

## Ultrasonic Effect on pH, Electric Conductivity, and Tissue Surface of Button Mushrooms, Brussels Sprouts and Cauliflower

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### Abstract

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The aim of this work was to use ultrasound pre-treatment as a potential method prior to the subsequent processing in the food industry, for button mushrooms, Brussels sprouts, and cauliflower in order to observe the impact of ultrasound on the vegetable surrounding media properties in the processing conditions. The samples treated with 20 kHz probe and 40 kHz bath for 3 and 10 min were compared with blanched (80°C/3 min) and untreated samples. The effect was followed of ultrasound and blanching treatments on pH, electrical conductivity, and temperature changes. The effect of ultrasound on the sample tissue surface was also studied. The pH decreased after the ultrasound treatment with the probe, the largest change having been observed after using a 20 kHz probe for 10 min in all samples as compared with the blanching treatment, whereas it increased in mushroom and cauliflower and decreased in Brussels sprouts. Electric conductivity of the surrounding water before and after the ultrasound and blanching treatments of vegetables increased with all the treatments suggesting the loss of electrolyte. The highest increase was observed with the blanching treatment in all samples, followed by the treatments using an ultrasonic bath (10 min > 3 min) and an ultrasonic probe (3 min > 10 min). The temperature increase in the surrounding water during the ultrasonic treatments was by 1°C using the bath, and by 25°C using the probe. Staining of cauliflower and button mushroom tissues surfaces carried out for the damage determination showed that cavitation damage (blue spots) was present after the ultrasonical treatment with 20 kHz probe for 3 min, followed by 20 kHz probe for 10 min, while very little cavitation damage occurred after sonication with 40 kHz bath for 3 and 10 min. In Brussels sprouts, the results showed cavitation after sonication with 20 kHz probe for 3 min, followed by 20 kHz probe for 10 min, but no cavitation was present after sonication with 40 kHz bath.

**Keywords:** ultrasonic pre-treatment; ultrasound probe; ultrasound bath; vegetable; functional properties; surface damage

Many pre-treatments in food industry have been used in order to preserve and extend the shelf life of food products such as fruits and vegetables,

prior to subsequent processing. Blanching is a common method that is used in food industry as a pre-treatment before canning, drying and free-

zing. Unfortunately, the whole process is often accompanied by an impairment of nutritional and functional properties due to the electrolyte and nutrient losses during the selected blanching type. Customers are also attracted to buying food products as a result of their visual perception of specific goods. Consumers demand minimally processed, fresh-like food with superior sensory and nutritional properties, so non-thermal food processing and preservation gain importance. The consumption of mushrooms has increased substantially due to their delicacy, flavour, and nutritional value. Mushrooms are an excellent source of several essential amino acids, vitamins (B<sub>2</sub>, niacin, and folates) and minerals (potassium, phosphorus, zinc, and copper) (MANZI *et al.* 2001; MATILA *et al.* 2001). Brassica vegetables belong to the Cruciferous family; they include different species of cabbage (white, red, savoy, swamp, chinese), cauliflower, broccoli, Brussels sprouts, and kale. These vegetables possess both antioxidant and anticarcinogenic properties (VERHOEVEN *et al.* 1997; COHEN *et al.* 2000; CHU *et al.* 2002). During blanching and other processing steps, the tissue is damaged, initiating chlorophyll degradation (HEATON & MARANGONI 1996).

Traditional processes for the pre-treatment of plant materials involve thermal processing (blanching, etc.), however, the emergence of novel non-thermal processes provides the possibility of producing minimally processed foods. Some of these non-thermal processes (high pressure – HP, high intensity electric field pulses – HELP, ultrasound – US, supercritical carbon dioxide, etc.) were applied in the processing of food materials. The results showed an enhanced mass transfer during osmodehydration of HP treated pineapples, HELP treated carrots and coconut, etc. (RASTOGI & NIRANJAN 1998; RASTOGI *et al.* 1999; ADE-OMOWAYE *et al.* 2000).

High power ultrasound field leads to the generation of cavitation bubbles. During ultrasonic treatment, high frequency acoustic signals are used to initiate the cavitation process. The ultrasonic field applied leads to the breakdown of cohesive forces of the liquid molecules, resulting in the generation of cavitation bubbles. During the collapse of the cavitation bubbles shock waves arise which propagate in the surrounding medium forming jet streams that cause the disruption of cells. Earlier work showed the deterioration effects of ultrasonics on microbiological activities

(SCHLAFFER *et al.* 2000). Ultrasonically induced cavitation is defined as the formation, growth, and collapse of gaseous or vaporous bubbles under the influence of ultrasound. It is used widely in ultrasonic cleaners for industry, in cell disruptions for biological studies etc. Current research issues include the applications of this phenomenon on sonochemistry and chemical processing (PESTMAN *et al.* 1994; LAUTERBORN & OHL 1998). However, the chemical effects of ultrasound originate from hot spots formed during the collapse of acoustic cavitation bubbles (HATANAKA *et al.* 2001).

