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Effect of ultrasound bath and probe combined to brine and brine-polyphosphate solutions on the qualitative and textural properties of beef meat

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Abstract

Various mechanical, enzymatic and chemical techniques are used to improve the quality levels of meat. Such techniques have disadvantages such as being time-consuming and damaging to the meat quality indicators. Ultrasound is used as an effective method to modify technological properties and tenderize the meat. The meat samples (Flank area) were put into brine solution or a mixture of the phosphate-brine solution under the ultrasound bath (at a frequency of 37 kHz) and probes (20 kHz) in 20, 25 and 30 minutes at 30, 40 and 50°C. The changes in the technological and textural properties of meat samples were then investigated. The results showed an increase in pH (from 5.55 for control up to 7.14), water-holding capacity (from 20.00 % for control up to 38.15 %), water-binding capacity (from 12.63 % for control up to 31.65 %) and a reduction in the drip loss (from 12.50 % for control up to 3.21 %), cooking loss (from 36.70 % for control up to 16.46 %), hardness and chewiness, whereas showed an increase in tenderness. In general, ultrasound treatment probe in a solution of mixed polyphosphate-brine was more efficient. It is concluded that ultrasound is an effective technique to improve the meat quality.

Keywords: Beef meat, Technological properties, Tenderize, Textural properties, Ultrasound

Introduction:

The quality of meat is evaluated by the parameters such as chemical composition, physical properties, organoleptic properties and appearance. Organoleptic properties and the overall quality of meat are mainly affected by the tenderness, flavor and water-holding capacity (WHC). In general, the functional and technological properties of meat such as WHC, water-binding capacity (WBC) and emulsifying properties are affected by the myofibrillar proteins. Tenderness is affected by the myofibrils protein composition and structure of the skeletal muscle. Many factors cause the loss of technological quality and palatability of meat. For example, it has been found that with increasing age of the animal, the covalent cross-links between collagen building blocks (tropocollagen) increased and result in decreased collagen solubility. Such a problem directly reduces the technological quality of meat. So, in such cases the use of various mechanical, enzymatic and chemical methods is necessary to improve the meat quality. Some techniques have disadvantages such as being time-consuming or damage to the meat quality indicators. In such cases, it is required to use new technologies with minimal damage to the product (Xiong *et al.,* 2012).

Ultrasound is an innovative technology with applications in food industry. In this technique, the sound waves are applied to the frequencies higher than those of which the human ears are capable of hearing (20 kHz) (Alarcon-Rojo *et al.,* 2015). This process causes condensation and expansion of the particles in the environment and thereby creating of voids or bubbles. These voids grow during ultrasound cycles and eventually become unstable and shatter and release high temperature and pressure. The bubble shattering affects biological materials in micro and macro scales (Alarcon-Rojo *et al.,* 2015).

In the meat industry, the ultrasound technology is currently applied as a fast alternative technique, relatively inexpensive, simple, and reliable to improve meat quality

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criteria, such as tenderness, extraction of proteins and gelatin. It can also help to create the texture in the meat products, reduce the salt, and enhance the curing process (Turantaş *et al*., 2015).

Brining is an old prserving and processing method of meat in which meat was cut out into pieces and put in a salt solution. This process improved durability, flavor, juiciness and tenderness of meat products. The salt release occurred slowly in the meat matrices, but can be improved by injection. However, in these circumstances, the products have lower quality. It is observed that permeability of muscle texture increases with ultrasound, thereby it can be used to enhance the penetration of salt used in brining process (Leal-Ramos *et al*., 2011).

Studies have been published on the use of low-frequency ultrasound in order to tenderize meat. Jayasooriya et al. (2007) investigated the effects of ultrasonic treatment (24 kHz, Wcm-2 12 and a maximum of 4 minutes) on the qualitative properties of beef muscle in the brine. Based on their results, ultrasound leads to a reduced hardness and an increased brittleness and pH of the meat. Ultrasound treatment had no effect on color parameters (L *, a *, b *, Chroma, hue); however it increases the cooking loss and also the total loss. Siró et al. (2009) examined three brining methods of static conduction, under vacuum conduction and low-frequency ultrasound (20 kHz and extremely Wcm^{-2} 2-4) to the curing process of pork lower back muscles with salt (at 5 °C). They reported that WBC parameter and textural characteristics are more improved by ultrasound in comparison with the other two treatments. The chewiness reduces based on the results of the ultrasound treatment and consequently the brittleness increases. In general, the lower sound intensities were more effective. The Ultrasound treatment significantly results in increased penetration index of salt compared to the brining under static conditions. It was also shown that the penetration rate increases randomly with increasing the ultrasound intensity.

Several studies indicated the significant and profound impact of polyphosphate in small amounts on the juiciness and tenderness of the meat. A reduction in the curing quality loss was also reported which leads to extensive use of polyphosphate (often combined with salt) in the processed meat production and minced meat products such as burgers and sausages (Sheard *et al*., 1999).

This study aiming at determining the effects of ultrasound bath (at a frequency of 37 kHz) and probes (20 kHz) combined with brine solution (40 gr/L of water) and polyphosphate (20 gr/L of water) for 20, 25 and 30 minutes at 30, 40 and 50°C on the qualitative characteristics of meat was conducted. To the best of our knowledge, no study on using ultrasound combined to polyphosphates for meat products has been conducted.

Materials and Methods

Meat production and preparation of samples

Beef meat (Flank area) was purchased from the local market (Sari, Iran) (48 hours after slaughter). Three meat samples with the weight of 50 grams were prepared from the central parts of the flank muscle (preferably without fat) in the same shape and dimensions (length, width and thickness of 50, 30 and 10 mm, respectively) with the help of a sharp knife. The samples were covered with waterproof coatings and kept at 4°C until the time of testing (Ozuna *et al*., 2013).

Prepare salt water solution and a solution containing salt and polyphosphate

A solution of brine was prepared by dissolving 40 grams of salt in one liter of distilled water. Also, the solution mixture of brine-polyphosphate was prepared by dissolving 20 grams of NaCl and 20 grams of polyphosphate in one liter of distilled water. The ratio of meat to the solutions for all mixtures was 10 : 1 (w/w) (Siró *et al*., 2009).

