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Effect of ultrasound application on the growth of *S. xylosus* inoculated in by-products from the poultry industry

Priscila Nehring [a], José Manuel Lorenzo [b], Suelen Priscila Santos [a], Roger Wagner [a], Cristiano Ragagnin de Menezes [a], Bibiana Alves dos Santos [a], Juliano Smanioto Barin [a], Paulo Cezar Bastianello Campagnol [a], Alexandre José Cichoski [a,*]

[a] Federal University of Santa Maria, CEP 97105-900, Santa Maria, Rio Grande do Sul, Brazil
[b] Meat Technological Center of Galicia, San Cibrao das Viñas, 32900, Ourense, Spain

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ABSTRACT

A wide variety of by-products are produced by the industry when animals are slaughtered. However, the proteins present in these by-products, are not being fully useable, in the elaboration of value-added products. *Staphylococcus xylosus* is commonly used as a starter culture in meat products subjected to ripening for a long period, as it produces proteolytic and lipolytic enzymes that improve the sensory quality of the products. Ultrasound (US) has been arousing interest in the meat industry, as it reduces processing time and also improves the technological and sensory quality of meat products. However, the stimulate effect of US on the growth of *S. xylosus* in by-products from the poultry industry is still unknown. Thus, this study aimed to evaluate the stimulate effect of US on the growth of *S. xylosus* inoculated in by-products from the poultry industry. *S. xylosus* was inoculated (5.63 log CFU/g) in sterilized by-products from the poultry, which were then sonicated at 37 °C for 0, 15, 30, and 45 min according to the following parameters: frequencies of 130 and 35 kHz, amplitudes of 50% and 80% and normal and degas operating modes. The sonicated samples were incubated at 37 °C for 0, 24, 48, and 72 h. Soon after sonication, no stimulate effect of US was observed on the growth of *S. xylosus*. However, after 24 h of incubation, the samples sonicated for 15 and 30 min in normal mode, at 35 and 130 kHz, and amplitudes of 50 and 80% exhibited better stimulate effect at the growth *S. xylosus* counts ($p < 0.01$) when compared to the Control, with values of 8.23 and 7.77 log CFU/g, respectively. These results can be exploited to obtain new added-value products, having as raw material by-products from the poultry industry.

1. Introduction

A wide range of by-products (blood, bones, tendons, meat trimmings, fat or lard, hides, hooves, horns, internal organs and viscera, cartilages, ears, and feet), are produced by the meat industry when animals are slaughtered. However, the proteins present in these by-products, are not being fully utilized in obtaining value-added products (Mora et al., 2019). Food ingredients, enzymes with a certain functional property (Chernukha et al., 2015; Lasekan et al., 2013), bioactive peptides (Martínez-Alvarez et al., 2015; Mora et al., 2014), as well as products pharmaceutical, chemicals and medicals (Toldrá et al., 2016) can be generated from by-products.

Staphylococcus xylosus is gram-positive cocci, circular in shape, with rigid and thick cell walls due to the presence of a thin layer of peptidoglycans (Hua et al., 2019). It is often found in meat products and is commonly used as a starter culture in the manufacture of fermented and dry-cured meat products. This microorganism adapts well to the nutrients present in meat, as it uses different substrates as sources of carbon and energy, as well as different sources of nitrogen. *S. xylosus* produces proteolytic and lipolytic enzymes that act on proteins and lipids, respectively, producing polypeptides, oligopeptides and peptides, and free fatty acids. In addition, *S. xylosus* helps to control fatty acid oxidation by producing the enzyme catalase (Xiao et al., 2020; Hua et al., 2019; Leroy et al., 2017).

The use of green technologies in the food industry, such as ultrasound (US), has gained popularity for providing reduced processing time without changing food quality (Chemat et al., 2020; Fu et al., 2020). Our research group has already successfully used US for sausage

* Corresponding author.
E-mail address: alejoci@pq.cnpq.br (A.J. Cichoski).

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Table 1
US parameters used in the sonication of the samples.

Treatments	Frequency (kHz)	Amplitude (%)	Operation mode	Sonication time (min)	Incubation time (h) at 37 °C
Control[a]	–	–	–	–	24, 48 and 72
TUS130N50A	130	50	Normal	15, 30 and 45	24, 48 and 72
TUS130N80A	130	80	Normal	15, 30 and 45	24, 48 and 72
TUS130D50A	130	50	Degas	15, 30 and 45	24, 48 and 72
TUS130D80A	130	80	Degas	15, 30 and 45	24, 48 and 72
TUS35N50A	35	50	Normal	15, 30 and 45	24, 48 and 72
TUS35N80A	35	80	Normal	15, 30 and 45	24, 48 and 72
TUS35D50A	35	50	Degas	15, 30 and 45	24, 48 and 72
TUS35D80A	35	80	Degas	15, 30 and 45	24, 48 and 72

[a] **Note:** Control samples were not sonicated, but were kept in water (37 °C) for 15, 30 and 45 min.

pasteurization (Cichoski et al., 2015), pre-chilling of chicken (Cichoski et al., 2019; Flores et al., 2018) and mortadella cooking (Da Silva et al., 2020).

