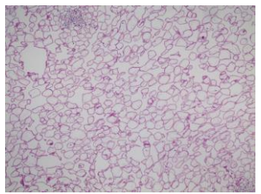
Highlights

- Ultrasound effects in samples of varying porosity and microstructure were evaluated
- Ultrasound had no significant effect on the cell size of eggplant (highly porous)
- Apple (medium porosity and largest cells) was the most affected by ultrasound
- All samples showed larger cells after sonication in the vegetable juice
- Generally, ultrasound provoked cell swelling and disruption

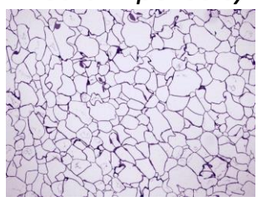
Graphical Abstract revised

Raw matter (R)

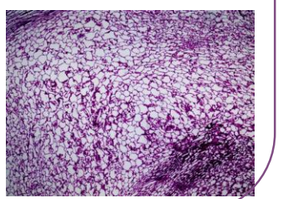
Eggplant (E) *High porosity*



Apple (A) *Medium porosity*

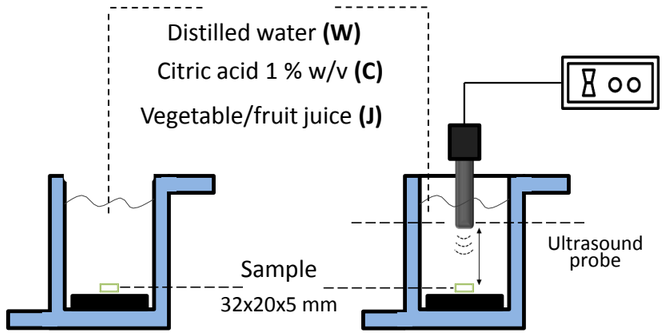


Beetroot (B) *Low porosity*



Immersion treatment

Immersion media



Without ultrasound **(S)**

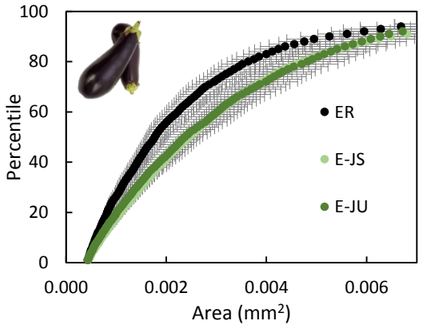
With ultrasound 192 ± 6 W/L **(U)**

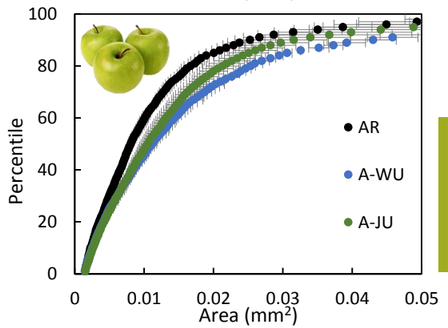
25 °C

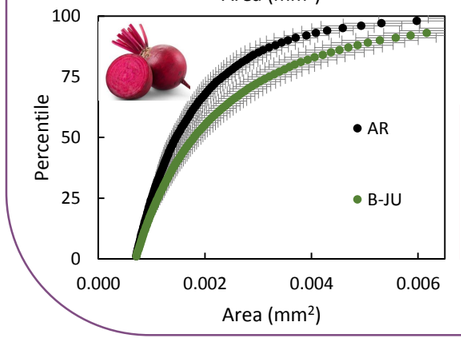
5 min

Results

Cell area percentile profiles







Cell swelling and/or rupture was observed…

when the sample was treated in the vegetable juice

with application of ultrasound in water and fruit juice

with application of ultrasound in vegetable juice

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

Mónica UMAÑA, Marina CALAHORRO, Valeria EIM, Carmen ROSSELLÓ, Susana SIMAL*

Department of Chemistry, University of the Balearic Islands, Ctra. Valldemossa, km. 7.5, 07122 Palma de Mallorca, Spain.

*Corresponding author: Tel: +34 971 172757. E-mail address: susana.simal@uib.es

Permanent address: Ctra Valldemossa km 7.5, 07122. University of the Balearic Islands, Palma de Mallorca, Spain.

Declarations of interest: none

[1]

[1] Abbreviations: A: apple samples, B: beetroot samples, E: eggplant samples, C: immersion treatment in citric acid, cells/area= number of cells per area (number of cells/mm^2), Cp: heat capacity (J/ kg °C), J: immersion treatment in the juice of the vegetable/fruit, m: mass (kg), OM: Optical microscopy, P: power (W), R: raw samples (control), S: immersion treatment carried out without high power ultrasound application, SEM: Scanning electron microscopy, T= temperature (°C), t: time (s), U: immersion treatment carried out with high power ultrasound application, US: High power ultrasound, W: immersion treatment in distilled water.

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 areas 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.

Keywords: Ultrasound, microstructure, vegetables, image analysis

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 and 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/cm^2) 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 surrender 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 32x20x5 mm rectangular sheet was extracted. The samples were immediately freeze-dried by frozen 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.

$$Porosity = \frac{m_{sat} - m_d}{\rho V} \quad (1)$$

Where m_{sat} is the weight of the sample saturated with ethanol (g), m_d is the weight of the freeze-dried sample (g), ρ 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 (Crison, 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 (32x20x5

123 mm) was obtained as described before for the porosity analysis. After cutting, samples
124 were immediately used for the experiments.

125 2.3 Immersion media

126 The immersion media used in the study were distilled water (W), a 1 % (w/v) citric acid
127 solution (C), and the juices (J) obtained from each product, using a common blender,
128 immediately before performing the experiments. The distilled water was chosen as a
129 solvent to evaluate the effect of a hypotonic immersion medium. The citric acid was
130 selected to determine the effect of a low-pH solvent since it has been previously
131 reported that citric acid solution can provoke damage to cell walls [12,29], and the juices
132 of the vegetables were used to evaluate the effect of an isotonic solvent. The pH and
133 the total soluble solids content of the immersion media were determined with a pH-
134 meter (Crison, pH 25, Spain) and a refractometer Abbe 325 (Zuzi, Spain), respectively, at
135 room temperature (~22 °C). The density of the immersion media was determined at 25
136 °C with a pycnometer. Finally, the viscosity was obtained with a J. P Selecta rotational
137 viscometer (ST-DIGIT R, Spain) at 25 °C using a spindle with a 35 mm diameter. The
138 relative viscosity was calculated by taking the viscosity of water as a reference. Finally,
139 the heat capacity (Cp) of the immersion media was determined with a differential
140 scanning calorimeter (DSC) (Mettler Toledo, DSC 3, USA) equipped with an intracooler
141 SP (Huber, TC100, Germany) using the dynamic methodology described by Ferrer et al.
142 [30] with some modifications. Briefly, three measurements were carried out, a blank
143 measurement using an empty crucible (aluminium 25 µL), a sapphire measurement (as
144 a reference), and the measurement of the sample. Samples were weighed (about 15
145 mg), subjected to an isotherm for 5 min at 5 °C, then heated (10 °C/min) till 35 °C, and

146 subjected to another isotherm for 5 min at 35 °C. The immersion medium Cp (at 25 °C)
147 was calculated from Eq 2.

$$Cp = \frac{y}{y'} x \frac{m'}{m} x Cp' \qquad 2$$

148 where Cp is the heat capacity of the sample (J/ kg °C), y is the difference between the
149 heat flux (W) of the sample and the blank, y' is the difference between the heat flux of
150 the sapphire and the blank (W), m' is the mass of sapphire (kg), m is the mass of the
151 sample (kg) and Cp' is the heat capacity of the sapphire at 25 °C (J/ kg °C).

152 2.4 Immersion treatment

153 The immersion treatment was carried out without (S) and with high-power ultrasound
154 assistance (U). Each sample was immersed for 5 min in 400 mL of the corresponding
155 immersion media (distilled water, citric acid solution, or juice of the vegetable/fruit)
156 within a jacketed glass vessel. This time was chosen since a previous study demonstrated
157 that this time (5 min) of ultrasound application produced microstructural changes in a
158 plant material (apple samples) and also intensified a mass transfer process (drying) [12].
159 The sample was clamped with forceps to prevent floating. The temperature was
160 maintained at 25 °C by driving ethylene glycol through the jacketed vessel with a chiller
161 unit (Frigedor, J.P. Selecta, Barcelona, Spain). Each experiment was performed at least 6
162 times.

163 The U immersion treatment was carried out using an ultrasonic generator UP400S
164 (Hielscher Ultrasonics GmbH, Schwabach, Germany) with 400 W, connected to a probe
165 (diameter of 22 mm), the amplitude and pulse being established at 100 % and cycles of
166 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 Figure 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 the raw matter: E (eggplant), A (apple), and B (beetroot); next an R for raw samples (control) or a letter indicating the immersion medium: W (distilled water), C (citric acid), and J (juice) followed by a letter to indicate if the process was (U) or not (S) acoustically assisted.

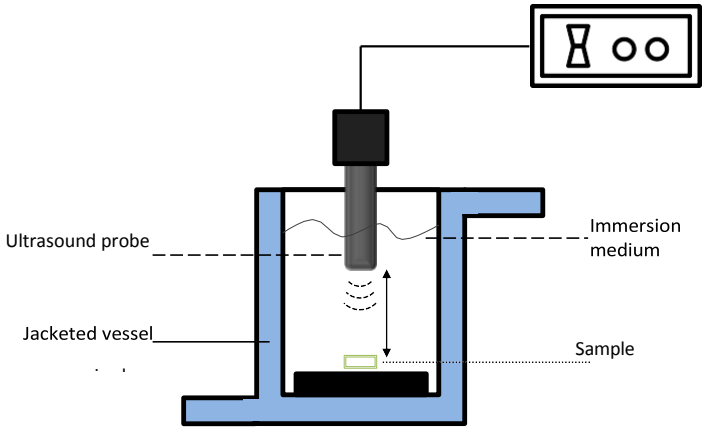


Figure 1. Schematic representation of the setup for the experiments carried out with ultrasound application.

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.

$$P = M \cdot C_p \cdot \frac{dT}{dt} \qquad 3$$

where M is the mass of the solvent (kg), Cp is the heat capacity of the liquid (J/kg °C), T is the temperature (°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 ± 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 °C freezer (IngClimas, Spain), for about 3 h and thereafter they were introduced in a freeze-dryer (Telstar LyoQuest, Spain) at -50 °C and vacuum pressure of 30 Pa. Samples were immediately observed by SEM after removal from the freeze dryer. A HITACHI S-3400N 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 50x magnification.

Samples (raw and treated samples) were prepared for optical microscopy as described by Vallespir et al. [31]. Briefly, samples were fixed 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 50x magnification

with a BX60 optical microscope (Olympus, Japan) connected to a Moticam 3 digital camera (Motic, China).

2.6 Image analysis

To quantify the effects of the immersion treatment, the images obtained by optical microscopy were processed with the free software ImageJ 1.52k (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 4.2×10^{-4} mm^2 were excluded from the analysis of eggplant to prevent structural imperfections from being detected as cells. This limit was settled at 1.4×10^{-3} mm^2 and 7.0×10^{-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 "PERCENTIL.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 (R Core Team 2017). 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 (de Mendiburu 2016).

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 Cp of the immersion media in Table 2.

Table 1. Porosity, pH, and soluble solids content of the raw matter.

Raw matter	Porosity	pH	Soluble solids (°Brix)
Eggplant	0.759 ± 0.106^{a}	5.46 ± 0.07^{b}	2.5 ± 0.2^{c}
Apple	0.313 ± 0.012^{b}	3.14 ± 0.06^{c}	13.3 ± 0.3^{a}
Beetroot	0.135 ± 0.015^{c}	5.87 ± 0.09^{a}	8.3 ± 0.3^{b}

244

245 **Table 2.** Characteristics of the immersion media.

Immersion medium	pH	Soluble solids (°Brix)	Density (kg/m^3)	Relative viscosity	Cp (J/Kg °C)
Distilled water	6.05 ± 0.06^b	--	995 ± 0^e	1.0 ± 0.0^c	4105 ± 215^a
Citric acid (1% w/v)	2.02 ± 0.03^e	0.6 ± 0.1^d	998 ± 1^d	1.0 ± 0.1^c	3990 ± 124^a
Eggplant juice	5.42 ± 0.04^c	2.4 ± 0.1^c	1013 ± 1^c	1.5 ± 0.0^b	3941 ± 70^a
Apple juice	3.13 ± 0.01^d	13.0 ± 0.1^a	1046 ± 1^b	1.6 ± 0.1^b	3776 ± 50^{ab}
Beetroot juice	6.03 ± 0.01^a	8.4 ± 0.0^b	1055 ± 1^a	2.2 ± 0.1^a	3594 ± 84^b

246 *Different letters for the same parameter and raw matter indicate significant
247 differences ($p < 0.05$)

248

249 As can be seen, the three raw materials and their juices exhibited significant ($p < 0.05$)
250 differences among them in the analyzed parameters. The three samples presented
251 significantly different porosity ($p < 0.05$), eggplant presented the higher value, followed
252 by apple, and beetroot was the least porous sample. The experimental values obtained
253 are similar but larger than that reported in the bibliography, 0.641 for eggplants, which
254 are classified as high-porosity vegetables, 0.210 for apples, and 0.043 for beetroots
255 which are considered low-porosity vegetables [35]. Differences with the bibliography
256 could be related to the area of the fruit or vegetable where the sample was obtained,
257 the variety of the plant, and the method used to measure this parameter. However, the
258 trend observed coincided with that reported in the bibliography and confirm the high
259 difference among the microstructure of the samples.

260 The apple sample presented the lowest pH as well as its juice, while the beetroot sample
261 and its juice showed the highest. Apples have a relatively high content of organic acids

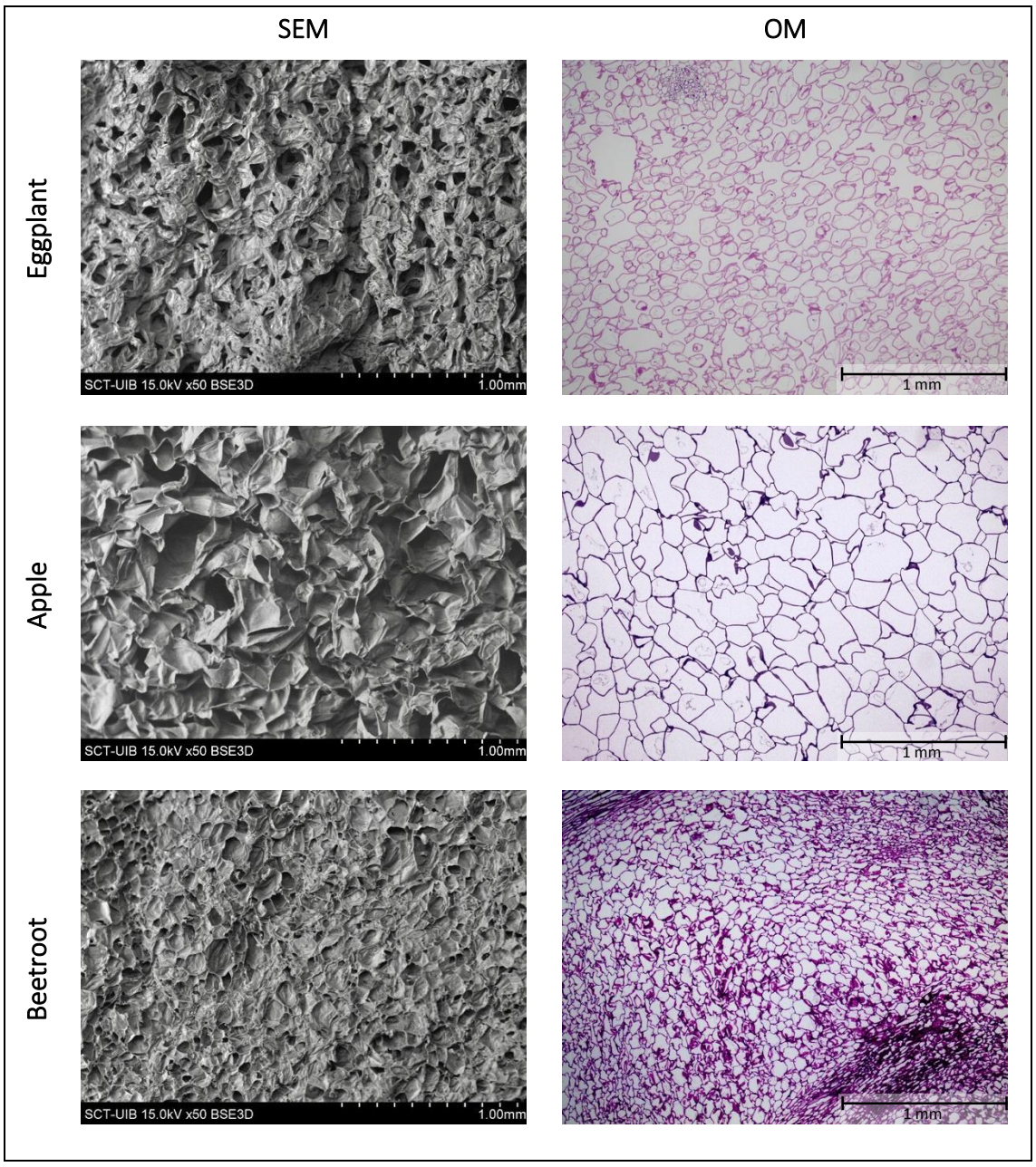
262 [36]. For instance, apples', beetroots', and eggplants' main organic acid is malic acid, but
263 apples can present up to 30 mg/g of fresh weight (fw) of this compound (depending on
264 the variety and ripening) [37] while eggplants and beetroots show about 1.3 and 3.6
265 mg/g fw, respectively [38,39]. Concerning the soluble solids content, the eggplant
266 sample and its juice showed the lowest value and apples showed the highest. Apples
267 are rich in sucrose and fructose [36], and beetroot is known as a source of sucrose [40].
268 Generally, the values of pH and total soluble solids are similar to those previously
269 reported in the literature for the three products [41–46]. Among the solvents, distilled
270 water and citric acid presented the lowest viscosity and densities. The juices were
271 significantly ($p < 0.05$) denser and more viscous, which was expected, as they contained
272 higher soluble solid concentrations and particles in suspension (such as non-soluble
273 fibre). Eggplant juice was the least dense and viscous among the juices. It is known that
274 apple and beetroot are rich in pectins and other soluble fibres which increase the
275 viscosity of liquids [47,48]. Finally, all the immersion media presented Cp figures similar
276 to that of water. However, the beetroot juice showed a significantly ($p < 0.05$) lower
277 value. This could be related to the soluble and non-soluble solids concentration of this
278 juice [49]. It is well known that ultrasound waves propagation can be affected by the
279 properties of the medium [16,25,50]. Thus, it could be expected that the effects of
280 ultrasound on the microstructure would be different according to the liquid media and
281 solids characteristics.