FERNANDEZ-MORENO (2005) showed that the damage of green pepper surface is caused by the effect of cavitation bubbles. When using the ultrasonic probe, a more aggressive effect upon the surface was observed. It caused larger damaged areas, more extensive, deep and in more localised way. The surface was more damaged where probe tip was near to the pepper surface, decreasing with the increasing distance between the tip and the point on the surface. Otherwise, the effect of ultrasonic bath was more superficial and less aggressive for the tissue, and the damaged areas appeared on the surface more randomly.

The aim of this work was to use ultrasound as a pre-treatment method in order to observe the impact of ultrasound on the vegetable surrounding media properties in the processing conditions. The use of ultrasound is a non-thermal process that can be used in food processing to achieve better products. Parameters such as pH, electric conductivity, and temperature changes upon sonication and blanching treatments were measured. The effect of ultrasound on the sample tissue surfaces was also studied.

## MATERIAL AND METHODS

**Raw material.** Button mushrooms, *Agaricus biosporus* (moisture 9.7 g/100 g sample), Brussels sprout, *Brassica oleracea* var. *gemmifera* (moisture 5.6 g/100 g sample), and cauliflower, *Brassica oleracea* var. *botrytis* (moisture 10.9 g/100 g sample) were purchased in the local supermarket Sainsbury's in Coventry, UK. Brussels sprout and cauliflower were washed, and button mushrooms were wiped before the ultrasound treatment. Mushrooms were very small, so they were not cut (dimensions: 2 cm × 1.5 cm), but Brussels sprout (in half) and cauliflower were cut to small fragments with knife (dimensions: 1.5 × 1.5 cm). As

the comparative method, the blanching treatment was introduced. For the blanching treatment, 10 g of each sample were placed separately in 400 ml beaker and 150 ml of hot water was added each weighed sample. Blanching was performed at 80°C for 3 min, maintaining the temperature constant with HEAT STIR Labline Pyro-Magnestir 1266 hotplate stirrer.

**Ultrasound treatment.** 10 g of each sample were placed separately in 400 ml beaker and 150 ml of distilled water of the temperature of 18°C was added to each weighed sample. They were sonicated for 3 and 10 min with power ultrasound, high intensity and low frequency, 20 kHz probe (Sonics & Materials Inc., Danbury, CT., USA, Model V1A, power 600 W) attached to a transducer so that a high power intensity could be obtained (Jencons Scientific Ltd. – Ultrasonic processor). The probe had a vibrating titanium tip 1.2 cm in diameter and was immersed in the liquid near the sample (1–2 cm distance). The liquid was irradiated with an ultrasonic wave directly from the horn tip. The ultrasonic power in this ultrasonic experiment was 39–43 W/cm<sup>2</sup>, as measured calorimetrically by thermocouple Hanna Instruments, model HI 9063. Since the ultrasonic irradiation of a liquid produces heat, recording the temperature as a function of time leads to the acoustic power estimation (in W) by the equation:

$$P = mC_p (dT/dt) \quad (1)$$

where:

- $m$  – mass of the sonicated liquid (g)
- $C_p$  – its specific heat at a constant pressure (J/gK)
- $dT/dt$  – slope at the origin of the curve. It is expressed in watts per unit area of the emitting surface (W/cm<sup>2</sup>), or in watts per unit volume of the sonicated solution (W/cm<sup>3</sup>)

For the ultrasound treatment with ultrasound bath (40 kHz), 50 g of each vegetable sample were weighed and placed directly into the cleaning bath and sonicated for 3 and 10 minutes. The samples were treated with 40 kHz bath (Sonomatic, Model SO375T, HF-Pk-power 300 W). An ultrasonic transducer was attached to the outer surface of the liquid container and the liquid was irradiated with an ultrasonic wave from the surface of the liquid container. A standing wave of an ultrasonic wave is formed inside the liquid. The typical acoustic amplitude in a standing wave type sonochemical reactor is much smaller than that in a horn-type

sonochemical reactor (TUZIUTI *et al.* 2002). In this ultrasonic experiment, the ultrasonic power was 0.5 W/cm<sup>2</sup>, as measured calorimetrically by thermocouple Hanna Instruments, model HI 9063. After the ultrasound treatment, the temperature was raised by 1°C–4°C with the bath and by 7°C to 25°C with the probe as shown in Table 3.

