Measurement of microstructural changes promoted by ultrasound application on plant materials with different porosity

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ABSTRACT

This research investigated the effects of ultrasound application (192 ± 6 W/L) on the microstructure of vegetables/fruits with different porosities, cell sizes and patterns (eggplants, beetroots, and apples), submitted to an immersion treatment in different liquids: distilled water, citric acid (1% w/v), and the vegetable/fruit juice, at 25 °C during 5 min. The ultrasound application did not significantly (p > 0.05) affect the size of the cells of the most porous material (eggplant) compared to the samples immersed without ultrasound assistance. The apple samples (with a middle-high porosity and the largest cells) were the most affected by ultrasound application. The median cell area of samples treated with ultrasound in water and apple juice were 26 and 20% larger than those of samples treated without ultrasound, mainly because of cell wall disruption which caused the cells to merge into bigger clusters, but no effect was observed with the citric acid. Ultrasound application significantly (p < 0.05) increased the median cell area of the less porous raw matter (beetroot) only when the treatment was carried out in the vegetable juice (cells were 26% larger after treatment assisted with ultrasound than without it). Thus, the effects of ultrasound differ in materials with initially different characteristics.

1. Introduction

Currently, there is a growing interest in the food industry in process intensification, mainly focused on energy sustainability [1,2]. In this context, researchers are constantly investigating new technologies for their application on plants among them, high-power ultrasound (US) stands out because of its simplicity of operation and relatively inexpensive equipment [3]. This type of ultrasound has high intensity (10–1000 W/cm²) and low frequency (20–100 kHz) [4] and has been applied to numerous unit operations in the food industry. These operations include extraction [5,6], osmotic dehydration [7], impregnation [8], drying [9], emulsification [10], defoaming [11] and so forth. US has also been applied as an immersion pre-treatment for further processes such as drying [12], hydro distillation of essential oil [13], physicochemical modification of starches [14] and others. Most of these operations take advantage of the capacity of US to intensify mass transfer processes.

Materials such as vegetable tissues show a natural resistance to mass transfer because of the rigidity of their cell walls. But ultrasonic acoustic waves can modify this natural resistance by altering the microstructure of the material [15]. This is a consequence of mechanisms directly or indirectly promoted by US. The direct effects are mainly due to the “sponge effect” which occurs when the acoustic waves travel through a material causing a fast altering compression and expansion of the tissue [16]. Indirect effects of US are related to cavitation. In solid–liquid systems, which are extensively used in processes such as extraction, impregnation, or pre-treatments of immersion, the main effects are due to cavitation. Cavitation consists of the formation of microbubbles in the suspender liquid, because of the constant pressure change. The bubbles grow during the rarefaction cycles and eventually implode. These implosions generate shear forces, temperature increases, turbulence, and microjets formation [17]. When this occurs close to the solid it can provoke the disruption of the solid surface [17,18]. These effects can cause damage to the cell walls and cell membranes in vegetable materials, and the creation of microchannels [19].

The study of the microstructural changes promoted by US greatly aids in understanding the mechanisms involved and their effects on different raw materials [20]. Some methods such as optical microscopy are relatively inexpensive and with adequate image analysis, it is possible to obtain quantitative information. Several studies have investigated the effect of US on the microstructure of different food materials, such as vegetables or fruits including kiwifruit, potato, apple, and carrot [21–24] and meat [25]. However, there are a limited number...
of studies that have evaluated how the characteristics of the initial raw matter affect the changes caused by the US application. For instance, Miano et al. [16] observed that US is more effective in intensifying mass transfer in products with higher water activity and porosity. Moreover, in solid–liquid processes, the type of solvent is critical to obtaining the desired results. For instance, it is known that cavitation occurs more easily in less viscous and dense liquids [26]. In vegetable tissues, the cellular membrane is semipermeable, thus mass transfer can occur because of the chemical difference between the intercellular fluid and the immersion medium [12]. Furthermore, the same solvent may have different effects on different raw materials. Therefore, this work aims to investigate the microstructural changes promoted by US when applied in an immersion treatment to plant materials with different initial microstructure and porosity. In addition, the effect of US when using different types of solvent has also been evaluated. Thus, two vegetables (eggplant and beetroot) and one fruit (apple) were chosen because of their different cell patterns, tissue structures, and porosity [27]. These samples were subjected to an immersion treatment with and without US using different immersion media, including distilled water, citric acid, and the juice extracted from the vegetable/fruit. The samples were analyzed by using both scanning electron microscopy (SEM) and optical microscopy (OM) before and after the treatment and quantitative information was obtained by image analysis. Therefore, to the best of our knowledge, this study reports for the first time, a quantitative comparison of the microstructural changes promoted by US in plant materials with different initial characteristics and different types of solvents.

2. Materials and methods

2.1. Chemical reagents

Citric acid 1-hydrate and Formaldehyde (37–38% v/v) were purchased from Panreac (Barcelona, Spain), and absolute ethanol from Scharlau (Barcelona, Spain).