US is defined as a high-frequency wave that exceeds the limit of human hearing (20 kHz) (Higuera-Barraza et al., 2016; Alarcon-Rojo et al., 2015). It is a type of mechanical energy called sound, which propagates through a conductive medium, in which the longitudinal wave produces alternating compression (high-pressure regions) and rarefaction (low-pressure regions) (Picó, 2015), forming bubbles that implode and generate the cavitation phenomenon (Alarcon-Rojo et al., 2015). Ultrasound waves of frequencies between 18 and 100 kHz and power between 10 and 1000W are classified as low frequency and high-intensity waves, while frequencies above 100 kHz and powers below 1 W are classified as high frequency and low-intensity waves (Picó, 2015). The effects of US on food depend on the process conditions, such as the type of equipment, operation mode, amplitude, intensity, exposure time, and frequency, as these factors change the temperature of the environment and affect the composition and quality of food (Marchesini et al., 2015).

Several studies have been carried out to elucidate the impact of US on the physicochemical, sensory, and biochemical characteristics of meat and meat products. In addition, the positive effect of US in reducing spoilage bacteria in meat and meat products is also well documented (Leães et al., 2021; Alves et al., 2018, 2020; Pinton et al., 2019, 2020; Cichoski et al., 2015). However, there is a gap in the literature about the effect of US on microorganisms that can be useful to produce value-added compounds from industrial waste. Thus, this study aimed to evaluate for the first time the stimulant effect of US on the growth of *S. xylosus* inoculated in by-products from the poultry industry, thus opening a new field of application for this green technology.

2. Materials and methods

2.1. Preparation of the meat matrices and application of US

By-product from the poultry industry was donated by a slaughterhouse located in southern Brazil (12.5 g meat trimmings of chicken breast and 12.5 g of meat trimmings of drumstick) were cut into 1 cm cubes and placed in 500 mL Erlenmeyer flasks. Then, 225 mL of 0.1% peptone water (Himedia, India) with 0.85% NaCl (Dinâmica, Brazil) was added (Silva et al., 2017; Stahnke, 1995). The mixture (by-product and peptone water) capped was sterilized in an autoclave at 121 °C for 15 min (Prismatec, CS150 L, 6000 W, 600 W × 720 L × 1400 mm H, Brazil). The mixture was left on the bench until reaching 37 °C. After, the mixture wax inoculated with 0.01g of pure culture of *S. xylosus* (Lyocarni SXH-01, Sacco, France) which matched the initial count of 5.63 log CFU/g. The flasks were manually homogenized for 2 min and then placed in a US bath (Ultrasonic power effective 200W, 8.6 L, 300 W × 240 D × 150 H, model TI-H 10, Elma, Germany) and sonicated according to the conditions shown in Table 1. Then, the flasks were incubated at 37 °C for 72 h.

2.2. Microbiological characterization

The enumeration of *S. xylosus* was performed after 0, 24, 48, and 72 h of incubation. The samples were serially diluted (10^{-1} a 10^{-6}) with 0.1% peptone water. After, 1 mL of each dilution was placed on the plates and MSA (Manitol Salt Phenol-red Agar) (Merck, Germany) was added using pour plated method. The samples were incubated at 37 °C for 72 h (Bouaziz et al., 2011; Stahnke, 1995). The results were expressed in log CFU/g of sample.

Table 2
S. xylosus counts (log CFU/g) in the samples sonicated at 130 kHz and incubated for 24, 48 and 72 h at 37 °C.