**pH determination.** pH of the surrounding water before and after the ultrasound and the blanching treatments of vegetables were determined by pH meter, Pye Model 292, Pye Unicam. The measurements were conducted by placing pH electrode in the surrounding water of vegetables.

**Electric conductivity determination.** Conductivities of the surrounding water before and after the ultrasound and the blanching treatments of vegetables were determined by Conductometer (PTI-8 Digital Conductivity Meter, Scientific Industries International Inc., UK). The measurements were conducted on placing the electrode of the instrument into the surrounding water of vegetables.

Staining of samples for cavitation damage determination. After the ultrasound treatment, the samples were stained to determine the cavitation damage. For the staining procedure, the method for the cell death determination by KOCH and SLUSARENKO (1990) was used with some modifications. The damaged areas were stained with lactophenol trypan blue solution (20 g phenol, 20 ml lactic acid, 20 ml glycerine, 20 ml water and 0.1% trypan blue (w/v) in 80 ml). The tissues were boiled for 1 min in lactophenol trypan blue. Then, the samples were cleared in 25% chloralhydrate solution. The solution was changed twice during the next hour. The samples were stored in 25% chloralhydrate solution (ATIA *et al.* 2004).

The sample pieces were placed under a magnifying microscope (SDZ Stereo Zoom Microscope, Kyowa Optical Co, Ltd. Japan) with transmitted light and oblique surface illuminator, and pictures of each were taken by Digital Microscope Eyepiece (DME) attached to the computer and microscope, using Ulead<sup>®</sup> Photo Explorer 7.0 software.

**Statistical analyses.** The whole study was repeated and each value represents the mean of three measurements from two independent ultrasound treatments. The effect of the ultrasound treatment on the properties investigated was determined by analysis of variance, using statistical analyses with SPSS for Windows version 13.0 (SPSS Inc., Chicago). Analysis of variance (One-Way ANOVA)

was carried out to assess whether the different treatments led to statistically different results for the variables evaluated. To test significant differences between the means of measurements, Duncan's Multiple Range Test was used. The significant level used was 5% ( $\alpha = 0.05$ ).

## RESULTS AND DISCUSSION

In the present study, the parameters for pH and electric conductivity changes of the surrounding water of vegetables before and after the treatments were measured. pH decreased ( $P < 0.05$ ) after the ultrasound treatments with the probe and the bath (for Brussels sprouts), the highest decrease occurring with 20 kHz probe for 10 min in all samples (Table 1). The leaching of the cell constituents during the ultrasound treatment and the extent of the electrolyte release into the solution under atmospheric pressure were similar to those in osmotic dehydration (TAIWO *et al.* 2003). The decrease in pH was the highest with the ultrasound treatment with 20 kHz probe for 10 min because the probe is more powerful than the 40 kHz bath. Due to cavitation, the ultrasound produces a series of effects when it travels across a medium. That

can affect the mass transfer (KNORR *et al.* 2004), so the vegetable samples are more apt for the leakage of organic acids from their interior. The high intensity (20 kHz probe) of the waves generates the growth and collapse of bubbles inside liquids, a phenomenon known as cavitation (MASON & CORDEMAN 1996). This phenomenon produced by sonication consists of the formation of bubbles in the liquid which can explosively collapse and generate a localised pressure (MASON 1990), thereby causing the leaching of the cell constituents. The blanching treatment caused increased pH values of the surrounding water of mushrooms and cauliflower, and decreased pH values in the case of Brussels sprouts.

Electric conductivity of the surrounding water of vegetables increased ( $P < 0.05$ ) with all treatments suggesting electrolyte losses (Table 2), since the electric conductivity of intercellular juice is significantly higher than the conductivity of the plasmatic membranes that cover the cells which are disrupted by ultrasound and thermal treatments. The highest increase was observed with the blanching treatment in all samples ( $P < 0.05$ ), because heating causes an increase in the electric conductivity due to the thermal denaturation of

