactive compounds. Further investigations are needed to study the effect of US parameters follow by drying process and monitor the target bioactive compounds of nopal cladodes step by step.

5. Acknowledges

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