282 3.2 Microstructure of the samples

283 Figure 2 shows representative photographs of the raw samples (before the immersion
284 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 Figure 2, apple presented the largest cells. This was also confirmed with the cell area percentile profiles obtained by image analysis and presented in Figure 3. In this figure, percentiles indicate the percentage of cells with an area equal to or smaller than the obtained value. As shown in Figure 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.

306



307 **Figure 2.** Representative photographs of raw eggplant, apple, and beetroot obtained by

308 scanning electron microscopy (SEM) and optical microscopy (OM).

309

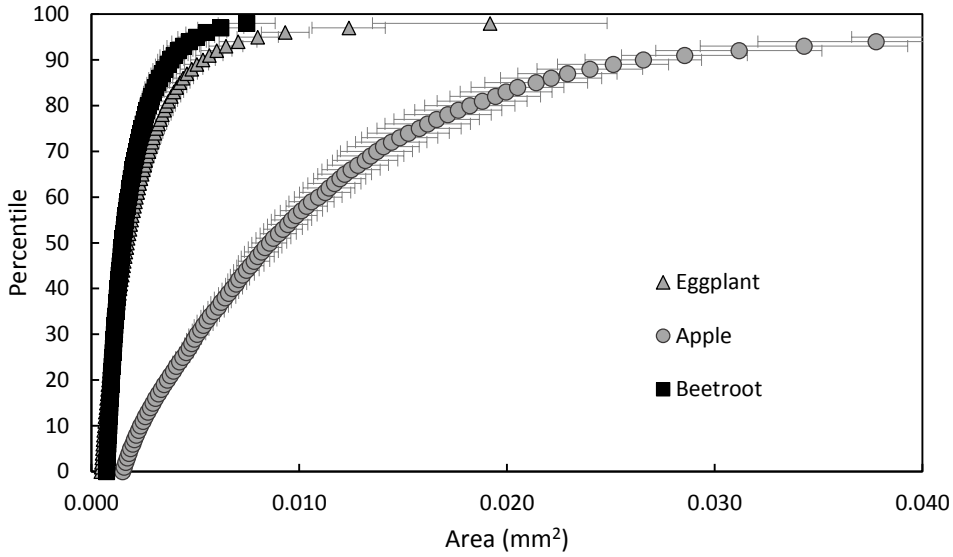


Figure 3. Cell area percentile profiles of raw eggplant, apple, and beetroot.

Table 3. Median cell area (d50) and the number of cells per area (cells/area) of eggplant, apple, and beetroot samples, untreated (raw, R) and subjected to an immersion treatment in water (W), citric acid (C), and the vegetable/fruit juice (J) without (S) and with ultrasound application (U) at 192 ± 6 W/L.

	Treatment	d50 (10^3) (mm^2)	Number of cells/area (cells/mm^2)
Eggplant	R	1.68 ± 0.18^{c}	372 ± 31^{a}
	WS	1.78 ± 0.16^{c}	350 ± 15^{ab}
	WU	2.01 ± 0.22^{bc}	307 ± 33^{b}
	CS	1.81 ± 0.26^{c}	381 ± 45^{a}
	CU	2.00 ± 0.18^{bc}	376 ± 34^{a}
	JS	2.46 ± 0.18^{a}	305 ± 34^{b}
	JU	2.37 ± 0.31^{ab}	306 ± 20^{b}
Apple	R	8.34 ± 0.80^{c}	76 ± 10^{a}
	WS	8.87 ± 0.60^{bc}	76 ± 9^{a}
	WU	11.16 ± 0.95^{a}	52 ± 6^{b}
	CS	8.91 ± 1.16^{bc}	72 ± 7^{a}
	CU	8.96 ± 1.39^{bc}	76 ± 10^{a}
	JS	8.77 ± 1.05^{bc}	73 ± 9^{a}
	JU	10.53 ± 0.96^{ab}	62 ± 8^{b}
Beetroot	R	1.45 ± 0.13^{b}	521 ± 71^{a}
	WS	1.55 ± 0.18^{b}	456 ± 60^{ab}
	WU	1.63 ± 0.07^{ab}	434 ± 22^{ab}
	CS	1.59 ± 0.07^{ab}	466 ± 36^{ab}
	CU	1.56 ± 0.12^{b}	496 ± 43^{bc}
	JS	1.44 ± 0.09^{b}	512 ± 51^{a}
	JU	1.80 ± 0.16^{a}	385 ± 40^{b}

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

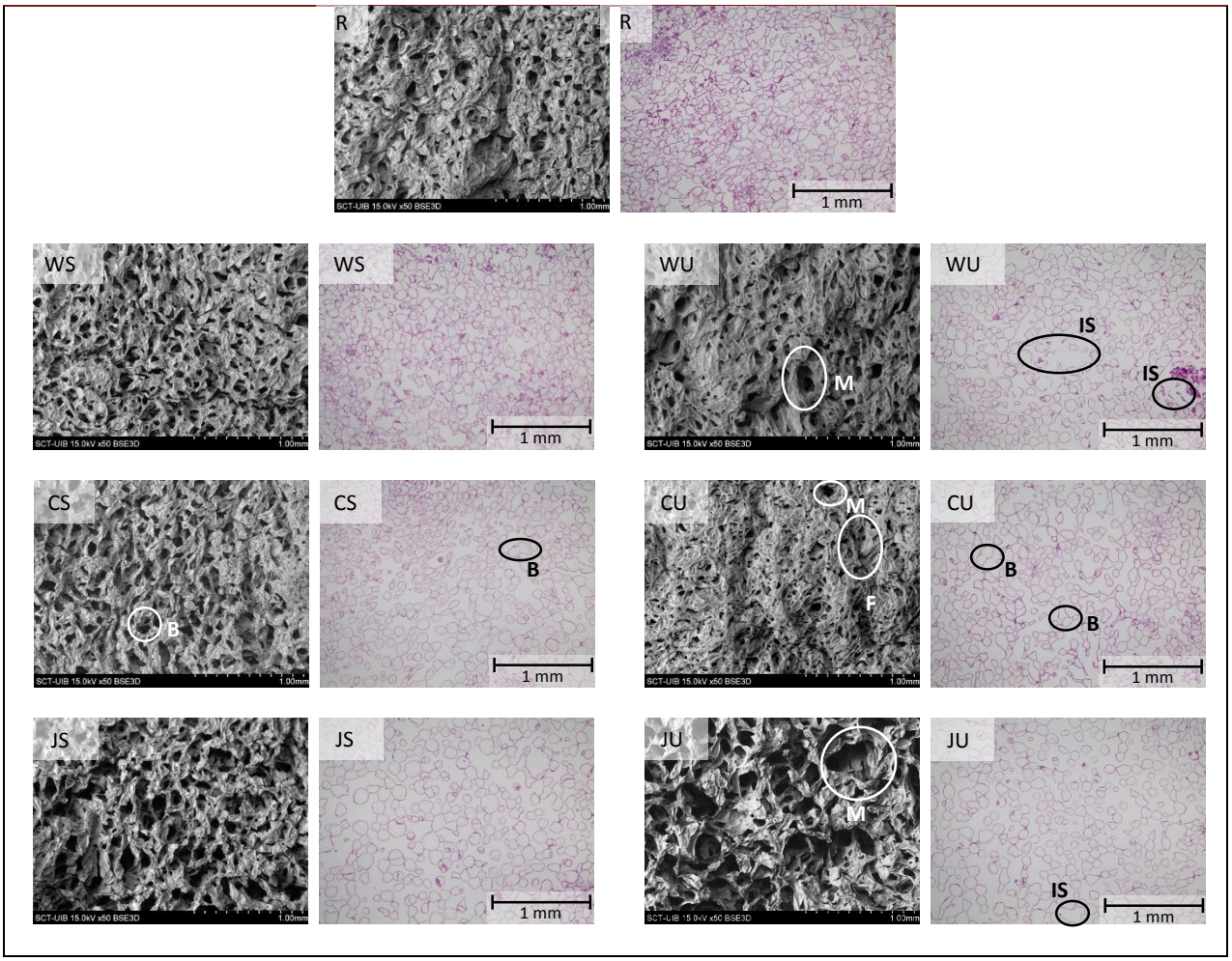
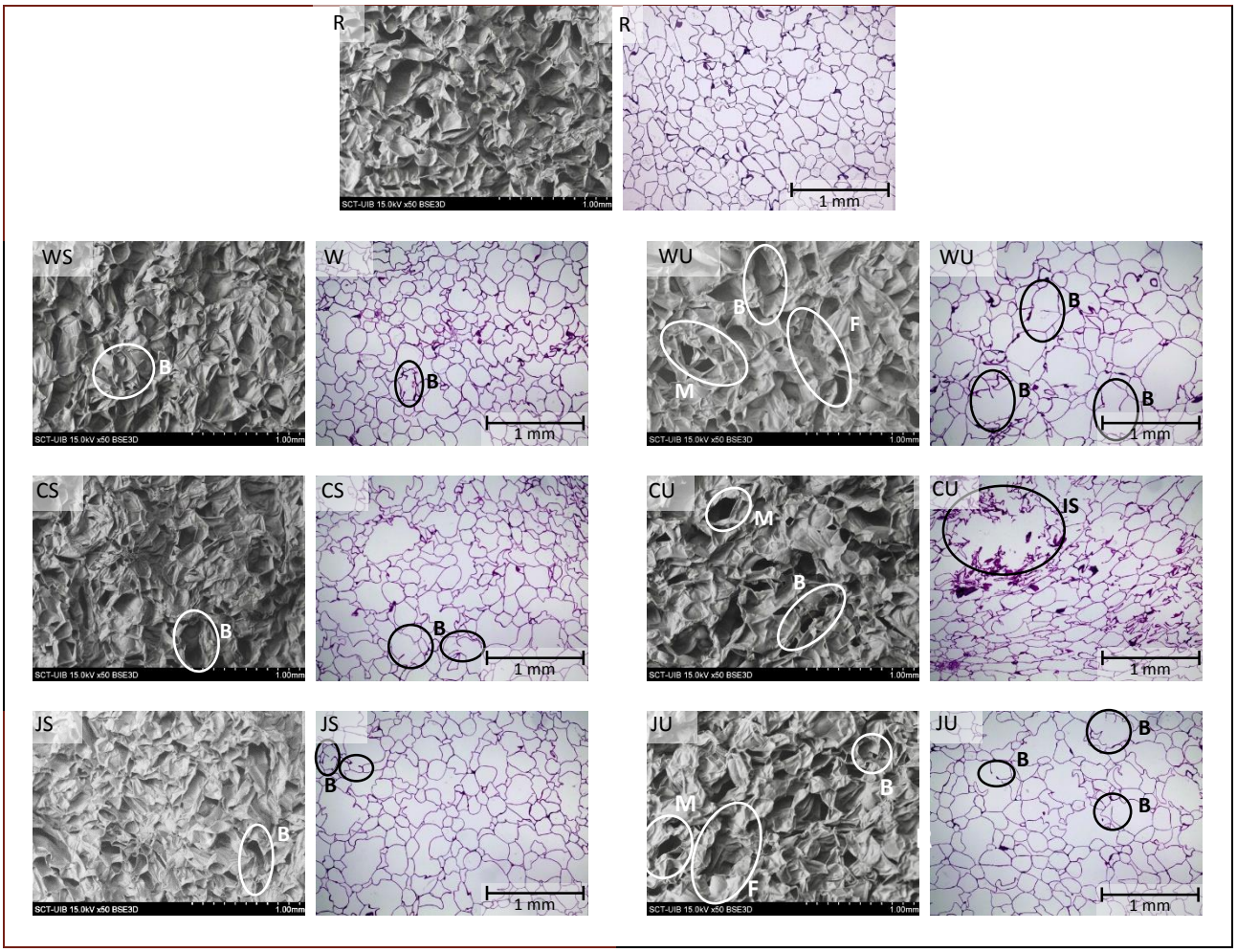
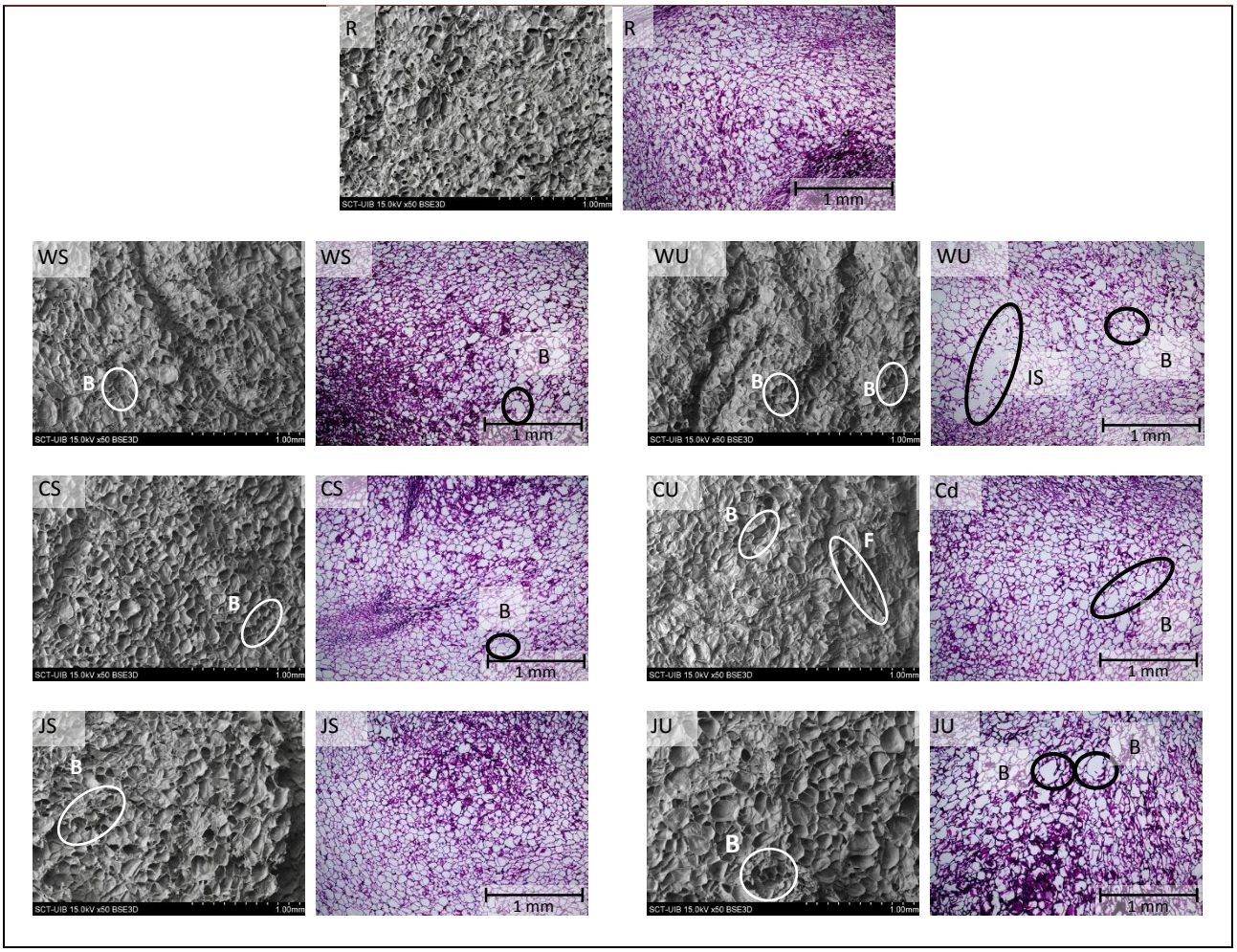


Figure 4. Scanning electron and optical micrographs of eggplant samples: raw (control: R) and subjected to an immersion treatment in water (W), citric acid (C), and eggplant juice (J) without (S) and with ultrasound application (U) at 192 ± 6 W/L. The images show the areas where cell breakdowns occurred promoting the merge of cells (B) and the formation of intercellular spaces (IS), fissures (F), and microchannels (M).