2.2. Raw matter preparation

Eggplants (Solanum melongena var. Black enorma), apples (Malus Domestica var. Granny Smith) and beetroots (Beta Vulgaris var. Conditiva), used as raw matter, were purchased at a local market in Palma de Mallorca (Spain) and stored at 2 °C for a maximum of about 1 week until the experiments were carried out. The selection of these raw materials was carried out considering their different cell patterns and microstructure.

The porosity of the samples was obtained according to the ethanol saturation method described by Baniasadi et al. [28]. First, the samples were cut into slices of 5 mm of thickness, in the case of apple and beetroot, the samples were obtained from the sides of the product, avoiding the presence of seeds or irregularities. For eggplant, the sample was obtained from the top of the vegetable. From each slice, a 32 × 20 × 5 mm rectangular sheet was extracted. The samples were immediately freeze-dried by vacuum freezing them in a −80 °C freezer (IngClima, Spain) for 3 h and thereafter, they were introduced in a freeze-dryer (Telstar LyoQuest, Spain) at −50 °C and vacuum pressure of 30 Pa for about 72 h. The freeze-dried samples were weight and introduced in a beaker with absolute ethanol (20 mL) for 48 h and the change in the weight was monitored. The porosity was calculated from Eq. (1).

\[ \text{Porosity} = \frac{m_{\text{sat}} - m_s}{\rho V} \]  

where \( m_{\text{sat}} \) is the weight of the sample saturated with ethanol (g), \( m_s \) is the weight of the freeze-dried sample (g), \( \rho \) is the density of ethanol (0.789 g/mL at 25 °C) and \( V \) is the apparent volume (cm\(^3\)) of the structure.

The pH of the samples (eggplant, apple, and beetroot) was determined with a pH meter (Gison, pH 25, Spain) by introducing the probe into a perforation of the vegetable/fruit. The total soluble solids content was obtained with a refractometer Abbe 325 (Zuzi, Spain) by manually extracting a few droplets from the samples. Both analyses were carried out at room temperature (~22 °C). Then, products without visible defects and with colour uniformity and similar ripening stage (pH of 5.40–5.55 and soluble solids of 2.3–2.7 °Brix for eggplant, pH of 3.10–3.20 and soluble solids of 13.0–13.6 °Brix for apple, and pH of 5.75–5.95 and soluble solids of 8.0–8.6 °Brix for beetroot) were selected, washed, and peeled. The samples were cut into slices, and a rectangular sheet (32 × 20 × 5 mm) was obtained as described before for the porosity analysis. After cutting, samples were immediately used for the experiments.

2.3. Immersion media

The immersion media used in the study were distilled water (W), a 1% (w/v) citric acid solution (C), and the juices (J) obtained from each product, using a common blender, immediately before performing the experiments. The distilled water was chosen as a solvent to evaluate the effect of a hypotonic immersion medium. The citric acid was selected to determine the effect of a low-pH solvent since it has been previously reported that citric acid solution can provoke damage to cell walls [12,29], and the juices of the vegetables were used to evaluate the effect of an isotonic solvent. The pH and the total soluble solids content of the immersion media were determined with a pH-meter (Gison, pH 25, Spain) and a refractometer Abbe 325 (Zuzi, Spain), respectively, at room temperature (~22 °C). The density of the immersion media was determined at 25 °C with a pycnometer. Finally, the viscosity was obtained with a J. P Selecta rotational viscometer (ST-DIGIT R, Spain) at 25 °C using a spindle with a 35 mm diameter. The relative viscosity was calculated by taking the viscosity of water as a reference. Finally, the heat capacity (\( C_p \)) of the immersion media was determined with a differential scanning calorimeter (DSC) (Mettler Toledo, DSC 3, USA) equipped with an intracooler SP (Huber, TC100, Germany) using the dynamic methodology described by Ferrer et al. [30] with some modifications. Briefly, three measurements were carried out, a blank measurement using an empty crucible (aluminium 25 µL), a sapphire measurement (as a reference), and the measurement of the sample. Samples were weighed (about 15 mg), subjected to an isotherm for 5 min at 5 °C, then heated (10 °C/min) to 35 °C, and subjected to another isotherm for 5 min at 35 °C. The immersion medium \( C_p \) (at 25 °C) was calculated from Eq. (2).

\[ C_p = \frac{1}{y} \frac{m^*}{m} C_p^* \]  

where \( C_p \) is the heat capacity of the sample (J/kg °C), \( y \) is the difference between the heat flux (W) of the sample and the blank, \( y^* \) is the difference between the heat flux of the sapphire and the blank (W), \( m^* \) is the mass of sapphire (kg), \( m \) is the mass of the sample (kg) and \( C_p^* \) is the heat capacity of the sapphire at 25 °C (J/kg °C).

