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Research Article

Effect of Acid Marination Assisted by Power Ultrasound on the Quality of Rabbit Meat

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The consumption of rabbit meat is scarce in Mexico, despite its good taste and high protein content. Acid marination may be an option to introduce this meat into new markets and add value to this food. The use of ultrasound has been reported as an accelerator in the impregnation process and improving the product quality. The objective of this study was to evaluate the effect of acid marination assisted by power ultrasound on quality of rabbit meat. Marinating solutions were prepared with 70, 140, or 200 g NaCl/L, 3 g $NaNO_2$, and 1.5% citric acid. Rabbit meat slices were immersed in every solution in static and ultrasonic bath at 40 kHz and 110 W of output power. Marinating time, NaCl concentration in the marinating solution, and US application increased weight losses and meat hardness but reduced water content, WHC, and pH. The color parameters L^* and a^* increased with the NaCl concentration in marinating solution and with the ultrasound application. NaCl content increased in meat with increased marinating time, NaCl concentration in marinating solutions, and higher rate in ultrasonic treatment. Application of ultrasound has high potential to be employed in the marinating of rabbit meat samples, diminishing the immersion time, and maintaining the quality.

1. Introduction

The consumption of rabbit meat is scarce in Mexico, despite its good taste and high protein content. In recent years, the rabbit meat production has been considered as a potential sector to contribute to the development of different rural zones in Mexico [1]. In comparison with cattle, sheep, goats, pigs, and poultry, rabbit meat and its products are less consumed, which leads to rabbit meat producers to diversify their products with the purpose to increase its value and consumption [2].

The most commonly used technique to add value to meat products is the acid marination. This technique may be an option to introduce the rabbit meat into new markets and add value to this food. In this process, an aqueous or oily acid solution is incorporated in the meat; the solution contains different ingredients (salt, phosphate, protein, organic acids, and others), improving the flavor, softness, color, juiciness in the meat, and extension of shelf life [3]. During meat marination the sodium chloride uptake and distribution are important factors to be considered since they represent the amount of brine absorbed during the marinating process and retained after the process, respectively. In such a process, both factors must be maximized to increase yield. For this reason, it is essential to control the marinating process, that is, the amount of salt and the marinating time.

The study of the transfer mechanism of sodium chloride through the meat structure is essential to control marinating. The mass transfer mechanism during this process is relatively slow resulting in a significant increase in processing time and costs [1]. Currently the use of power ultrasound (US) in the meat process is based on the advantage over traditional processes by reducing processing time, improving the product quality, and accelerating mass transfer, due to the effects of cavitation, compression, and expansion produced by the waves [4]. Some examples are the salting of beef [5], curing of meat pork [6], salting of meat pork [4, 7], drying of salted cod [8] and marinating of chicken breast [9]. The acceleration of mass transfer in the impregnation process of meat is generated through the phenomena described, notably cavitation, which is the implosion of microscopic gas bubbles close to a solid surface generating microjets in the direction of the surface that can enhance mass transfer by disturbing the boundary layer [4]. The violent implosion of cavities in solid surface may produce meat tissue damage and therefore affect the hardness, water holding capacity (WHC), and tenderization of the meat [5]. Likewise, US treatment can induce free radicals that are generated from water molecules as a result of cavitation and causing hydrolysis or oxidation of lipid [10, 11], reactions which affected the color of the meat [12]. Regarding compression and expansion, these mechanisms may also affect the mass transfer and the structure of the meat. The pressure fluctuations between the compression and rarefaction cycles of the sound wave cause a mechanical squeezing and releasing of the sample or the so-called "sponge-effect" [4] and this may lead to better distribution of the brine within the meat. Previously, we applied ultrasound for marination of rabbit meat in order to evaluate the diffusion of salt in the meat [13]. Following the Fick's second law, the ultrasound treatment increased the diffusion, with a coefficient ranging between 4 and $9.69 \times 10^{-7}\,m^2/s$, in comparison with the static bath with values from 1.36 to $6.37 \times 10^{-7}\,m^2/s$, depending on the NaCl concentration in the marinating solution. Now, the objective of this work was to study the effect of power ultrasound on the quality of rabbit meat, which has lower tenderness than other kinds of meat.