Ultrasound treatment

The meat samples were subjected to ultrasound and placed in the brine based on two methods of bath and probe for 20, 25 and

30 minutes at 30, 40 and 50°C. In the probe method, an ultrasound cell disruptor (Model KS-250F, China, Ningbo Zhejiang) was used with a frequency of 20 kHz, amplitude of 45% and a power of 250 watts. For bath method Elma Ultrasonic cleaning device (model S 30 H, Germany) was used with a frequency of 37 kHz and a power of 280 watts. Untreated meat sample was considered as a control. After the ultrasound, the pieces of meat were wrapped in a filter paper, dried and packed until analyses at 4 °C (Chang *et al*., 2012).

Measuring pH:

PH of the samples was measured with the digital pH meter electrode penetration PB 11 (Sartorius, Germany).

Water-holding capacity (WHC)

WHC was measured based on pressing the filter paper method for 24 hours after the treatment. For this purpose, small pieces of meat (2 g) were cut from the samples and were pressed on a filter paper (Whatman No. 1) with the means of a 2 kg lifting weight for 5 minutes. Then, WHC percentage was calculated by the following equation (1):

$$\%WHC = \frac{W_{ap}}{W_{bp}} \times 100 \quad (1)$$

Where W_{ap} is the sample weight after pressing and W_{bp} is the sample weight before pressing (Savadkoohi *et al*., 2014).

Water binding capacity (WBC)

WBC for 50 g of the samples was calculated immediately after soaking them in brine by the following equation (2):

$$\%\mathrm{WBC} = \frac{m_{\mathrm{brine}} - m_{\mathrm{brine0}}}{m_{\mathrm{brine0}}} \times 100 \quad (2)$$

Where m_{brine0} is the initial brine solution weight and m_{brine} is the brine solution weight after treatment (Siró *et al*., 2009). The control sample of WBC was calculated according to the above formula based on static brining at 30°C and 20 minutes.

Drip loss

The drip loss of meat sample was determined 7 days after ultrasound treatment (stored at -18°C). For this purpose, after removing the samples from inside of the package, thawing process was performed at a temperature of 20°C, then the samples were dried completely with filter paper and drip loss percentage was calculated by the equation 3 (Xiong *et al*., 2012):

$$\%\,Drip\ loss = \frac{Initial\ weight - Drip\ weight}{Initial\ weight} \times 100 \quad (3)$$

Cooking loss (curing loss)

To determine the amount of cooking loss, the samples were thawed in a warm water bath (Ben Murray) to perform the baking operation at 80 °C. The cooking operations continued for 30 minutes, and the surface of the cooked meat was then well dried with filter paper and weighed. Cooking loss percentage was calculated according to the following equation (Xiong et al., 2012):

$$\%\,Cook\ loss = \frac{Drip\ weight - Cook\ weight}{Drip\ weight} \times 100 \quad (4)$$

Texture profile analysis (TPA)

To study the changes resulting from the ultrasound on the textural characteristics of the samples, texture profile analysis (TPA) was conducted by Brookfield CT3 Texture Analyzer (Brookfield Engineering Laboratories, USA) during the compression cycle of the cylindrical probe (diameter 10 mm) to 40% of the original thickness. The pre-test speed, speed test and returning speed was 1 mm/minute, 2 mm and 100 mm/minute, respectively. The texture qualitative characteristics were evaluated as Hardness; Resilience; Cohesiveness; Adhesiveness, Springiness; Chewiness (Jayasooriya *et al*., 2007).

Statistical analysis

All experiments were carried out in triplicate and presented as a mean ±standard deviation. Analysis of variance (ANOVA) of samples followed by Duncan test was carried out at the significant level of $p<0.05$ by SAS software (version 9, SAS Institute).

Results and Discussion

The effects of ultrasound treatment on pH

pH as the most important quality parameters of meat, has a direct impact on many technological properties such as WHC, WBC and the meat quality (tenderness). For example, the amount of water within the muscle tissue is dependent on the available space between actin fibers, which depends on pH. In general, higher and lower pH value of the isoelectric point would greatly increase WHC of meat. However, the role of pH levels above the isoelectric point is more considerable. A decrease in the pH value of the isoelectric point causes a WHC decrease in the meat.

At the complex actomyosin isoelectric point (with a pH value of 2.5) most groups of COOH are in form of COO^- anions and most groups of NH^2 are in form of NH^{3+}. In this case, the positive and negative ions attract each other and a tightly bound protein molecule will be created with a net charge of zero. As a result, only a very small amount of water can be attached to the protein. The increase of both positive and negative charged groups in the protein molecule by creating more space between protein molecules leads to an increase in the WHC. When the negative charge is higher than the positive charge, pH values will also be higher than the isoelectric point of the protein; therefore WHC increased (Feiner, 2006).

Fig. 1, shows the effects of different temperatures and times of ultrasound treatment on the pH of beef in a brine solution (Fig. 1a) and brine and polyphosphate solution (Fig. 1b).

As can be seen in Fig. 1, generally, with increasing temperature and ultrasound treatment time, a significant increase in the pH was observed ($p<0.05$). Meat pH increase as a result of the ultrasound treatment can vary because of several different mechanisms. The main mechanisms can be the damage of cell structure, the release of ions into the cytosol, and the covering of the hidden acidic group by the protein strands. As a result of heat and high pressure in the cavitation, meat proteins are partially denatured, which lead to hiding acid groups in the field of protein and consequently an increase in the pH. With increasing temperature caused by converting ultrasound energy to heat, an increase in pH was observed (Jayasooriya *et al.*, 2007). Jayasooriya et al. (2007) also reported that ultrasound treatment with increasing of time from 25 to 250 seconds (24 kHz, Wcm-2 12 and room temperature) was able to increase the pH of beef. Ma & Ledward (2004) evaluated the effects of high pressure (200-800 MPa) at different temperatures (20–70°C) for 20 min on post-rigor beef. They reported an increase in the pH of beef muscle, which may be related to heat and pressure generated by the ultrasound cavitation phenomenon. They also reported that with increasing temperature to 40, 60 and 80 °C, pH value increases to 0.60, 0.11 and 0.14, respectively.