354 **Figure 5.** Scanning electron and optical micrographs of apple samples: raw (control: R)
355 and subjected to an immersion treatment in water (W), citric acid (C), and apple juice (J)
356 without (S) and with ultrasound application (U) at 192 ± 6 W/L. The images show the
357 areas where cell breakdowns occurred promoting the merge of cells (B) and the
358 formation of intercellular spaces (IS), fissures (F), and microchannels (M).

359



360 **Figure 6.** Scanning electron and optical micrographs of beetroot samples:
361 raw (control: R) and subjected to an immersion treatment in water (W), citric
362 acid (C), and beetroot juice (J) without (S) and with ultrasound application
363 (U) at 192 ± 6 W/L. The images show the areas where cell breakdowns
364 occurred promoting the merge of cells (B) and the formation of intercellular
365 spaces (IS), and fissures (F).

366

367

368 The acoustic energy of US is known to provoke damage to vegetable tissues through
369 different mechanisms, such as the sponge effect, absorption of acoustic energy, and
370 cavitation and its consequences [19]. Similar effects of the application of power US have
371 been reported in different vegetable tissues. For instance, several investigations have
372 demonstrated the formation of microchannels in vegetable tissues subjected to US
373 application. Miano et al. [22] studied the effect of US (ultrasonic bath of 91 W/L) applied
374 for 120 min to cylindrical samples of potatoes. They observed the formation of
375 microchannels inside the potato tissue and considerable surface erosion. Nowacka &
376 Wedzik [24] applied US (3-4 W/m^2) from 10 to 30 min to hermetically packed carrot
377 samples immersed in 1 L of distilled water. They observed that after this treatment, the
378 cells of carrot tissue were distorted, damaged and merged together, and several large
379 spaces were observed (especially after 30 min). They also reported the formation of
380 microchannels and larger cells in samples treated with US. In our research, the tissue
381 damage was not as great as that reported by Nowacka & Wedzik [24]. This might be
382 explained by the fact that considerably shorter times were used in this research (5 min).
383 In the investigation of Nowacka & Wedzik [24], the outcomes caused by the US
384 treatment were mainly due to the “sponge effect” since the sample was not in direct
385 contact with the solvent because of the vacuum packaging. In our research, the sample
386 was in direct contact with the solvent, and it is known that the results of US application
387 in a solid-liquid system are mainly due to the cavitation effect [19]. The implosion of
388 cavitation bubbles improves the solvent penetration into the solid through several
389 mechanisms such as microjet formation [53]. The solvent penetration could cause
390 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 Figure 7 and Table 3. Figure 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 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.

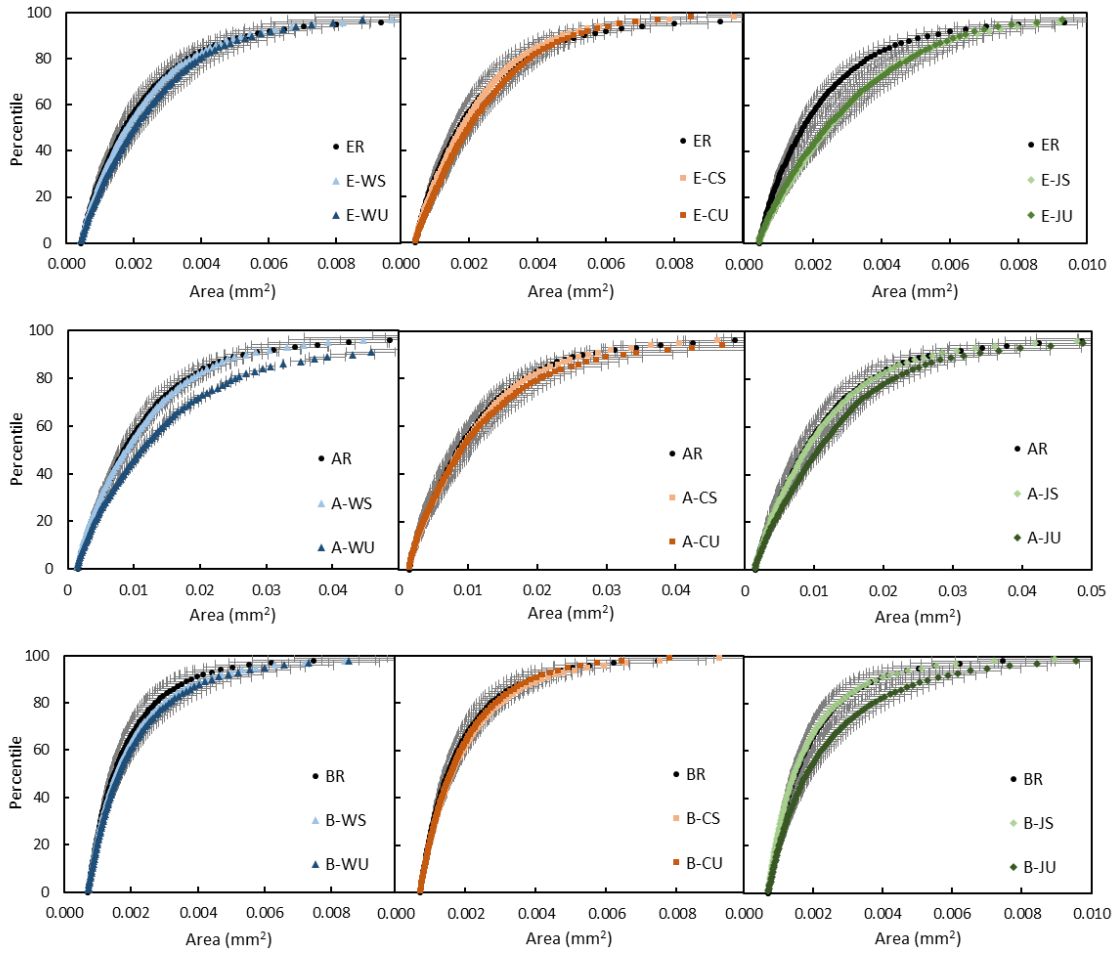


Figure 7. Cell area percentile profiles of eggplant (E), apple (A), and beetroot (B) samples: raw (control: ER, AR, and BR respectively) and subjected to an immersion treatment in water (W), citric acid (C), and the juice of the vegetable/fruit (J) without (S) and with ultrasound application (U) at 192 ± 6 W/L. The raw sample profile was added to all the charts to facilitate the comparison.

411 According to the cell area percentiles of eggplant (Figure 7), the profiles of the samples
412 treated with water and with citric acid without US application (E-WS and E-CS) practically
413 coincided with that of the control sample (ER). Thus, practically no osmosis was
414 observed with the distilled water, which would have been expected considering that the
415 solvent was hypotonic. Moreover, the citric acid, which was the solvent with the lowest
416 pH, was not able to significantly affect the cell walls of this sample under the studied
417 conditions. These results are also reflected in the d50 and the cells/area (Table 3). As
418 can be seen, there were no significant differences ($p > 0.05$) among the values of these
419 parameters on eggplant samples when comparing ER with the E-WS and E-CS samples.
420 A slight increase in the d50 was observed when comparing these samples (E-WS and E-
421 CS) with those that were subjected to US application (E-WU and E-CU). However, these
422 differences were not statistically significant ($p > 0.05$). On the other hand, there was a
423 significant ($p < 0.05$) increase in the area of the cells of the eggplants treated with the
424 eggplant juice without and with US (E-JS and E-JU), which can be observed in Figure 7
425 and the d50 figures (Table 3). This value was about 46 and 41 % larger for E-JS and E-JU
426 compared with ER, respectively. Also, significantly ($p < 0.05$) fewer cells/area were
427 observed on the samples treated with the eggplant juice (without and with US)
428 compared to the ER sample. This is consistent with the characteristics observed by SEM
429 for these samples. Overall, samples treated with US presented significantly ($p < 0.05$)
430 larger cells than ER only when the treatment was carried out in the vegetable juice.
431 However, this parameter in the E-JU sample was not significantly ($p > 0.05$) different to
432 that of the sample treated without US (E-JS). Therefore, in eggplant, the US did not
433 exhibit a significant ($p > 0.05$) effect on the size of the cells. This was probably because
434 eggplant was the most porous material, so even if US had boosted the solvent

435 penetration, it mostly occupied the intercellular spaces. Oladejo et al. [54] carried out
436 an osmotic dehydration pre-treatment of potato samples in distilled water with US (300
437 W for 20-60 min). They observed that the samples treated with US did not lose their
438 firmness because they had gained water which filled the intercellular spaces of the
439 potato, and this effect was not observed without US.

440 On the other hand, eggplant samples treated with US presented significantly ($p < 0.05$)
441 fewer cells/area when the treatment was carried out in water (E-WU) and eggplant juice
442 (E-JU) compared to ER. The decrease in the cells/area parameter without an increase in
443 the size of the cells, observed in E-WU, might be explained by the formation of more
444 intercellular space. It should be considered that, due to the large intercellular space in
445 eggplant microstructure, if some cell wall breakdowns occurred it did not always result
446 in the merger of two cells to form a larger cluster, but it would just probably cause the
447 formation of bigger intercellular spaces. Some examples of this effect are highlighted in
448 Figure 4 as IS (intercellular space) for samples treated with US in water and eggplant
449 juice. Rodrigues et al. [55] studied the effect of an immersion pre-treatment on papaya
450 samples with US application (10-30 min at 4870 W/m^2). They reported that papaya
451 tissue did not present intercellular space originally, but the application of US for 10 min
452 resulted in the formation of several large cell interspaces. Fernandes et al. [56], also
453 observed a significant increase of the intercellular space in pineapple samples when they
454 were subjected to an osmotic treatment with US application (30 min at 4870 W/m^2).
455 They reported that the US application resulted in the loss of adhesion among the cells
456 because of the solubilization of pectins of the middle lamella.

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

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

481 significantly ($p < 0.05$) fewer cells were observed in the samples treated with US in water
482 and apple juice than in the control sample.

483 The larger cells observed on apples in samples A-WU and A-JU could be a consequence
484 of the swelling of the cells because of solvent penetration but also of the cell wall
485 breakdowns that result in two or more cells merging into one larger cluster. Several
486 examples of this effect are highlighted in Figure 5 as merged cells (B). Nowacka & Wedzik
487 [24] also deduced from the percentile area profile of carrot samples, that an increase in
488 the cell size occurred because of the US application (3-4 W/m^2 for 10 to 30 min). In our
489 research, in the case of using water as a solvent, the US application probably intensified
490 the water transfer to the cells because of osmosis since the distilled water was a
491 hypotonic solution. Moreover, water was the less dense and viscous solvent used with
492 apple samples (Table 2). Thus, the cavitation bubbles were probably formed more easily
493 in this liquid [26]. The intensification of water transfer from a hypotonic solvent into
494 vegetable cells because of US application has already been reported by other authors.
495 For instance, Vasile et al. [8], who subjected apple samples to an immersion treatment
496 in water enriched with cyanocobalamin, observed a water gain with US application (200
497 W/L for 15 min) larger than that observed without US. Among the three investigated
498 materials, apple was the most affected when using water as an immersion medium. This
499 was probably because apple presented the highest concentration of soluble solids when
500 compared with beetroot and eggplant (Table 1), which means a higher difference in
501 osmotic pressure between the sample and water. The mass transfer intensification and
502 cell wall breakdown could be a consequence of the microjets promoted by the cavitation
503 bubbles that improve the solvent penetration into the solid and of the “sponge effect”
504 that keeps microchannels and pores free and promotes mass transfer through pumping

505 [16]. On the other hand, an important effect of the US application was also observed in
506 the apple juice. This could not be attributed to the physical characteristics of this solvent
507 since it was more viscous and denser than the water and the citric acid. Rodríguez et al.
508 [12] investigated an immersion pre-treatment for drying carried out with US application
509 (2-12 W/cm^2 for 5 min) and reported more evident damage of apple tissues when it was
510 carried out with the apple juice and with citric acid than with water, attributing it to the
511 low pH of these solvents. However, in this investigation, according to the image analysis
512 results, when applying US, the treatment with the apple juice caused larger cells than
513 the treatment with the citric acid, even when the latter had a lower pH. Therefore, as
514 occurred with the eggplant samples, the higher similarity of the solvent with the extra
515 and the intracellular fluid seemed to be the explanation for better solvent penetration.
516 For instance, the most abundant organic acid in apples is not citric acid but malic acid
517 [57], which should be present in apple juice [58]. The apple juice composition in
518 combination with the US application probably promoted degradation of the pectin
519 compounds of the apple cell walls enhancing the cell wall disruption and the liquid
520 entrance. In addition, in these immersion treatments, there is a multidirectional mass
521 exchange, including the transfer of water from the solvent to the sample or vice versa,
522 but also the penetration of low-molecular substances such as vitamins, saccharides, and
523 others [19]. This transfer of substances from the solvent to the solid must be more
524 significant when using the fruit juice as a solvent than when using water or citric acid
525 considering their composition.

526 Regarding the beetroot samples, the area percentile profiles of the control (BR) and the
527 samples treated with beetroot juice without US (B-JS) practically coincided (Figure 7).
528 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 (Figure 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

553 no significant ($p > 0.05$) differences between the samples treated with US and without
554 them in this solvent. Thus, these results indicate that the application of US to materials
555 with a lot of intercellular space (such as eggplants), under the conditions used in this
556 study, does not promote a significant change in the size of the cells, probably because
557 the solvent introduced into the material by the cavitation effect stays in the intercellular
558 space or generates even more porosity [59]. On the other hand, samples with a medium-
559 high porosity (apple) treated with US application, presented a significant ($p < 0.05$)
560 increase in the size of the cells and a decrease in the cells/area (compared with the
561 control and with samples treated without US) in two solvents (water and apple juice).
562 For the low-porosity material (beetroot), the US effect was only observed in the sample
563 juice. Therefore, apple samples were the most affected by the US application. Pieczywek
564 et al. [23] investigated the effect of US application (7.5-30 min at 10 kWh/kg) on the cell
565 wall stiffness of cylindrical apple samples. They observed that larger times of US
566 exposure resulted in lower cell wall stiffness. They also observed solubilization of pectin
567 material. Apple presented the largest cells among all the samples, thus, in comparison
568 with beetroot, apple presented lower density in “cell wall material”, making this tissue
569 more fragile and susceptible to US application.

570 4 Conclusions

571 This study evaluated the effect of US application in the microstructure of vegetables
572 with different tissue structures and porosity. The results indicate that US has different
573 effects depending on the initial microstructure of the raw matter. Overall, US application
574 stimulated solvent penetration into the vegetable cells, increasing their sizes and/or
575 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.

5 Acknowledgements

The authors would like to acknowledge the Spanish research agency (AEI) MCIN/AEI/10.13039/501100011033 for the financial support for the project (PID2019-106148RR-C43). We would also like to thank Dr. Fernando Hierro from the “Serveis Cientificotècnics” of the University of the Balearic Islands for his assistance with SEM and to Dr. Carlos Saus from the “Hospital Son Espases” for his assistance with samples preparation for optical microscopy.

6 Bibliography

[1] S.E. Demirel, J. Li, M.F. Hasan, Systematic process intensification, Curr. Opin. Chem. Eng. 25 (2019) 108–113. https://doi.org/10.1016/J.COCHE.2018.12.001.

[2] B. Khadhraoui, V. Ummat, B.K. Tiwari, A.S. Fabiano-Tixier, F. Chemat, Review of ultrasound combinations with hybrid and innovative techniques for extraction and processing of food and natural products, Ultrason. Sonochem. 76 (2021) 105625. https://doi.org/10.1016/J.ULTSONCH.2021.105625.