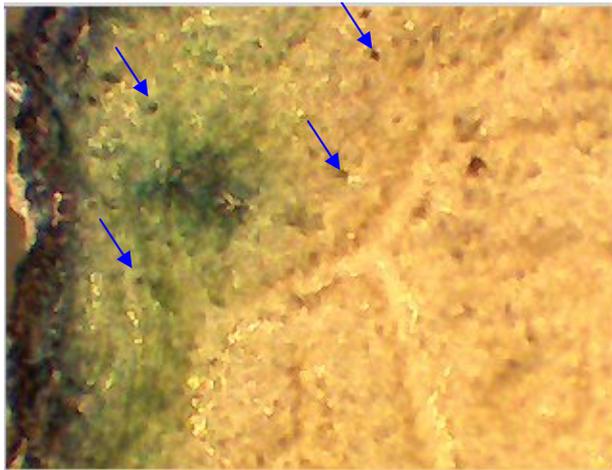
Table 1. pH values of surrounding water before and after ultrasound and blanching treatments of vegetables

Samples	Button mushrooms	Brussels sprouts	Cauliflower	Distilled water without samples
No ultrasound	5.1 <sup>a</sup>	6.7 <sup>d</sup>	5.5 <sup>c</sup>	4.3 <sup>b</sup>
20 kHz probe – 3 min	5.0 <sup>a</sup>	5.1 <sup>a</sup>	5.2 <sup>a</sup>	4.3 <sup>b</sup>
– 10 min	4.4 <sup>b</sup>	4.5 <sup>b</sup>	4.8 <sup>b</sup>	4.6 <sup>c</sup>
40 kHz bath – 3 min	5.2 <sup>c</sup>	6.5 <sup>d</sup>	5.6 <sup>c</sup>	4.3 <sup>b</sup>
– 10 min	5.4 <sup>c</sup>	6.3 <sup>c</sup>	5.9 <sup>d</sup>	4.4 <sup>b</sup>
Blanching	5.8 <sup>d</sup>	5.7 <sup>e</sup>	6.2 <sup>e</sup>	

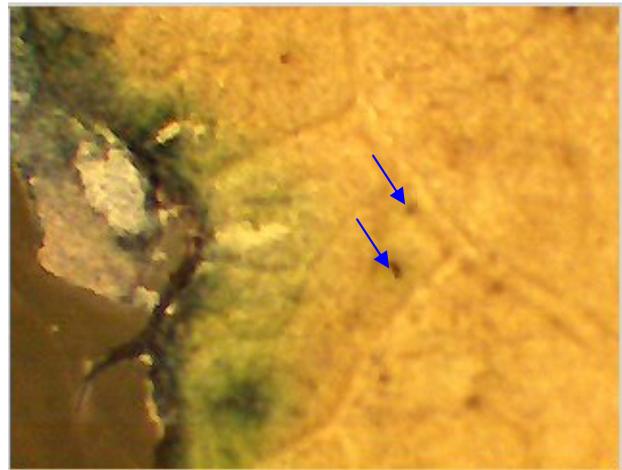
The mean values in the column are significantly different ( $P < 0.05$ ) if they are not marked with the same letter (Duncan's Multiple Range Test)

Analysis of variance (ANOVA) to assess whether the different treatments led to statistically different results for the variables evaluated

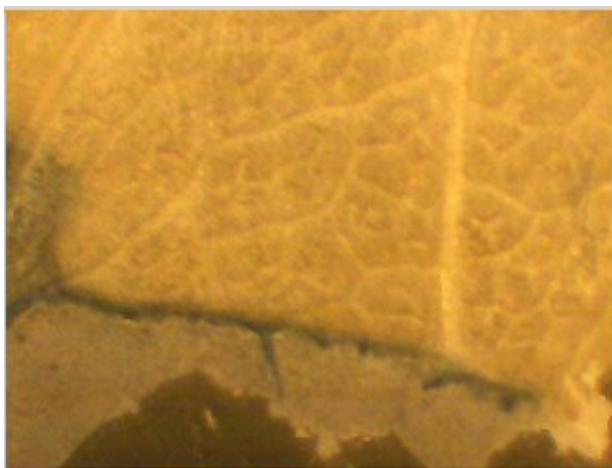
Source of variation	SS	df	MS	F	P-value	F <sub>crit</sub>
Treatments	4.402778	5	0.880556	5.351114	0.011901	3.325835
Samples	1.281111	2	0.640556	3.89264	0.056195	4.102821
Error	1.645556	10	0.164556			
Total	7.329444	17				