2.4. Immersion treatment

The immersion treatment was carried out without (S) and with high-power ultrasound assistance (U). Each sample was immersed for 5 min in 400 mL of the corresponding immersion media (distilled water, citric acid solution, or juice of the vegetable/fruit) within a jacketed glass vessel. This time was chosen since a previous study demonstrated that this time (5 min) of ultrasound application produced microstructural changes in a plant material (apple samples) and also intensified a mass transfer process (drying) [12]. The sample was clamped with forceps to prevent floating. The temperature was maintained at 25 °C by driving ethylene glycol through the jacketed vessel with a chiller unit (Frigedor, J.P. Selecta, Barcelona, Spain). Each experiment was performed at least
The U immersion treatment was carried out using an ultrasonic generator UP400S (Hielser Ultrasonics GmbH, Schwabach, Germany) with 400 W, connected to a probe (diameter of 22 mm), the amplitude and pulse being established at 100% and cycles of 0.5 s, respectively. The probe was immersed in the immersion medium 1 cm from the liquid interface, reaching a distance of 4 cm above the sample. The sample was placed on a grid centered in relation to the ultrasound probe. The setup of the U immersion treatment is depicted in Fig. 1. The S experiments were carried out in the same way but without the US probe. The nomenclature used to name the samples was as follows: a first letter indicating if the process was (U) or not (S) acoustically assisted. an R for raw samples (control) or a letter indicating the immersion indicating the raw matter: E (eggplant), A (apple), and B (beetroot); next of the U immersion treatment is depicted in Fig. 1. The S experiments were carried out in the same way but without the US probe. The nomenclature used to name the samples was as follows: a first letter indicating if the process was (U) or not (S) acoustically assisted. an R for raw samples (control) or a letter indicating the immersion indicating the raw matter: E (eggplant), A (apple), and B (beetroot); next

A calorimetric method was used to determine the effective ultrasound power density applied to each immersion medium [12]. Thus, the increment of temperature during 150 s of US application (dT/dt) was measured and the effective ultrasound power (P, W) was calculated from Eq. (3).

\[
P = MC \frac{dT}{dt}
\]

where \( M \) is the mass of the solvent (kg), \( C_p \) is the heat capacity of the liquid (J/kg \( ^\circ \)C), \( T \) is the temperature (\( ^\circ \)C), and \( t \) is the time (s). No significant (\( p > 0.05 \)) differences were observed among the P values obtained for the different immersion media.

Then, the acoustic density was obtained as power by litre with an average value of 192 \( \pm \) 6 W/L.

2.5. Microstructure

The microstructure of the samples before (raw, R) and after the immersion treatment was evaluated by scanning electron microscopy (SEM) and optical microscopy (OM). From each slab, a disc 16 mm in diameter and 5 mm thick was cut, discarding the corners of the square sheet. Half of this disc was used for the SEM analysis and the rest for optical microscopy. Before observing the samples by SEM, they were freeze-dried. First, samples were frozen in a \(-80^\circ\)C freezer (IngClimas, Spain), for about 3 h and thereafter they were introduced in a freeze-dryer (Telstar LyoQuest, Spain) at \(-50^\circ\)C and vacuum pressure of 30 Pa. Samples were immediately observed by SEM after removal from the freeze dryer. A HITACHI S-3400 N microscope (Germany), accelerated at 15 kV and under vacuum pressure of 40 Pa, was used. At least 12 micrographs of each replicate were taken at \( \times 50 \) magnification. Samples (raw and treated samples) were prepared for optical microscopy as described by Vallespir et al. [31]. Briefly, samples were fixed 6 times.

Fig. 1. Schematic representation of the setup for the experiments carried out with ultrasound application.

To quantify the effects of the immersion treatment, the images obtained by optical microscopy were processed with the free software ImageJ 1.52 k (National Institutes of Health, USA) by determining the cell number per unit area and the areas of cells in each replicate. For this purpose, the contrast of each image was enhanced, and the image was converted to 8 bits. Thereafter, the commands “Make binary” and “Dilate” were applied in order to convert the micrographs into binary (black and white), and to make the cell wall wider, respectively. Subsequently, the “Threshold” function was used to transform the interior of the cell to a black colour and delimit the perimeter of the cell. Then, both the number of cells in a specific area and the area of each cell were automatically obtained by using the “Analyze particle” command. For this, a scale was settled by using a standard with a known size (1 mm = 840.66 pixels). The image analysis was slightly different for each type of sample (eggplant, apple, and beetroot). Thus, in the case of eggplants and apples, the function “Dilate” was applied twice to obtain edges wide enough to be detected by the software. Particles smaller than \( 4.2 \times 10^{-4} \) mm\(^2\) were excluded from the analysis of eggplant to prevent structural imperfections from being detected as cells. This limit was settled at 1.4 \( \times 10^{-3} \) mm\(^2\) and 7.0 \( \times 10^{-4} \) mm\(^2\) for apple and beetroot, respectively, because of the different cell sizes of these products. In the case of eggplant, the option “include holes” of the “analyze particles” function was deactivated since this vegetable has a large intercellular space.

