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Quality of beef after application of high-intensity ultrasound

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Abstract

BACKGROUND: The application of high-intensity ultrasound causes changes in physical and chemical properties of biological materials including meat. In this study the physicochemical and microbiological characteristics of beef after the application of high-intensity ultrasound for 60 and 90 min and subsequently stored at 4 °C for six varying time periods, namely 0, 2, 4, 6, 8 and 10 days, were evaluated.

RESULTS: The ultrasound-treated meat showed higher (P <0.05) pH and luminosity than the control with no difference (P >0.05) between sonication times. The meat redness of ultrasound treated meat was lower than the control meat however no difference (P >0.05) was observed after day 8 of storage. The 90 min ultrasound treated meat was higher (P <0.05) in yellowness and this

remained during the entire storage period. Ultrasound decreased (P <0.05) coliform, mesophilic and psychrophilic bacteria in the meat throughout the whole storage period, however the original microbial loads increased constantly during refrigeration. The 90 min ultrasound treated samples showed the greatest reduction in microbial load during storage. Coliforms and psychrophilic bacteria were the most affected by ultrasound.

CONCLUSION: The application of high-intensityultrasound to beef semitendinosus stored at 4 °C decreased bacterial growth and mantained the physicochemical quality of meat.

Keywords: High-intensity ultrasound; Beef; Physicochemical properties; Bacteria.

INTRODUCTION

Ultrasound is considered as being one of the few processing technologies with the potential to diversify into a range of industry sectors, as such it has been widely investigated over the last decade. Extensive review articles have shown the multiple applications of ultrasound in the food industry (1-12) most being focused to improving the product quality and extending the shelf life of fresh and processed products (3, 9).

Some research has indicated that ultrasound can aid in the extraction, gelation, and restructuring of meat proteins (13, 14) and to accelerate the brining process by an increase of NaCl transfer into the meat structure (15, 13, 15, 16, 17).

The application of ultrasound in meat increases the tenderness of beef (18-21), and also the pH, particularly during aging (22), with an additional improvement in color (21) and water-holding capacity of the meat (18, 22). However other workers failed to find any variation in meat quality as a result of the application of ultrasound (23, 24). Therefore more information and research on this subject, in particular with respect to meat physicochemical quality, is still needed.

Other well known applications of ultrasound include the killing or inhibiting of bacterial

growth. It has been reported that high-intensity ultrasound within the frequency range of 20–100 kHz and of energy intensity 10–100 Wcm^{-1} generates gradients of intense pressure that can alter the structure of bacteria in food. Microbial inactivation has been observed using ultrasound in fruits and vegetables (25, 26) and skimmed milk (27-28). The effect of ultrasound on microorganisms is complex, but the alteration of cell membranes and DNA chains is believed to be the main cause of the lethal or deactivation effect. A study carried out with vacuum-packed meat demonstrated that ultrasound treatment caused an immediate reduction in the number of viable bacteria using low energy intensity (1.55 W cm^{-2}); however, after 5 days the number of microorganisms grew back to the same levels as in the untreated meat (21). When it is used in combination with moderate heat, ultrasound can accelerate the decontamination rate of food, and reduce both the duration and intensity of heat treatments and their resulting damage (29).

It has also been reported that gram-positive bacteria are more resistant to ultrasound treatments than gram-negative bacteria (29, 30, 31, 32). Research studies have mainly focused on the deactivation of *Listeria monocytogenes* (33, 34)*, Escherichia* (33, 35, 36, 37, 38), a series of *Salmonella spp.* (36, 39), *Staphylococcus aureus,* (40) and some other microorganisms (41).

In this work the characteristics of the physicochemical and microbiological quality of beef treated with high-intensity ultrasound were evaluated in order to determine the effect of different sonication treatment times on the beef during storage.

EXPERIMENTAL

Sample preparation

Semitendinosus muscle samples were randomly collected from the carcasses of five adult cows from a commercial establishment. The visible fat was removed and each muscle was cut in 18 slices, each 1.7 cm thick. Three ultrasound treatments (0, 60, and 90 min) and five storage periods at 4 °C (2, 4, 6, 8, and 10 days) were applied. Six slices were assigned per treatment and each slice was vacuum-packed individually for each storage period. The treatments studied were (1) 0

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min control sample, sample not treated with ultrasound, (2) 60 min, meat treated with ultrasound for 60 min; and (3) 90 min, meat treated with ultrasound for 90 min.