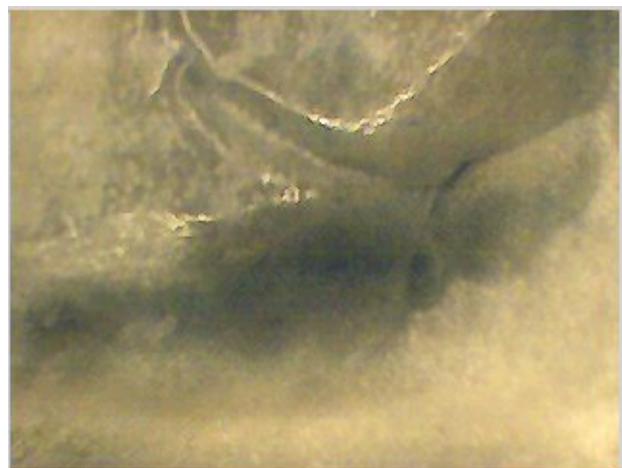
20 kHz probe – 3 min



20 kHz probe – 10 min



40 kHz bath – 3 min



40 kHz bath – 10 min



Untreated

the vegetable tissues. This increase in the electric conductivity could be attributed to the observed progressive enrichment of the surrounding water with mineral salts and organic acids coming from the samples.

The conductivity of the surrounding water of vegetables was higher on using ultrasonic

Figure 1. Staining of Brussels sprouts surface tissue for cavitation damage after ultrasound treatment and of untreated one (2× magnification)

bath (10 min > 3 min) than ultrasonic probe (3 min > 10 min), because of the larger surface exposed to ultrasonic waves in the bath where samples were placed directly into the cleaning bath. That does not mean that the bath ruptures the surface more extensively than the probe, but with the probe, conductivity was increased be-

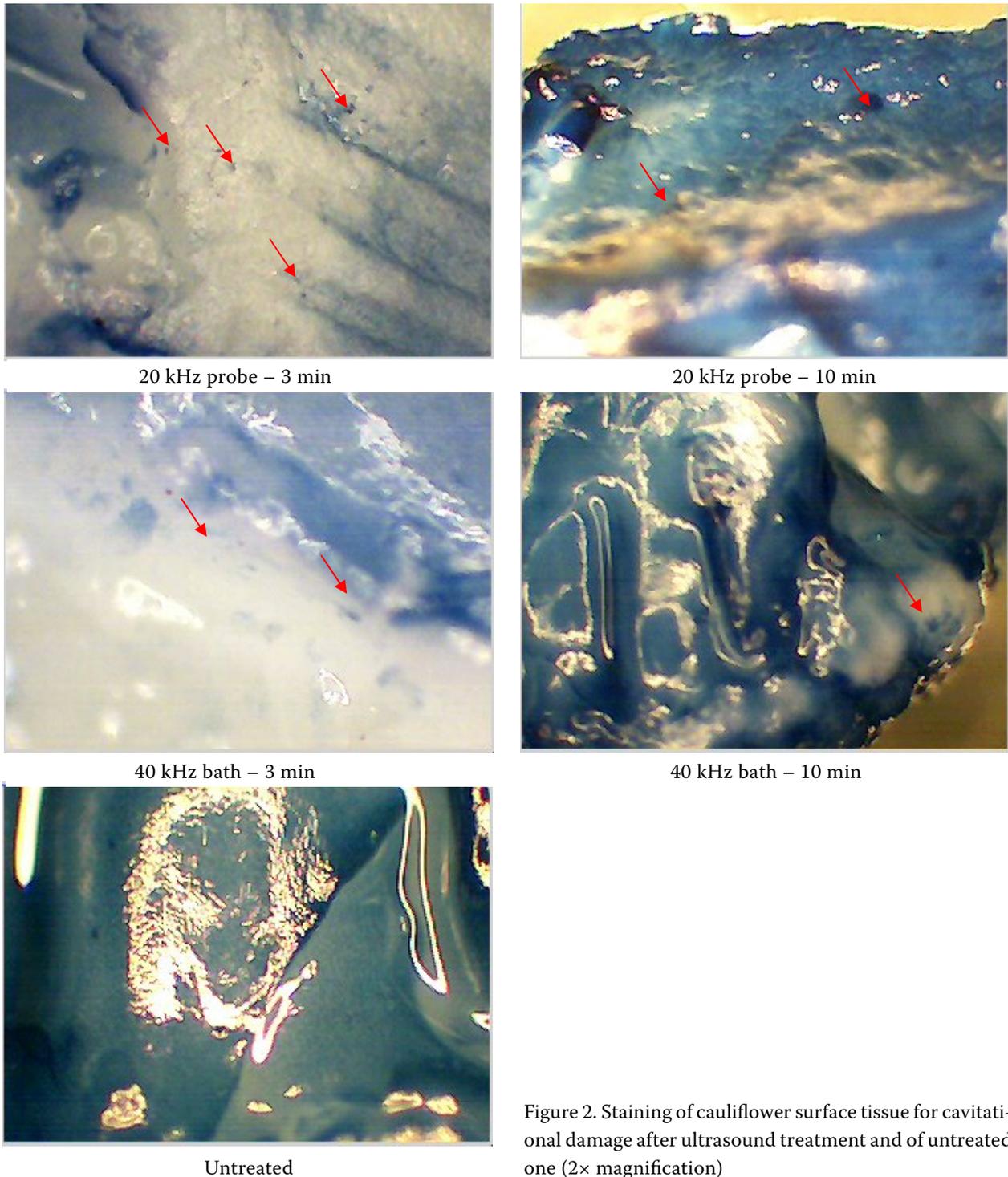


Figure 2. Staining of cauliflower surface tissue for cavitation damage after ultrasound treatment and of untreated one (2× magnification)