[3] Y.C. Cheung, K.C. Siu, J.Y. Wu, Kinetic Models for Ultrasound-Assisted Extraction of Water-Soluble Components and Polysaccharides from Medicinal Fungi, Food Bioprocess Technol. 6 (2013) 2659–2665. https://doi.org/10.1007/s11947-012-0929-z.

[4] N. Muñoz-Almagro, E. Morales-Soriano, M. Villamiel, L. Condezo-Hoyos, Hybrid high-intensity ultrasound and microwave treatment: A review on its effect on quality and bioactivity of foods, Ultrason. Sonochem. 80 (2021) 105835. https://doi.org/10.1016/J.ULTSONCH.2021.105835.

[5] C. Reche, C. Rosselló, M.M. Umaña, V. Eim, S. Simal, Mathematical Modelling of Ultrasound-Assisted Extraction Kinetics of Bioactive Compounds from Artichoke By-Products, Foods 2021, Vol. 10, Page 931. 10 (2021) 931. https://doi.org/10.3390/FOODS10050931.

[6] M. Umaña, V. Eim, C. Garau, C. Rosselló, S. Simal, Ultrasound-assisted extraction of ergosterol and antioxidant components from mushroom by-products and the attainment of a β-glucan rich residue, Food Chem. 332 (2020) 127390.

https://doi.org/10.1016/j.foodchem.2020.127390.

[7] F.A.N. Fernandes, T.R. Braga, E.O. Silva, S. Rodrigues, Use of ultrasound for dehydration of mangoes (Mangifera indica L.): kinetic modeling of ultrasound-assisted osmotic dehydration and convective air-drying, J. Food Sci. Technol. 56 (2019) 1793. https://doi.org/10.1007/S13197-019-03622-Y.

[8] F.E. Vasile, S. Simal, C. Rosselló, V.S. Eim, Power Ultrasound-Assisted Impregnation of Apple Cubes with Vitamin B12, Food Bioprocess Technol. 15 (2022) 219–229. https://doi.org/10.1007/S11947-021-02752-6/FIGURES/5.

[9] F. Vallespir, Ó. Rodríguez, J.A. Cárcel, C. Rosselló, S. Simal, Ultrasound assisted low-temperature drying of kiwifruit: Effects on drying kinetics, bioactive compounds and antioxidant activity, J. Sci. Food Agric. 99 (2019) 2901–2909. https://doi.org/10.1002/JSFA.9503.

[10] F. Abbasi, F. Samadi, S.M. Jafari, S. Ramezanpour, M. Shams Shargh, Ultrasound-assisted preparation of flaxseed oil nanoemulsions coated with alginate-whey protein for targeted delivery of omega-3 fatty acids into the lower sections of gastrointestinal tract to enrich broiler meat, Ultrason. Sonochem. 50 (2019) 208–217. https://doi.org/10.1016/J.ULTSONCH.2018.09.014.

[11] J.A. Gallego-Juárez, G. Rodríguez, E. Riera, A. Cardoni, Ultrasonic defoaming and debubbling in food processing and other applications, Power Ultrason. Appl. High-Intensity Ultrasound. (2015) 793–814. https://doi.org/10.1016/B978-1-78242-028-6.00026-0.

[12] Ó. Rodríguez, P.J. Llabrés, S. Simal, A. Femenia, C. Rosselló, Intensification of

643 Predrying Treatments by Means of Ultrasonic Assistance: Effects on Water
644 Mobility, PPO Activity, Microstructure, and Drying Kinetics of Apple, Food
645 Bioprocess Technol. 8 (2015) 503–515. https://doi.org/10.1007/s11947-014-
646 1424-5.

647 [13] F. Chen, S. Liu, Z. Zhao, W. Gao, Y. Ma, X. Wang, S. Yan, D. Luo, Ultrasound pre-
648 treatment combined with microwave-assisted hydrodistillation of essential oils
649 from Perilla frutescens (L.) Britt. leaves and its chemical composition and
650 biological activity, Ind. Crops Prod. 143 (2020) 111908.
651 https://doi.org/10.1016/J.INDCROP.2019.111908.

652 [14] L. Han, S. Cao, Y. Yu, X. Xu, X. Cao, W. Chen, Modification in physicochemical,
653 structural and digestive properties of pea starch during heat-moisture process
654 assisted by pre- and post-treatment of ultrasound, Food Chem. 360 (2021)
655 129929. https://doi.org/10.1016/J.FOODCHEM.2021.129929.

656 [15] J.E. González-Pérez, N. Ramírez-Corona, A. López-Malo, Mass Transfer During
657 Osmotic Dehydration of Fruits and Vegetables: Process Factors and Non-Thermal
658 Methods, Food Eng. Rev. 13 (2021) 344–374. https://doi.org/10.1007/S12393-
659 020-09276-3.

660 [16] A.C. Miano, A. Ibarz, P.E.D. Augusto, Mechanisms for improving mass transfer in
661 food with ultrasound technology: Describing the phenomena in two model cases,
662 Ultrason. Sonochem. 29 (2016) 413–419.
663 https://doi.org/10.1016/J.ULTSONCH.2015.10.020.

664 [17] C. Wen, J. Zhang, H. Zhang, C.S. Dzah, M. Zandile, Y. Duan, H. Ma, X. Luo, Advances
665 in ultrasound assisted extraction of bioactive compounds from cash crops – A

review, Ultrason. Sonochem. 48 (2018) 538–549. https://doi.org/10.1016/j.ultsonch.2018.07.018.

[18] S. Zhao, C. Yao, Q. Zhang, G. Chen, Q. Yuan, Acoustic cavitation and ultrasound-assisted nitration process in ultrasonic microreactors: The effects of channel dimension, solvent properties and temperature, Chem. Eng. J. 374 (2019) 68–78. https://doi.org/10.1016/j.cej.2019.05.157.

[19] M. Nowacka, M. Dadan, U. Tylewicz, Current Applications of Ultrasound in Fruit and Vegetables Osmotic Dehydration Processes, Appl. Sci. 2021, Vol. 11, Page 1269. 11 (2021) 1269. https://doi.org/10.3390/APP11031269.

[20] V.M. Karizaki, S. Sahin, G. Sumnu, M.T.H. Mosavian, A. Luca, Effect of Ultrasound-Assisted Osmotic Dehydration as a Pretreatment on Deep Fat Frying of Potatoes, Food Bioprocess Technol. 6 (2013) 3554–3563. https://doi.org/10.1007/S11947-012-1012-5/FIGURES/10.

[21] M. Nowacka, U. Tylewicz, L. Laghi, M. Dalla Rosa, D. Witrowa-Rajchert, Effect of ultrasound treatment on the water state in kiwifruit during osmotic dehydration, Food Chem. 144 (2014) 18–25. https://doi.org/10.1016/J.FOODCHEM.2013.05.129.

[22] A.C. Miano, M.L. Rojas, P.E.D. Augusto, Structural changes caused by ultrasound pretreatment: Direct and indirect demonstration in potato cylinders, Ultrason. Sonochem. 52 (2019) 176–183. https://doi.org/10.1016/J.ULTSONCH.2018.11.015.

[23] P.M. Pieczywek, A. Kozioł, D. Konopacka, J. Cybulska, A. Zdunek, Changes in cell

688 wall stiffness and microstructure in ultrasonically treated apple, J. Food Eng. 197
689 (2017) 1–8. https://doi.org/10.1016/j.jfoodeng.2016.10.028.

690 [24] M. Nowacka, M. Wedzik, Effect of ultrasound treatment on microstructure,
691 colour and carotenoid content in fresh and dried carrot tissue, Appl. Acoust. 103
692 (2016) 163–171. https://doi.org/10.1016/j.apacoust.2015.06.011.

693 [25] G.M. Gonzalez, Effects of power ultrasound treatments on properties of
694 Longissimus beef muscle, 2003. https://lib.dr.iastate.edu/rtd/1432 (accessed
695 April 23, 2021).

696 [26] J.P. Lorimer, T.J. Mason, Sonochemistry. Part 1—The physical aspects, Chem. Soc.
697 Rev. 16 (1987) 239–274. https://doi.org/10.1039/CS9871600239.

698 [27] F. Vallespir, Ó. Rodríguez, V.S. Eim, C. Rosselló, S. Simal, Freezing pre-treatments
699 on the intensification of the drying process of vegetables with different
700 structures, J. Food Eng. 239 (2018) 83–91.
701 https://doi.org/10.1016/J.JFOODENG.2018.07.008.

702 [28] H. Baniasadi, R. Ajdary, J. Trifol, O.J. Rojas, J. Seppälä, Direct ink writing of aloe
703 vera/cellulose nanofibrils bio-hydrogels, Carbohydr. Polym. 266 (2021) 118114.
704 https://doi.org/10.1016/J.CARBPOL.2021.118114.

705 [29] K.K. Valladares-Diestra, L. Porto de Souza Vandenberghe, L.A. Zevallos Torres, A.
706 Zandoná Filho, A. Lorenci Woiciechowski, C. Ricardo Soccol, Citric acid assisted
707 hydrothermal pretreatment for the extraction of pectin and xylooligosaccharides
708 production from cocoa pod husks, Bioresour. Technol. 343 (2022) 126074.
709 https://doi.org/10.1016/J.BIORTECH.2021.126074.

710 [30] G. Ferrer, C. Barreneche, A. Solé, I. Martorell, L.F. Cabeza, New proposed
711 methodology for specific heat capacity determination of materials for thermal
712 energy storage (TES) by DSC, J. Energy Storage. 11 (2017) 1–6.
713 https://doi.org/10.1016/J.EST.2017.02.002.

714 [31] F. Vallespir, Ó. Rodríguez, V.S. Eim, C. Rosselló, S. Simal, Effects of freezing
715 treatments before convective drying on quality parameters: Vegetables with
716 different microstructures, J. Food Eng. 249 (2019) 15–24.
717 https://doi.org/10.1016/j.jfoodeng.2019.01.006.

718 [32] R Core Team, R: A language and environment for statistical computing, (2017).

719 [33] F. de Mendiburu, Agricolae: Statistical Procedures for Agricultural Research,
720 (2016). https://cran.r-project.org/package=agricolae.

721 [34] R Core Team, Foreign: Read Data Stored by Minitab, S, SAS, SPSS, Stata, Systat,
722 Weka, dBase, (2017). https://cran.r-project.org/package=foreign.

723 [35] C.J. Boukouvalas, M.K. Krokida, Z.B. Maroulis, D. Marinos-Kouris, Density and
724 Porosity: Literature Data Compilation for Foodstuffs, Int. J. Food Prop. 9 (2006)
725 715–746. https://doi.org/10.1080/10942910600575690.

726 [36] B. Ma, J. Chen, H. Zheng, T. Fang, C. Ogutu, S. Li, Y. Han, B. Wu, Comparative
727 assessment of sugar and malic acid composition in cultivated and wild apples,
728 Food Chem. 172 (2015) 86–91.
729 https://doi.org/10.1016/J.FOODCHEM.2014.09.032.

730 [37] B. Ma, Y. Yuan, M. Gao, C. Li, C. Ogutu, M. Li, F. Ma, Determination of Predominant
731 Organic Acid Components in Malus Species: Correlation with Apple

732 Domestication, Metabolites. 8 (2018).
733 https://doi.org/10.3390/METABO8040074.

734 [38] E. Rosa-Martínez, M.D. García-Martínez, A.M. Adalid-Martínez, L. Pereira-Dias, C.
735 Casanova, E. Soler, M.R. Figàs, M.D. Raigón, M. Plazas, S. Soler, J. Prohens, Fruit
736 composition profile of pepper, tomato and eggplant varieties grown under
737 uniform conditions, Food Res. Int. 147 (2021) 110531.
738 https://doi.org/10.1016/J.FOODRES.2021.110531.

739 [39] J. Vasconcellos, C. Conte-Junior, D. Silva, A.P. Pierucci, V. Paschoalin, T.S. Alvares,
740 Comparison of total antioxidant potential, and total phenolic, nitrate, sugar, and
741 organic acid contents in beetroot juice, chips, powder, and cooked beetroot, Food
742 Sci. Biotechnol. 25 (2016) 79–84. https://doi.org/10.1007/S10068-016-0011-0.

743 [40] M.L. Tan, S.B.S. Hamid, Beetroot as a Potential Functional Food for Cancer
744 Chemoprevention, a Narrative Review, J. Cancer Prev. 26 (2021) 1–17.
745 https://doi.org/10.15430/JCP.2021.26.1.1.

746 [41] M. Schmutzler, C.W. Huck, Simultaneous detection of total antioxidant capacity
747 and total soluble solids content by Fourier transform near-infrared (FT-NIR)
748 spectroscopy: A quick and sensitive method for on-site analyses of apples, Food
749 Control. 66 (2016) 27–37. https://doi.org/10.1016/j.foodcont.2016.01.026.

750 [42] J. Prohens, A. Rodríguez-Burruezo, M.D. Raigón, F. Nuez, Total phenolic
751 concentration and browning susceptibility in a collection of different varietal
752 types and hybrids of eggplant: Implications for breeding for higher nutritional
753 quality and reduced browning, J. Am. Soc. Hortic. Sci. 132 (2007) 638–646.
754 https://doi.org/10.21273/jashs.132.5.638.

755 [43] M. Šlosár, A. Hegedusova, Yield parameters, antioxidant activity, polyphenol and
756 total soluble solids content of beetroot cultivars with different flesh colours, Folia
757 Hortic. 2 (2020) 351–362. https://doi.org/10.2478/fhort-2020-0030.

758 [44] E.Z. Al Anazi, Dental erosion caused by Granny Smith apples: An evidence-based
759 case report and 1-year follow-up, Clin. Case Reports. 6 (2018) 1689–1696.
760 https://doi.org/10.1002/ccr3.1702.

761 [45] R.C. Osidacz, M.C.B. Ambrosio-Ugri, Análise da qualidade físico-química de
762 berinjela desidratada com variações no pré-tratamento, Acta Sci. - Technol. 35
763 (2013) 175–179. https://doi.org/10.4025/actascitechnol.v35i1.10551.

764 [46] D. Trishitman, P.S. Negi, N.K. Rastogi, Concentration of beetroot juice colorant
765 (betalains) by forward osmosis and its comparison with thermal processing, LWT.
766 145 (2021) 111522. https://doi.org/10.1016/j.lwt.2021.111522.

767 [47] M. Teresa Pacheco, M. Villamiel, R. Moreno, F.J. Moreno, Structural and
768 Rheological Properties of Pectins Extracted from Industrial Sugar Beet By-
769 Products, Molecules. 24 (2019). https://doi.org/10.3390/MOLECULES24030392.

770 [48] J. Zheng, H. Li, D. Wang, R. Li, S. Wang, B. Ling, Radio frequency assisted extraction
771 of pectin from apple pomace: Process optimization and comparison with
772 microwave and conventional methods, Food Hydrocoll. 121 (2021) 107031.
773 https://doi.org/10.1016/J.FOODHYD.2021.107031.

774 [49] Y.R. Sekhar, K. V. Sharma, Study of viscosity and specific heat capacity
775 characteristics of water-based Al2O3 nanofluids at low particle concentrations,
776 Https://Doi.Org/10.1080/17458080.2013.796595. 10 (2014) 86–102.

https://doi.org/10.1080/17458080.2013.796595.

[50] S.D. Jayasooriya, B.R. Bhandari, P.& B.R. Torley, Effect of High Power Ultrasound Waves on Properties of Meat: A Review, Int. J. Food Prop. 7 (2004) 301–319. https://doi.org/10.1081/JFP-120030039.

[51] A. Puig, I. Perez-Munuera, J.A. Carcel, I. Hernando, J. V. Garcia-Perez, Moisture loss kinetics and microstructural changes in eggplant (Solanum melongena L.) during conventional and ultrasonically assisted convective drying, Food Bioprod. Process. 90 (2012) 624–632. https://doi.org/10.1016/J.FBP.2012.07.001.

[52] F. Vallespir, J.A. Cárcel, F. Marra, V.S. Eim, S. Simal, Improvement of Mass Transfer by Freezing Pre-treatment and Ultrasound Application on the Convective Drying of Beetroot (Beta vulgaris L.), Food Bioprocess Technol. 11 (2018) 72–83. https://doi.org/10.1007/S11947-017-1999-8/FIGURES/6.

[53] T. Mason, F. Chemat, M. Vinatoru, The Extraction of Natural Products using Ultrasound or Microwaves, Curr. Org. Chem. 15 (2011) 237–247. https://doi.org/10.2174/138527211793979871.

[54] A.O. Oladejo, H. Ma, W. Qu, C. Zhou, B. Wu, X. Yang, Influence of ultrasound pretreatments on diffusion coefficients, texture and colour of osmodehydrated sweet potato (Ipomea batatas), Int. J. Food Sci. Technol. 52 (2017) 888–896. https://doi.org/10.1111/IJFS.13352.

[55] S. Rodrigues, F.I.P. Oliveira, M.I. Gallão, F.A.N. Fernandes, Effect of immersion time in osmosis and ultrasound on papaya cell structure during dehydration, Dry. Technol. 27 (2009) 220–225. https://doi.org/10.1080/07373930802605883.