2.7. Statistical analyses

The cell areas obtained from the image analysis were used to obtain a percentile profile for each replicate with the “PERCENTILE.EXC” function of Microsoft Excel v.2201. From the percentile profile, the percentile 50 (median of the distribution, d50) was obtained as a representative value for each replicate. The rest of the statistical analyses were performed using R software [32]. An average of the d50 and the number of cells per area (cells/area) for each sample was obtained from the replicates and reported with the standard deviation. These results were compared by using a parametric analysis of variance (ANOVA) test to determine the existence of significant differences (\( p < 0.05 \)) among the samples, and the Tukey’s test to compare the means [33].

3. Results and discussion

3.1. Raw matter and immersion media characteristics

The porosity, pH, and the soluble solids content of the raw matter (eggplant, apple, and beetroot) are shown in Table 1, and the pH, the soluble solids content, density, relative viscosity, and \( C_p \) of the immersion media in Table 2.

As can be seen, the three raw materials and their juices exhibited in formaldehyde (10%), dehydrated, embedded in paraffin (60 °C for 3 h) and sectioned by a microtome Finesse 325 (Thermo Shandon, UK) to obtain pieces of 4–5 μm. The sections were stained with Periodic Acid-Schiff to observe the cell walls. The micrographs were obtained at 50× magnification with a BX60 optical microscope (Olympus, Japan) connected to a Moticam 3 digital camera (Motic, China).

2.6. Image analysis

Table 1

| Raw matter | Porosity | pH     | Soluble solids (°Brix) |
|------------|----------|--------|------------------------|
| Eggplant   | 0.759 ± 0.106° | 5.46 ± 0.07° | 2.5 ± 0.2°               |
| Apple      | 0.313 ± 0.012° | 3.14 ± 0.06° | 13.3 ± 0.3°               |
| Beetroot   | 0.135 ± 0.015° | 5.87 ± 0.09° | 8.3 ± 0.3°               |

*Different letters for the same parameter indicate significant differences (\( p < 0.05 \)).
significant (p < 0.05) differences among them in the analyzed parameters. The three samples presented significantly different porosity (p < 0.05), eggplant presented the higher value, followed by apple, and beetroot was the least porous sample. The experimental values obtained are similar but larger than that reported in the bibliography, 0.641 for eggplants, which are classified as high-porosity vegetables, 0.210 for apples, and 0.043 for beetroot's which are considered low-porosity vegetables [35]. Differences with the bibliography could be related to the area of the fruit or vegetable where the sample was obtained, the variety of the plant, and the method used to measure this parameter. However, the trend observed coincided with that reported in the bibliography and confirm the high difference among the microstructure of the samples.

The apple sample presented the lowest pH as well as its juice, while the beetroot sample and its juice showed the highest. Apples have a relatively high content of organic acids [36]. For instance, apples', beetroot's, and eggplants' main organic acid is malic acid, but apples can present up to 30 mg/g of fresh weight (fw) of this compound (depending on the variety and ripening) [37] while eggplants and

| Immersion medium | pH       | Soluble solids (°Brix) | Density (kg/m³) | Relative viscosity | Cp (J/Kg °C) |
|------------------|----------|------------------------|-----------------|--------------------|-------------|
| Distilled water  | 6.05 ± 0.06 | 995 ± 0.06 | 1.0 ± 0.00 | 4105 ± 1105 | 215° ± 715° |
| Citric acid (1% w/v) | 2.02 ± 0.02 | 998 ± 0.10 | 1.0 ± 0.10 | 3990 ± 1390 | 124° ± 524° |
| Eggplant juice   | 5.42 ± 0.04 | 1013 ± 0.10 | 1.5 ± 0.00 | 3941 ± 1391 | 70° ± 370° |
| Apple juice      | 3.13 ± 0.01 | 1046 ± 0.10 | 1.6 ± 0.10 | 3776 ± 1377 | 59° ± 299° |
| Beetroot juice   | 6.03 ± 0.01 | 1055 ± 0.10 | 2.2 ± 0.10 | 3594 ± 1359 | 84° ± 484° |

*Different letters for the same parameter indicate significant differences (p < 0.05).
beetroots show about 1.3 and 3.6 mg/g fw, respectively [38,39]. Concerning the soluble solids content, the eggplant sample and its juice showed the lowest value and apples showed the highest. Apples are rich in sucrose and fructose [36], and beetroot is known as a source of sucrose [40]. Generally, the values of pH and total soluble solids are similar to those previously reported in the literature for the three products [41–46]. Among the solvents, distilled water and citric acid presented the lowest viscosity and densities. The juices were significantly (p < 0.05) denser and more viscous, which was expected, as they contained higher solid soluble concentrations and particles in suspension (such as non-soluble fibre). Eggplant juice was the least dense and viscous among the juices. It is known that apple and beetroot are rich in pectins and other soluble fibres which increase the viscosity of liquids [47,48]. Finally, all the immersion media presented Cp figures similar to that of water. However, the beetroot juice showed a significantly (p < 0.05) lower value. This could be related to the soluble and non-soluble solids concentration of this juice [49]. It is well known that ultrasound waves propagation can be affected by the properties of the medium [16,25,50]. Thus, it could be expected that the effects of ultrasound on the microstructure would be different according to the liquid media and solids characteristics.