cause of acoustic cavitation (expansion and rapid adiabatic collapse of gas bubbles in solution) that can generate sufficient energy to produce radicals, high pressure, and temperature elevation in the gas phase of the bubbles, in the core of the bubble, and in its liquid interface. Conductivity of the surrounding water of cauliflower and button

mushrooms was lower for ultrasonic probe after 10 min than after 3 min, because a longer exposure of the material to ultrasound waves can cause the closure of pores, on the surface of the material, therefore the mass transfer (MASON 1990) and the cell constituents leakage are slower. This was not shown for Brussels sprouts because it possesses

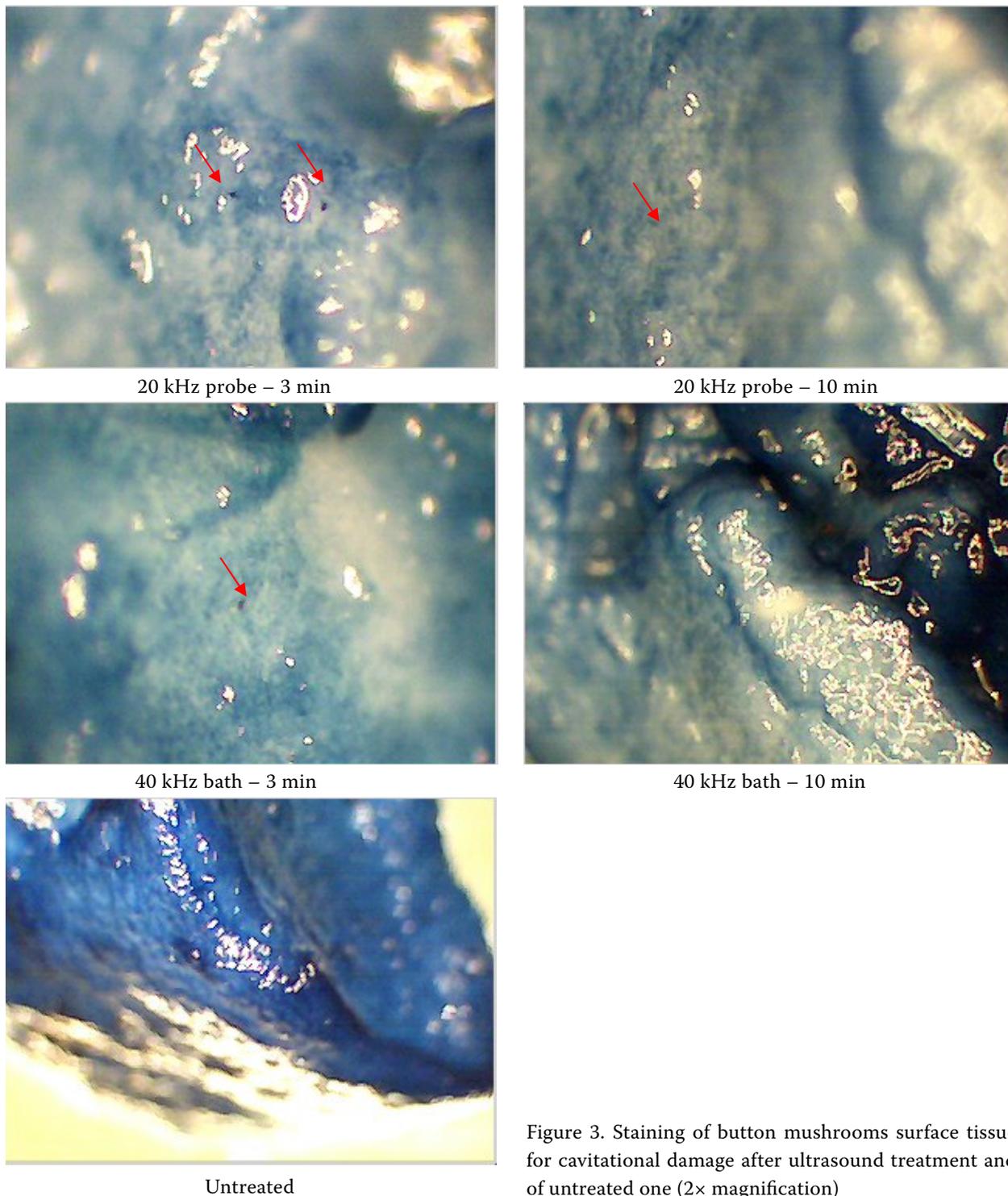


Figure 3. Staining of button mushrooms surface tissue for cavitation damage after ultrasound treatment and of untreated one (2× magnification)