[56] F.A.N. Fernandes, M.I. Gallão, S. Rodrigues, Effect of osmosis and ultrasound on pineapple cell tissue structure during dehydration, J. Food Eng. 90 (2009) 186–190. https://doi.org/10.1016/J.JFOODENG.2008.06.021.

[57] Y. Han, Y. Wang, J. Li, J. Du, Z. Su, Evaluating the effect of bentonite, malic acid on pectin methyl esterase, methanol in fermented apple juice, J. Food Compos. Anal. (2022) 104468. https://doi.org/10.1016/J.JFCA.2022.104468.

[58] J. Dobrowolska-Iwanek, M. Gąstoł, A. Adamska, M. Krośniak, P. Zagrodzki, Traditional versus modern apple cultivars – a comparison of juice composition, Folia Hortic. 27 (2015) 33–41. https://doi.org/10.1515/FHORT-2015-0012.

[59] E.K. Méndez-Calderón, J.C. Ocampo-Castaño, C.E. Orrego, Optimization of convective drying assisted by ultrasound for Mango Tommy (Mangifera indica L.), J. Food Process Eng. 41 (2018) e12634. https://doi.org/10.1111/JFPE.12634.

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

Mónica UMAÑA, Marina CALAHORRO, Valeria EIM, Carmen ROSSELLÓ, Susana SIMAL*

Department of Chemistry, University of the Balearic Islands, Ctra. Valldemossa, km. 7.5, 07122 Palma de Mallorca, Spain.

*Corresponding author: Tel: +34 971 172757. E-mail address: susana.simal@uib.es

Permanent address: Ctra Valldemossa km 7.5, 07122. University of the Balearic Islands, Palma de Mallorca, Spain.

Declarations of interest: none

[1]

[1] Abbreviations: A: apple samples, B: beetroot samples, E: eggplant samples, C: immersion treatment in citric acid, cells/area= number of cells per area (number of cells/mm^2), Cp: heat capacity (J/ kg °C), J: immersion treatment in the juice of the vegetable/fruit, m: mass (kg), OM: Optical microscopy, P: power (W), R: raw samples (control), S: immersion treatment carried out without high power ultrasound application, SEM: Scanning electron microscopy, T= temperature (°C), t: time (s), U: immersion treatment carried out with high power ultrasound application, US: High power ultrasound, W: immersion treatment in distilled water.

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 areas 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.

Keywords: Ultrasound, microstructure, vegetables, image analysis

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 and 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/cm^2) 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 surrender 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 32x20x5 mm rectangular sheet was extracted. The samples were immediately freeze-dried by frozen 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.

$$Porosity = \frac{m_{sat} - m_d}{\rho V} \quad (1)$$

Where m_{sat} is the weight of the sample saturated with ethanol (g), m_d is the weight of the freeze-dried sample (g), ρ 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 (Crison, 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 (32x20x5

123 mm) was obtained as described before for the porosity analysis. After cutting, samples
124 were immediately used for the experiments.

125 2.3 Immersion media

126 The immersion media used in the study were distilled water (W), a 1 % (w/v) citric acid
127 solution (C), and the juices (J) obtained from each product, using a common blender,
128 immediately before performing the experiments. The distilled water was chosen as a
129 solvent to evaluate the effect of a hypotonic immersion medium. The citric acid was
130 selected to determine the effect of a low-pH solvent since it has been previously
131 reported that citric acid solution can provoke damage to cell walls [12,29], and the juices
132 of the vegetables were used to evaluate the effect of an isotonic solvent. The pH and
133 the total soluble solids content of the immersion media were determined with a pH-
134 meter (Crison, pH 25, Spain) and a refractometer Abbe 325 (Zuzi, Spain), respectively, at
135 room temperature (~22 °C). The density of the immersion media was determined at 25
136 °C with a pycnometer. Finally, the viscosity was obtained with a J. P Selecta rotational
137 viscometer (ST-DIGIT R, Spain) at 25 °C using a spindle with a 35 mm diameter. The
138 relative viscosity was calculated by taking the viscosity of water as a reference. Finally,
139 the heat capacity (Cp) of the immersion media was determined with a differential
140 scanning calorimeter (DSC) (Mettler Toledo, DSC 3, USA) equipped with an intracooler
141 SP (Huber, TC100, Germany) using the dynamic methodology described by Ferrer et al.
142 [30] with some modifications. Briefly, three measurements were carried out, a blank
143 measurement using an empty crucible (aluminium 25 µL), a sapphire measurement (as
144 a reference), and the measurement of the sample. Samples were weighed (about 15
145 mg), subjected to an isotherm for 5 min at 5 °C, then heated (10 °C/min) till 35 °C, and

146 subjected to another isotherm for 5 min at 35 °C. The immersion medium Cp (at 25 °C)
147 was calculated from Eq 2.

$$Cp = \frac{y}{y'} x \frac{m'}{m} x Cp' \qquad 2$$

148 where Cp is the heat capacity of the sample (J/ kg °C), y is the difference between the
149 heat flux (W) of the sample and the blank, y' is the difference between the heat flux of
150 the sapphire and the blank (W), m' is the mass of sapphire (kg), m is the mass of the
151 sample (kg) and Cp' is the heat capacity of the sapphire at 25 °C (J/ kg °C).

152 2.4 Immersion treatment

153 The immersion treatment was carried out without (S) and with high-power ultrasound
154 assistance (U). Each sample was immersed for 5 min in 400 mL of the corresponding
155 immersion media (distilled water, citric acid solution, or juice of the vegetable/fruit)
156 within a jacketed glass vessel. This time was chosen since a previous study demonstrated
157 that this time (5 min) of ultrasound application produced microstructural changes in a
158 plant material (apple samples) and also intensified a mass transfer process (drying) [12].
159 The sample was clamped with forceps to prevent floating. The temperature was
160 maintained at 25 °C by driving ethylene glycol through the jacketed vessel with a chiller
161 unit (Frigedor, J.P. Selecta, Barcelona, Spain). Each experiment was performed at least 6
162 times.

163 The U immersion treatment was carried out using an ultrasonic generator UP400S
164 (Hielscher Ultrasonics GmbH, Schwabach, Germany) with 400 W, connected to a probe
165 (diameter of 22 mm), the amplitude and pulse being established at 100 % and cycles of
166 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 Figure 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 the raw matter: E (eggplant), A (apple), and B (beetroot); next an R for raw samples (control) or a letter indicating the immersion medium: W (distilled water), C (citric acid), and J (juice) followed by a letter to indicate if the process was (U) or not (S) acoustically assisted.

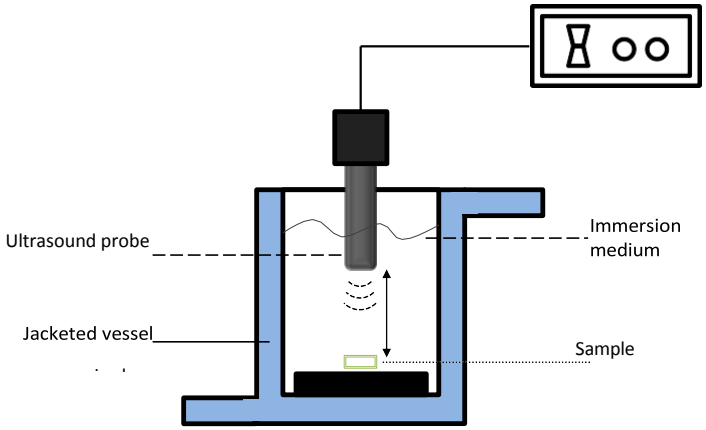


Figure 1. Schematic representation of the setup for the experiments carried out with ultrasound application.

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.

$$P = M \cdot C_p \cdot \frac{dT}{dt} \qquad 3$$

where M is the mass of the solvent (kg), Cp is the heat capacity of the liquid (J/kg °C), T is the temperature (°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 ± 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 °C freezer (IngClimas, Spain), for about 3 h and thereafter they were introduced in a freeze-dryer (Telstar LyoQuest, Spain) at -50 °C and vacuum pressure of 30 Pa. Samples were immediately observed by SEM after removal from the freeze dryer. A HITACHI S-3400N 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 50x magnification.

Samples (raw and treated samples) were prepared for optical microscopy as described by Vallespir et al. [31]. Briefly, samples were fixed 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 50x magnification

with a BX60 optical microscope (Olympus, Japan) connected to a Moticam 3 digital camera (Motic, China).

2.6 Image analysis

To quantify the effects of the immersion treatment, the images obtained by optical microscopy were processed with the free software ImageJ 1.52k (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 4.2×10^{-4} mm^2 were excluded from the analysis of eggplant to prevent structural imperfections from being detected as cells. This limit was settled at 1.4×10^{-3} mm^2 and 7.0×10^{-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 "PERCENTIL.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 (R Core Team 2017). 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 (de Mendiburu 2016).

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 Cp of the immersion media in Table 2.

Table 1. Porosity, pH, and soluble solids content of the raw matter.

Raw matter	Porosity	pH	Soluble solids (°Brix)
Eggplant	0.759 ± 0.106^{a}	5.46 ± 0.07^{b}	2.5 ± 0.2^{c}
Apple	0.313 ± 0.012^{b}	3.14 ± 0.06^{c}	13.3 ± 0.3^{a}
Beetroot	0.135 ± 0.015^{c}	5.87 ± 0.09^{a}	8.3 ± 0.3^{b}

244

245 **Table 2.** Characteristics of the immersion media.

Immersion medium	pH	Soluble solids (°Brix)	Density (kg/m³)	Relative viscosity	Cp (J/Kg °C)
Distilled water	6.05 ± 0.06^{b}	--	995 ± 0^{e}	1.0 ± 0.0^{c}	4105 ± 215^{a}
Citric acid (1% w/v)	2.02 ± 0.03^{e}	0.6 ± 0.1^{d}	998 ± 1^{d}	1.0 ± 0.1^{c}	3990 ± 124^{a}
Eggplant juice	5.42 ± 0.04^{c}	2.4 ± 0.1^{c}	1013 ± 1^{c}	1.5 ± 0.0^{b}	3941 ± 70^{a}
Apple juice	3.13 ± 0.01^{d}	13.0 ± 0.1^{a}	1046 ± 1^{b}	1.6 ± 0.1^{b}	3776 ± 50^{ab}
Beetroot juice	6.03 ± 0.01^{a}	8.4 ± 0.0^{b}	1055 ± 1^{a}	2.2 ± 0.1^{a}	3594 ± 84^{b}

246 *Different letters for the same parameter and raw matter indicate significant
247 differences ($p < 0.05$)

248

249 As can be seen, the three raw materials and their juices exhibited significant ($p < 0.05$)
250 differences among them in the analyzed parameters. The three samples presented
251 significantly different porosity ($p < 0.05$), eggplant presented the higher value, followed
252 by apple, and beetroot was the least porous sample. The experimental values obtained
253 are similar but larger than that reported in the bibliography, 0.641 for eggplants, which
254 are classified as high-porosity vegetables, 0.210 for apples, and 0.043 for beetroots
255 which are considered low-porosity vegetables [35]. Differences with the bibliography
256 could be related to the area of the fruit or vegetable where the sample was obtained,
257 the variety of the plant, and the method used to measure this parameter. However, the
258 trend observed coincided with that reported in the bibliography and confirm the high
259 difference among the microstructure of the samples.

260 The apple sample presented the lowest pH as well as its juice, while the beetroot sample
261 and its juice showed the highest. Apples have a relatively high content of organic acids

262 [36]. For instance, apples', beetroots', and eggplants' main organic acid is malic acid, but
263 apples can present up to 30 mg/g of fresh weight (fw) of this compound (depending on
264 the variety and ripening) [37] while eggplants and beetroots show about 1.3 and 3.6
265 mg/g fw, respectively [38,39]. Concerning the soluble solids content, the eggplant
266 sample and its juice showed the lowest value and apples showed the highest. Apples
267 are rich in sucrose and fructose [36], and beetroot is known as a source of sucrose [40].
268 Generally, the values of pH and total soluble solids are similar to those previously
269 reported in the literature for the three products [41–46]. Among the solvents, distilled
270 water and citric acid presented the lowest viscosity and densities. The juices were
271 significantly ($p < 0.05$) denser and more viscous, which was expected, as they contained
272 higher soluble solid concentrations and particles in suspension (such as non-soluble
273 fibre). Eggplant juice was the least dense and viscous among the juices. It is known that
274 apple and beetroot are rich in pectins and other soluble fibres which increase the
275 viscosity of liquids [47,48]. Finally, all the immersion media presented Cp figures similar
276 to that of water. However, the beetroot juice showed a significantly ($p < 0.05$) lower
277 value. This could be related to the soluble and non-soluble solids concentration of this
278 juice [49]. It is well known that ultrasound waves propagation can be affected by the
279 properties of the medium [16,25,50]. Thus, it could be expected that the effects of
280 ultrasound on the microstructure would be different according to the liquid media and
281 solids characteristics.

282 3.2 Microstructure of the samples

283 Figure 2 shows representative photographs of the raw samples (before the immersion
284 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 Figure 2, apple presented the largest cells. This was also confirmed with the cell area percentile profiles obtained by image analysis and presented in Figure 3. In this figure, percentiles indicate the percentage of cells with an area equal to or smaller than the obtained value. As shown in Figure 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.

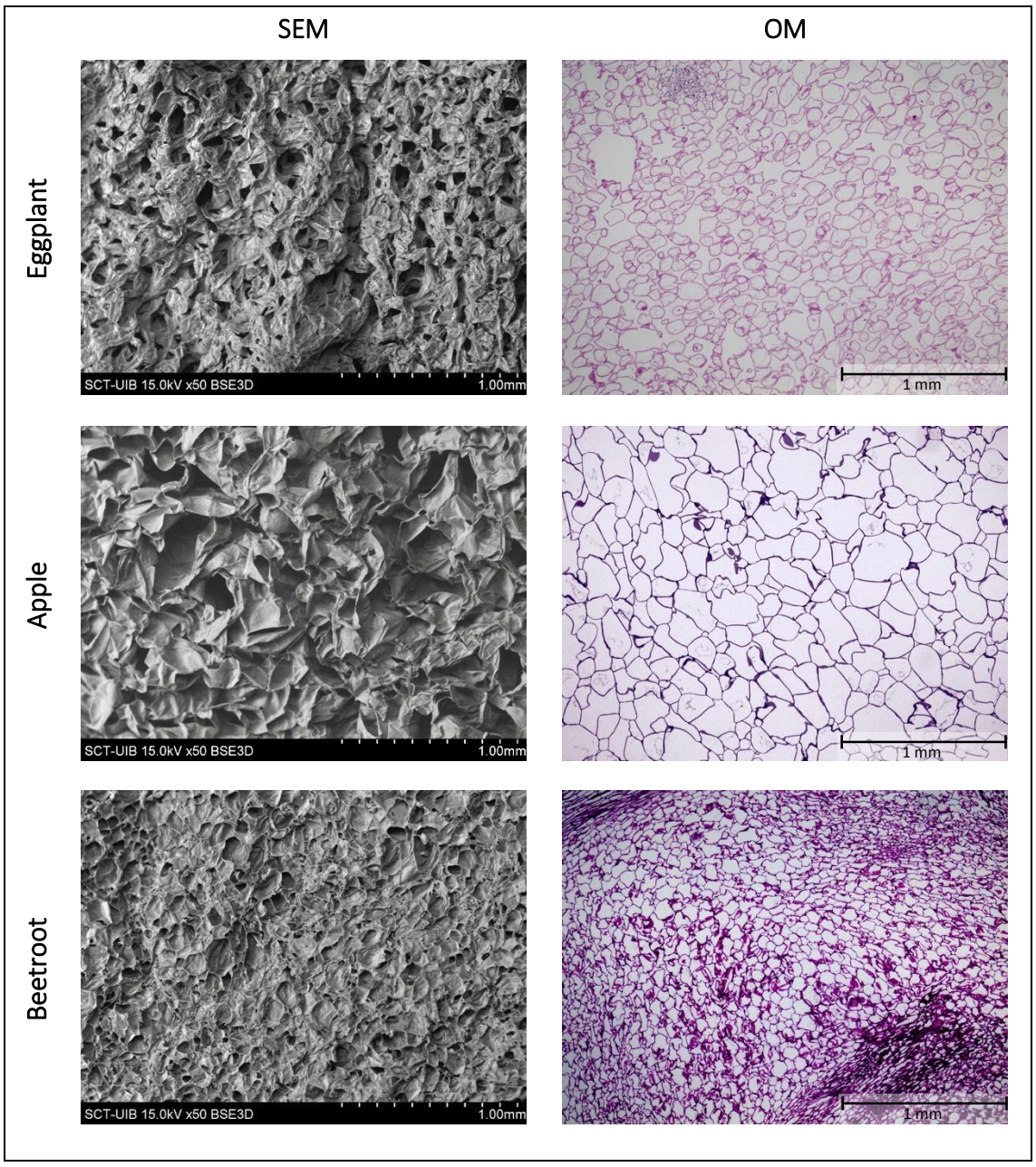
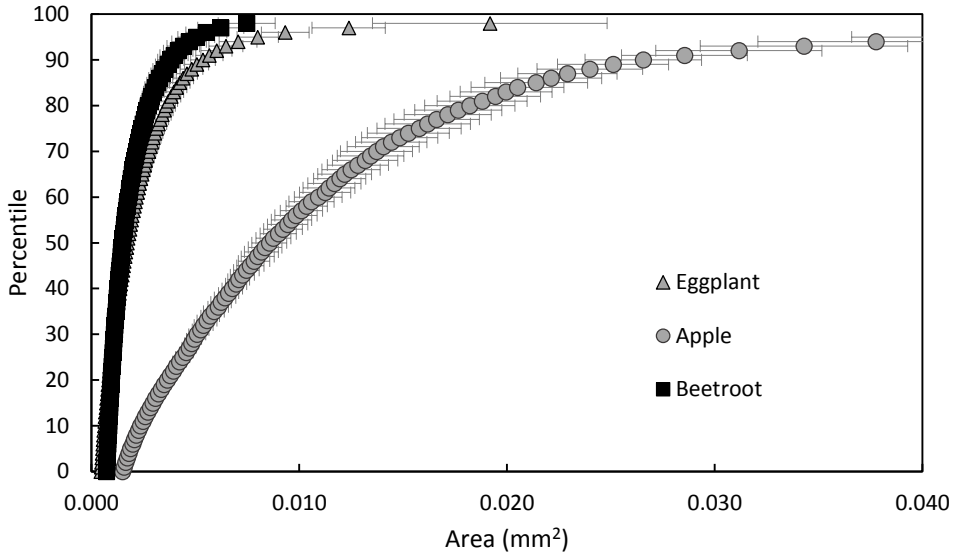


Figure 2. Representative photographs of raw eggplant, apple, and beetroot obtained by scanning electron microscopy (SEM) and optical microscopy (OM).