3.2. Microstructure of the samples

Fig. 2 shows representative photographs of the raw samples (before the immersion treatment) obtained by SEM and by OM. In the case of the eggplant, rounded cells with large intercellular space were observed; similar observations were reported by Puig et al. [51] for fresh eggplant. The apple sample presented a relatively well-arranged structure with an anisotropic pattern coinciding with previous observations reported in the literature for raw apples [12]. The beetroot sample, on the other hand, presented polyhedral cells with very few intercellular spaces, similar to the description of the beetroot structure reported by Vallespir et al. [52]. Among the three samples, eggplant presented the largest intercellular spaces and beetroot the smallest. This is related to the porosity of the samples, which is high for eggplant and low for beetroot [35]. As can be observed in Fig. 2, apple presented the largest cells. This was also confirmed with the cell area percentile profiles obtained by image analysis and presented in Fig. 3. In this figure, percentiles indicate the percentage of cells with an area equal to or smaller than the obtained value. As shown in Fig. 3, each product presented a different percentage distribution. Apple’s profile is shifted to the right, meaning the presence of larger cells. Beetroot and eggplant presented similar profiles, but only slightly shifted to the right in the case of the eggplant and with some larger cells. The median area (d50) for each sample is shown in Table 3. The median area of the raw apple cells was about 4 and 4.8-fold higher than that of raw eggplant and raw beetroot, respectively. This difference can also be observed in the number of cells per area unit, since this value was about 3.9 and 5.6-fold lower for raw apple than for raw eggplant and raw beetroot, respectively.

Table 3

| Treatment | d50 (10^2) (mm²) | Number of cells/area (cells/mm²) |
|-----------|----------------|---------------------------------|
| Eggplant  |                |                                 |
| R         | 1.68 ± 0.18    | 372 ± 31                        |
| WS        | 1.78 ± 0.16    | 350 ± 15ab                      |
| WU        | 2.01 ± 0.22    | 307 ± 33                        |
| CS        | 1.81 ± 0.26    | 381 ± 45                        |
| CU        | 2.00 ± 0.18    | 376 ± 34ab                      |
| JS        | 2.46 ± 0.31    | 306 ± 20b                       |
| JU        | 2.37 ± 0.31    | 306 ± 20b                       |
| Apple     |                |                                 |
| R         | 8.34 ± 0.80    | 76 ± 10                         |
| WS        | 8.87 ± 0.60    | 76 ± 9                          |
| WU        | 11.16 ± 0.95   | 52 ± 6                          |
| CS        | 8.91 ± 1.16    | 72 ± 7                          |
| CU        | 8.96 ± 1.29    | 76 ± 10                         |
| JS        | 8.77 ± 1.05    | 73 ± 9                          |
| JU        | 10.53 ± 0.96   | 62 ± 8                          |
| Beetroot  |                |                                 |
| R         | 1.45 ± 0.13    | 521 ± 71                        |
| WS        | 1.55 ± 0.18    | 456 ± 60ab                      |
| WU        | 1.63 ± 0.07    | 434 ± 22ab                      |
| CS        | 1.59 ± 0.07    | 466 ± 36ab                      |
| CU        | 1.56 ± 0.12    | 496 ± 45ab                      |
| JS        | 1.44 ± 0.09    | 512 ± 51ab                      |
| JU        | 1.80 ± 0.16    | 385 ± 40ab                      |

*Different letters for the same parameter and raw matter indicate significant differences (p < 0.05).
3.3. Effect of the immersion treatment on the microstructure

To study the effect of the immersion treatment with and without US, micrographs of the samples were obtained by SEM and OM. Figs. 4, 5, and 6 show representative micrographs obtained by SEM and OM for eggplant, apple, and beetroot after the immersion treatment, the images of the raw samples were also included to facilitate the comparison. It can be observed that the immersion treatment modified the microstructure of all the samples. The images show areas where cell breakdown occurred causing the merger of cells (B) and the formation of intercellular spaces (IS), fissures (F) and microchannels (M) were also observed.

For eggplant (Fig. 4), the cells were dilated after the treatment with the vegetable juice without and with US. Also, eggplant samples subjected to the immersion treatments presented larger intercellular spaces than the control.

Apple (Fig. 5) presented larger fissures and microchannels than the other materials as well as several cell breakdowns. These breakdowns in apple samples were more numerous when the samples were treated with US. Larger cells were observed in apple samples treated with water and apple juice with US (A-WU and A-JU) than with the rest of the treatments.

In beetroot (Fig. 6), practically no microchannels were observed and the cells were notably larger when treated with the vegetable juice especially when US was applied.