Some studies reported no significant change in meat pH as a result of ultrasound treatment which is different from the present results. It can be explained by different ultrasound treatment conditions (Dolatowski *et al.*, 2007). Dolatowski & Stadnik (2007) demonstrated no significant difference between beef meat treated with ultrasound (frequency 45 kHz, power 2 W/cm^2, 120 seconds at 4 °C) and the meat stored for 72 and 96 hours. They reported no significant difference between the pH of beef meat treated with ultrasound 24, 72 and 96 hours after rigor mortis. This is most likely due to different ultrasound conditions applied such as frequency, power and especially the temperature.

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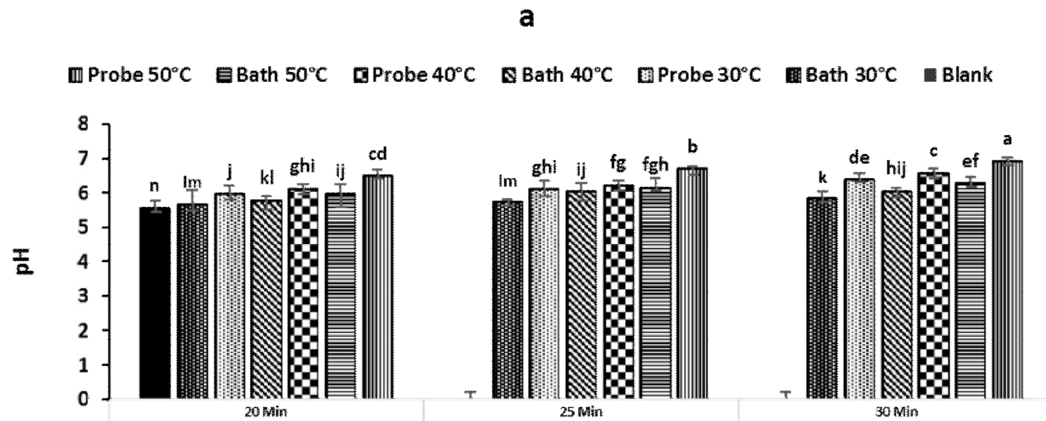
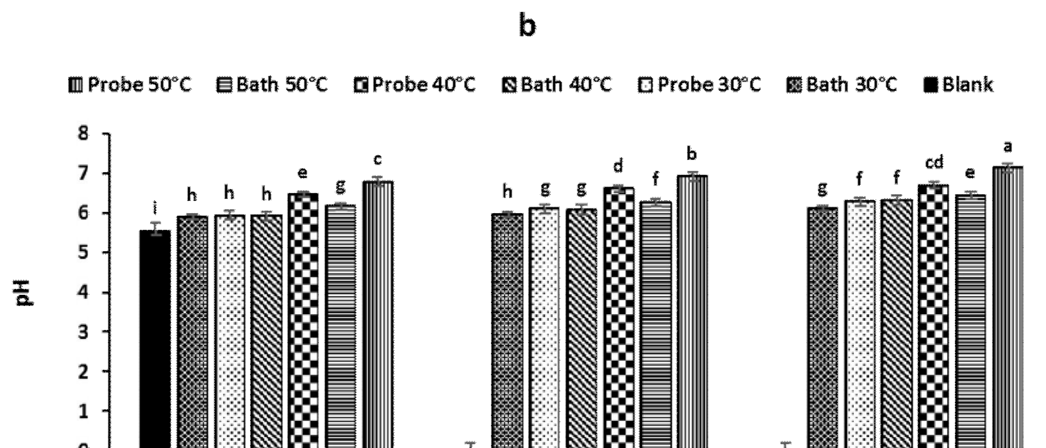
As can be seen in Fig. 1, generally, the treated samples with a brine-polyphosphate solution has a higher pH than those of brine solution (p<0.05). Almost all kinds of phosphates and phosphate mixtures used in the meat industry (alkaline and alkaline phosphatase) leads to an increasethe pH value in meat. The increase of pH causes an increase of electrostatic repulsion between actin and myosin and therefore WHC and WBC increased (Feiner, 2006).



	20 Min	25 Min	30 Min
Probe 50°C	6.48	6.69	6.91
Bath 50°C	5.98	6.16	6.27
Probe 40°C	6.11	6.22	6.57
Bath 40°C	5.76	6.03	6.05
Probe 30°C	5.97	6.1	6.38
Bath 30°C	5.67	5.71	5.84
Blank	5.55	0	0



	20 Min	25 Min	30 Min
Probe 50°C	6.79	6.92	7.14
Bath 50°C	6.16	6.27	6.45
Probe 40°C	6.47	6.62	6.7
Bath 40°C	5.92	6.09	6.32
Probe 30°C	5.94	6.1	6.29
Bath 30°C	5.89	5.97	6.1
Blank	5.55	0	0

Fig. 1. The effect of ultrasound on the pH of beef meat treated in a solution of brine (a) treated with a mixed solution of brine and polyphosphate (b).

Sheard *et al*. (1999) injected an aqueous solution of polyphosphate (3 and 5%) into pork. The results showed that injection of an aqueous solution of polyphosphate leads to significant increase in meat pH. They also stated that pH increased proportionally by increasing polyphosphates in meat.

The increase in meat pH as a result of ultrasound can have important potential implications in meat tenderness. The optimal pH of the protease-cathepsin system is relatively acidic. Hence, it can be expected that the ultrasound treatment with an increase in pH and thus an increase in the activity of protease calpain system, will enhance meat tenderization (Jayasooriya *et al*., 2007).

The effects of ultrasound treatment on water holding capacity (WHC) and water binding capacity (WBC) of meat

WHC is a term used to describe the ability of a muscle to hold water within a set of conditions (such as cutting, pressing, packing, baking, heat treatment, etc.). WHC is relevant to some sensory characteristics of meat, such as juiciness, texture and flavor. In general, it has been proven that the forces led to retention of free water in the structure of the muscle or meat products are a kind of surface tension (Siró *et al*., 2009; Cheng & Sun, 2008).

WHC increases after rigor mortis and relative decomposition of Actomyosin proteins. Meat sonication after slaughter or during rigor mortis can be a useful technique to modify water-protein interactions (Ahmed *et al*., 2009).