Ultrasound treatment

The samples were sonicated for the appropriate time (0 (control samples), 60, 90 mins) in a Branson 1510 ultrasonic cleaner (Branson Ultrasonics, Emerson Industrial Automation, San Louis, MO) at a 40 kHz frequency and an intensity of 11 Wcm^{-2}, using triple distilled water as the diffusion medium.

Physicochemical analysis

For each storage time, the wrapping was removed from the sonicated meat slice, relating to each specific treatment, and then the sample was tested to evaluate pH, color (L*, a*, and b*, defined below), water-holding capacity (WHC) and drip loss (DL). pH was measured with a digital hand-held meat pH meter (Sentron, Model 1001, Sentron Technologies, Roden, The Netherlands) with the electrode inserted into the muscle. Measurement of pH was carried out at the time of performing the other physicochemical tests for meat quality. All measurements were carried out in triplicate. To determine color, first, the connective tissue and the visible fat from the muscle surface were removed, the surface was exposed to air for 10 min, and then the measurements of the color parameters were carried out using a Minolta CR400 colorimeter. The values were expressed as L* (luminosity), a* (redness), and b* (yellowness). WHC, was determined by the press method (42) as modified by Tsai and Ockerman (43). A sample of approximately 0.3 g was weighed on an analytical balance and placed between two filter papers, which in turn were placed between two plexiglass plates, upon which a constant weight pressure of 5 kg was exerted. WHC was later calculated by weight difference and expressed as a percentage.

Drip loss was evaluated by the method of Honikel and Hamm (44) using a portion of semitendinosus muscle of approximately 3 g to later introduce it to a plastic container, suspended

by a thread which was then sealed and stored at 4 °C for 48 h to later calculate DL by difference and expressed as a percentage.

Microbiological analysis

A sample of exudate was taken from each of the packed meat portions. The external part of the bag was disinfected to prevent contamination of the sample and then the package was opened. An amount of 0.25 mL of exudate was taken from each sample and 2.5 mL of diluent was added. The samples were then repacked before proceeding with the application of high-intensity ultrasound (as it corresponded to each treatment). The collection of exudate samples was carried out in the same way immediately after the ultrasound treatment and after 2, 4, 6, 8, and 10 days of storage at 4 °C. After collecting exudate, a series of dilutions from 1:10 to 1:10,000,000 were prepared in maximum recovery diluent (MRD; Oxoid) broth. For the recounting of *psychrophilic* and mesophilic bacteria, we used the spread plate technique (45). From each of the dilutions, 100 μL was inoculated in the corresponding culture medium. For counting mesophilic and *psychrophilic* bacteria, the dilutions were inoculated onto agar plates (plate count agar, Oxoid). The plates for mesophilic bacteria were incubated at 35 ± 2 °C for 48 ± 2 h and the plates for *psychrophilic* bacteria were incubated at 5 ± 2 °C for 7 days. Determination of *E. coli* was made on a solid medium, by inoculating the dilutions in MacConkey agar (Oxoid) samples and incubating the plates at 44 ± 1 °C for 18 to 24 h. Finally, the microbial count of each petri dish was carried out. The following equation was used to calculate the number of colony forming units per milliliter (CFU/mL^{-1}) in the exudate of each semitendinosus muscle sample:

$$\frac{CFU}{mL} = A \cdot DF \cdot 10$$

where

A: average number of colonies in a dilution

DF: dilution factor, which depends on the dilution used, as follows: 1 for 1:0, 10 for 1:10, 100 for 1:100, 1000 for 1:1000, 10000 for 1:10000, 100000 for 1:100000 and 1000000 for 1:1000000.

Microbiological characteristics

To measure the growth of mesophilic, psychrophilic and coliform bacteria present in meat, the $CFUmL^{-1}$ value was determined for each of the six sampling times. Each value obtained in $CFUmL^{-1}$ was transformed to units of log cycles (log_{10}).