310

311 **Figure 3.** Cell area percentile profiles of raw eggplant, apple, and beetroot.

312

313

Table 3. Median cell area (d50) and the number of cells per area (cells/area) of eggplant, apple, and beetroot samples, untreated (raw, R) and subjected to an immersion treatment in water (W), citric acid (C), and the vegetable/fruit juice (J) without (S) and with ultrasound application (U) at 192 ± 6 W/L.

	Treatment	d50 (10^3) (mm^2)	Number of cells/area (cells/mm^2)
Eggplant	R	1.68 ± 0.18^{c}	372 ± 31^{a}
	WS	1.78 ± 0.16^{c}	350 ± 15^{ab}
	WU	2.01 ± 0.22^{bc}	307 ± 33^{b}
	CS	1.81 ± 0.26^{c}	381 ± 45^{a}
	CU	2.00 ± 0.18^{bc}	376 ± 34^{a}
	JS	2.46 ± 0.18^{a}	305 ± 34^{b}
	JU	2.37 ± 0.31^{ab}	306 ± 20^{b}
Apple	R	8.34 ± 0.80^{c}	76 ± 10^{a}
	WS	8.87 ± 0.60^{bc}	76 ± 9^{a}
	WU	11.16 ± 0.95^{a}	52 ± 6^{b}
	CS	8.91 ± 1.16^{bc}	72 ± 7^{a}
	CU	8.96 ± 1.39^{bc}	76 ± 10^{a}
	JS	8.77 ± 1.05^{bc}	73 ± 9^{a}
	JU	10.53 ± 0.96^{ab}	62 ± 8^{b}
Beetroot	R	1.45 ± 0.13^{b}	521 ± 71^{a}
	WS	1.55 ± 0.18^{b}	456 ± 60^{ab}
	WU	1.63 ± 0.07^{ab}	434 ± 22^{ab}
	CS	1.59 ± 0.07^{ab}	466 ± 36^{ab}
	CU	1.56 ± 0.12^{b}	496 ± 43^{bc}
	JS	1.44 ± 0.09^{b}	512 ± 51^{a}
	JU	1.80 ± 0.16^{a}	385 ± 40^{b}

*Different letters for the same parameter and raw matter indicate significant differences ($p < 0.05$)

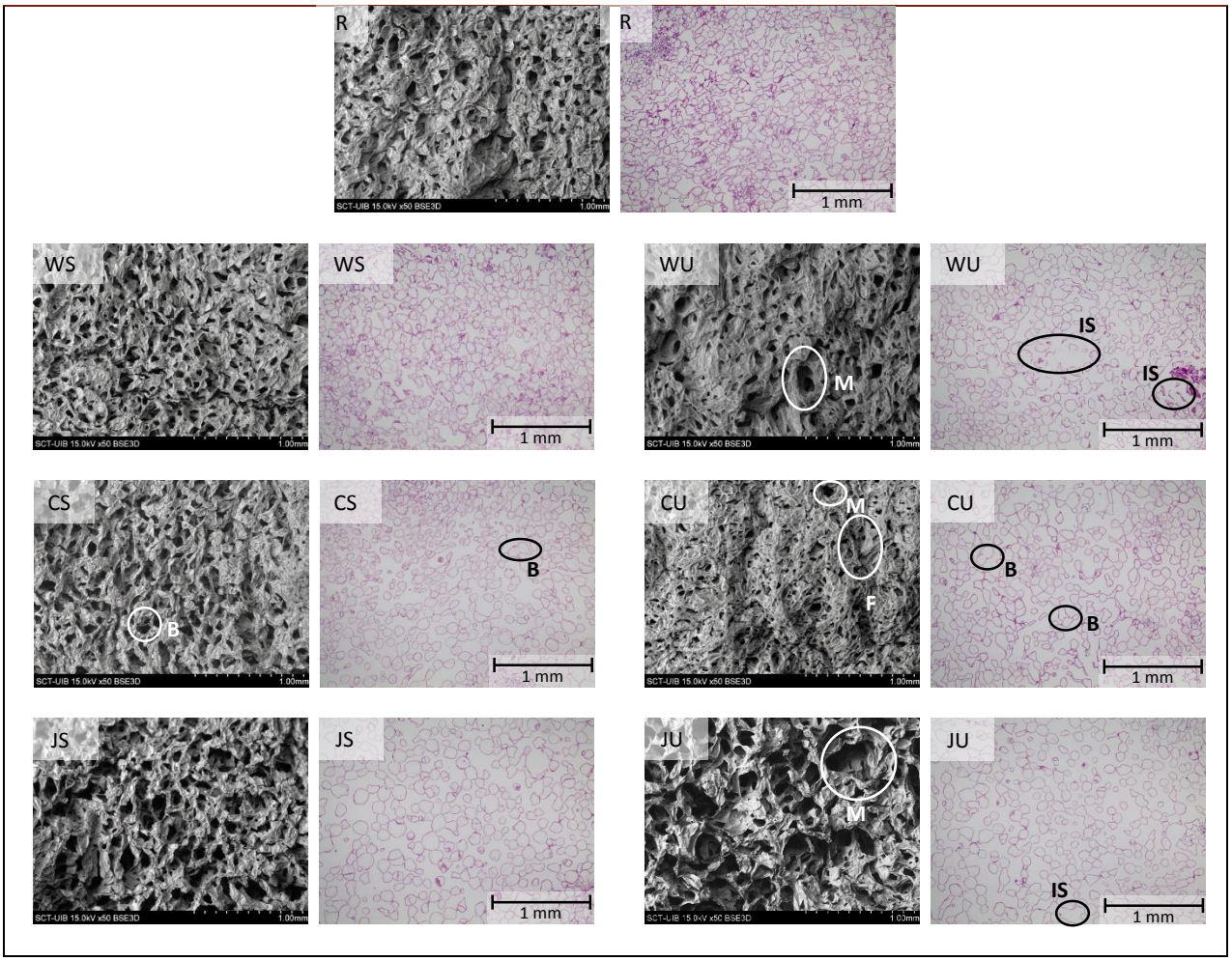
3.3 Effect of the immersion treatment on the microstructure

To study the effect of the immersion treatment with and without US, micrographs of the samples were obtained by SEM and OM. Figures 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 (figure 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 (figure 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 (figure 6), practically no microchannels were observed and the cells were notably larger when treated with the vegetable juice especially when US was applied.



344 **Figure 4.** Scanning electron and optical micrographs of eggplant samples: raw (control:
345 R) and subjected to an immersion treatment in water (W), citric acid (C), and eggplant
346 juice (J) without (S) and with ultrasound application (U) at 192 ± 6 W/L. The images show
347 the areas where cell breakdowns occurred promoting the merge of cells (B) and the
348 formation of intercellular spaces (IS), fissures (F), and microchannels (M).

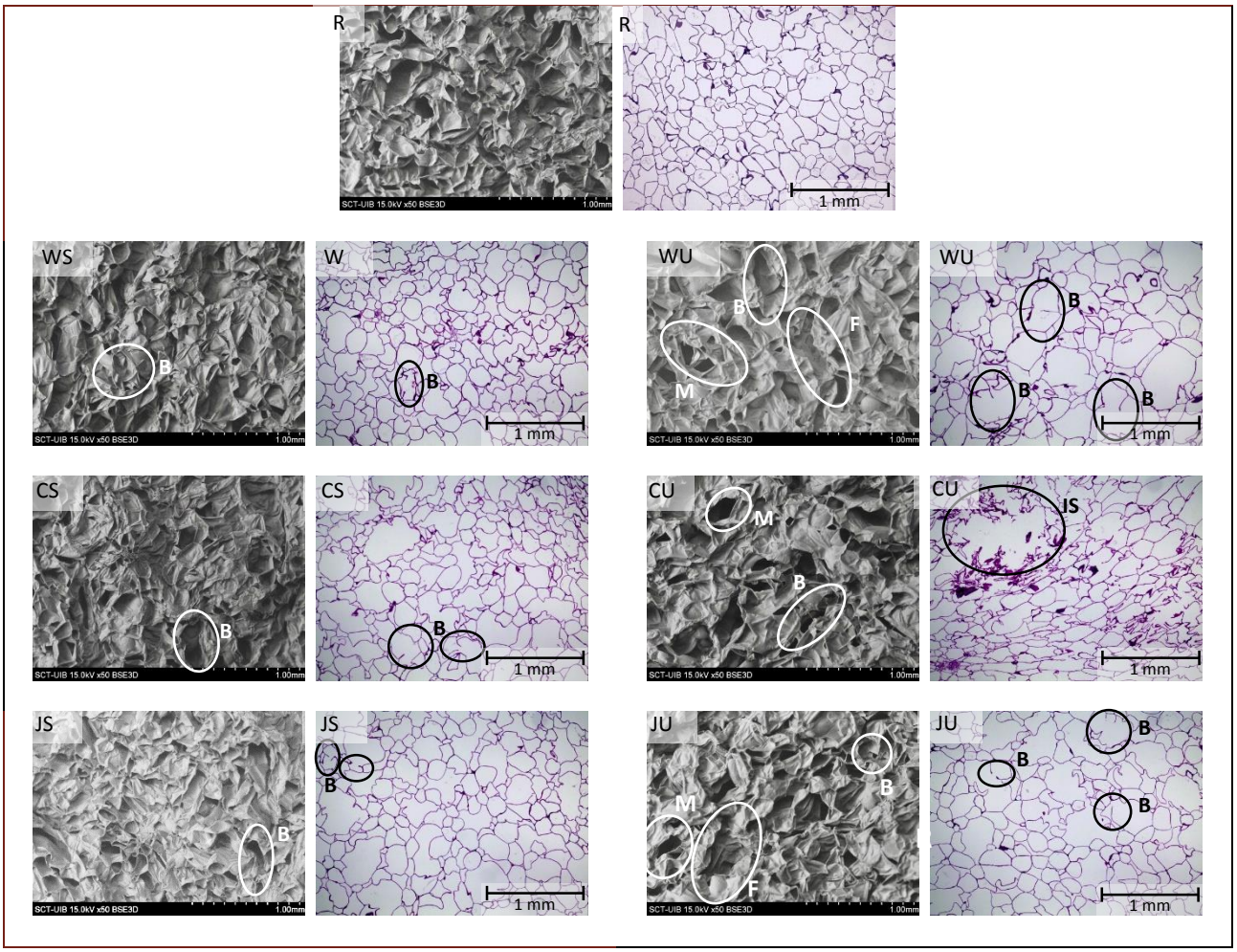
349

350

351

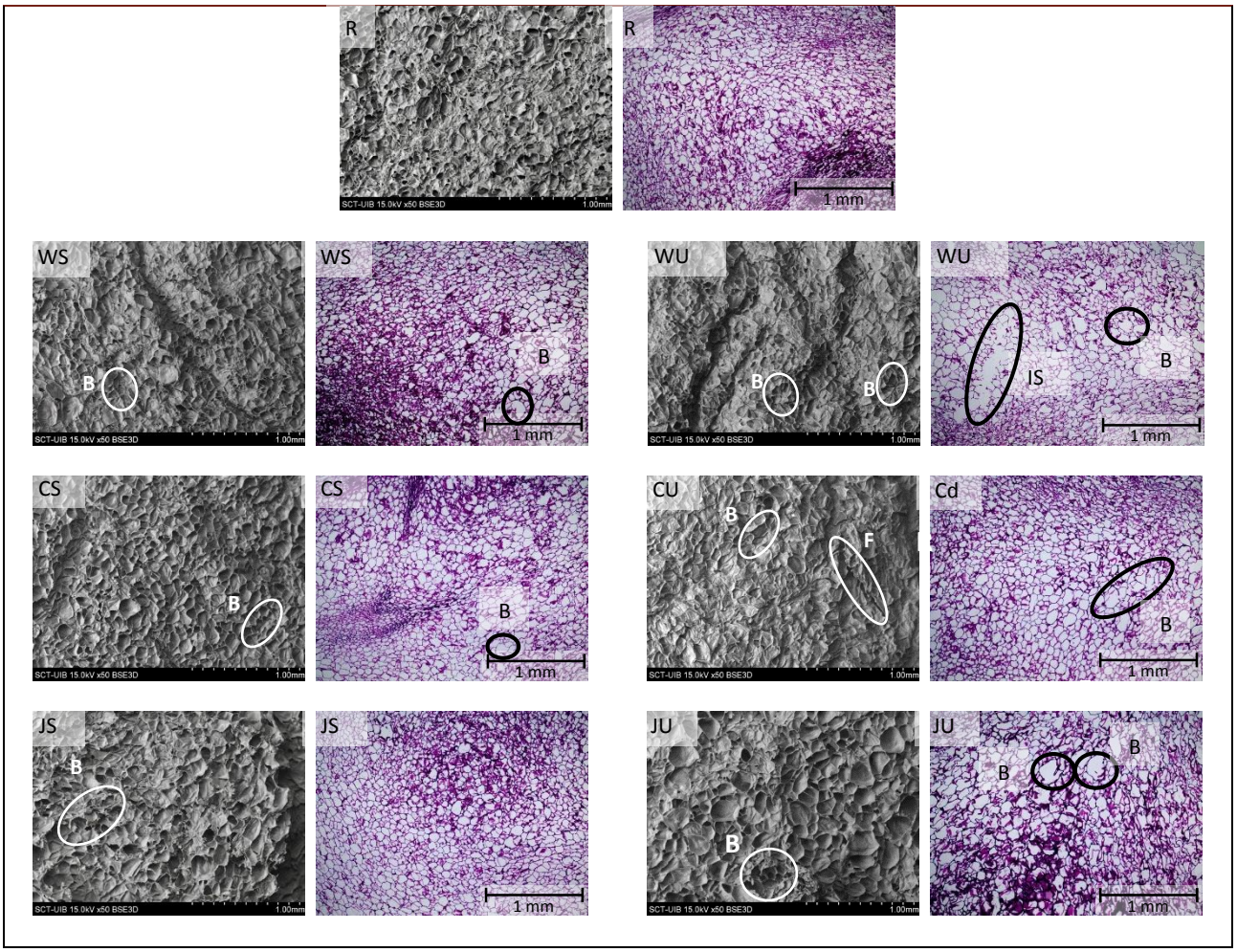
352

353



354 **Figure 5.** Scanning electron and optical micrographs of apple samples: raw (control: R)
355 and subjected to an immersion treatment in water (W), citric acid (C), and apple juice (J)
356 without (S) and with ultrasound application (U) at 192 ± 6 W/L. The images show the
357 areas where cell breakdowns occurred promoting the merge of cells (B) and the
358 formation of intercellular spaces (IS), fissures (F), and microchannels (M).