Tables 1 and 2 showed the WHC amounts of the control samples and the treated meat with ultrasound in a brine solution and brine-polyphosphate solution, respectively. The amount of WHC in control was found to be 22.00%. In general, ultrasound treatment caused a significant increase in WHC compared to the control ($p<0.05$). The WHC amount of treated samples with ultrasound was in the range of 13.27 to 38.15 % (Tables 1 and 2). As can be seen in Tables 1 and 2, with the temperature increase of ultrasound from 30 to 50 °C, WHC significantly declines. WHC of the treatments conducted at 50°C was lower than that of the control ($p<0.05$). Also, at all experiment conditions, with increasing ultrasound treatment time, WHC decreased significantly ($p<0.05$). The reason for these results is related to meat protein denaturation in higher ultrasound time and also an increased temperature which can reduce the ability of meat to hold water.

Table 1. Water holding capacity (WHC), water binding capacity (WBC), drip loss and cooking loss of beef meat treated with ultrasound in a solution of brine

Type ultrasound		WHC (%)	WBC (%)	Drip Loss (%)	Cooking Loos (%)
Bath	20 min, 30 °C	28.11 ± 1.22^{c}	16.84 ± 1.42^{b}	4.96 ± 0.24^{q}	19.24 ± 1.18^{o}
	20 min, 40 °C	26.04 ± 2.35^{g}	10.96 ± 0.60^{i}	7.25 ± 0.35^{m}	20.58 ± 0.32^{k}
	20 min, 50 °C	23.26 ± 2.17^{j}	8.72 ± 1.23^{m}	8.22 ± 0.17^{k}	21.68 ± 1.23^{i}
	25 min, 30 °C	27.96 ± 2.11^{d}	12.80 ± 1.31^{g}	6.52 ± 0.42^{n}	20.45 ± 0.99^{l}
	25 min, 40 °C	24.33 ± 1.24^{i}	9.66 ± 0.22^{k}	9.13 ± 0.08^{h}	21.86 ± 0.35^{h}
	25 min, 50 °C	18.97 ± 1.31^{m}	7.89 ± 0.85^{n}	10.18 ± 0.10^{f}	22.90 ± 0.65^{f}
	30 min, 30 °C	26.77 ± 1.33^{e}	11.47 ± 0.52^{h}	8.75 ± 0.51^{j}	21.88 ± 0.34^{h}
	30 min, 40 °C	22.40 ± 0.62^{k}	9.07 ± 1.40^{l}	10.95 ± 0.47^{d}	23.25 ± 0.26^{e}
	30 min, 50 °C	16.76 ± 1.40^{o}	6.15 ± 1.50^{q}	11.27 ± 1.32^{c}	24.85 ± 0.63^{c}
Probe	20 min, 30 °C	31.97 ± 1.42^{a}	20.37 ± 2.12^{a}	3.88 ± 0.50^{r}	18.15 ± 0.11^{p}
	20 min, 40 °C	26.42 ± 2.20^{f}	14.41 ± 1.62^{d}	5.84 ± 0.33^{o}	19.52 ± 0.24^{m}
	20 min, 50 °C	22.16 ± 1.42^{l}	7.22 ± 0.13^{o}	8.93 ± 0.17^{i}	22.82 ± 1.19^{f}
	25 min, 30 °C	29.27 ± 2.16^{b}	16.56 ± 0.71^{c}	5.45 ± 0.16^{p}	19.33 ± 0.40^{n}
	25 min, 40 °C	25.04 ± 2.15^{h}	12.89 ± 0.46^{f}	7.71 ± 0.51^{l}	20.74 ± 0.53^{j}
	25 min, 50 °C	17.62 ± 1.33^{n}	6.48 ± 0.33^{p}	10.82 ± 1.30^{e}	24.00 ± 0.60^{d}
	30 min, 30 °C	26.47 ± 1.21^{f}	14.11 ± 1.23^{e}	7.66 ± 0.23^{l}	20.76 ± 1.21^{k}
	30 min, 40 °C	23.35 ± 1.16^{j}	10.62 ± 1.34^{j}	9.46 ± 0.25^{g}	22.15 ± 0.44^{g}
	30 min, 50 °C	13.27 ± 0.51^{p}	5.73 ± 0.50^{r}	12.03 ± 1.13^{b}	25.32 ± 0.21^{b}
Untreated samples		20.00 ± 1.70^{l}	12.63 ± 1.53^{g}	12.50 ± 1.42^{a}	26.70 ± 1.02^{a}

Average values (n= ±3) standard deviation, Different letters indicate significant differences in each column ($p<0.05$).

Table 2. Water holding capacity (WHC), water binding capacity (WBC), drip loss and cooking loss of beef meat treated with ultrasound in a mixture solution of brine-polyphosphate

Type ultrasound		WHC (%)	WBC (%)	Drip Loss (%)	Cooking Loos (%)
Bath	20 min, 30 °C	35.02 ± 0.33^{b}	24.97 ± 1.71^{e}	4.02 ± 0.31^{p}	17.57 ± 0.24^{l}
	20 min, 40 °C	32.65 ± 0.20^{e}	19.23 ± 1.20^{k}	6.24 ± 0.25^{l}	19.04 ± 0.32^{i}
	20 min, 50 °C	26.10 ± 0.15^{m}	13.48 ± 1.22^{o}	7.12 ± 0.27^{i}	19.96 ± 0.30^{h}
	25 min, 30 °C	33.32 ± 0.26^{d}	23.15 ± 2.10^{g}	5.60 ± 0.40^{m}	18.84 ± 1.19^{j}
	25 min, 40 °C	29.79 ± 0.16^{i}	17.08 ± 1.30^{l}	7.77 ± 0.19^{h}	20.31 ± 0.05^{g}
	25 min, 50 °C	32.87 ± 0.19^{o}	11.20 ± 0.15^{q}	8.89 ± 0.15^{f}	21.35 ± 1.17^{f}
	30 min, 30 °C	32.15 ± 0.33^{f}	21.32 ± 1.23^{i}	7.83 ± 0.32^{h}	20.33 ± 1.14^{g}
	30 min, 40 °C	26.97 ± 0.21^{k}	15.19 ± 0.44^{n}	10.03 ± 0.26^{d}	21.84 ± 0.34^{p}
	30 min, 50 °C	19.89 ± 0.53^{r}	9.70 ± 1.22^{r}	10.16 ± 0.30^{c}	22.06 ± 2.19^{d}
Probe	20 min, 30 °C	38.15 ± 0.11^{a}	31.65 ± 1.35^{a}	3.21 ± 0.20^{q}	16.46 ± 0.31^{m}
	20 min, 40 °C	31.77 ± 0.09^{g}	26.84 ± 1.40^{c}	5.18 ± 0.13^{n}	17.82 ± 1.13^{k}
	20 min, 50 °C	26.64 ± 0.23^{n}	20.61 ± 1.23^{j}	8.23 ± 0.31^{g}	21.22 ± 0.88^{f}
	25 min, 30 °C	33.70 ± 0.36^{c}	27.24 ± 2.64^{b}	4.78 ± 0.26^{o}	17.69 ± 0.52^{jl}
	25 min, 40 °C	28.75 ± 0.15^{j}	23.77 ± 1.55^{f}	6.83 ± 0.41^{k}	19.12 ± 1.20^{i}
	25 min, 50 °C	20.83 ± 0.19^{p}	17.05 ± 1.30^{l}	10.05 ± 0.10^{d}	22.44 ± 0.20^{c}
	30 min, 30 °C	31.24 ± 0.21^{h}	25.76 ± 1.28^{d}	6.95 ± 0.25^{k}	19.03 ± 1.23^{i}
	30 min, 40 °C	26.20 ± 0.18^{l}	22.08 ± 1.15^{h}	9.13 ± 0.14^{e}	20.38 ± 0.14^{g}
	30 min, 50 °C	16.55 ± 0.20^{s}	15.35 ± 1.16^{m}	11.31 ± 0.23^{b}	23.95 ± 0.80^{b}
Untreated samples		20.00 ± 1.70^{q}	12.63 ± 1.53^{p}	12.50 ± 1.42^{a}	26.70 ± 1.02^{a}