The acoustic energy of US is known to provoke damage to vegetable tissues through different mechanisms, such as the sponge effect, absorption of acoustic energy, and cavitation and its consequences [19]. Similar effects of the application of power US have been reported in different vegetable tissues. For instance, several investigations have demonstrated the formation of microchannels in vegetable tissues subjected to US application. Miano et al. [22] studied the effect of US (ultrasonic bath of 91 W/L) applied for 120 min to cylindrical samples of potatoes. They observed the formation of microchannels inside the potato tissue and considerable surface erosion. Nowacka & Wedzik [24] applied US (3–4 W/m²) from 10 to 30 min to hermetically packed carrot samples immersed in 1 L of distilled water. They observed that after this treatment, the cells of carrot tissue were distorted, damaged and merged together, and several large spaces were observed (especially after 30 min). They also reported the formation of microchannels and larger cells in samples treated with US. In our research, the tissue damage was not as great as that reported by Nowacka & Wedzik [24]. This might be explained by the fact that considerably shorter times were used in this research (5 min). In the investigation of Nowacka & Wedzik [24], the outcomes caused by the US treatment were mainly due to the “sponge effect” since the sample was not in direct contact with the solvent because of the vacuum packaging. In our research, the sample was in direct contact with the solvent, and it is known that the results of US application in a solid–liquid system are mainly due to the cavitation effect [19]. The implosion of cavitation bubbles improves the solvent penetration into the solid through several mechanisms such as microjet formation [53]. The solvent penetration could cause swelling of the cells and/or cell disruption as observed in several samples.

3.3.1. Quantitative results

A more detailed analysis of the effect of the treatments on the microstructure of the samples can be made using the quantitative data obtained by image analysis of the OM pictures. The results of such analysis are depicted in Fig. 7 and Table 3. Fig. 7 shows the cell area percentile profiles of eggplant, apple, and beetroot raw samples (R), and then subjected to an immersion treatment in water (W), citric acid (C), and eggplant juice (J) without (S) and with ultrasound application (U) at 192 ± 6 W/L. The images show the areas where cell breakdowns occurred promoting the merge of cells (B) and the formation of intercellular spaces (IS), fissures (F), and microchannels (M).
and the vegetable/fruit juice (J) without (S) and with (U) US application. Table 3 shows the median area (d50) and the number of cells per area (cells/area) for the control samples and those subjected to all the treatments studied.

According to the cell area percentiles of eggplant (Fig. 7), the profiles of the samples treated with water and with citric acid without US application (E-WS and E-CS) practically coincided with that of the control sample (ER). Thus, practically no osmosis was observed with the distilled water, which would have been expected considering that the solvent was hypotonic. Moreover, the citric acid, which was the solvent with the lowest pH, was not able to significantly affect the cell walls of this sample under the studied conditions. These results are also reflected in the d50 and the cells/area (Table 3). As can be seen, there were no significant differences (p > 0.05) among the values of these parameters on eggplant samples when comparing ER with the E-WS and E-CS samples. A slight increase in the d50 was observed when comparing these samples (E-WS and E-CS) with those that were subjected to US application (E-WU and E-CU). However, these differences were not statistically significant (p > 0.05). On the other hand, there was a significant (p < 0.05) increase in the area of the cells of the eggplants treated with the eggplant juice without and with US (E-JS and E-JU), which can be observed in Fig. 7 and the d50 figures (Table 3). This value was about 46 and 41% larger for E-JS and E-JU compared with ER, respectively. Also, significantly (p < 0.05) fewer cells/area were observed on the samples treated with the eggplant juice (without and with US) compared to the ER sample. This is consistent with the characteristics observed by SEM for these samples. Overall, samples treated with US presented significantly (p < 0.05) larger cells than ER only when the treatment was carried out in the vegetable juice. However, this parameter in the E-JU sample was not significantly (p > 0.05) different to that of the sample treated without US (E-JS). Therefore, in eggplant, the US did not exhibit a significant (p > 0.05) effect on the size of the cells. This was probably because eggplant was the most porous material, so even if US had boosted the solvent penetration, it mostly occupied the intercellular spaces. Oladejo et al. [54] carried out an osmotic dehydration pre-treatment of potato samples in distilled water with US (300 W for 20–60 min). They observed that the samples treated with US did not lose their firmness because they had gained water which filled the intercellular spaces of the potato, and this effect was not observed without US.

On the other hand, eggplant samples treated with US presented significantly (p < 0.05) fewer cells/area when the treatment was carried out in water (E-WU) and eggplant juice (E-JU) compared to ER. The decrease in the cells/area parameter without an increase in the size of the cells, observed in E-WU, might be explained by the formation of more intercellular space. It should be considered that, due to the large intercellular space in eggplant microstructure, if some cell wall breakdowns occurred it did not always result in the merger of two cells to form a larger cluster, but it would just probably cause the formation of bigger intercellular spaces. Some examples of this effect are highlighted in

Fig. 5. Scanning electron and optical micrographs of apple samples: raw (control: R) and subjected to an immersion treatment in water (W), citric acid (C), and apple juice (J) without (S) and with ultrasound application (U) at 192 ± 6 W/L. The images show the areas where cell breakdowns occurred promoting the merge of cells (B) and the formation of intercellular spaces (IS), fissures (F), and microchannels (M).
Fig. 4 as IS (intercellular space) for samples treated with US in water and eggplant juice. Rodrigues et al. [55] studied the effect of an immersion pre-treatment on papaya samples with US application (10–30 min at 4870 W/m²). They reported that papaya tissue did not present intercellular space originally, but the application of US for 10 min resulted in the formation of several large cell interspaces. Fernandes et al. [56], also observed a significant increase of the intercellular space in pineapple samples when they were subjected to an osmotic treatment with US application (30 min at 4870 W/m²). They reported that the US application resulted in the loss of adhesion among the cells because of the solubilization of pectins of the middle lamella.

