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Direct contact ultrasound assisted freezing of chicken breast samples

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ABSTRACT

Nowadays, rapid freezing is sought to favor the formation of small ice crystals. Several studies have shown that the application of ultrasounds (US) accelerates the processes of energy and mass transfer when they are applied through immersion systems. However, there are hardly any studies on its application in direct systems without the use of a liquid medium for its transmission. Therefore, the objective of this work was to evaluate the potential of the application of US for improving the freezing process of chicken breast samples. First, the application of intermittent US treatments at different net sonication times of 7, 17, 37, 50 and 67% during the freezing of distilled water samples in a conventional freezer was evaluated. It was observed that net sonication times of 37, 50 and 67% reduced the phase change period by 30.0, 21.4, 27.0%, respectively. The effective freezing time was also reduced by 12.4 and 12.8% by applying net sonication times of 37 and 50%. Considering these results, an intermittent US treatment with a net sonication time of 37% was chosen for chicken breast freezing in an air-forced cooling tunnel at ambient temperatures from −13 to −22 °C. The length of all the freezing phases was reduced upon application of US, leading to an overall process time reduction of approx. 11%. On the other hand, no significant differences were found either in the Water Holding Capacity (WHC) or Cooking Loss (CL) values between control and US assisted frozen chicken breast samples. Furthermore, *in vitro* experiments showed that US-assisted freezing did not influence protein digestibility of chicken meat samples.

This study demonstrates the potential of the application of US by direct contact to favor energy transfer processes during freezing of water and chicken breasts samples. However, its effect on the quality of the frozen products should be further studied.

1. Introduction

Freezing is one of the most traditional food preservation processes used in the food industry as it extends the shelf life of food by inhibiting microbial growth and slowing down chemical and enzymatic spoilage reactions [1]. It consists of decreasing the temperature of food below the freezing point of water causing the conversion of liquid water to ice crystals. Therefore, freezing implies a process of heat/energy transfer. During freezing, microstructural changes potentially affecting the quality, texture and shelf life of frozen food occur. The magnitude of these changes largely dependents on the size and shape of the ice crystals [2–4]. Thus, the quality of frozen food is highly determined by the size distribution, location and shape of the ice crystals [5,6]. When freezing occurs at slow speeds, the formed ice crystals are large, with sharp edges and mainly located extracellularly causing severe cellular damages, and leading to different processes such as cell contraction, dehydration and thawing loss [4]. However, when freezing speed rates are higher, the generated ice crystals are smaller, more numerous and both located intra- and extracellularly minimizing product quality losses [4,7]. Currently, quick freezing processes are sought although this increase in speed implies higher energy and economic costs. For this reason, technologies capable of improving the energy transfer and, consequently reducing the costs of freezing processes are being investigated. Various emerging technologies have been investigated for this purpose, including high hydrostatic pressure, dehydro-freezing, magnetic field freezing and ultrasound (US) assisted freezing [7].

In this work US technology was chosen due to its high potential in the food industry for improving mass and energy transfer processes [8]. US is included within the technologies considered as “Green Food Processing” [9] since it allows to reduce processing time and energy

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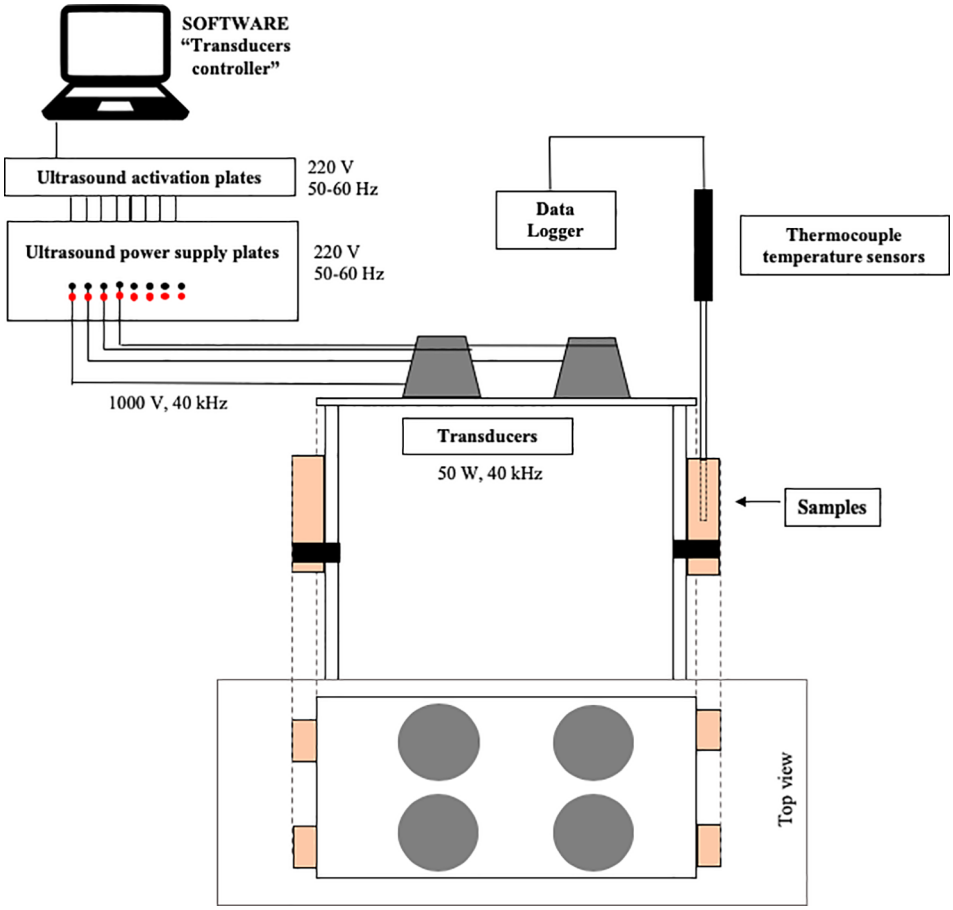

Fig. 1. System for applying US by direct contact to the samples.