a harder structure than cauliflower and button mushrooms which have a thinner surface structure, so the filling of pores proceeds faster than in Brussels sprouts. Thereby, ultrasound disrupts biological membranes, probably by a combination of the cavitation phenomena and the associated shear disruption, localised heating, and the free radical

formation. In the aqueous medium, water molecules decompose to form the  $H^+$  and  $OH^-$  radicals as the main primary radicals (MCLEAN & MORTIMER 1988; SCHNEIDER *et al.* 2006). Different values of pH and conductivity after the ultrasound probe and the bath treatment can be explained on the basis of the main factors limiting acoustic cavita-

Table 2. Electric conductivity ( $\mu\text{S}/\text{cm}$ ) values of surrounding water before and after ultrasound and blanching treatments of vegetables

Samples	Button mushrooms	Brussels sprouts	Cauliflower	Distilled water without samples
No ultrasound	40 <sup>a</sup>	20 <sup>a</sup>	30 <sup>a</sup>	2.40 <sup>a</sup>
20 kHz probe – 3 min	100 <sup>b</sup>	30 <sup>b</sup>	80 <sup>c</sup>	3.72 <sup>b</sup>
– 10 min	40 <sup>a</sup>	50 <sup>c</sup>	50 <sup>b</sup>	3.28 <sup>b</sup>
40 kHz bath – 3 min	200 <sup>c</sup>	150 <sup>d</sup>	190 <sup>d</sup>	2.13 <sup>c</sup>
– 10 min	450 <sup>d</sup>	340 <sup>e</sup>	500 <sup>e</sup>	2.50 <sup>a</sup>
Blanching	900 <sup>e</sup>	560 <sup>f</sup>	1270 <sup>f</sup>	

The mean values in the column are significantly different ( $P < 0.05$ ) if they are not marked with the same letter (Duncan's Multiple Range Test)

Analysis of variance (ANOVA) to assess whether the different treatments led to statistically different results for the variables evaluated

Source of variation	SS	df	MS	$F$	$P$ -value	$F_{\text{crit}}$
Treatments	1771244	5	354248.9	18.60008	8.917E-05	3.325835
Samples	79411.11	2	39705.56	2.084768	0.1750741	4.102821
Error	190455.6	10	19045.56			
Total	2041111	17				

tion like degassing of liquid, high atmospheric pressure, temperature elevation, and irradiation with increasing US frequencies. The temperature increase ( $^{\circ}\text{C}$ ) of the surrounding water before and after the ultrasound and the blanching treatments of vegetables was on the ultrasonic treatments  $1^{\circ}\text{C}$  using the bath, and  $25^{\circ}\text{C}$  using the probe (Table 3). The temperature rise caused by the probe treatment was higher because of the higher power introduced in the system as compared to the bath. With the ultrasound bath, the temperature of the surrounding water of vegetables was raised after ultrasonic treatments by  $1^{\circ}\text{C}$  or by  $4^{\circ}\text{C}$  (Table 3), and there was lesser damage to the cell membrane (LEBOVKA *et al.* 2000).

From the results shown, and according to the pictures taken after the staining of Brussels sprouts (Figure 1), cauliflower (Figure 2) and button mushrooms (Figure 3) showing the cavitation damage after the most powerful ultrasound treatment (20 kHz for 3 min), it is obvious that the vibrations do indeed disturb the cell walls and thereby facilitate the removal of the cell contents. Acoustic cavitation (spots) that is shown for the probe treatment for 3 min and 10 min, leads to cellular

damage or to the destruction of cells in close proximity to the collapsing bubble, *via* microstreaming leading to a direct mechanical effect by immediate membrane damage and by the high pressure and shearing forces that are generated by the propagating shock wave.

Ultrasonic waves can alternatively create positive and negative pressures in a liquid. The positive pressure generated by ultrasonic waves can compress liquid molecules, while on the other hand, the negative pressure generated by ultrasonic waves will expand or bulge liquid molecules. When the pressure produced by ultrasonic waves becomes larger than the corresponding liquid hydrostatic pressure, the liquid molecules become twisted and hollowed out to form cavities (Figures 1–3).