Statistical analysis

The data from the physicochemical variables were analyzed using a model for a completely randomized design with repeated measurements over time, which included as fixed effects the application of ultrasound, storage time, and their possible interaction. The Proc Mixed procedures of SAS version 8.0 (46) was used according to the following statistical model:

$Y_{ijk} = \mu + U_i + T_j + U^*T_{ij} + \varepsilon_{ijk}$

where

Y_{ijk}: response variable, measured in observation k for the exposure period to ultrasound i, at the storage time j

μ: overall means

U_i: effect of the exposure period to ultrasound (i = 0, 60, and 90 min)

T_j: effect of storage time j (j = 0, 2, 4, 6, 8, and 10 days)

$U^*T_{(ij)}$: effect of the interaction between exposure period to ultrasound i and storage time j

ε_{ijk}: random error measured in observation k for the exposure period to ultrasound i, at the storage time j.

Differences among treatment means were determined by orthogonal contrasts (SAS Institute, 2003).

RESULTS AND DISCUSSION

Physicochemical analysis

In general, meat pH tended to decrease (Table 1) over storage time at 4 °C (P < 0.05) and for all the periods of exposure to ultrasound (P < 0.05). It is well known that pH is one of the parameters with great influence on meat quality and it is directly related to characteristics such as WHC, DL, color and texture of meat (47). Therefore, it is an important quality indicator of fresh and processed meat. However, the pH values observed in this study were within the normal values of meat; they ranged from 5.4 to 5.6 (48). In other work, (22) did not find effect on the pH after sonication of meat, and observed an increase of pH with increasing ageing time up to 4 days.

Table 1 shows that L*of meat from the control group was lower (P < 0.05) than that of meat treated with ultrasound for 60 or 90 min, but no significant difference was detected between the two sonication times (P > 0.05). L*of meat during storage was similar in all treatments. It tended to increase until day 6, at which the maximum value was reached, then it decreased, and by day 10 the value of L* was similar to that at time 0 (P > 0.05). It is possible that these changes are related to pH, which increases until day 4 of storage and then decreases to lower than the initial value.

In Figure 1 significant differences were observed among treatments (P < 0.05) for the color characteristics (a* and b*). Regarding red tendency (a*), the values of the control sample were higher than those of meat treated with either of the two ultrasound periods as these showed a negative tendency until day 6 of storage (Figure 1), with an increase on day 8, and they then remained unchanged until day 10 of storage. This indicated that the control sample retained a red tendency better than the sonicated samples during refrigerated storage. Similar result were found by Pohlman et al. (21) who observed that ultrasonic treatment caused muscles to be lighter, less red and more yellow colored, i.e. L* and a* increased whereas a* decreased.

Regarding the values of yellow intensity (b*) (see Figure 2), there was a difference between treated sample type only on day 0, where the control showed a higher value compared to the sonicated samples, but from days 2 to10 there were no differences among the three treatments.

In meat, the color may be influenced by intrinsic factors (type of muscle, breed, gender, susceptibility to stress) and extrinsic factors (food, pre-slaughter stress, enzymatic reduction of myoglobin, pH, temperature, storage, and oxidation) (49). Furthermore, meat color depends on physicochemical aspects such as pH decrease, since color evolves from the moment of slaughter, produced by the transformation of glycogen into lactic acid, which can transform the optical properties of meat into an opaque or clear solid (50).

Significant differences (P < 0.05) were found in the WHC over time (Figure 3). The treatments presented a tendency to increase WHC during the storage time without showing differences among them (P > 0.05). WHC presented the highest percentages on day 6 of storage in all of the three treatments. It showed a decrease on days 8 and 10 of storage.

It is known that WHC in any muscle is minimal at low pH, but it tends to increase with maturation due to protein degradation and changes in electric charges by intramolecular rearrangement (48). In the present study, the performance of WHC can be explained by the pH decrease observed throughout the storage period, which might cause shrinkage of the network of polypeptide chains and decrease the number of free ionic groups available to bind water. If the WHC is low, the weight loss during storage is greater because of surface evaporation and exudation of the cutting surface. WHC is related to many physicochemical characteristics of myofibrillar and protein components. The results of the present study agree partially with the results of others (17, 22) who also found an increase in WHC of sonicated meat.

Significant differences (P < 0.05) were found in DL over time (Figure 4). The ultrasound treatment for 90 min had the highest values (P < 0.05) from day 2 of storage and they remained stable until day 10, while the DL of the control sample and the one treated for 60 min decreased with storage. DL results indicated a decrease according to the storage time. This is related to the

increase of WHC observed in treatments (Figure 3), as there is an inverse relationship between WHC and DL. This means that if WHC is low, humidity loss or loss of weight during storage is greater because of exudation of the surface (50). In this study, the samples with high WHC showed the lowest DLs.