consumption resulting in more sustainable food processing. US can be defined as sound waves at a frequency above 16 kHz, which is near the upper limit of the human hearing range. The application of high intensity ultrasounds (> 1 W/cm^2) in the food industry has been investigated for cleaning processes, inactivation of microorganisms, degassing, atomisation, homogenisation and emulsification, drying, dehydration, and extraction, among others [10–12]. The transmission and effect of US depends basically on the propagation medium of acoustic waves (liquid, solid or gas), the process conditions (temperature, pressure and intensity) and the structure of the product [13]. Most of the investigations carried out on the application of US for food processing have been done using immersion systems (ultrasonic baths) where the product is immersed in a liquid medium (i.e. water). In these types of systems, the sound waves are transmitted through the liquid medium and the effects that occur are mainly physical (cavitation, formation of microjets and microchannels), but also chemical effects can occur depending on the frequency of the baths. There are several studies dealing with US assisted immersion freezing of food and the results show that this technology can accelerate the freezing process by promoting the initiation of nucleation, the control of ice crystal growth and the increase of heat and mass transfer [8,9]. Sun et al [14] observed that by applying US-assisted immersion freezing on samples of common carp the freezing time was reduced by 37.2% at frequencies of 30 and 175 kHz achieving cooking loss values similar to those of the fresh product (8.9% with US vs 7.9% in fresh product). In another study, Zhang et al. [15] evaluated the effect of immersion freezing US on pork loin and observed that at an ultrasonic power of 180 W and frequency of 30 kHz freezing times were reduced by 12.6%. Smaller and more uniformly distributed ice crystals were formed and thawing and cooking losses were reduced by 61% and 12.32%, respectively, as compared to the forced air freezing system.

However, there are very few studies on the effect of the microvibration generated by the acoustic waves when applied in a system in which they are in direct contact with the food without using a fluid medium (liquid or gas) for its propagation [8]. Prototypes designed for direct transmission of the acoustic waves have been evaluated mainly for improving drying and freeze-drying processes [16–19]. These systems are based on the presence of a transducer or ultrasonic horn attached to a plate where the samples are placed so that the vibration generated is transmitted directly to the samples. The obtained results showed an improvement in mass and energy transfer when US is applied. For example, Liu et al. [17] observed that the direct application of the acoustic wave (28 kHz) in the drying of pear slices made it possible to reduce drying times at 35 °C by 40 and 60% at 24 and 48 W, respectively. This new US application system is interesting for food freezing since it will not require a liquid propagation medium and would facilitate the implantation of this technology in currently static freezing lines used in the industry or even at home. In addition, another potential advantage of direct contact US is that whereas it has been demonstrated that the application of US in food can lead to its degradation and, especially, to lipid oxidation [20] in direct contact US the transmission of US is through solid materials and therefore, chemical effects will be minimized since cavitation is very unlikely to occur.

Therefore, the aim of this work was to evaluate the potential of direct contact US for improving the freezing process of chicken breasts. At the same time, preservation of important product quality factors such as water holding capacity, cook loss and *in vitro* protein digestibility, were investigated.

Table 1
Ultrasonic applied treatments.

Ultrasonic protocol (throughout the freezing)	Net sonication time (%) based on Schössler et al [19]
1 s on / 14 s off	7
1 s on / 5 s off	17
3 s on / 5 s off	37
2 s on / 2 s off	50
2 s on / 1 s off	67

2. Materials and methods

2.1. Samples

Two different matrices were used to carry out this work: distilled water and chicken breast. For freezing experiments, 3 mL of distilled water or 3.1 × 1.6 cm (height × diameter) cylinders of chicken breast were placed inside –at the bottom- of 10 mL glass test tubes.

2.2. US assisted-freezing

2.2.1. US equipment

The US equipment consists of 3 main components (Fig. 1): an US activation system developed by the Electronic Instrumentation Service of University of Zaragoza (Spain) that includes 4 activation plates which generate each a 220 V signal to connect or disconnect the corresponding ultrasound generator plate of the power supply. The second component is a power supply with 4 ultrasound generator plates (Allendale, United Kingdom) which transforms the 220 V activation signal of the activation plates into 1000 V signal at 40 kHz supplied to the corresponding 40 kHz transducer. Finally, the third element is a support (US-support) with the corresponding transducers of 40 kHz and 50 W each (Allendale, United Kingdom). Each transducer is connected with its corresponding 40 kHz ultrasound generator plate. The activation plates are connected to a computer from which the protocol for the application of the US is established by means of a software ("Transducers Controller software") developed by the Electronic Instrumentation Service of University of Zaragoza (Spain). The actual energy consumption of the US equipment was 135 ± 0.2 W and it was measured using a wattmeter (NETBSEM5, Velleman, Flanders, Belgium).

The US-support (Fig. 1) consists of an aluminum plate of 29 cm long by 13 cm wide with four legs of 20 cm length. The four transducers are fixed to the aluminum plate with cold welding (Special high temperature, Ceys, Spain). Glass test tubes with the corresponding samples were attached to the legs of the support by clamps to achieve maximum vibration.

To characterize the vibration created in the sample tubes by US, calorimetry measurements [21,22] and the pressure created in 3 mL-water samples by using a hydrophone TC4013 (Teledyne- Reson, Denmark) connected to an oscilloscope TDS 3012 (Tektronix, EE. UU.) were determined. An acoustic pressure value of 0.159 ± 0.062 atm and a transmitted power of 0.012 ± 0.042 W were measured. Also, the amplitude of the displacement caused by the vibration was measured using an accelerometer (Type 8339-001, Brüel & Kjaer, Skodsborgvej, Denmark) which was located in the zone of the US-support where the samples were placed. The signal was recorded using front-end LMS SCADAS Recorder SCR01 (LMS International, Leuven, Belgium) and Signature de TestLAB V12 software (Siemens, Plano, USA). The obtained data was subsequently processed employing the software ME'-Scope VES 4.0 (Vibrant Technology, Inc) determining the Auto Power Spectrum (APS) in order to know how the power of a signal is distributed in the frequency domain. The Fast Fourier Transform (FFT) algorithm was used to calculate a Digital Fourier Transform (DFT) of a time waveform. An Auto Power Spectrum was calculated by multiplying the DFT by its own complex conjugate. Based on the APS [23] a displacement RMS value of 0.0085 μm was determined.