359



360 **Figure 6.** Scanning electron and optical micrographs of beetroot samples:
361 raw (control: R) and subjected to an immersion treatment in water (W), citric
362 acid (C), and beetroot juice (J) without (S) and with ultrasound application
363 (U) at 192 ± 6 W/L. The images show the areas where cell breakdowns
364 occurred promoting the merge of cells (B) and the formation of intercellular
365 spaces (IS), and fissures (F).

366

367

368 The acoustic energy of US is known to provoke damage to vegetable tissues through
369 different mechanisms, such as the sponge effect, absorption of acoustic energy, and
370 cavitation and its consequences [19]. Similar effects of the application of power US have
371 been reported in different vegetable tissues. For instance, several investigations have
372 demonstrated the formation of microchannels in vegetable tissues subjected to US
373 application. Miano et al. [22] studied the effect of US (ultrasonic bath of 91 W/L) applied
374 for 120 min to cylindrical samples of potatoes. They observed the formation of
375 microchannels inside the potato tissue and considerable surface erosion. Nowacka &
376 Wedzik [24] applied US (3-4 W/m^2) from 10 to 30 min to hermetically packed carrot
377 samples immersed in 1 L of distilled water. They observed that after this treatment, the
378 cells of carrot tissue were distorted, damaged and merged together, and several large
379 spaces were observed (especially after 30 min). They also reported the formation of
380 microchannels and larger cells in samples treated with US. In our research, the tissue
381 damage was not as great as that reported by Nowacka & Wedzik [24]. This might be
382 explained by the fact that considerably shorter times were used in this research (5 min).
383 In the investigation of Nowacka & Wedzik [24], the outcomes caused by the US
384 treatment were mainly due to the "sponge effect" since the sample was not in direct
385 contact with the solvent because of the vacuum packaging. In our research, the sample
386 was in direct contact with the solvent, and it is known that the results of US application
387 in a solid-liquid system are mainly due to the cavitation effect [19]. The implosion of
388 cavitation bubbles improves the solvent penetration into the solid through several
389 mechanisms such as microjet formation [53]. The solvent penetration could cause
390 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 Figure 7 and Table 3. Figure 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 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.

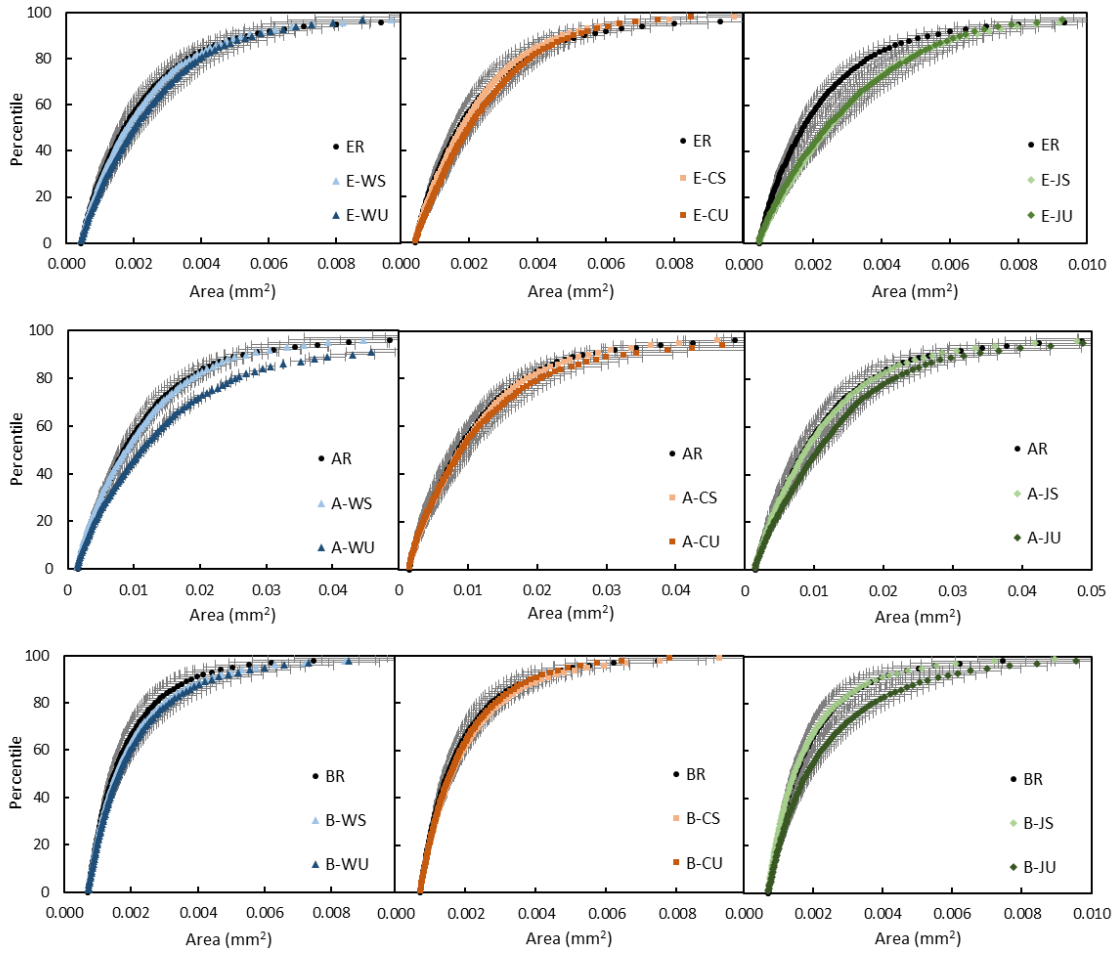


Figure 7. Cell area percentile profiles of eggplant (E), apple (A), and beetroot (B) samples: raw (control: ER, AR, and BR respectively) and subjected to an immersion treatment in water (W), citric acid (C), and the juice of the vegetable/fruit (J) without (S) and with ultrasound application (U) at 192 ± 6 W/L. The raw sample profile was added to all the charts to facilitate the comparison.

411 According to the cell area percentiles of eggplant (Figure 7), the profiles of the samples
412 treated with water and with citric acid without US application (E-WS and E-CS) practically
413 coincided with that of the control sample (ER). Thus, practically no osmosis was
414 observed with the distilled water, which would have been expected considering that the
415 solvent was hypotonic. Moreover, the citric acid, which was the solvent with the lowest
416 pH, was not able to significantly affect the cell walls of this sample under the studied
417 conditions. These results are also reflected in the d50 and the cells/area (Table 3). As
418 can be seen, there were no significant differences ($p > 0.05$) among the values of these
419 parameters on eggplant samples when comparing ER with the E-WS and E-CS samples.
420 A slight increase in the d50 was observed when comparing these samples (E-WS and E-
421 CS) with those that were subjected to US application (E-WU and E-CU). However, these
422 differences were not statistically significant ($p > 0.05$). On the other hand, there was a
423 significant ($p < 0.05$) increase in the area of the cells of the eggplants treated with the
424 eggplant juice without and with US (E-JS and E-JU), which can be observed in Figure 7
425 and the d50 figures (Table 3). This value was about 46 and 41 % larger for E-JS and E-JU
426 compared with ER, respectively. Also, significantly ($p < 0.05$) fewer cells/area were
427 observed on the samples treated with the eggplant juice (without and with US)
428 compared to the ER sample. This is consistent with the characteristics observed by SEM
429 for these samples. Overall, samples treated with US presented significantly ($p < 0.05$)
430 larger cells than ER only when the treatment was carried out in the vegetable juice.
431 However, this parameter in the E-JU sample was not significantly ($p > 0.05$) different to
432 that of the sample treated without US (E-JS). Therefore, in eggplant, the US did not
433 exhibit a significant ($p > 0.05$) effect on the size of the cells. This was probably because
434 eggplant was the most porous material, so even if US had boosted the solvent

435 penetration, it mostly occupied the intercellular spaces. Oladejo et al. [54] carried out
436 an osmotic dehydration pre-treatment of potato samples in distilled water with US (300
437 W for 20-60 min). They observed that the samples treated with US did not lose their
438 firmness because they had gained water which filled the intercellular spaces of the
439 potato, and this effect was not observed without US.

440 On the other hand, eggplant samples treated with US presented significantly ($p < 0.05$)
441 fewer cells/area when the treatment was carried out in water (E-WU) and eggplant juice
442 (E-JU) compared to ER. The decrease in the cells/area parameter without an increase in
443 the size of the cells, observed in E-WU, might be explained by the formation of more
444 intercellular space. It should be considered that, due to the large intercellular space in
445 eggplant microstructure, if some cell wall breakdowns occurred it did not always result
446 in the merger of two cells to form a larger cluster, but it would just probably cause the
447 formation of bigger intercellular spaces. Some examples of this effect are highlighted in
448 Figure 4 as IS (intercellular space) for samples treated with US in water and eggplant
449 juice. Rodrigues et al. [55] studied the effect of an immersion pre-treatment on papaya
450 samples with US application (10-30 min at 4870 W/m^2). They reported that papaya
451 tissue did not present intercellular space originally, but the application of US for 10 min
452 resulted in the formation of several large cell interspaces. Fernandes et al. [56], also
453 observed a significant increase of the intercellular space in pineapple samples when they
454 were subjected to an osmotic treatment with US application (30 min at 4870 W/m^2).
455 They reported that the US application resulted in the loss of adhesion among the cells
456 because of the solubilization of pectins of the middle lamella.

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

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

481 significantly ($p < 0.05$) fewer cells were observed in the samples treated with US in water
482 and apple juice than in the control sample.

483 The larger cells observed on apples in samples A-WU and A-JU could be a consequence
484 of the swelling of the cells because of solvent penetration but also of the cell wall
485 breakdowns that result in two or more cells merging into one larger cluster. Several
486 examples of this effect are highlighted in Figure 5 as merged cells (B). Nowacka & Wedzik
487 [24] also deduced from the percentile area profile of carrot samples, that an increase in
488 the cell size occurred because of the US application (3-4 W/m^2 for 10 to 30 min). In our
489 research, in the case of using water as a solvent, the US application probably intensified
490 the water transfer to the cells because of osmosis since the distilled water was a
491 hypotonic solution. Moreover, water was the less dense and viscous solvent used with
492 apple samples (Table 2). Thus, the cavitation bubbles were probably formed more easily
493 in this liquid [26]. The intensification of water transfer from a hypotonic solvent into
494 vegetable cells because of US application has already been reported by other authors.
495 For instance, Vasile et al. [8], who subjected apple samples to an immersion treatment
496 in water enriched with cyanocobalamin, observed a water gain with US application (200
497 W/L for 15 min) larger than that observed without US. Among the three investigated
498 materials, apple was the most affected when using water as an immersion medium. This
499 was probably because apple presented the highest concentration of soluble solids when
500 compared with beetroot and eggplant (Table 1), which means a higher difference in
501 osmotic pressure between the sample and water. The mass transfer intensification and
502 cell wall breakdown could be a consequence of the microjets promoted by the cavitation
503 bubbles that improve the solvent penetration into the solid and of the “sponge effect”
504 that keeps microchannels and pores free and promotes mass transfer through pumping

505 [16]. On the other hand, an important effect of the US application was also observed in
506 the apple juice. This could not be attributed to the physical characteristics of this solvent
507 since it was more viscous and denser than the water and the citric acid. Rodríguez et al.
508 [12] investigated an immersion pre-treatment for drying carried out with US application
509 (2-12 W/cm^2 for 5 min) and reported more evident damage of apple tissues when it was
510 carried out with the apple juice and with citric acid than with water, attributing it to the
511 low pH of these solvents. However, in this investigation, according to the image analysis
512 results, when applying US, the treatment with the apple juice caused larger cells than
513 the treatment with the citric acid, even when the latter had a lower pH. Therefore, as
514 occurred with the eggplant samples, the higher similarity of the solvent with the extra
515 and the intracellular fluid seemed to be the explanation for better solvent penetration.
516 For instance, the most abundant organic acid in apples is not citric acid but malic acid
517 [57], which should be present in apple juice [58]. The apple juice composition in
518 combination with the US application probably promoted degradation of the pectin
519 compounds of the apple cell walls enhancing the cell wall disruption and the liquid
520 entrance. In addition, in these immersion treatments, there is a multidirectional mass
521 exchange, including the transfer of water from the solvent to the sample or vice versa,
522 but also the penetration of low-molecular substances such as vitamins, saccharides, and
523 others [19]. This transfer of substances from the solvent to the solid must be more
524 significant when using the fruit juice as a solvent than when using water or citric acid
525 considering their composition.

526 Regarding the beetroot samples, the area percentile profiles of the control (BR) and the
527 samples treated with beetroot juice without US (B-JS) practically coincided (Figure 7).
528 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 (Figure 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

553 no significant ($p > 0.05$) differences between the samples treated with US and without
554 them in this solvent. Thus, these results indicate that the application of US to materials
555 with a lot of intercellular space (such as eggplants), under the conditions used in this
556 study, does not promote a significant change in the size of the cells, probably because
557 the solvent introduced into the material by the cavitation effect stays in the intercellular
558 space or generates even more porosity [59]. On the other hand, samples with a medium-
559 high porosity (apple) treated with US application, presented a significant ($p < 0.05$)
560 increase in the size of the cells and a decrease in the cells/area (compared with the
561 control and with samples treated without US) in two solvents (water and apple juice).
562 For the low-porosity material (beetroot), the US effect was only observed in the sample
563 juice. Therefore, apple samples were the most affected by the US application. Pieczywek
564 et al. [23] investigated the effect of US application (7.5-30 min at 10 kWh/kg) on the cell
565 wall stiffness of cylindrical apple samples. They observed that larger times of US
566 exposure resulted in lower cell wall stiffness. They also observed solubilization of pectin
567 material. Apple presented the largest cells among all the samples, thus, in comparison
568 with beetroot, apple presented lower density in “cell wall material”, making this tissue
569 more fragile and susceptible to US application.

570 4 Conclusions

571 This study evaluated the effect of US application in the microstructure of vegetables
572 with different tissue structures and porosity. The results indicate that US has different
573 effects depending on the initial microstructure of the raw matter. Overall, US application
574 stimulated solvent penetration into the vegetable cells, increasing their sizes and/or
575 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.

5 Acknowledgements

The authors would like to acknowledge the Spanish research agency (AEI) MCIN/AEI/10.13039/501100011033 for the financial support for the project (PID2019-106148RR-C43). We would also like to thank Dr. Fernando Hierro from the “Serveis Cientificotècnics” of the University of the Balearic Islands for his assistance with SEM and to Dr. Carlos Saus from the “Hospital Son Espases” for his assistance with samples preparation for optical microscopy.

6 Bibliography

[1] S.E. Demirel, J. Li, M.F. Hasan, Systematic process intensification, Curr. Opin. Chem. Eng. 25 (2019) 108–113. https://doi.org/10.1016/J.COCHE.2018.12.001.

[2] B. Khadhraoui, V. Ummat, B.K. Tiwari, A.S. Fabiano-Tixier, F. Chemat, Review of ultrasound combinations with hybrid and innovative techniques for extraction and processing of food and natural products, Ultrason. Sonochem. 76 (2021) 105625. https://doi.org/10.1016/J.ULTSONCH.2021.105625.

[3] Y.C. Cheung, K.C. Siu, J.Y. Wu, Kinetic Models for Ultrasound-Assisted Extraction of Water-Soluble Components and Polysaccharides from Medicinal Fungi, Food Bioprocess Technol. 6 (2013) 2659–2665. https://doi.org/10.1007/s11947-012-0929-z.

[4] N. Muñoz-Almagro, E. Morales-Soriano, M. Villamiel, L. Condezo-Hoyos, Hybrid high-intensity ultrasound and microwave treatment: A review on its effect on quality and bioactivity of foods, Ultrason. Sonochem. 80 (2021) 105835. https://doi.org/10.1016/J.ULTSONCH.2021.105835.

[5] C. Reche, C. Rosselló, M.M. Umaña, V. Eim, S. Simal, Mathematical Modelling of Ultrasound-Assisted Extraction Kinetics of Bioactive Compounds from Artichoke By-Products, Foods 2021, Vol. 10, Page 931. 10 (2021) 931. https://doi.org/10.3390/FOODS10050931.

[6] M. Umaña, V. Eim, C. Garau, C. Rosselló, S. Simal, Ultrasound-assisted extraction of ergosterol and antioxidant components from mushroom by-products and the attainment of a β-glucan rich residue, Food Chem. 332 (2020) 127390.

https://doi.org/10.1016/j.foodchem.2020.127390.

[7] F.A.N. Fernandes, T.R. Braga, E.O. Silva, S. Rodrigues, Use of ultrasound for dehydration of mangoes (Mangifera indica L.): kinetic modeling of ultrasound-assisted osmotic dehydration and convective air-drying, J. Food Sci. Technol. 56 (2019) 1793. https://doi.org/10.1007/S13197-019-03622-Y.

[8] F.E. Vasile, S. Simal, C. Rosselló, V.S. Eim, Power Ultrasound-Assisted Impregnation of Apple Cubes with Vitamin B12, Food Bioprocess Technol. 15 (2022) 219–229. https://doi.org/10.1007/S11947-021-02752-6/FIGURES/5.