Average values (n= ±3) standard deviation, Different letters indicate significant differences in each column ($p<0.05$).

In the meantime, the treated samples by ultrasound probe at 30 and 40°C have greater WHC in comparison with samples treated with ultrasound bath ($p<0.05$). However, samples treated by ultrasound probe at 50°C, have less WHC than samples treated with ultrasonic bath (at the same temperature and time). This finding could be related to the more damaging impact of ultrasound probes on meat protein and also more denaturation compared to ultrasonic bath treatment at 50°C. In general, it can be stated that maximum WHC was obtained during 20 minutes at 30°C by the ultrasound probe (38.15 %). Diluted brine solution (up to 5 % salt) leads to improvement of water absorption and swelling of the meat proteins by increasing similar loads in myofibrillar proteins. This phenomenon is called salting-in (Feiner, 2006). Data indicate that ultrasound treatment can lead to an increasethe distribution of salt and thus accelerating the process of brining and the uniform distribution of salt in meat which also helps to increase the WHC and WBC of the meat (Siró *et al.*, 2009). Recently, Inguglia et al. (2017) investigated the effect of geometric parameters of the ultrasound instrument during meat salting in order to enhance salt diffusion and salt distribution in pork meat on a lab scale. They investigated the effects of probe size (Ø 2.5 and 1.3 cm) and of different distances between the transducer and the meat sample (0.3, 0.5, and 0.8 cm) on NaCl diffusion. Their results showed that 0.3 cm was the most efficient distance between the probe and the sample to ensure a higher salt diffusion rate. A distance of 0.5 cm was considered as trade-off distance to ensure salt diffusion and maintenance of meat quality parameters. The enhancement of salt diffusion by ultrasound was observed to be decreased with increasing horizontal distance from the probe.

Polyphosphate has a wide applications in the meat industry. The polyphosphate disturbs the actin and myosin complex through a decrease in electrostatic force, which leads to an increase the solubility and water absorption of meat. The separation of actin and myosin is due to the connection of negative charged phosphate ions with the positive ions of Mg^{+2} and $Ca^{+\ 2}$, because the positive ions of Mg^{+2} and $Ca^{+\ 2}$ have a vital role in muscle contraction (Feiner, 2006).

Pohlman *et al*. (1997) reported that during the ultrasound treatment of meat with a water bath at refrigerator temperature, the ultrasound intensity increase did not cause the WHC to change. They stated that the reason for no dramatic change in WHC is connected to the lack of meat thermal denaturation due to low temperature applied in the experiment.

Siró *et al*. (2009) reported that with respect to WHC, there is no significant difference between the static brining and brining treated with ultrasound (20 kHz, 2-2 Wcm^{-2}, 30 to 180 minutes and at a temperature of 4°C). They also reported that the intensity and ultrasound treatment did not affect the WHC; which can be attributed to the low temperature used in this research.

WBC is one of the very important quality parameters during the production of meat. Indeed, WBC represents the ability of connection and absorption of water. It is known that soluble proteins in salt like actin and myosin have better emulsifying properties and have higher WBC than water-soluble proteins. The importance of increasing WBC in meat products is because of the improvement in the sensory quality of the final product in addition to creating more affordable products (Feiner, 2006).

The WBC of control sample during the static brining was 12.63 % for 20 minutes at a temperature of 30 °C. The WBC of treated meat with ultrasound can be seen in Tables 1 and 2 for brine and a mixture of brine-polyphosphate solutions, respectively. In general, it can be stated that the effect of ultrasound treatment on WBC was similar to that of WHC. Ultrasound treatment significantly increased WBC compared to the control sample ($p<0.05$). The WBC amounts of treated samples with ultrasound were in the range of 5.73 to 31.65 % (Tables 1 and 2). Similar to the result reported for WHC, by increasing the ultrasound treatment temperature from 30 to 50°C, WBC was significantly decreased. However, the WBC of all treatments was more than the control sample ($p<0.05$).

With increasing the time of ultrasound treatment in all test conditions, WBC significantly reduced ($p<0.05$), as reported for WHC. The decrease of WBC with increasing temperature and time can be attributed to meat protein denaturation. The denaturation of proteins leads to the opening of proteins and increasing of the surface of hydrophobic groups and therefore reducing WBC of meat (Feiner, 2006).

At 30 to 40°C and the same treating time , the treated samples by probe ultrasound had greater WBC than those samples treated with bath ultrasound ($p<0.05$). However, samples treated by probe ultrasound at 50°C, have less WBC than those of bath ultrasound (at the same treating time). In fact, the ultrasound probe due to exert higher power than the bath ultrasound can be more effective on the meat texture and consequently the salt and polyphosphate penetrate more greatly into meat. However, the ultrasound probe at higher temperatures intensifies the effects of heat on meat protein denaturation and thus decreasing the WBC. As reported for WHC, the highest amount of WBC was found to be 31.65 % for probe ultrasound (20 minutes at 30°C).