Throughout the research, intermittent ultrasonic treatments were applied using different protocols depending on the on/off activation time of the transducers. The application mode was as follows: each time ultrasound was applied, a single transducer was turned on while the other 3 were off; once the first transducer was off, the next one following the clockwise direction was activated, acting therefore the transducers in a rotatory manner to minimize the heat generated by the operation of the transducers. The US treatment was defined as the "net sonication time" [19] calculated as the operating time of the transducer over the total processing time. This parameter is considered for the whole set-up.

$$\%\ \ net\ \ \ sonication\ \ \ time = \left(\frac{t_{on}}{t_{on} + t_{off}}\right) \times 100 \quad (1)$$

t_{on} is the operating time of the transducers; t_{off} is the switched time of the transducer. The applicated net sonication times are shown in Table 1.

2.2.2. Freezing equipment

The freezing processes of the samples were studied using two different facilities: a static conventional freezer and a forced-air tunnel. The freezer (Comfort NoFrost, LIEBHERR, Zaragoza, Spain) was used to perform the initial freezing studies with water samples. For these experiments, the regime temperature was −18.5 °C. The freezing tunnel (1.0 kW; ITA 100-SYE-H, TARRE S.A, Spain) enable the control of temperature (from 10 °C to −35 °C) and air speed (up to 13 m/s). Experiments carried out in the tunnel, freezing temperatures ranging from −13 °C to −25 °C were used. In all cases, the air speed was the minimum selectable (< 0.4 m/s) measured with an anemometer (FVAD 15, Almemo, Ahlborn, Germany).

In order to establish meaningful comparisons between US-assisted and control (no US) freezing conditions, two supports were constructed, one with the transducers fixed -for ultrasound-assisted freezing- and another with non-transducers -for control samples. In each experiment, both the control sample support and the ultrasonic sample support were introduced into the freezing system ensuring that there was no contact between them to avoid transmission of vibration and that, in the case of the freezing tunnel, the air speed was the same for both (US and control) types of samples.

2.3. Freezing curves analyses

During the freezing process of the samples, temperature was recorded over time and freezing curves were obtained. The final point of the freezing process was established when the temperature of the samples was 2 °C above the freezer ambient temperature measurement was carried out using type K thermocouple temperature sensors (Almemo, Ahlborn, Germany) which were covered with a plastic insulating material to prevent deviations in the measurement and placed in the center of the samples. Also, the temperature of the air of the freezing system was monitored. These thermocouples were connected to a data logger (Data logger 710, Almemo Ahlborn, Germany) which allowed recording the temperatures of all samples in real time. In the initial studies with water samples, each processing condition was performed at least in duplicate, recording the temperature of 2 samples in each replicate. For meat samples, at least two replicates of each experimental condition were carried out. In this case, in each replicate the temperature of 4–5 samples was recorded.

The analysis of the freezing curves (temperature *versus* the processing time) of chicken samples was carried out after dividing them into three zones, similarly to Xu et al. [24]: 1) the initial cooling period -from the beginning of the experiment until the sample reached one degree above freezing temperature-; 2) the phase change period -from one degree above the freezing temperature to one degree below the

freezing temperature-; and 3) the completion of freezing period -from one degree below the freezing temperature to two degrees above the ambient temperature at that phase. For the initial cooling period and the completion of freezing period, the cooling rate (˚C/min) was calculated, expressed as the temperature change (ΔT) expressed in ˚C divided by the time required for this ΔT. In the phase change period, the time duration was calculated and expressed in min. On the other hand, the effective freezing time of each of the samples was established as the time required by that sample to reach a temperature of −10 ˚C.

2.4. Product analyses

In order to evaluate the effects of the treatment on the quality of the chicken samples, firstly samples were defrosted before the analyses were carried out. For this purpose, once the freezing process was completed, the air temperature of the equipment was increased up to 4 ˚C, and the temperature of each of the samples was recorded. The samples were considered to be thawed when their temperature at the thermal centre was 2 ± 0.5 ˚C.

2.4.1. Water holding capacity (WHC)

This parameter was analyzed using the centrifugation-based technique [25]. For this purpose, 5–6 g samples were prepared and wrapped in a gauze and placed in a Falcon tube with glass pearls. These tubes were centrifuged at 1,300 rpm for 15 min in a centrifuge (MEGAFUGE 1.0 R, Kendro, Germany). Then the meat was weighed after centrifugation. To calculate the % WHC, Equation (2) was used.

$$WHC = 100 - \left(\frac{Wi - Wf}{Wi} \cdot 100\right) \quad (2)$$

where W_i is the initial weight in grams; and W_f is the final weight of the sample in grams.

The same protocol was applied to non-treated samples that were used as control. Three replicates were carried out for each sample.

2.4.2. Cook-loss (CL)

Samples of 2 g were weighed and placed in test tubes and immersed in boiling water until a final sample temperature of 75 ˚C at the thermal centre was reached. Afterwards, the meat portions were recovered, the surface water was removed with paper and they were weighed again [26]. Equation (3) was used to calculate the % loss by cooking:

$$\%\ Cook - loss = \frac{W_i - W_f}{W_i} \cdot 100 \quad (3)$$

where W_i is the initial weight in grams, and W_f is the final weight of the sample in grams.

The same protocol was applied to non-treated samples that were used as control. Three replicates were carried out for each sample.