2. Materials and Methods

2.1. Raw Material and Shaping Samples. Fifty rabbits (California race) were provided by the National Center of Rabbit located in the city of Irapuato, Mexico. Individual animal was not considered as a factor in the statistical analysis, because of homogeneity in weight (±50 g), age (±2 days born), and gender (males). Only legs were employed for the study. The meat (24 h postmortem and $pH_{24} = 5.9 \pm 0.1$) was packed in plastic film and frozen for 13-14 h before separating the samples. Parallelepiped-shaped samples (length 50 mm, width 30 mm, and thickness 10 mm) were obtained from the central part of the rabbit legs using a sharp knife. After cutting, the samples were wrapped in plastic waterproof film and stored at $-18 \pm 0.5^{\circ}C$ until the marinating experiments. Before experiments, samples were slowly thawed at temperature 2°C for 24 h.

2.2. Experimental Conditions. The samples were weighted and subjected to different treatments in liquid medium. These were marinated by immersion in solutions that containing different concentrations of NaCl (70, 140, and 200 g/L), with an addition of 3 g/l $NaNO_2$, 1.5% citric acid, and deionized water. Fourteen of the fifteen rabbit meat slices were immersed in every solution (1 : 5 sample : solution) at 4°C during 120 min in static bath. The remaining slice was used to characterize the initial conditions of the meat marinating. Samples were taken out of the solutions at 0, 15, 30, 45, 60, 90, and 120 min (two samples by time), rinsed in distilled water for 20 s in order to eliminate the solution adhered to the surface, and blotted with tissue paper to remove surface water. Then samples were weighted and wrapped in plastic waterproof film and frozen ($-18 \pm 0.5^{\circ}C$) until the physical chemical determinations.

The same procedure was carried out in samples immersed in an ultrasonic bath (Branson M2800, Danbury, USA) at 40 kHz and 110 W of output power. The bath temperature was maintained constant at 4°C during the marinating with US application. Ice gel cubes were added to the bath surface to keep a constant temperature and water temperature was monitored with a thermocouple. A calorimetric method was used to determine the acoustic intensity applied to the marinating solutions. This method involved the determination of the temperature increase in the first 180 s of ultrasound application [4]. The value of $\delta T/\delta t$ was estimated from the graph of temperature as a function of time:

$$P = MC_p \frac{\partial T}{\partial t}, \quad (1)$$

where P is the ultrasonic power, M is the mass of the fluid undergoing US (kg), C_p is the calorific capacity of the fluid at constant pressure (J/g°C), and $\delta T/\delta t$ is the increase of temperature. The acoustic intensity (W/cm^2) was determined dividing the ultrasonic power by the emitting surface (referred to the surface of the US bath).

2.3. Quality Techniques for Rabbit Meat

2.3.1. NaCl Uptake. After the marinating treatment, the procedure for NaCl determination was performed from the Mohr method [14]. For this purpose, 5 g of meat tissue previously triturated was cooked for 2 min in 50 mL of distilled water. Afterwards, the homogenate was diluted with water (100 mL) and filtered (Whatman No. 4) to obtain the meat extracts. From the final solution, 5 mL was taken and mixed with the reagent, $K_2Cr_2O_7$, and titrated with $AgNO_3$ (0.1 N). Finally, the percentage of NaCl was calculated. The NaCl content of each sample was also determined in triplicate.

2.3.2. Moisture Content. The evolution of the water content of each slice over time was determined by drying the sample until constant weight at 103 ± 2°C [10].

2.3.3. Water Holding Capacity (WHC). The effect of acid marinating at different concentration of NaCl on WHC at marinating times was calculated as the variation between the water content after immersion the sample in the acid marination solutions (M_{water}) and the initial water content (M_{water0}) referred to this last value [15]:

$$\text{WHC} = \frac{M_{water} - M_{water0}}{M_{water}}. \quad (2)$$

2.3.4. pH Determination. The pH was measured using a digital pH meter (Model pH120, Conductronic, Mexico City) by direct immersion of the electrode into marinating samples (1 : 10 tissue : deionized water). pH meter was calibrated with buffer 7.0 and 4.0.