Siró et al. (2009) reported that brining under ultrasound treatment leads to more effectively improve WBC and increase penetration coefficient of sodium chloride, than static brining and soaking into the brine solution. They reported that ultrasound treatment is able to increase the distance between the fibers because of charging of membrane myofibril proteins.

The effects of ultrasound treatment on drip loss and cooking loss of meat:

The amount of drip and cooking loss of control and ultrasound treated samples in brine and brine-polyphosphate are shown in Tables 1 and 2, respectively. Drip and cooking loss of control samples were 12.5 and 26.70 %, respectively. Drip and cooking loss of samples treated with ultrasound was in the range of 3.21-12.03 % and 16.46-25.32 %, respectively. There is a close relationship between WHC and WBC results with drip and cooking loss in such a way that the higher WHC and WBC of samples showed lower drip and cooking loss. As can be seen in Tables 1 and 2, generally, applying ultrasound reduces drip and cooking loss ($p<0.05$). With increasing time and temperature of ultrasound treatment, an increase in drip and cooking loss were observed. The reason for this phenomenon could be explained by denaturation the meat myofibrillar proteins which led to a reduction in the meat WHC and WBC.

Ojha *et al*. (2016) investigated the effect of a combined sonication (at power levels of 9.0 and 54.9 W cm^{-2} for 120 min) and 5 % NaCl on the cooking loss of pork meat. They reported a reduction in cooking losses with an increase in brining time. They also reported that samples treated with ultrasound had lower cook loss than the control sample which might be due to an increase in the sodium content in pork samples.

The effects of ultrasound treatment on the meat texture

The texture is one of the most important organoleptic characteristics of meat which is related to brittleness and mouthfeel. Meat textures depend on factors such as tenderness, WHC or the juiciness as well as the level of meat ripeness and animal age (amount and quality of the connective texture). Texture profile analysis (TPA) is a very important indicator for determining the changes in meat texture (Xiong *et al.,* 2012; Chang *et al*., 2012).

TPA parameters (hardness, resilience, adhesion, cohesion, springiness and potential for chewing) of the control sample and meat treated with ultrasound in brine and a brine-polyphosphate solution are mentioned in Tables 3 and 4, respectively.

Hardness represents the amount of force necessary to achieve the desired deformation in the sample. The hardness of control samples, the samples treated with ultrasound in brine and a mixture of brine-polyphosphate solution were in the range of 77.93 N, 18.24-48.87 N and 12.37-42.49 N, respectively (Tables 3 and 4). Ultrasound treatment at higher temperatures leads to reduce the meat hardness ($p<0.05$). This most likely due to the more meat proteins denaturation at further temperatures and times. Siró et al. (2009) reported that higher intensity of ultrasound treatment led to denaturation of proteins and consequently a reduction in the WHC and hardness of meat.

Table 3. Parameters of texture samples treated with ultrasound in a solution of brine

Type ultrasound		TPA parameters					
		Hardness (N)	Resilience	Adhesiveness (mJ)	Cohesiveness	Springiness (mm)	Chewiness (mJ)
Bath	20 min, 30 °C	20.98 ± 4.33^{o}	0.20 ± 0.01^{bc}	0.90 ± 0.11^{c}	0.51 ± 0.12^{bcd}	5.78 ± 0.32^{g}	61.60 ± 6.24^{m}
	20 min, 40 °C	29.90 ± 3.20^{h}	0.22 ± 0.06^{b}	1.10 ± 0.25^{b}	0.50 ± 0.03^{bcd}	5.06 ± 0.23^{k}	75.20 ± 7.32^{j}
	20 min, 50 °C	45.05 ± 5.15^{d}	0.18 ± 0.04^{bcd}	0.40 ± 0.17^{e}	0.48 ± 0.02^{bcd}	5.48 ± 0.27^{i}	119.30 ± 4.31^{e}
	25 min, 30 °C	22.62 ± 1.26^{l}	0.23 ± 0.05^{b}	0.20 ± 0.06^{f}	0.54 ± 0.10^{bc}	6.68 ± 0.44^{a}	81.60 ± 5.19^{h}
	25 min, 40 °C	33.04 ± 2.16^{f}	0.23 ± 0.04^{b}	1.00 ± 0.19^{bc}	0.56 ± 0.30^{ab}	6.03 ± 0.19^{f}	111.70 ± 6.05^{f}
	25 min, 50 °C	48.32 ± 5.19^{c}	0.23 ± 0.06^{b}	0.10 ± 0.15^{fg}	0.48 ± 0.15^{bcd}	6.28 ± 0.17^{cd}	145.80 ± 2.18^{c}
	30 min, 30 °C	28.79 ± 2.33^{i}	0.18 ± 0.03^{bcd}	0.90 ± 0.22^{c}	0.42 ± 0.23^{def}	6.44 ± 0.30^{b}	77.30 ± 5.14^{i}
	30 min, 40 °C	42.49 ± 1.21^{e}	0.32 ± 0.04^{a}	0.01 ± 0.01^{g}	0.65 ± 0.34^{a}	6.66 ± 0.26^{a}	111.72 ± 5.34^{f}
	30 min, 50 °C	48.87 ± 3.53^{b}	0.19 ± 0.02^{bcd}	0.70 ± 0.20^{d}	0.48 ± 0.22^{bcd}	6.22 ± 0.31^{de}	145.20 ± 3.19^{d}
Probe	20 min, 30 °C	18.24 ± 1.11^{q}	0.23 ± 0.04^{b}	0.20 ± 0.20^{f}	0.47 ± 0.15^{bcd}	5.66 ± 0.23^{h}	48.60 ± 3.31^{p}
	20 min, 40 °C	22.49 ± 2.09^{m}	0.14 ± 0.20^{d}	1.00 ± 0.85^{bc}	0.36 ± 0.06^{ef}	5.57 ± 0.13^{hi}	45.00 ± 2.13^{q}
	20 min, 50 °C	30.15 ± 3.23^{g}	0.18 ± 0.09^{bcd}	0.90 ± 0.37^{c}	0.43 ± 0.13^{cdef}	5.14 ± 0.32^{k}	66.00 ± 1.28^{l}
	25 min, 30 °C	20.67 ± 1.36^{p}	0.15 ± 0.10^{cd}	0.40 ± 0.10^{e}	0.45 ± 0.12^{bcde}	5.30 ± 0.36^{g}	49.40 ± 2.52^{jo}
	25 min, 40 °C	26.90 ± 2.15^{k}	0.21 ± 0.04^{b}	1.10 ± 0.29^{b}	0.49 ± 0.15^{bcd}	6.37 ± 0.41^{bc}	84.10 ± 2.20^{g}
	25 min, 50 °C	33.04 ± 4.19^{f}	0.23 ± 0.05^{b}	1.00 ± 0.25^{bc}	0.56 ± 0.30^{ab}	6.03 ± 0.19^{f}	111.70 ± 3.20^{f}
	30 min, 30 °C	21.96 ± 1.21^{n}	0.23 ± 0.03^{b}	0.40 ± 0.32^{e}	0.43 ± 0.28^{cdef}	6.15 ± 0.25^{e}	57.60 ± 1.23^{n}
	30 min, 40 °C	28.05 ± 3.18^{m}	0.21 ± 0.04^{b}	0.40 ± 0.26^{e}	0.48 ± 0.15^{bcd}	5.17 ± 0.17^{k}	69.40 ± 2.14^{k}
	30 min, 50 °C	42.49 ± 3.20^{e}	0.32 ± 0.08^{a}	0.01 ± 0.01^{g}	0.65 ± 0.16^{bcde}	6.66 ± 0.23^{a}	182.70 ± 4.80^{a}
Untreated samples		77.93 ± 4.85^{a}	0.15 ± 0.02^{cd}	1.60 ± 0.27^{a}	0.33 ± 0.02^{f}	6.48 ± 0.67^{b}	164.70 ± 3.06^{b}