2.4.3. In vitro protein digestibility

Frozen chicken breast filet samples were vacuum packed, shipped frozen and stored at −80 ˚C before analysis. Samples were thawed in the fridge overnight. Since chicken meat is generally eaten cooked, one vacuum pack per treatment (US vs control) was subjected to heat treatment prior to digestion by placing the thawed samples in a water bath at 70 ˚C for 30 min. Samples were minced in a food processor and protein digestibility was estimated using a static *in vitro* digestion model (INFOGEST) [27,28] as previously described [29]. Digestion was performed on 1 g minced samples weighed into 50 mL centrifuge tubes with individual tubes for each time point. All digestions were performed in parallel (two tubes per time point). The oral-, gastric- and intestinal phases were simulated using commercial enzymes and porcine bile and pancreatin. Digestion of samples from the gastric phase was terminated by pH adjustment to 7.0, while samples from the intestinal phase were heated in a water bath at 90 ˚C for 10 min. After stopping the enzymatic reactions, tubes were centrifuged at 4000 rpm for 10 min (Heraeus Multifuge 4 KR). The supernatant was filtered through 0.45 μm syringe filters into HPLC vials and peptide size distribution was determined by Size exclusion chromatography as previously described [30]. With the employed chromatographic set up, an elution time of 9.4 min corresponds to an average molecular weight of approximately 900 g/mol and all peptides eluting after 9.4 min were defined as small peptides. Protein degradation, i.e. the % of small peptides (based on total dissolved protein) was calculated by comparing the area under the chromatographic peaks from 9.4 to 15 min (small peptides) with the total area (5 to 15 min). PSS WinGPC Unichrome with multi area settings was used to process chromatographic data. The protein content of raw materials and supernatants after digestion was determined by combustion using a Vario EL cube (Elementar, Langenselbold, Germany) operated in CNS mode. Raw and heat treated chicken breast samples were analyzed after freeze drying. Approximately 5 mg of each solid sample were weighed into tin foils. Liquid samples (supernatants after *in vitro* digestion) were pipetted (50 μL) into double tin foils before complete evaporation of water over night at room temperature prior to combustion. Protein contents were calculated by using the standard Nitrogen to protein conversion factor of 6.25.

2.5. Statistical analyses

GraphPad PRISM software was used for statistical analyses (one-way ANOVA with Tukey post-test and Student *t* test) ($p = 0.05$). Error bars in the figures correspond to the mean standard deviation. Throughout the text, the standard deviation has been used to express the dispersion of the freezing times and the confidence intervals are used when referring to the percentage improvement when applying US. Minitab software version19 was used for statistical analysis of *in vitro* digestion data. For ANOVA a general linear model with two categorial variables (C1 = raw/cooked and C2 = control/US) and one continuous variable (digestion time) was fitted to the data including first order interaction terms C1*C2, C1*time, C2*time, C1*C2*time. Comparisons between samples and time points were made with the post hoc Tukey pairwise comparison test at a confidence interval of 95%.

3. Results and discussion

In a first approach, the effect of direct contact US on the freezing rates of water has been investigated to evaluate the effect of US in this configuration. Then further research was carried out on a food matrix such as chicken breasts. Lastly it was studied the impact on the final quality of chicken breast samples frozen by this process.

3.1. Direct contact US effects in water freezing process

3.1.1. Freezing curves of water samples

In order to evaluate in a simple way the effect of direct contact US in the freezing process, the first studies were carried out using distilled water. For this purpose, test probes with 3 mL of water were introduced into a conventional freezer at – 18.5 ˚C and an intermittent US treatment at 50% net sonication time was applied according to the following protocol: 2 s on / 2 s off throughout all the freezing process. Simultaneously, control samples (no US) were also frozen. Fig. 2 shows the obtained freezing curves for both US and no US samples. As observed, the application of US allowed reducing the effective freezing time by 15.2% compared to control time (66.6 ± 0.7 min for US samples vs 78.6 ± 0.9 min for control samples). Moreover, the time of the phase change period was 9.6% shorter in US samples (35.1 ± 0.2 min for US samples vs 38.9 ± 2.3 min for control samples). No differences were observed in the first (cooling) and third (from freezing to the end of the curve) part of the curve, probably due to the small volume of water. The obtained results indicated that the

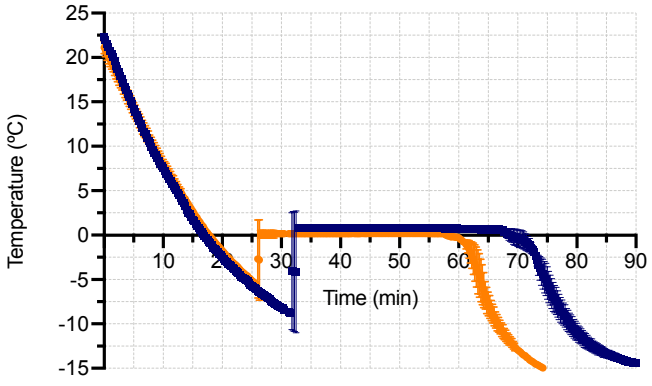

Fig. 2. Freezing curves of water samples applying (●) or not (■) direct contact US at net sonication of 50% (2 s on / 2 s off throughout freezing).

application of US by direct contact would increase the freezing rate of water affecting mainly to the transition phase. However, it would be interesting to investigate whether these results are still observed or improved when applying treatments of different US intensities.

3.1.2. Influence of different net sonication times

In this part of the study, different net sonication times (7, 17, 37, 50 and 67%) were applied to water samples during freezing in a conventional freezer. Non-US assisted samples (control) and US assisted samples were frozen at the same time in different supports and the temperatures of both control and US samples were registered. The corresponding freezing curves were obtained similarly to that shown in Fig. 1 (data not shown). As stated above, the most critical phase of the freezing curve is the phase change in which the crystallization process takes place, as it determines the morphology and size of the crystal which are crucial for product quality. Therefore, the faster this phase occurs, the better. Fig. 3 shows the length/duration of the phase change (Fig. 3A), the freezer temperature (Fig. 3B), and the effective freezing time (Fig. 3C) for each net sonication time. As observed in Fig. 3A, when low (7 and 17%) net sonication times were applied, no statistically significant differences between the length (minutes) of the transition phase of US and control samples were found. However, at net sonication times of 37, 50 and 67% the length of the phase change decreased 23.8–38.0%, 14.5–22.1% and 11.8–41.5%, respectively, compared to the control samples under the same freezing conditions. Fig. 3 also shows that the length for the transition phase increases with net sonication time, mainly for control samples, and scarcely 4 min for US ones (from 0 to 67% net sonication time). This increment in the length of the transition phase would be due to the increase of the temperature of the air inside the freezer when applying higher net sonication times (Fig. 3B) due to the warming up of the transducers when working. The higher net sonication time, the higher temperature increment of the air of the freezer for both US and control samples. It should be reminded that, as described in materials and methods section, US and control samples were frozen at the same time but placed in difference supports, with and without transducers. This would explain why, the length phase change period in control samples increased with higher net sonication time.