[9] F. Vallespir, Ó. Rodríguez, J.A. Cárcel, C. Rosselló, S. Simal, Ultrasound assisted low-temperature drying of kiwifruit: Effects on drying kinetics, bioactive compounds and antioxidant activity, J. Sci. Food Agric. 99 (2019) 2901–2909. https://doi.org/10.1002/JSFA.9503.

[10] F. Abbasi, F. Samadi, S.M. Jafari, S. Ramezanpour, M. Shams Shargh, Ultrasound-assisted preparation of flaxseed oil nanoemulsions coated with alginate-whey protein for targeted delivery of omega-3 fatty acids into the lower sections of gastrointestinal tract to enrich broiler meat, Ultrason. Sonochem. 50 (2019) 208–217. https://doi.org/10.1016/J.ULTSONCH.2018.09.014.

[11] J.A. Gallego-Juárez, G. Rodríguez, E. Riera, A. Cardoni, Ultrasonic defoaming and debubbling in food processing and other applications, Power Ultrason. Appl. High-Intensity Ultrasound. (2015) 793–814. https://doi.org/10.1016/B978-1-78242-028-6.00026-0.

[12] Ó. Rodríguez, P.J. Llabrés, S. Simal, A. Femenia, C. Rosselló, Intensification of

643 Predrying Treatments by Means of Ultrasonic Assistance: Effects on Water
644 Mobility, PPO Activity, Microstructure, and Drying Kinetics of Apple, Food
645 Bioprocess Technol. 8 (2015) 503–515. https://doi.org/10.1007/s11947-014-
646 1424-5.

647 [13] F. Chen, S. Liu, Z. Zhao, W. Gao, Y. Ma, X. Wang, S. Yan, D. Luo, Ultrasound pre-
648 treatment combined with microwave-assisted hydrodistillation of essential oils
649 from Perilla frutescens (L.) Britt. leaves and its chemical composition and
650 biological activity, Ind. Crops Prod. 143 (2020) 111908.
651 https://doi.org/10.1016/J.INDCROP.2019.111908.

652 [14] L. Han, S. Cao, Y. Yu, X. Xu, X. Cao, W. Chen, Modification in physicochemical,
653 structural and digestive properties of pea starch during heat-moisture process
654 assisted by pre- and post-treatment of ultrasound, Food Chem. 360 (2021)
655 129929. https://doi.org/10.1016/J.FOODCHEM.2021.129929.

656 [15] J.E. González-Pérez, N. Ramírez-Corona, A. López-Malo, Mass Transfer During
657 Osmotic Dehydration of Fruits and Vegetables: Process Factors and Non-Thermal
658 Methods, Food Eng. Rev. 13 (2021) 344–374. https://doi.org/10.1007/S12393-
659 020-09276-3.

660 [16] A.C. Miano, A. Ibarz, P.E.D. Augusto, Mechanisms for improving mass transfer in
661 food with ultrasound technology: Describing the phenomena in two model cases,
662 Ultrason. Sonochem. 29 (2016) 413–419.
663 https://doi.org/10.1016/J.ULTSONCH.2015.10.020.

664 [17] C. Wen, J. Zhang, H. Zhang, C.S. Dzah, M. Zandile, Y. Duan, H. Ma, X. Luo, Advances
665 in ultrasound assisted extraction of bioactive compounds from cash crops – A

review, Ultrason. Sonochem. 48 (2018) 538–549. https://doi.org/10.1016/j.ultsonch.2018.07.018.

[18] S. Zhao, C. Yao, Q. Zhang, G. Chen, Q. Yuan, Acoustic cavitation and ultrasound-assisted nitration process in ultrasonic microreactors: The effects of channel dimension, solvent properties and temperature, Chem. Eng. J. 374 (2019) 68–78. https://doi.org/10.1016/j.cej.2019.05.157.

[19] M. Nowacka, M. Dadan, U. Tylewicz, Current Applications of Ultrasound in Fruit and Vegetables Osmotic Dehydration Processes, Appl. Sci. 2021, Vol. 11, Page 1269. 11 (2021) 1269. https://doi.org/10.3390/APP11031269.

[20] V.M. Karizaki, S. Sahin, G. Sumnu, M.T.H. Mosavian, A. Luca, Effect of Ultrasound-Assisted Osmotic Dehydration as a Pretreatment on Deep Fat Frying of Potatoes, Food Bioprocess Technol. 6 (2013) 3554–3563. https://doi.org/10.1007/S11947-012-1012-5/FIGURES/10.

[21] M. Nowacka, U. Tylewicz, L. Laghi, M. Dalla Rosa, D. Witrowa-Rajchert, Effect of ultrasound treatment on the water state in kiwifruit during osmotic dehydration, Food Chem. 144 (2014) 18–25. https://doi.org/10.1016/J.FOODCHEM.2013.05.129.

[22] A.C. Miano, M.L. Rojas, P.E.D. Augusto, Structural changes caused by ultrasound pretreatment: Direct and indirect demonstration in potato cylinders, Ultrason. Sonochem. 52 (2019) 176–183. https://doi.org/10.1016/J.ULTSONCH.2018.11.015.

[23] P.M. Pieczywek, A. Kozioł, D. Konopacka, J. Cybulska, A. Zdunek, Changes in cell

688 wall stiffness and microstructure in ultrasonically treated apple, J. Food Eng. 197
689 (2017) 1–8. https://doi.org/10.1016/j.jfoodeng.2016.10.028.

690 [24] M. Nowacka, M. Wedzik, Effect of ultrasound treatment on microstructure,
691 colour and carotenoid content in fresh and dried carrot tissue, Appl. Acoust. 103
692 (2016) 163–171. https://doi.org/10.1016/j.apacoust.2015.06.011.

693 [25] G.M. Gonzalez, Effects of power ultrasound treatments on properties of
694 Longissimus beef muscle, 2003. https://lib.dr.iastate.edu/rtd/1432 (accessed
695 April 23, 2021).

696 [26] J.P. Lorimer, T.J. Mason, Sonochemistry. Part 1—The physical aspects, Chem. Soc.
697 Rev. 16 (1987) 239–274. https://doi.org/10.1039/CS9871600239.

698 [27] F. Vallespir, Ó. Rodríguez, V.S. Eim, C. Rosselló, S. Simal, Freezing pre-treatments
699 on the intensification of the drying process of vegetables with different
700 structures, J. Food Eng. 239 (2018) 83–91.
701 https://doi.org/10.1016/J.JFOODENG.2018.07.008.

702 [28] H. Baniasadi, R. Ajdary, J. Trifol, O.J. Rojas, J. Seppälä, Direct ink writing of aloe
703 vera/cellulose nanofibrils bio-hydrogels, Carbohydr. Polym. 266 (2021) 118114.
704 https://doi.org/10.1016/J.CARBPOL.2021.118114.

705 [29] K.K. Valladares-Diestra, L. Porto de Souza Vandenberghe, L.A. Zevallos Torres, A.
706 Zandoná Filho, A. Lorenci Woiciechowski, C. Ricardo Soccol, Citric acid assisted
707 hydrothermal pretreatment for the extraction of pectin and xylooligosaccharides
708 production from cocoa pod husks, Bioresour. Technol. 343 (2022) 126074.
709 https://doi.org/10.1016/J.BIORTECH.2021.126074.

710 [30] G. Ferrer, C. Barreneche, A. Solé, I. Martorell, L.F. Cabeza, New proposed
711 methodology for specific heat capacity determination of materials for thermal
712 energy storage (TES) by DSC, J. Energy Storage. 11 (2017) 1–6.
713 https://doi.org/10.1016/J.EST.2017.02.002.

714 [31] F. Vallespir, Ó. Rodríguez, V.S. Eim, C. Rosselló, S. Simal, Effects of freezing
715 treatments before convective drying on quality parameters: Vegetables with
716 different microstructures, J. Food Eng. 249 (2019) 15–24.
717 https://doi.org/10.1016/j.jfoodeng.2019.01.006.

718 [32] R Core Team, R: A language and environment for statistical computing, (2017).

719 [33] F. de Mendiburu, Agricolae: Statistical Procedures for Agricultural Research,
720 (2016). https://cran.r-project.org/package=agricolae.

721 [34] R Core Team, Foreign: Read Data Stored by Minitab, S, SAS, SPSS, Stata, Systat,
722 Weka, dBase, (2017). https://cran.r-project.org/package=foreign.

723 [35] C.J. Boukouvalas, M.K. Krokida, Z.B. Maroulis, D. Marinos-Kouris, Density and
724 Porosity: Literature Data Compilation for Foodstuffs, Int. J. Food Prop. 9 (2006)
725 715–746. https://doi.org/10.1080/10942910600575690.

726 [36] B. Ma, J. Chen, H. Zheng, T. Fang, C. Ogutu, S. Li, Y. Han, B. Wu, Comparative
727 assessment of sugar and malic acid composition in cultivated and wild apples,
728 Food Chem. 172 (2015) 86–91.
729 https://doi.org/10.1016/J.FOODCHEM.2014.09.032.

730 [37] B. Ma, Y. Yuan, M. Gao, C. Li, C. Ogutu, M. Li, F. Ma, Determination of Predominant
731 Organic Acid Components in Malus Species: Correlation with Apple

732 Domestication, Metabolites. 8 (2018).
733 https://doi.org/10.3390/METABO8040074.

734 [38] E. Rosa-Martínez, M.D. García-Martínez, A.M. Adalid-Martínez, L. Pereira-Dias, C.
735 Casanova, E. Soler, M.R. Figàs, M.D. Raigón, M. Plazas, S. Soler, J. Prohens, Fruit
736 composition profile of pepper, tomato and eggplant varieties grown under
737 uniform conditions, Food Res. Int. 147 (2021) 110531.
738 https://doi.org/10.1016/J.FOODRES.2021.110531.

739 [39] J. Vasconcellos, C. Conte-Junior, D. Silva, A.P. Pierucci, V. Paschoalin, T.S. Alvares,
740 Comparison of total antioxidant potential, and total phenolic, nitrate, sugar, and
741 organic acid contents in beetroot juice, chips, powder, and cooked beetroot, Food
742 Sci. Biotechnol. 25 (2016) 79–84. https://doi.org/10.1007/S10068-016-0011-0.

743 [40] M.L. Tan, S.B.S. Hamid, Beetroot as a Potential Functional Food for Cancer
744 Chemoprevention, a Narrative Review, J. Cancer Prev. 26 (2021) 1–17.
745 https://doi.org/10.15430/JCP.2021.26.1.1.

746 [41] M. Schmutzler, C.W. Huck, Simultaneous detection of total antioxidant capacity
747 and total soluble solids content by Fourier transform near-infrared (FT-NIR)
748 spectroscopy: A quick and sensitive method for on-site analyses of apples, Food
749 Control. 66 (2016) 27–37. https://doi.org/10.1016/j.foodcont.2016.01.026.

750 [42] J. Prohens, A. Rodríguez-Burruezo, M.D. Raigón, F. Nuez, Total phenolic
751 concentration and browning susceptibility in a collection of different varietal
752 types and hybrids of eggplant: Implications for breeding for higher nutritional
753 quality and reduced browning, J. Am. Soc. Hortic. Sci. 132 (2007) 638–646.
754 https://doi.org/10.21273/jashs.132.5.638.

755 [43] M. Šlosár, A. Hegedusova, Yield parameters, antioxidant activity, polyphenol and
756 total soluble solids content of beetroot cultivars with different flesh colours, Folia
757 Hortic. 2 (2020) 351–362. https://doi.org/10.2478/fhort-2020-0030.

758 [44] E.Z. Al Anazi, Dental erosion caused by Granny Smith apples: An evidence-based
759 case report and 1-year follow-up, Clin. Case Reports. 6 (2018) 1689–1696.
760 https://doi.org/10.1002/ccr3.1702.

761 [45] R.C. Osidacz, M.C.B. Ambrosio-Ugri, Análise da qualidade físico-química de
762 berinjela desidratada com variações no pré-tratamento, Acta Sci. - Technol. 35
763 (2013) 175–179. https://doi.org/10.4025/actascitechnol.v35i1.10551.

764 [46] D. Trishitman, P.S. Negi, N.K. Rastogi, Concentration of beetroot juice colorant
765 (betalains) by forward osmosis and its comparison with thermal processing, LWT.
766 145 (2021) 111522. https://doi.org/10.1016/j.lwt.2021.111522.

767 [47] M. Teresa Pacheco, M. Villamiel, R. Moreno, F.J. Moreno, Structural and
768 Rheological Properties of Pectins Extracted from Industrial Sugar Beet By-
769 Products, Molecules. 24 (2019). https://doi.org/10.3390/MOLECULES24030392.

770 [48] J. Zheng, H. Li, D. Wang, R. Li, S. Wang, B. Ling, Radio frequency assisted extraction
771 of pectin from apple pomace: Process optimization and comparison with
772 microwave and conventional methods, Food Hydrocoll. 121 (2021) 107031.
773 https://doi.org/10.1016/J.FOODHYD.2021.107031.

774 [49] Y.R. Sekhar, K. V. Sharma, Study of viscosity and specific heat capacity
775 characteristics of water-based Al2O3 nanofluids at low particle concentrations,
776 Https://Doi.Org/10.1080/17458080.2013.796595. 10 (2014) 86–102.

https://doi.org/10.1080/17458080.2013.796595.

[50] S.D. Jayasooriya, B.R. Bhandari, P.& B.R. Torley, Effect of High Power Ultrasound Waves on Properties of Meat: A Review, Int. J. Food Prop. 7 (2004) 301–319. https://doi.org/10.1081/JFP-120030039.

[51] A. Puig, I. Perez-Munuera, J.A. Carcel, I. Hernando, J. V. Garcia-Perez, Moisture loss kinetics and microstructural changes in eggplant (Solanum melongena L.) during conventional and ultrasonically assisted convective drying, Food Bioprod. Process. 90 (2012) 624–632. https://doi.org/10.1016/J.FBP.2012.07.001.

[52] F. Vallespir, J.A. Cárcel, F. Marra, V.S. Eim, S. Simal, Improvement of Mass Transfer by Freezing Pre-treatment and Ultrasound Application on the Convective Drying of Beetroot (Beta vulgaris L.), Food Bioprocess Technol. 11 (2018) 72–83. https://doi.org/10.1007/S11947-017-1999-8/FIGURES/6.

[53] T. Mason, F. Chemat, M. Vinatoru, The Extraction of Natural Products using Ultrasound or Microwaves, Curr. Org. Chem. 15 (2011) 237–247. https://doi.org/10.2174/138527211793979871.

[54] A.O. Oladejo, H. Ma, W. Qu, C. Zhou, B. Wu, X. Yang, Influence of ultrasound pretreatments on diffusion coefficients, texture and colour of osmodehydrated sweet potato (Ipomea batatas), Int. J. Food Sci. Technol. 52 (2017) 888–896. https://doi.org/10.1111/IJFS.13352.

[55] S. Rodrigues, F.I.P. Oliveira, M.I. Gallão, F.A.N. Fernandes, Effect of immersion time in osmosis and ultrasound on papaya cell structure during dehydration, Dry. Technol. 27 (2009) 220–225. https://doi.org/10.1080/07373930802605883.

[56] F.A.N. Fernandes, M.I. Gallão, S. Rodrigues, Effect of osmosis and ultrasound on pineapple cell tissue structure during dehydration, J. Food Eng. 90 (2009) 186–190. https://doi.org/10.1016/J.JFOODENG.2008.06.021.

[57] Y. Han, Y. Wang, J. Li, J. Du, Z. Su, Evaluating the effect of bentonite, malic acid on pectin methyl esterase, methanol in fermented apple juice, J. Food Compos. Anal. (2022) 104468. https://doi.org/10.1016/J.JFCA.2022.104468.

[58] J. Dobrowolska-Iwanek, M. Gąstoł, A. Adamska, M. Krośniak, P. Zagrodzki, Traditional versus modern apple cultivars – a comparison of juice composition, Folia Hortic. 27 (2015) 33–41. https://doi.org/10.1515/FHORT-2015-0012.

[59] E.K. Méndez-Calderón, J.C. Ocampo-Castaño, C.E. Orrego, Optimization of convective drying assisted by ultrasound for Mango Tommy (Mangifera indica L.), J. Food Process Eng. 41 (2018) e12634. https://doi.org/10.1111/JFPE.12634.

Declaration of interests

☒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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

CRediT author statement

Mónica UMAÑA: Investigation, Data curation, Formal analysis, Writing-original draft, Visualization. **Marina CALAHORRO:** Investigation, Software, Validation, Data curation. **Valeria EIM:** Conceptualization, Methodology, Supervision. **Carmen ROSSELLÓ:** Resources, Writing-Review & Editing, Supervision. **Susana SIMAL:** Conceptualization, Formal analysis, Writing - Review & Editing, Supervision, Funding acquisition, Project administration.