Average values (n= ±3) standard deviation, Different letters indicate significant differences in each column ($p<0.05$).

Table 4. Parameters of texture samples treated with ultrasound in a mixture solution of brine-polyphosphate

Type ultrasound		TPA parameters					
		Hardness (N)	Resilience	Adhesiveness (mJ)	Cohesiveness	Springiness (mm)	Chewiness (mJ)
Bath	20 min, 30 °C	16.75±0.32^{p}	0.19±0.01^{cd}	0.50±0.31^{d}	0.44±0.07^{f}	5.53±0.21^{ij}	41.10±2.24^{k}
	20 min, 40 °C	20.99±0.27^{l}	0.20±0.03^{cd}	0.90±0.26^{c}	0.42±0.20^{f}	5.64±0.25^{h}	49.80±2.32^{i}
	20 min, 50 °C	28.95±0.15^{e}	0.22±0.02^{cd}	0.10±0.37^{gh}	0.50±0.22^{def}	9.79±0.67^{a}	143.00±5.30^{k}
	25 min, 30 °C	19.37±0.46^{m}	0.18±0.04^{cd}	0.10±0.42^{gh}	0.49±0.11^{def}	5.38±0.40^{k}	51.60±3.19^{i}
	25 min, 40 °C	24.24±0.56^{h}	0.17±0.05^{d}	0.20±0.19^{fg}	0.49±0.10^{def}	6.82±0.29^{c}	80.90±3.05^{de}
	25 min, 50 °C	30.15±0.19^{c}	0.18±0.05^{cd}	0.90±0.15^{c}	0.43±0.15^{f}	5.14±0.25^{l}	66.00±4.17^{g}
	30 min, 30 °C	22.21±0.13^{j}	0.33±0.03^{a}	0.10±0.32^{gh}	0.65±0.23^{ab}	5.72±0.42^{h}	82.40±2.14^{d}
	30 min, 40 °C	28.79±0.21^{f}	0.18±0.14^{cd}	0.90±0.20^{c}	0.42±0.34^{fg}	6.44±0.26^{e}	77.30±1.34^{e}
	30 min, 50 °C	42.49±0.53^{b}	0.32±0.05^{ab}	0.00±0.00^{h}	0.65±0.28^{ab}	6.66±0.34^{d}	182.70±2.19^{a}
Probe	20 min, 30 °C	12.37±0.18^{r}	0.20±0.03^{cd}	0.30±0.31^{ef}	0.46±0.35^{ef}	7.97±0.20^{b}	44.90±0.31^{jk}
	20 min, 40 °C	17.38±0.09^{n}	0.28±0.03^{abc}	0.40±0.25^{de}	0.71±0.40^{a}	6.27±0.48^{f}	82.60±2.13^{d}
	20 min, 50 °C	23.35±0.13^{i}	0.18±0.07^{cd}	0.20±0.27^{fg}	0.51±0.25^{def}	5.51±0.11^{g}	72.90±3.28^{f}
	25 min, 30 °C	14.29±0.26^{q}	0.22±0.04^{cd}	0.10±0.40^{gh}	0.55±0.44^{cde}	6.09±0.26^{g}	48.00±2.52^{ij}
	25 min, 40 °C	21.71±0.15^{k}	0.17±0.04^{d}	0.10±0.19^{gh}	0.47±0.25^{def}	5.63±0.47^{hi}	58.00±1.20^{h}
	25 min, 50 °C	25.45±0.16^{g}	0.20±0.05^{cd}	0.50±0.15^{d}	0.51±0.30^{def}	5.00±0.16^{m}	64.60±4.00^{g}
	30 min, 30 °C	16.97±0.21^{o}	0.17±0.03^{d}	0.40±0.32^{de}	0.44±0.29^{f}	4.36±0.25^{k}	32.60±2.23^{l}
	30 min, 40 °C	23.35±0.19^{i}	0.18±0.04^{cd}	0.20±0.26^{fg}	0.57±0.15^{bsd}	5.51±0.24^{g}	72.90±4.14^{f}
	30 min, 50 °C	29.81±0.22^{d}	0.23±0.02^{cd}	1.80±0.30^{a}	0.61±0.16^{abc}	6.89±0.33^{c}	125.40±6.80^{c}
Untreated samples		77.93±4.85^{a}	0.15±0.02^{d}	1.60±0.27^{b}	0.33±0.02^{g}	6.48±0.67^{e}	164.70±3.06^{b}

Average values (n= ±3) standard deviation, Different letters indicate significant differences in each column ($p<0.05$).