In any case, results obtained demonstrate that the application of US over 17% net sonication time allowed a reduction in phase change time compared to control samples. Similar conclusions concerning the temperature increment due to the application of US were obtained by [19]. They studied the influence of net sonication time (10, 14 and 25%) and excitation amplitude (4.9, 6.0 and 6.7 μm) in the freeze-drying of red bell pepper in order to minimize the heat provided by US treatment. They observed that by applying intermittent US treatments at 10% net sonication time and an excitation amplitude of 4.9 μm the temperature of the samples did not increase because of US. Moreover, in this study

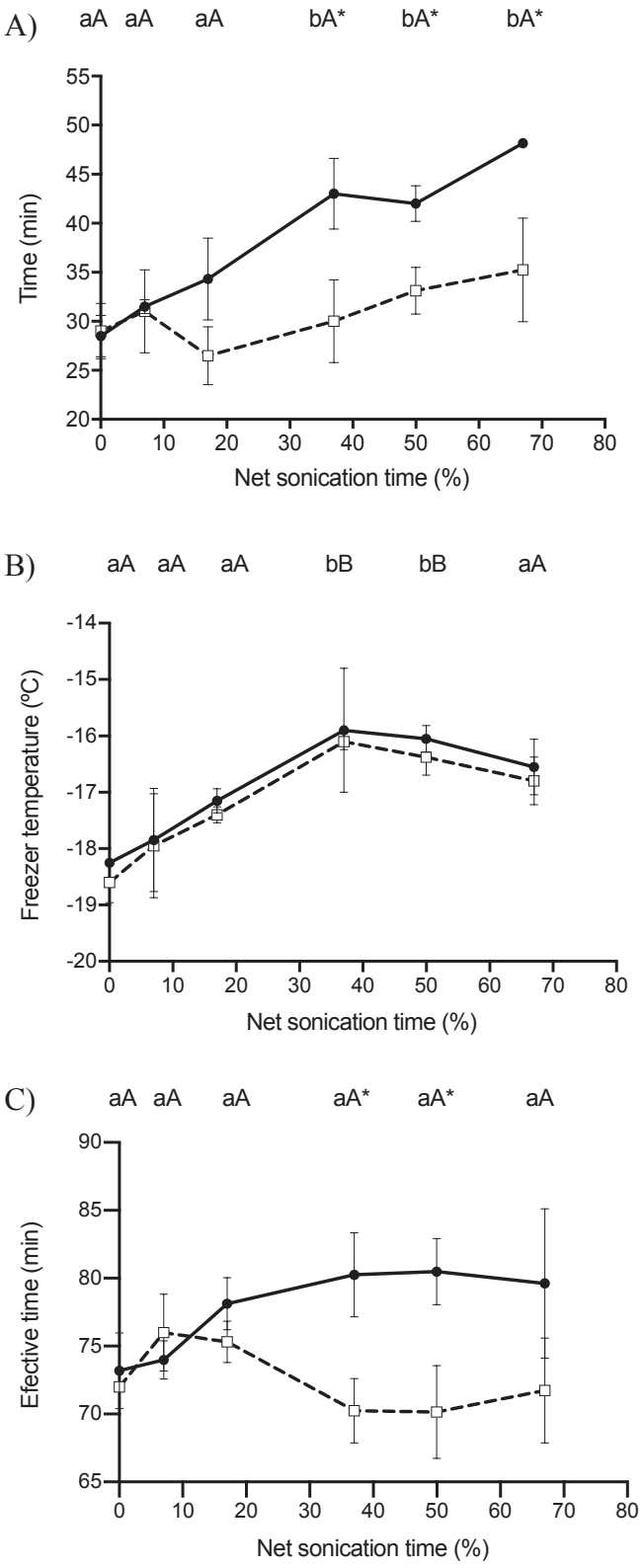

Fig. 3. Freezing process of water samples applying (□) or not (●) different net sonication times (7, 17, 37, 50, 67%). A) Phase change time (min); B) Freezer ambient temperature (°C); C) Effective time (min). The same letter in the upper part of the graph indicates that there are no significant differences ($p = 0.05$) for control samples when different net sonication times were applied. The same capital letter indicates that there are no significant differences ($p = 0.05$) for US samples when different net sonication times were applied. An asterisk indicates significant differences ($p = 0.05$) between the US and control samples for the same net sonication time.

the freeze-drying time could be reduced by 11.5% when US was applied.

Finally, a similar behavior of the length of the phase change period was observed for the effective time required for freezing the different samples (with and without the applications of US) (Fig. 3C). As observed, US treatments with a net sonication time over 17% were required in order to find significant reduction in the effective freezing time. Thus, the higher differences were found at 37 and 50% net sonication time, which led to an effective time reduction of 12.0–12.9% and 11.6–14.2%, respectively. From these results it can be concluded that the application of direct contact US assisted freezing can help to reduce the phase change time and the effective time of water samples, but a certain intensity of US is required. In this case and for the size of the samples, a net sonication time of 37% would be required.

Once demonstrated the effect of US in reducing the freezing time of water samples, the next studies were focused on the evaluation of these effects in a food matrix such as chicken meat (chicken breasts).

3.2. Application of direct contact US assisted freezing to chicken samples

Given the already demonstrated capability of US to increase the freezing rates of food, it would be of the highest interest to study the effect of direct contact US on the freezing process of meat products. It might help not only to shorten processing times and to obtain meat products of a higher quality but also would facilitate the upscaling of equipment and the transfer of this technology (US) to the meat industry.