Microbiological tests

Effects ($P < 0.05$) of the ultrasound exposure period on the concentration of microorganisms were observed (Table 2). The response varied ($P < 0.05$) according to the type of microorganism being evaluated. Storage time affected the concentration of microorganisms ($P > 0.05$). All the types of microorganisms ($P > 0.05$) and all the exposure periods to ultrasound ($P > 0.05$) were affected in the same way (without interaction).

Mesophilic bacteria

For mesophilic bacteria no significant difference ($P > 0.05$) was found for the storage time (Figure 5 and Table 2) but a significant difference was detected for ultrasound treatment ($P < 0.05$). For all the storage times, the control treatment showed a higher number of $CFUmL^{-1}$, since all the samples treated with ultrasound showed a count well below the control treatment. This is also consistent with the results obtained by Dolatowski and Stasiak (51) which indicated that large numbers of bacteria can be controlled by high-intensity ultrasound treatment. This effect has also been corroborated by other research (35, 52, 33, 53). Furthermore, it has been recently reported that ultrasound's capacity to control microbial growth depends directly on its intensity and its frequency (54). In research carried out by Jayasooriya *et al.* (18) and Lyng et al. (55), it was observed that vacuum packing hinders the penetration of high-intensity ultrasound to the sample; consequently the ultrasound effect decreases. However in subsequent research, Birk and Knøchel (34) found that when the intensity and duration of treatment are adequate, the effect of packing may not be important to the effect of ultrasound.

Psychrophilic bacteria

Significant differences (P < 0.05) were found in psychrophilic bacteria for the effect of ultrasound exposure (Table 2). The control samples showed a higher number of $CFUmL^{-1}$ than the values shown by the samples that were subjected to ultrasound treatment. On the counts, psychrophilic bacteria showed a greater decrease (P < 0.05) than mesophilic bacteria (Figure 5). The results of the present study do not agree with the results of Vilkhu *et al*. (56) who pointed out that ultrasound is an efficient method for the extraction of various food components, and this can contribute to the growth of psychrophilic bacteria. In contrast, Dolatowski and Stasiak (51) reported that samples stored under refrigeration did not show a significant reduction in bacteria even when they had been treated with ultrasound. In this regard, other authors (57, 58) also pointed out that some psychrophilic organisms grow easily in products that are vacuum packed and stored under refrigeration, this effect was not seen in the current study. In our study, we found an effect of ultrasound on psychrophilic bacteria and that it persisted throughout the storage time under refrigeration. The lowest psychrophilic values were registered at period 0 of storage (Figure 5), and there was a tendency for these values to increase on the last days of storage. Nonetheless, the samples treated with ultrasound had values of psychrophilic bacteria below those of the control sample.

Total coliforms

Significant differences were found on the coliform counts between ultrasound treatments (P < 0.05) (Table 2). These bacteria were the most affected by ultrasound treatment at each storage time. An effect could be observed in the decrease of $CFUmL^{-1}$ values in the two ultrasound exposure periods as well as in the storage periods in comparison with control samples (Figure 5). These results are similar to those reported by Nazari (59) where *E. coli* inhibition levels of approximately 49% in fruit syrup were reported. However, when the syrup was treated with

ultrasound, the inhibition of this organism increased to 81%. Joyce *et al*. (54) studied different ultrasound frequencies and found that *E. coli* was more sensitive to low frequency ultrasound (20 and 40 kHz) because a significant decrease in the counts at the end of treatment was observed. According to Drakopoulou *et al*. (60), the effect of high-intensity ultrasound on *E. coli* depends directly on the temperature at which the experiment is performed. At room temperature (~ 25 °C) or lower, the effectiveness of ultrasound treatment decreases; conversely, the elimination of bacteria such as *E. coli* at high temperatures remains constant because of the effect of ultrasound (61). This is demonstrated in the present study, in which temperature presented a tendency to increase when ultrasound was applied, thus limiting the growth of coliforms. At refrigeration temperatures of 4°C the growth of *E. coli* is practically nonexistent. These results suggest that the main effect of high-intensity ultrasound is the deagglomeration of bacterial colonies (62). Therefore, if there is cell deagglomeration, the number of $CFUmL^{-1}$ detected will be higher. Despite these observations, in the current study, the ultrasound method was very effective in the reduction of total coliforms, when applied to the meat for either 60 or 90 min, presenting an approximate reduction of three and four log cycles, respectively.