The type of solvent had a significant effect (p < 0.05) on the microstructure of the eggplant samples. Interestingly, the vegetable juice was more efficient in penetrating the cell walls by dilating them (without and with US), despite being an isotonic solution. Karizaki et al. [20] observed more cell damage in potato samples subjected to osmotic dehydration assisted by US (10–90 min at 20 kHz) when the process was carried out in solutions with higher concentrations of sugar. In our study, the juice of the vegetable was the most concentrated solvent. In addition, possibly, since the solvent (eggplant juice) was practically the same as the intra and extracellular fluid of the tissue of the sample, it has more affinity (e.g. in polarity) to penetrate the sample.

Regarding the apple samples, it can be observed in Fig. 7 that all the treatments carried out without US application presented percentile profiles very similar to that of the control (AR). This can also be observed on the d50 and cells/area data (Table 3). Thus, comparing the d50 of AR with that of the samples treated without US (A-WS, A-CS, and A-JS) no significant differences (p > 0.05) were observed. Also, the cells/area figures were not statistically different (p > 0.05) among AR and A-WS, A-CS, and A-JS samples. The application of US, on the other hand, did cause notable changes in the microstructure of the apple samples. Thus, when comparing the percentile profile (Fig. 7) of the raw sample with those of the samples treated with US (A-WU, A-CU, and A-JU), it can be observed how these last profiles are shifted to the right, meaning the presence of larger cells. This was more evident in the sample treated in water. In fact, the d50 (Table 3) was significantly (p < 0.05) higher in the samples treated with US in water and apple juice than in the raw sample, while the sample treated with citric acid did not present significant differences (p > 0.05). Thus, the d50 of A-WU and A-JU was about 34 and 26% higher than that of AR. According to the cells/area parameter, significantly (p < 0.05) fewer cells were observed in the samples treated with US in water and apple juice than in the control sample.

The larger cells observed on apples in samples A-WU and A-JU could be a consequence of the swelling of the cells because of solvent penetration but also of the cell wall breakdowns that result in two or more cells merging into one larger cluster. Several examples of this effect are highlighted in Fig. 5 as merged cells (B). Nowacka & Wedzik [24] also deduced from the percentile area profile of carrot samples, that an increase in the cell size occurred because of the US application (3–4 W/m²).
for 10 to 30 min). In our research, in the case of using water as a solvent, the US application probably intensified the water transfer to the cells because of osmosis since the distilled water was a hypotonic solution. Moreover, water was the less dense and viscous solvent used with apple samples (Table 2). Thus, the cavitation bubbles were probably formed more easily in this liquid [26]. The intensification of water transfer from a hypotonic solvent into vegetable cells because of US application has already been reported by other authors. For instance, Vasile et al. [8], who subjected apple samples to an immersion treatment in water enriched with cyanocobalamin, observed a water gain with US application (200 W/L for 15 min) larger than that observed without US. Among the three investigated materials, apple was the most affected when using water as an immersion medium. This was probably because apple presented the highest concentration of soluble solids when compared with beetroot and eggplant (Table 1), which means a higher difference in osmotic pressure between the sample and water. The mass transfer intensification and cell wall breakdown could be a consequence of the microjets promoted by the cavitation bubbles that improve the solvent penetration into the solid and of the “sponge effect” that keeps microchannels and pores free and promotes mass transfer through pumping [16]. On the other hand, an important effect of the US application was also observed in the apple juice. This could not be attributed to the physical characteristics of this solvent since it was more viscous and denser than the water and the citric acid. Rodríguez et al. [12] investigated an immersion pre-treatment for drying carried out with US application (2–12 W/cm² for 5 min) and reported more evident damage of apple tissues when it was carried out with the apple juice and with citric acid than with water, attributing it to the low pH of these solvents. However, in this investigation, according to the image analysis results, when applying US, the treatment with the apple juice caused larger cells than the treatment with the citric acid, even when the latter had a lower pH. Therefore, as occurred with the eggplant samples, the higher similarity of the solvent with the extra and the intracellular fluid seemed to be the explanation for better solvent penetration. For instance, the most abundant organic acid in apples is not citric acid but malic acid [57], which should be present in apple juice [58]. The apple juice composition in combination with the US application probably promoted degradation of the pectin compounds of the apple cell walls enhancing the cell wall disruption and the liquid entrance. In addition, in these immersion treatments, there is a multidirectional mass exchange, including the transfer of water from the solvent to the sample or vice versa, but also the penetration of low-molecular substances such as vitamins, saccharides, and others [19]. This transfer of substances from the solvent to the solid must be more significant when using the fruit juice as a solvent than when using water or citric acid considering their composition.