3.2.1. Freezing curves of chicken meat

A net sonication time of 37% (3 s on / 5 s off throughout freezing) was chosen on the basis of previous results in order to carry out the experiments on chicken breasts. Both control and US samples placed in different supports were introduced inside the conventional freezer at a temperature of −18.5 °C. Similar freezing curves to that shown in Fig. 1 were obtained (data not shown). The effective freezing time required to lower the temperature of the samples to −10 °C was 82.8 ± 2.4 min for control samples and 76.3 ± 3.6 min for US samples achieving a time reduction from 6.2 to 9.7% for US-assisted freezing samples. However, and as described above, in spite of reducing freezing times, the freezer temperature inside the freezer increased considerably when US was applied, reaching −15 °C instead of maintaining −18.5 °C. For this reason and in order to keep the temperature during the process constant when using US, the following experiments were carried out in an air forced tunnel, which is much more efficient than the conventional freezer for heat removal. Using this equipment, again similar freezing curves to that shown in Fig. 1 were obtained (Fig. 4). In this case, when US treatment of 37% was applied at an ambient temperature

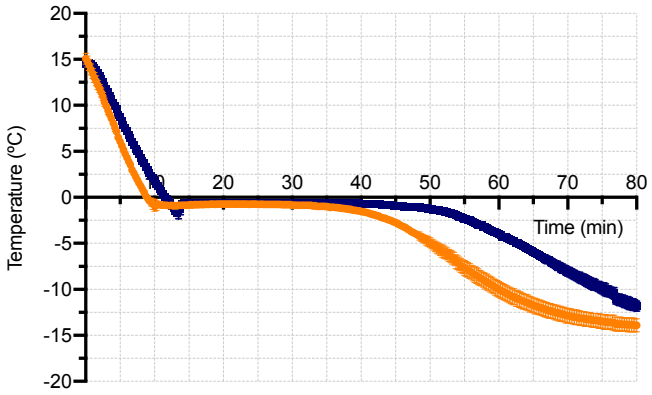

Fig. 4. Freezing curves of meat breast samples applying (●) or not (■) direct contact US at net sonication of 37% (3 s on / 5 s off throughout freezing) in an air forced tunnel at −15 °C.

of −15 °C the effective freezing time was reduced by 19.9% in US samples (75.1 ± 1.6 min control samples vs 60.2 ± 2.2 min US samples). In this case, the time of the transition phase was also shorter for US samples (33.3 ± 0.4 min) compared to control ones (41.2 ± 0.3 min), although differences were not statistically significant. Therefore, the application of US during the process was effective in reducing the freezing time in the chicken meat samples.

3.2.2. Influence of air freezing temperature on the effect of US

In an energy transfer process such as freezing, the process rate is highly influenced by the thermal gradient between the food and the environment: the greater the temperature difference between them, the faster the energy transfer speed [31]. Therefore, the effect of US can be influenced by the ambient temperature since at very low temperatures, where heat removal from food is faster, the ultrasonic effect on the freezing rate could be hidden. On the contrary, if the freezing efficiency is smaller (i.e. higher air freezing temperatures) where heat removal is lower, the heat generated by transducers could also distort the results becoming counterproductive. Hence, the influence of ambient temperature on direct contact US assisted freezing effect was evaluated.

In order to carry out this study, air temperatures from −13 to −26 °C were applied for freezing the chicken breasts samples using the previously described air forced tunnel at its minimum air speed (< 0.4 m/s). Fig. 5 shows the initial cooling rate (Fig. 5A) expressed in °C / min, the phase change period in min (Fig. 5B), the completion rate in °C/min (Fig. 5C), and the effective time to reduce sample's temperature up to –10 °C (Fig. 5D) at the different investigated temperatures. As observed, in Fig. 5A, the air temperature hardly influenced the freezing rate during the cooling period. There was a very low correlation between air temperature and freezing rate for both control and US samples (R^2 control = 0.132; R^2 US = 0.113). More interesting, in this initial part of the freezing curve the application of US throughout all temperatures always managed to improve the cooling rate, by 24.0–32.0 %. That is, the application of US enabled to reduce this cooling phase becoming more efficient in removing the sensitive heat from the food until the freezing temperature is reached.

In the case of the phase change period (Fig. 5B), the freezing time decreased linearly as the ambient temperature went down or as the thermal gradient increased (R^2 control = 0.736; R^2 US = 0.829) for both control and US assisted frozen samples. Results obtained also indicate that the magnitude of the decrease in the length of the phase change period caused by the application of US was higher the higher the air freezing temperature. This would mean that the effect of US would be more beneficial for reducing the length of this phase when the freezing temperature is higher or the thermal gradient is smaller. Finally, regarding the completion of freezing period (Fig. 5C), where the majority of water in the food is in an ice state, the effect of the temperature affected the freezing rate in a linear manner, increasing the freezing rate when the air temperature decreases (R^2 control = 0.885; R^2 US = 0.757). As in the previous phases, the application of US improved the cooling speeds (by 11.3–23.0 %) although in this case its effect was less marked. It should also be noted that, conversely to that indicated for the phase change period, the effect of US on this phase seems to be higher the lower the freezing temperature was. Altogether, obtained results indicate that the application of US at a net sonication time of 37% (40 kHz) allowed shortening the times of each freezing phases of the chicken breast samples improving energy transfer processes. This effect is clearly reflected in the effective time to decrease the temperature of the samples down to −10 °C (Fig. 4D) which was 9.9–11.3% lower for the US assisted frozen samples for all the ambient temperature range between −13 and −22 °C. This effect of US is constant and independent of the temperature since the regression lines were parallel, similarly to what was observed in the cooling phase.

There are very few studies related to the application of direct contact US in food freezing until now. Islam et al. [32] evaluated US assisted freezing of mushrooms by direct contact (300 W, 20 kHz) and