In addition, the cavities in the liquid are compressed by the ultrasonic wave and produce a collapsing explosion accompanied by heat generation among the liquid molecules (CHERNG-YUAN & LI-WEI 2006).

Cavitation-related tissue effects shown in Figures 1–3 after the probe treatment arise from a complex array of interactions between microbubbles and tissue which include concomitant processes of

Table 3. Temperature (°C) of surrounding water before and after ultrasound and blanching treatments of vegetables

Samples	Button mushrooms	Brussels sprouts	Cauliflower	Distilled water without samples
No ultrasound	16 <sup>a</sup>	16 <sup>a</sup>	16 <sup>a</sup>	18 <sup>a</sup>
20 kHz prob – 3 min	23 <sup>b</sup>	24 <sup>e</sup>	26 <sup>e</sup>	27 <sup>d</sup>
– 10 min	41 <sup>c</sup>	40 <sup>c</sup>	41 <sup>c</sup>	29 <sup>c</sup>
40 kHz bath – 3 min	17 <sup>a</sup>	17 <sup>a</sup>	17 <sup>a</sup>	20 <sup>a</sup>
– 10 min	20 <sup>b</sup>	20 <sup>b</sup>	20 <sup>b</sup>	22 <sup>b</sup>
Blanching	80 <sup>d</sup>	80 <sup>d</sup>	80 <sup>d</sup>	

The mean values in the column are significantly different ( $P < 0.05$ ) if they are not marked with the same letter (Duncan's Multiple Range Test)

Analysis of variance (ANOVA) to assess whether the different treatments led to statistically different results for the variables evaluated

Source of variation	SS	df	MS	<i>F</i>	<i>P</i> -value	<i>F</i> <sub>crit</sub>
Treatments	9170.667	5	1834.133	4232.615	2.76E-16	3.325835
Samples	1	2	0.5	1.153846	0.354093	4.102821
Error	4.333333	10	0.433333			
Total	9176	17				

mechanical damage resulting from microstreaming and microbubble oscillation, growth, and collapse behaviour, and thermal damage resulting from the enhanced local heating caused by absorption of acoustic energy by microbubbles present in the field.

Cavitation results after the treatment with 20 kHz probe, because when the ultrasonic power is increased, the cavitation bubbles enclosing the radiant surface of the transducer are larger and denser and form a more effective acoustic shielding, so more intensive ultrasonic attenuation happens and more ultrasonic energy is converted into heat energy than after the treatment with 40 kHz bath. Very little damage but no cavitation can be observed after sonication with 40 kHz bath for 3 and 10 min in Brussels sprouts (Figure 1) because of pectins, hemicellulose, and cellulose present in the vegetable sample that are strong cell constituents and act preventively to the mechanical damage which is opposite in the case of cauliflower and button mushrooms. They have smooth and tiny surfaces that had been already disrupted in general by customers in the supermarket and during handling in the laboratory due to which the whole surface was damaged (blue) (Figures 2 and 3).

## CONCLUSIONS

pH of the surrounding water of vegetables decreased after the ultrasound treatments with the probe, the highest decrease being with 20 kHz probe for 10 min in all samples as compared with the blanching treatment, whereas it increased in mushrooms and cauliflower, and decreased in Brussels sprouts. Conductivity of the surrounding water of vegetables increased on all the treatments suggesting electrolyte loss. The highest increase was observed on the blanching treatment in all samples, followed by ultrasonic bath (10 min > 3 min) and ultrasonic probe (3 min > 10 min).

The temperature of the surrounding water of vegetables was raised after ultrasonic treatments by 1°C (bath) to 25°C (probe), and it can be concluded that the ultrasonic effect on the system temperature was lesser than that of blanching. This is important because no damage caused by high temperature occurs to vegetable tissues due to sonication.

Staining of cauliflower and mushroom tissues surfaces in order to determine damage showed that cavitation damage (blue spots) was present after ultrasonical treatment with 20 kHz probe for 3 min, followed by 20 kHz probe for 10 min,

while very little damage occurred with sonication with 40 kHz bath for 3 and 10 min. For Brussels sprouts, the results showed cavitation present after sonication with 20 kHz probe for 3 min, followed by 20 kHz probe for 10 min, but no cavitation was present after sonication with 40 kHz bath. In the untreated samples, little damage was present because it was caused only during the preparation of the experiment, the handling during the transport, and by customers and employees in the supermarket.

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