Regarding the beetroot samples, the area percentile profiles of the control (BR) and the samples treated with beetroot juice without US (B-JS) practically coincided (Fig. 7). This, similar to that observed for apples, might be explained by the fact that the beetroot juice was an isotonic solvent. The profile of the samples treated with citric acid and water without US (B-WS and B-CS) were similar but slightly shifted to the right compared to that of BR sample. This indicates a small presence of larger cells probably because of the osmosis occurring in the cells immersed in those hypotonic solvents. According to the d50 and cells/
area parameters (Table 3), there were no significant (p > 0.05) differences among the BR and the samples treated without US (B-WS, B-CS, and B-JS). As for the application of US, it caused significant (p < 0.05) differences in the sample tissue when the treatment was carried out in the vegetable juice. This could be observed in the percentile profile (Fig. 7), in the d50, and in the cells/area parameters (Table 3). Thus, the d50 was about 24% higher and the cells/area parameter was about 26% lower in the B-JU sample than in the control. The cells/area parameter also showed a significant (p < 0.05) decrease compared to BR, on the samples treated with US in citric acid (B-CU). However, this sample did not present significant differences when compared with that treated without US (B-CS). Thus, the microstructural change was caused by the combination of both factors, the solvent and the US application.

There are very few studies investigating the application of US to food materials with different porosity. For instance, Miano et al. [16] studied the effect of US application (ultrasonic bath 28 W/l for 1–2.5 h) in a mass transfer process (inflow of a pigment) using melon cylinders and evaluated the effect of the porosity of the raw matter by perforating some of the samples with a needle. They observed that the samples with a higher porosity (previously perforated) presented a higher absorbance of the pigment with the US application than those with low porosity (unperforated). According to our results, the sample with the highest porosity (eggplant) only presented an increase in the cell sizes when the treatment was carried out in the eggplant juice and there were no significant (p > 0.05) differences between the samples treated with US and without them in this solvent. Thus, these results indicate that the application of US to materials with a lot of intercellular space (such as eggplants), under the conditions used in this study, does not promote a significant change in the size of the cells, probably because the solvent introduced into the material by the cavitation effect stays in the intercellular space or generates even more porosity [59]. On the other hand, samples with a medium–high porosity (apple) treated with US application, presented a significant (p < 0.05) increase in the size of the cells and a decrease in the cells/area (compared with the control and with samples treated without US) in two solvents (water and apple juice). For the low-porosity material (beetroot), the US effect was only observed in the sample juice. Therefore, apple samples were the most affected by the US application. Pieczywak et al. [23] investigated the effect of US application (7.5–30 min at 10 kWh/kg) on the cell wall stiffness of lycindrical apple samples. They observed that larger times of US exposure resulted in lower cell wall stiffness. They also observed solubilization of pectin material. Apple presented the largest cells among all the samples, thus, in comparison with beetroot, apple presented lower density in “cell wall material”, making this tissue more fragile and susceptible to US application.

4. Conclusions

This study evaluated the effect of US application in the microstructure of vegetables with different tissue structures and porosity. The results indicate that US has different effects depending on the initial microstructure of the raw matter. Overall, US application stimulated solvent penetration into the vegetable cells, increasing their sizes and/or disrupting the cell walls. But this effect was less appreciable in a high-porosity raw material, such as eggplant. In these samples, if the solvent penetrates the tissue, it probably remains in the intercellular space, since no swelling of the cells was observed with ultrasound application. Moreover, the breakdown of cell walls generates even more free spaces, which could be deduced from the reduction of the number of cells per area with no significant (p > 0.05) increase in the size of the cells with ultrasound application in water. This should be considered in the processes of impregnation. Further, the selection of the solvent is decisive in obtaining the desired effects from US applications. Solvents with lower viscosity and density are useful to intensify the effects of cavitation (such as water). But the similarity of the solvent with the inter and extracellular fluid of the raw matter was more crucial in facilitating penetration through the cell walls. Samples with larger cells and intermediate porosity (such as apple) are more susceptible to cell wall disruption caused by acoustic energy than samples with low porosity and smaller cells (such as beetroot). This is interesting for the process of solid–liquid extraction which benefits from cell breakdowns.

CRediT authorship contribution statement

Mónica Umaña: Investigation, Data curation, Formal analysis, Writing – original draft, Visualization. Marina Calahorrro: Investigation, Software, Validation, Data curation. Valeria Eim: Conceptualization, Methodology, Supervision. Carmen Rossello: Resources, Writing – review & editing, Supervision. Susana Simal: Conceptualization, Formal analysis, Writing – review & editing, Supervision, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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