2.3.5. Texture. Hardness, characterized as maximum penetration force, was evaluated in marinating samples using a Texture Analyzer (TAX-T2, Stable Micro System, United Kingdom). Penetration tests were conducted with a 2 mm flat cylinder probe (SMS P/2N), a crosshead speed of 1 mm/s, and a strain of 60% (penetration). The peak height of the force time curve was recorded as the hardness of the sample. In each meat slice, penetration tests were carried out at 6 points of the sample.

2.3.6. Color. External color of raw and marinating meat rabbit was measured by reflectance mode in a Color Gard System colorimeter (Color Flez, Hunter Lab, Reston VA), using the Hunter parameters L^*, a^*, and b^*. The instrument was calibrated using the black tile and the white tile standards.

2.4. Statistical Analysis. Statistical analyses were performed on means using the ANOVA-LSD test (Statgraphics Centurion, Statpoint Technologies, USA). The fixed factors were NaCl concentration in the marinating solution (three levels: 70, 140 and 200 g/L), marinating time (seven levels: 0, 15, 30, 45, 60, 90, and 120 min), and US application (two levels: without or with application). The responses were weight loss, moisture content, and NaCl content in the meat. To analyze the physicochemical and quality parameters (pH, WHC, hardness, and color) the fixed factors were NaCl concentration in the marinating solution and US application, leaving the marinating time fixed at the value of 120 min (end of the treatment). The $p < 0.05$ level was considered to be statistically significant.

3. Results and Discussion

3.1. Calorimetry. Calorimetry is usually employed to determine the efficiency of ultrasound energy transformation. The ultrasonic intensity measured was 12.25 W/cm^2 at ultrasound power of 110 W. This fact confirms that the electrical output of the ultrasonic generator does not provide enough information about the ultrasonic intensity applied to a medium and that, in the experiments, part of the ultrasound energy is converted to heat and reflected back to the transducer, while another part of energy could be absorbed by material and its surrounding medium [16, 17].

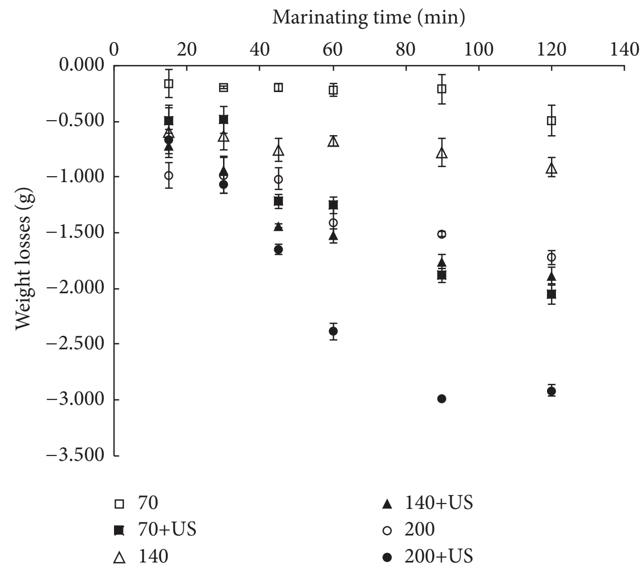

Figure 1: Weight losses during marinating of rabbit meat assisted by power ultrasound or in static bath at different salt concentrations (70, 140, and 200 g NaCl/L).

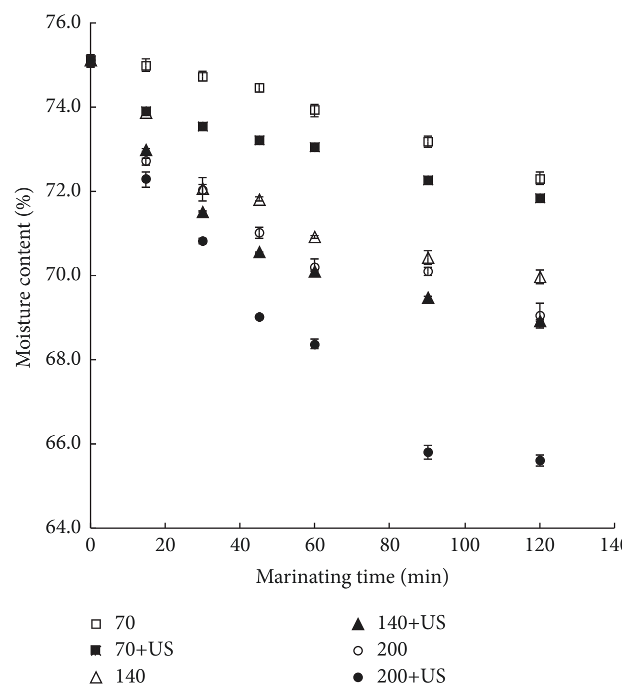

Figure 2: Moisture content in rabbit meat during marinating assisted by power ultrasound or in static bath at different salt concentrations (70, 140, and 200 g NaCl/L).