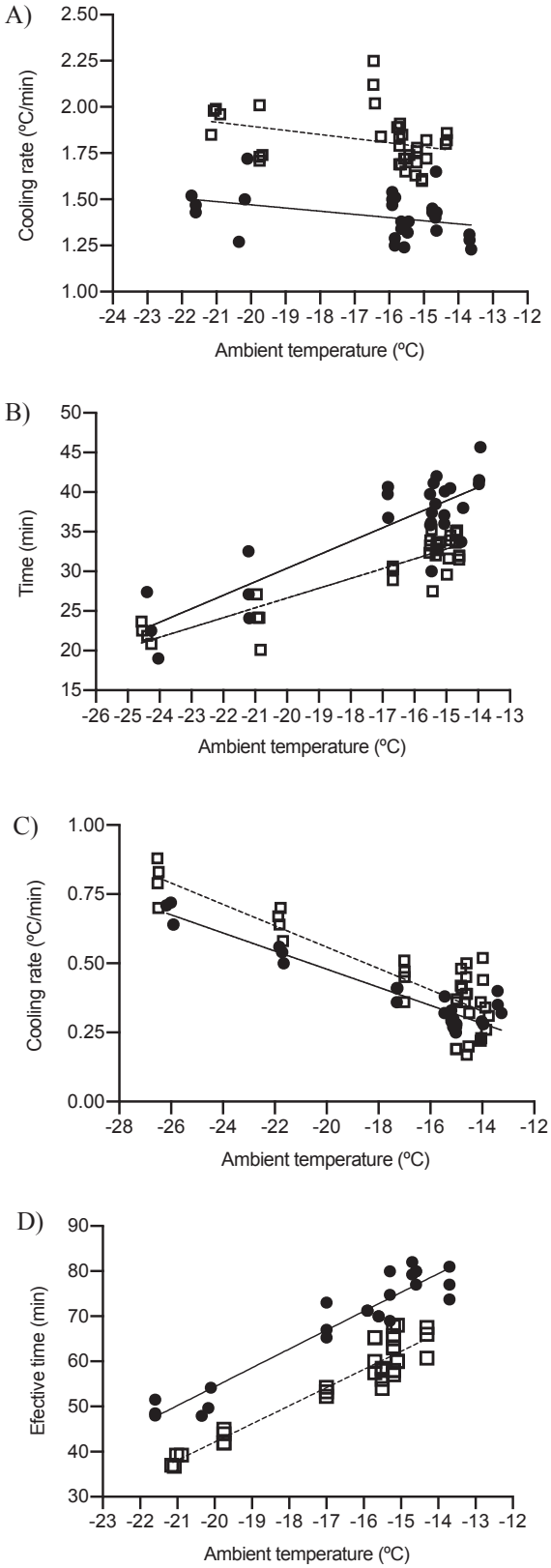

Fig. 5. Influence of air temperature in freezing of chicken breasts applying (□) or not US (●). A) initial cooling rate (˚C / min); B) phase change period (min); C) completion of freezing rate (˚C/min); D) effective time to reduce samples temperature up to –10 ˚C.

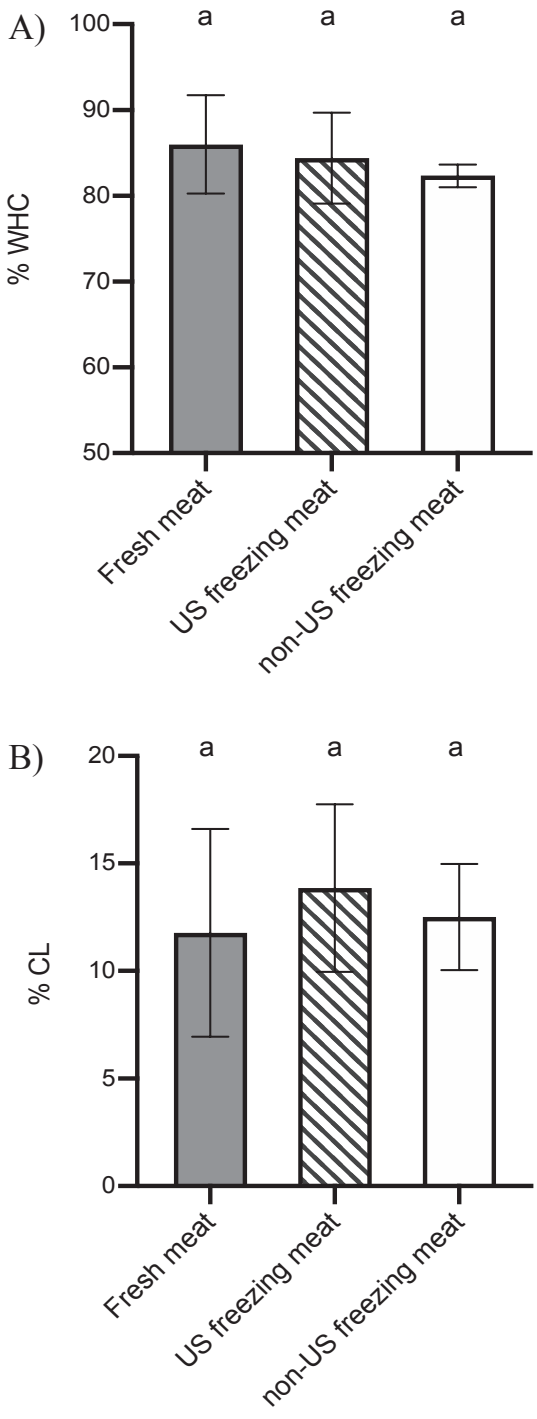

Fig. 6. WHC (A) and CL (B) values of fresh, US and non-US assisted freezing samples of chicken breasts after thawing at 4 ˚C. The same letter in the upper part of the graph indicates that there are no significant differences (p = 0.05) between the studied variables.

indicated that US favored earlier nucleation. Even if it́s not the same process, the improvement in energy transfer due to the application of contact direct US has been also observed in air dehydration process (45 ˚C). Liu et al. [17] dehydrated pear slices on an US radiation disk placed on a transducer (28 kHz) and determined that applying US power of 24 and 48 W the drying rates were increased by 33.3 and 140.1%, respectively. So, our results are in agreement to those published indicating the possibilities of the technology not only in plant based products but in animal based products.

3.2.3. Effect of freezing US on product quality

The impact of US on the quality of the thawed product was carried out by applying US at 37% net sonication time during freezing and defrosting at 4 °C as indicated in material & method section.

Fig. 6 shows the WHC and CL of chicken samples from fresh meat, and freeze-defrosted samples frozen with or without US. As observed, no significant differences were determined among the samples indicating that the process was mild enough not to impact on WHC or CL. The lack of differences could be due in part to the small dimensions of the sample, resulting in a very quick freezing-thawing process hardly affecting WHC and CL, which were similar to the ones of the fresh product. In addition, there was a high variability within each treatment including the fresh product (high standard deviation) and a higher number of samples might have been needed to identify differences. In any case, the obtained results are aligned to those described in literature for freezing meat samples by immersion in US baths. Thus Li et al. [33] observed that after 20 min of US treatment (20 kHz) an increase in the proportion of water retained within the myofibrillary proteins of chicken breast meat was determined, resulting in a higher WHC. On the other hand, Zhang et al. [34] observed that by applying US (180 W) to the freezing process of loin samples, the CL was reduced by 16.26% compared to samples frozen by air systems. To evaluate in more detail the possible effect of ultrasound when freezing meat samples, histocytochemistry and scanning electron microscopy of cells would result of interest as already evaluated [35] when studying the possible erosion that would generate ultrasound in vegetables. Considering the obtained results, in this case, ultrasound would hardly effect meat cells.