In general, samples treated with a brine-polyphosphate solution had a lower hardness in comparison with those of brine solution only. This is related to higher WHC and WBC of samples because the amounts of water attached to the meat make it softer (Cheng & Sun, 2008). Samples treated with probe ultrasound had a lower hardness than those of ultrasound bath, which attributed to the extremely damaging effect of the ultrasound probe on meat texture.

Resilience represents the capacity of a substance to store energy caused by stress in the linear elastic range. Higher resilience means that the substance has a higher resistance to permanent deformation (Chang *et al.*, 2012). According to Tables 3 and 4, the resilience of samples treated with ultrasound, in most cases was significantly increased compared to that of control samples ($p<0.05$). However, in some cases the increase was not significant.

Adhesion is a parameter to describe food sticking to the teeth by chewing. The amount of adhesion was noticeably reduced by ultrasound treatment ($p<0.05$).

Coherence parameter helps to have a comprehensive understanding of the viscoelastic properties, such as material tensile strength. As can be seen in Tables 3 and 4, generally, the coherence of samples treated with ultrasound was increased significantly (except for a few examples) ($p<0.05$). This indicates that the cohesion of samples was a little affected by ultrasound treatments and therefore samples maintained their cohesion. Zhong *et al.*, (2007) reported that increased meat cohesion is most likely due to the release of some components during the sonication.

Meat springiness is likely to be related to the degree of swelling of muscle fibers (diameter of muscle fibers). It seems that this parameter affects myosin denaturation and α-actinin (Chang *et al.*, 2012). According to Tables 3 and 4, the springiness of samples was independent from the temperature, the time and the type of ultrasound treatment.

Chewiness can be used to evaluate meat tenderness, which is obtained by multiplying indicators of hardness, cohesion and springiness. There is an indirect relationship between chewiness and meat tenderness in such a way that the lower chewiness value of meat, has the more tenderness value (Siró *et al.*, 2009). As can be seen in Tables 3 and 4, ultrasound treatment in most samples leads to a significant reduction in meat chewiness compared to the control samples. It represents an increase in meat tenderness treated with ultrasound ($p<0.05$). With increasing temperature and ultrasound treatment time, small reduction in chewiness was occuredin comparison with that of the control sample; which can be related to the denaturation of proteins at higher temperatures and longer times. The sample chewiness treated with probe ultrasound was lower than those of ultrasound bath ($p<0.05$). This indicates a greater ability of ultrasound probe to increase the meat tenderness. The chewiness of treated samples in a mixture solution of brine-polyphosphate was less than those of brine (Tables 3 and 4). It is attributed to higher WHC and WBC of this samples (Table 1 and 2), which resulted in their higher tenderness.

Similar to the present results, Siró *et al.* (2009) reported that ultrasound treatment reduces the hardness and the chewiness of meat. They also expressed that the increase in temperature and intensity of ultrasound treatment is due to denaturation of proteins that causes higher hardness and chewiness of meat compared to lower temperature and intensity of the ultrasound. Chang *et al.* (2012) showed that the use of ultrasound (40 kHz, 1500W) in beef meat for 10, 20, 30, 40, 50, and 60 minutes did not have a significant effect on color. In general, ultrasound treatment leads to a reduction in total meat hardness. In this regard, the lowest hardness of the samples was for those of under ultrasound treatment for 10 minutes.

The most important reasons for the increase in meat tenderness with ultrasound treatment can be directly connected to the physical degradation of the skeletal muscle (especially Nebulin grids) and bonded coating on the muscles (perimysium). It could also be indirectly related to activation of calcium-dependent proteolytic enzymes (for example calpains), through the release of calcium ions and cathepsin from the sarcoplasm network and other organelles (Jayasooriya *et al*., 2007; Xiong *et al*. 2012).

In general, the results showed that ultrasonic treatment can effectively improve meat quality such as increasing WHC and WBC and reducing drip, cooking loss, hardness, chewiness and tenderness of meat. It was also found that the probe ultrasound was more effective than ultrasound bath and a solution mixture of brine-polyphosphate is better than only brine solution. Furthermore, results showed that ultrasound treatment at lower temperatures and short times resulted in an improve in meat quality in comparison with longer ultrasound times and higher temperatures.

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اثر اولتراسوند حمام و پروب در ترکیب با محلول های آب نمک و آب نمک-پلی فسفات بر خواص کیفی و بافتی گوشت گاو

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چکیده

تکنیک‌های مختلف مکانیکی، آنزیمی و شیمیایی برای بهبود کیفیت گوشت استفاده می‌شود. چنین تکنیک‌هایی معایبی مانند زمان‌بر بودن و آسیب رساندن به شاخص‌های کیفی گوشت دارند. اولتراسوند به‌عنوان یک روش موثر برای اصلاح خواص تکنولوژیکی و ترد کردن گوشت استفاده می‌شود. نمونه‌های گوشت (از قسمت قلوه‌گاه) درون محلول نمکی یا مخلوطی از محلول آب نمک- پلی‌فسفات در التراسوند حمام (در فرکانس 37 کیلوهرتز) و پروب (20 کیلوهرتز) در دماهای 30، 40 و 50 درجه سانتی‌گراد به مدت 20، 25 و 30 دقیقه. تحت تیماردهی قرار گرفتند. تغییرات خواص تکنولوژیکی و بافتی نمونه‌های گوشتی مورد بررسی قرار گرفت.نتایج افزایش pH (از 5/55 برای نمونه کنترل تا 7/14)، ظرفیت نگهداری آب (WHC)(از 20/00% برای نمونه کنترل تا 38,15%)، ظرفیت اتصال آب (WBC) (از 12/63% برای نمونه کنترل تا 31/65%) و یک کاهش در افت خونابه (از 12/50% برای نمونه کنترل تا 3/21%)، افت پخت (از 36/70% برای کنترل تا 16/46%) را نشان داد.همچنین پارامترهای بافتی از جمله سختی و قابلیت جویدن کاهش، در حالی‌که تردی افزایش یافت. به‌طور کلی، تیمار اولتراسوند پروب درون محلول ترکیبی آب نمک- پلی‌فسفات کارآمدتر بود. می‌توان نتیجه گرفت که التراسوند یک روش موثر برای بهبود کیفیت گوشت می‌تواند باشد.

واژه‌های کلیدی: گوشت گاو، خواص تکنولوژیکی، تردی، ویژگی‌های بافتی، اولتراسوند

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