3.2. Effect of Treatments on Weight Losses and NaCl Content. Weight loss of rabbit meat during acid marinating is shown in Figure 1. The weight losses were influenced by marinating time and US application ($p < 0.05$); longer marinating times resulted in higher weight losses. In all experiments, the weight losses were higher when NaCl concentration in marinating solution was increased and when the samples were marinated with US. At the end of marinating, weight losses in samples treatment at 70, 140, and 200 g NaCl/L were 0.4, 0.9, and 1.7 g of sample, respectively. In comparison, the weight losses at the end of marinating with US were 2.05, 1.8, and 2.9 g of sample at 70,140, and 200 gNaCl/L, respectively, which is more than 2 times higher. These results are related to the water content of the rabbit meat samples. The initial water content of the rabbit meat slices was 75% (w/w). Figure 2 shows the evolution

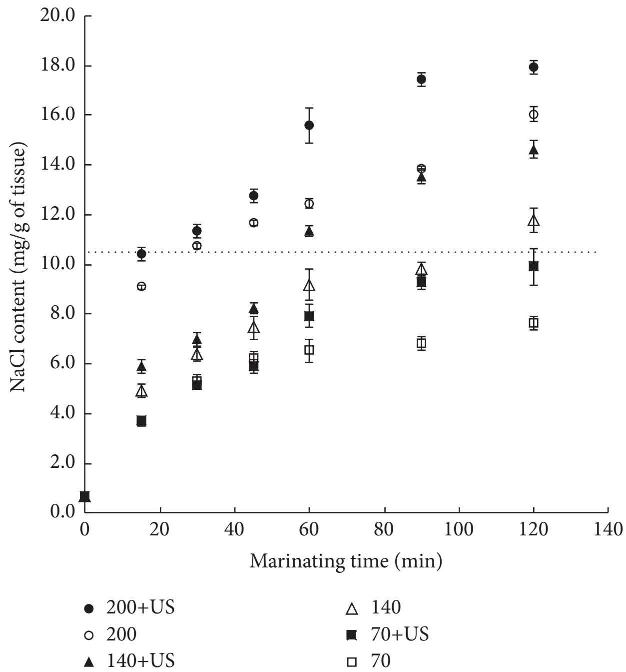

Figure 3: NaCl content in rabbit meat during marinating assisted by power ultrasound or in static bath at different salt concentrations (70, 140, and 200 g NaCl/L).

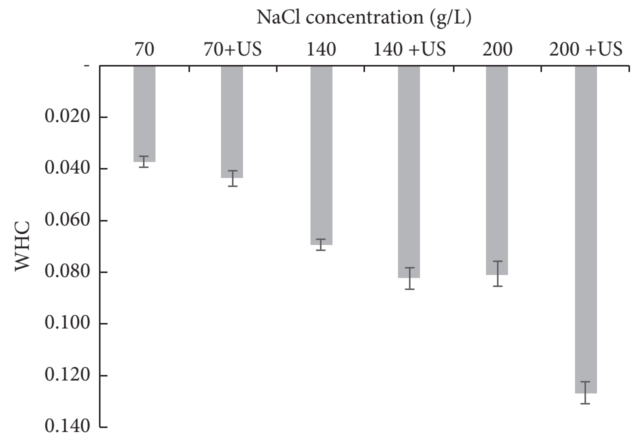

Figure 4: Effect of treatments on Water Holding Capacity (WHC) of rabbit meat after marinating assisted by power ultrasound or in static bath at different salt concentrations (70, 140, and 200 g NaCl/L).

of the experimental average moisture content of the slice samples during the experiments. The water content decreased in line when the NaCl concentration in marination solutions increased. The water content in the samples marinating at 70, 140, and 240 gNaCl/L was 72, 70, and 69% (w/w), respectively. These results are typical of meat salting by immersion and marinating, where a rapid penetration of salt occurs at the beginning of salting, following a tendency to uniformity at the end of the process [1]. The US affected significantly ($p < 0.05$) the water content in rabbit meat. The values of water content in samples at the end of marinating processes with US were 71, 69, and 65% (w/w) at 70, 140, and 200 g NaCl/L, respectively.