CONCLUSIONS

In conclusion ultrasound applied for 60 or 90 min to bovine semitendinosus muscle increases meat luminosity and lowers pH without affecting the redness or yellowness, or the water-holding or drip loss properties. Storage at 4 °C improves water holding and reduces drip loss. High-intensity ultrasound helps to control the growth of mesophilic and psychrophilic bacteria and total coliforms in beef stored at 4 °C. It was demonstrated that the use of high-intensity ultrasound shows immediate effects over the bacterial flora of meat. This method represents an alternative technique in order to control the growth of psychrophilic bacteria and coliforms in beef stored at 4 °C, without affecting its physicochemical properties. This is of great interest to the meat

industry and to the consumer, however further research is still needed in order to standardize the application of ultrasound at the industrial level.

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Table 1. Least squares means (± standard error) for L* and pH of the bovine semitendinosus muscle after application of high-intensity ultrasound

Characteristic	Days of storage					
	0	2	4	6	8	10
pH						
Control[x]	5.51 ± 0.02^{ac}	5.47 ± 0.02^{bc}	5.57 ± 0.02^{a}	5.44 ± 0.02^{c}	5.17 ± 0.02^{d}	5.18 ± 0.02^{d}
60 min[y]	5.35 ± 0.02^{ac}	5.36 ± 0.02^{bc}	5.52 ± 0.02^{a}	5.35 ± 0.02^{c}	5.12 ± 0.02^{d}	5.13 ± 0.02^{d}
90 min[y]	5.41 ± 0.02^{ac}	5.38 ± 0.02^{bc}	5.50 ± 0.02^{a}	5.31 ± 0.02^{c}	5.11 ± 0.02^{d}	5.07 ± 0.02^{d}
L*						
Control[x]	32.80 ± 0.84^{ac}	34.96 ± 0.84^{bc}	35.54 ± 0.84^{ab}	36.21 ± 0.84^{a}	32.37 ± 0.97^{c}	32.43 ± 0.97^{c}
60 min[y]	40.60 ± 0.84^{ac}	37.91 ± 0.84^{bc}	40.73 ± 0.84^{ab}	41.81 ± 0.84^{a}	38.42 ± 0.97^{c}	38.44 ± 0.97^{c}
90 min[y]	44.34 ± 0.84^{ac}	39.90 ± 0.84^{bc}	43.43 ± 0.84^{ab}	45.19 ± 0.84^{a}	37.75 ± 0.97^{c}	36.33 ± 0.97^{c}

[abcdxy] Different letters within the row or column indicate significant difference ($P < 0.05$).

Table 2. Lean squaremeans (Log CFUmL^{-1}± standard error) for bacteria growth during storage at 4 °C of bovine semitendinosus after application of high-intensity ultrasound

Sonication (min)	Storage time (days at 4 °C)						
	0	**2**	**4**	6	8	10	Average
Total coliforms							
0	2.86±0.33	3.62±0.31	4.51±0.31	6.09±0.31	5.97±0.31	5.61±0.43	4.78±0.16
60	0.66±0.33	0.66±0.33	0.98±0.33	2.55±0.33	2.75±0.33	2.81±0.43	1.73±0.16
90	0.66±0.33	0.66±0.33	0.66±0.33	1.26±0.33	1.97±0.33	2.38±0.43	1.26±0.16
Mesophilic							
0	4.56±0.33	4.12±0.37	4.55±0.37	5.52±0.37	6.58±0.37	7.60±0.43	5.49±0.17
60	2.43±0.33	3.24±0.33	3.51±0.33	5.25±0.33	5.76±0.33	6.50±0.43	4.45±0.16
90	1.65±0.33	2.85±0.33	3.23±0.33	4.48±0.33	5.16±0.33	5.47±0.33	3.81±0.16
Psychrophilic							
0	3.84±0.33	4.29±0.33	4.42±0.33	5.72±0.33	6.27±0.33	6.73±0.43	5.21±0.16
60	0.66±0.33	1.55±0.33	2.10±0.33	3.67±0.33	3.88±0.33	4.51±0.43	2.73±0.16
90	0.66±0.33	0.66±0.33	0.92±0.33	2.37±0.33	3.14±0.33	3.23±0.43	1.83±0.16