Although no differences were found in the WHC and CL of the samples after the freeze-thawing process, new studies about the possible impact on the final meat quality by using direct contact US were evaluated.

Chicken meat is considered to be a good source of high-quality protein. However, proteins differ in quality not only based on their amino acid content, but also digestibility. Processing, such as freezing, thawing and ultrasound treatment, may induce changes in protein structure that could affect their digestibility and bioavailability [36,37]. In this study, the protein digestibility of chicken meat samples was investigated using a static *in vitro* digestion model. Results showed that US-assisted freezing had no significant impact on protein digestibility of chicken breast filets (Fig. 7 A and B). However, heat treatment (cooking at 70 °C for 30 min) of the samples significantly decreased protein solubility for both control and US-assisted freezing, at the end of the stomach phase (120 min) and intestinal phase (240 min) (Fig. 7 A), but did not significantly influence the rate of protein degradation (% small peptides) (Fig. 7 B). The decreased protein solubility indicates that cooking may lower the digestibility of chicken meat proteins, which is in accordance with previous findings and depends on the extent of heat treatment [38]. US-assisted freezing showed some effect on protein digestion in the stomach phase (120 min), with tendencies for higher protein solubility (albeit not significant when comparing raw vs US raw, and cooked vs US cooked) and a higher share of small peptides (significant for raw, but not cooked samples) compared to the control. However, these differences were reduced both by heat treatment (cooking) and digestion time. For all practical purposes it can therefore be assumed that there was no difference in protein digestibility of US and non-US assisted freezing of chicken breast samples.

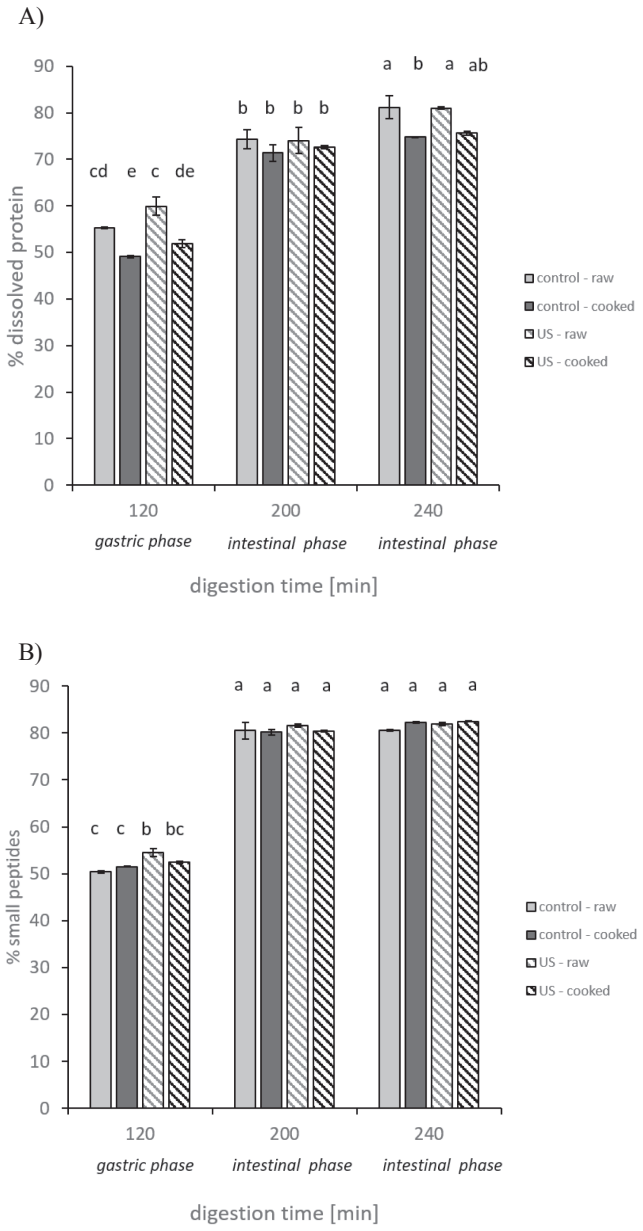

Fig. 7. Dissolved protein (A) and % small peptides (B) generated during simulated digestion of US and non-US assisted freezing (control) samples of chicken breast digested after thawing (raw) or heat treatment (cooked). Columns sharing the same letter are not significantly different ($p = 0.05$).

4. Conclusions

This article shows the potential of direct contact US for enhancing chicken meat freezing by increasing mass and energy transfer processes. US allowed to reduce the effective time to decrease samples' temperature to −10 °C compared to control samples. Indeed, the effect of US was reflected in each of the phases of the freezing curve which means that the application of the US could favor both the transfer of sensible heat in the initial and completion phases as well as the transfer of latent heat during the phase change period. Besides, for the phase change period, the effect of US was more noticeable at higher freezing temperatures. These results indicated that the application of US would enable to work with higher freezing temperatures or with minor thermal gradient between the freezing air and the sample what would lead to a minor economic cost. However, this is a point that needs more investigation.

Preliminary results of the impact of direct contact US freezing on the quality of frozen products showed no differences in the values of WHC and CL of control and ultrasonic chicken breast samples. US-assisted freezing did not influence *in vitro* protein digestibility of chicken meat samples.

Therefore, obtained results in this investigation indicate that the application of US by direct contact during freezing could be another way to improve the freezing capacity of current static freezing systems improving the energy transfer process. Further studies are needed to

evaluate the process with larger quantities of the product and their influence on meat quality based on sensorial analysis.

CRediT authorship contribution statement

L. Astráin-Redín: Investigation, Methodology, Formal analysis, Writing - original draft. **J. Abad:** Methodology, Writing - review & editing. **A. Rieder:** Investigation, Writing - review & editing. **B. Kirkhus:** Methodology, Writing - review & editing. **J. Raso:** . **G. Cebrián:** Conceptualization, Writing - review & editing. **I. Álvarez:** Conceptualization, Methodology, Writing - review & editing, Supervision.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2020.105319.

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