The NaCl content was influenced by marinating time and US application ($p < 0.05$). The NaCl content increases when the NaCl concentration in marination solutions increases (Figure 3). During the first 60 min of marinating, a faster increased in the NaCl content was observed at all experiments. The same behavior was observed by other authors when salting or marinating with sodium chloride, which was attributed to the large concentration gradient between solution of marinating and meat at the beginning of the salting process [18, 19]. When using a marinating solution of 70 g NaCl/L, the NaCl content in the meat after 120 min of process was almost two times lower than when using 140 and 200 g NaCl/L. The rabbit meat marinated with US showed a higher NaCl content than the marinated without US. The faster increases in the NaCl content during the first 60 min of marinating was more marked in samples marinating with US. As can be observed in Figure 3, the NaCl content was higher in the samples subjected to ultrasound during marinating. Other authors reported a similar effect of power ultrasound on the effective diffusivity of sodium chloride in meat [5]: the authors explain that this effect is caused by the cavitations created by the power ultrasound. The increasing in salt concentration on the surface of the meat sample in contact with the brine, besides the microinjection created by cavitation, causes a rupture of myofibril proteins [20] resulting in higher NaCl content.

Table 1 shows the average values of the maximum NaCl uptake at the end of the marinating experiments. The maximum NaCl uptake increases around of 15% with the application of US in the marinating compared to the samples without application of US ($p < 0.05$). The values of the NaCl content were higher than those obtained by other authors, such as Ozuna et al. [18] when studying the effect of high intensity ultrasound on the diffusion of NaCL during salting of pork meat (*Longissimus dorsi* muscle), with values of between 0.25 and 0.33 g NaCl/g dry basis. High values of the of NaCl may be related to the differences between rabbit and pork tissue, in addition to the application of citric acid in marinating.

Meat products commonly contain 10–28 mg NaCl/g of tissue at the end of the process; in this study, 10 mg NaCl/g was reached when rabbit meat was submitted at 200 g NaCl/L with US application (12.25 W/cm^2) for duration of 20 min (Figure 3).

3.3. Effect of Treatments on WHC. WHC was significantly affected by both NaCl concentration and ultrasound treatment ($p < 0.05$). In all experiments, the tissue lost water. The WHC decreases when the NaCl concentration in marination solutions increases and with application of US (Figure 4). WHC loss was higher in samples treated without US (overall average WHC loss of 8.1%) than with US (average of 6.2%). The maximum water loss was observed for NaCl concentrations at 200 g/L with US. These results are associated with the strong cavitation mechanisms produced by the US applied, which produces a cellular rupture and therefore grater movement of water out of the tissue [20].

Table 1: Effect of marinade treatments on NaCl uptake of rabbit meat at the end of marinating process (120 min).

Treatment (NaCl g/L)	Static Bath		US Bath	
	NaCl (mg/g of tissue)	± std. dev.	NaCl (mg/g of tissue)	± std. dev.
70	7.62[a]	0.26	9.92[d]	0.71
140	11.78[b]	0.47	14.63[e]	0.33
200	16.03[c]	0.53	17.91[f]	0.26

Different letters indicate statistically significant differences at $p < 0.05$.

Table 2: Effect of treatments on physicochemical properties of rabbit meat at different salt concentrations (marinating time = 120 min).

Treatment	Hardness (N)	Color			pH
		L^*	a^*	b^*	
Control	1.67 ± 0.13[a]	41.87 ± 0.62[a]	5.51 ± 0.54[a]	10.56 ± 0.59[a]	6.01 ± 0.09[a]
70 g NaCl/L	0.98 ± 0.04[b]	67.56 ± 0.04[b]	1.55 ± 0.02[b]	26.15 ± 0.01[b]	5.38 ± 0.05[b]
70 g NaCl/L + US	1.12 ± 0.08[b]	63.43 ± 0.01[c]	2.22 ± 0.01[c]	37.46 ± 0.02[c]	5.06 ± 0.02[c]
140 g NaCl/L	1.27 ± 0.05[bc]	55.98 ± 0.03[d]	0.90 ± 0.03[d]	18.36 ± 0.06[d]	5.05 ± 0.05[c]
140 g NaCl/L + US	1.46 ± 0.14[d]	62.92 ± 0.12[e]	2.02 ± 0.02[c]	36.85 ± 0.12[e]	5.05 ± 0.02[c]
200 g NaCl/L	1.69 ± 0.14[ae]	55.31 ± 0.01[f]	0.69 ± 0.02[d]	8.66 ± 0.01[f]	5.50 ± 0.04[d]
200 g NaCl/L + US	1.79 ± 0.09[e]	65.68 ± 0.03[g]	2.03 ± 0.02[c]	32.89 ± 0.05[g]	4.97 ± 0.01[c]

Different letters in the same column indicate statistically significant differences at $p < 0.05$.

Furthermore, with long duration immersions of meat in marinating solutions, there may be high loss of proteins in liquid tissue, of up to 30% according to Goli et al. [21], which reduces the strength of the tissue structure.

3.4. Effect of Treatments on Physicochemical Parameters. Table 2 shows the average values of the physicochemical parameters of meat samples at the end of marinating process. Hardness, ranging between 0.98 and 1.79 N, was significantly affected by both NaCl concentration and ultrasound treatment ($p < 0.05$). Increasing the NaCl concentration progressively between 70 and 140 g/L reduced the hardness, while treatments with 200 g/L increased the hardness. Similar trends were obtained by Ozuna et al. [18] for pork meat salting at different NaCl concentration in brine, in which this behavior is due to the high concentrations of NaCl in the tissue, which promoted changes in meat texture, leading to harder samples. On the other hand, the application of US during marinating increased the meat hardness. In the same NaCl treatments, hardness was higher for the samples marinating with US at 200 g/L than for those marinating at 70 and 140 g/L with US. Tenderness is an important quality parameter in the meat, and US was reported as reduced the hardness of bovine muscles, when applied at 24 kHz and 12 W/cm^2 [22]. Also, acidic marinade favors the tendering of meat, as was reported by Burke and Monahan [23] in shin beef.

The color parameters of marinating samples were significantly ($p < 0.05$) different, compared to fresh samples (control). The L^* values, ranging between 41.87 and 67.56, increased with the NaCl concentration in marinating solution and with the US application. This increase was greater in samples marinating at 70 g NaCl/L and for all treatments with US application. Regarding the a^* values (from 0.90 to 5.51), they decreased when NaCl concentration increased and with the US application. However, in the samples marinating with US, this decrease was small compared with the samples marinating in static bath. For b^* values (10.56 to 37.46), they increased with marinating at 70 and 140 g NaCl/L and with US application. The values in samples marinating with US were higher in comparison to control and static bath. These results coincide with those of Pohlman et al. [24], who reported that bovine *Pectoralis* treated at 22 W/cm^2 and 20 kHz became lighter (higher L^*), less red (lower a^*), and more yellow (higher b^*), when compared to controls. The change of the color parameters in meat samples can be generated by the effects of cavitation. This phenomenon during US treatment can induce free radicals and cause oxidation of lipid or protein [10, 11]. Wolff et al. [25] reported that the reactive oxygen species produced by lipid oxidation may modify or oxidize intracellular and membrane proteins in muscle. Ladeira et al. [26] suggested that the changes of redness index (a^*) are associated with the instability of heme pigments in the secondary products (alpha- and beta-aldehydes) of lipid oxidation, causing the decreased stability of oxymyoglobin.

4. Conclusions

Ultrasound-assisted marinating increased the NaCl uptake in rabbit meat in comparison with static marinating. Application of ultrasound has high potential to be employed in the marinating of rabbit meat, diminishing the immersion time, and increasing the meat hardness and water loss. Also, the application of ultrasound during marinating brought relevant effects on parameters of meat color, such as increase in the a^* values and a decrease in the b^* parameter. Further studies about effects of US in lipid oxidation of meat could increase the knowledge in the application of this technology in food process. These findings may help meat industry to a better management of the marinating process with the aim to develop high-value meat products.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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