Sonication time (min)	Incubation time (h) at 37 °C	Control	TUS130N50A	TUS130N80A	TUS130D50A	TUS130D80A	SEM	Sig.
15	0	5.63^{aB}	5.07^{abC}	4.89^{bE}	5.16^{abB}	5.44^{abD}	0.07	*
30	0	5.63^{aB}	5.01^{bC}	5.37^{abED}	5.12^{abB}	5.36^{abD}	0.06	*
45	0	5.63^{aB}	4.96^{bC}	5.27^{abED}	5.10^{bB}	5.20^{abD}	0.06	*
15	24	7.77^{abA}	8.05^{aA}	6.99^{cCB}	7.96^{abA}	7.65^{bAB}	0.07	*
30	24	7.77^{bA}	8.25^{aA}	7.64^{bBA}	7.90^{abA}	7.95^{abAB}	0.05	*
45	24	7.77^{aA}	7.82^{aA}	6.27^{bC}	7.83^{aA}	7.48^{aAB}	0.11	*
15	48	7.99^{abA}	8.19^{aA}	7.71^{abBA}	7.52^{bA}	7.62^{abAB}	0.06	*
30	48	7.99^{abA}	7.50^{abB}	8.21^{aBA}	7.43^{bA}	8.03^{abAB}	0.08	*
45	48	7.99^{aA}	7.41^{abB}	7.34^{abCBA}	7.45^{abA}	6.33^{bC}	0.14	*
15	72	7.35^{bA}	8.25^{abA}	8.47^{aA}	7.64^{abA}	8.10^{abA}	0.10	*
30	72	7.35^{aA}	7.98^{aA}	7.06^{aCB}	7.86^{aA}	7.73^{aAB}	0.14	n.s.
45	72	7.35^{aA}	7.55^{aAB}	7.45^{aCBA}	7.41^{aA}	7.16^{aBC}	0.09	n.s.
	SEM	0.21	0.16	0.15	0.14	0.13		
	Sig	*	*	*	*	*		

Note: Control samples were not sonicated, but were kept in water (37 °C) for 15, 30 and 45 min.
Means followed by the same lowercase letter (in the same line) not differ significantly.
Means followed by the same uppercase letter (in the same column) not differ significantly.
Treatments: see Table 1.
SEM: Standard error of the mean.
Sig: Significance level: *$p < 0.01$, n.s. (not significant).

Table 3
S. xylosus counts (log CFU/g) in the samples sonicated at 35 kHz and incubated for 24, 48 and 72 h at 37 °C.

Sonication time (min)	Incubation time (h) at 37 °C	Control	TUS35N50A	TUS35N80A	TUS35D50A	TUS35D80A	SEM	Sig.
15	0	5.63^{abB}	5.13^{bD}	5.18^{abB}	5.18^{abC}	5.37^{abB}	0.05	*
30	0	5.63^{aB}	5.27^{abD}	5.30^{abB}	5.14^{bC}	5.20^{abB}	0.04	*
45	0	5.63^{aB}	5.32^{abD}	5.28^{abB}	5.14^{bC}	5.18^{abB}	0.05	*
15	24	7.77^{bA}	7.52^{bA}	8.21^{aA}	7.49^{bA}	7.60^{bA}	0.06	*
30	24	7.77^{abA}	7.77^{abA}	7.96^{aA}	7.28^{bA}	7.56^{abA}	0.06	*
45	24	7.77^{bA}	7.51^{bA}	8.23^{aA}	7.61^{bA}	7.60^{bA}	0.05	*
15	48	7.99^{aA}	6.28^{bC}	8.24^{aA}	7.51^{aA}	7.53^{aA}	0.13	*
30	48	7.99^{aA}	7.58^{aA}	7.74^{aA}	7.32^{aA}	7.42^{aA}	0.08	n.s.
45	48	7.99^{aA}	6.66^{cBC}	7.78^{abA}	7.12^{bcA}	7.52^{abA}	0.10	*
15	72	7.35^{aA}	7.93^{aA}	7.68^{aA}	6.33^{bB}	7.68^{aA}	0.14	*
30	72	7.35^{abA}	7.51^{abA}	8.04^{aA}	6.93^{bAB}	7.45^{abA}	0.12	*
45	72	7.35^{aA}	7.19^{aAB}	8.13^{aA}	7.62^{aA}	7.31^{aA}	0.10	n.s.
	SEM	0.21	0.13	0.15	0.12	0.12		
	Sig	*	*	*	*	*		

Note: Control samples were not sonicated, but were kept in water (37 °C) for 15, 30 and 45 min.
Means followed by the same lowercase letter (in the same line) not differ significantly.
Means followed by the same uppercase letter (in the same column) not differ significantly.
Treatments: see Table 1.
SEM: Standard error of the mean.
Sig: Significance level: *$p < 0.01$, n.s. (not significant).

2.3. Statistical analysis

The experiment was carried out twice on different days, and the determinations were carried out in triplicate. A generalized linear mixed model was used to analyze the results. The variable "treatments" was considered as a fixed effect, and repetition was considered as a random effect. Tukey test was used to compare means, considering a significance level of 1% ($p < 0.01$). Statistical analysis was performed using the software IBM SPSS Statistics 22 for Windows (IBM Corporation, Armonk, NY, 2013).

3. Results and discussion

3.1. Staphylococcus xylosus counts (log CFU/g) in the samples by-product sonicated at 130 kHz

3.1.1. Influence of sonication time, amplitude and mode of operation of the US device on the growth of S. xylosus

The *S. xylosus* count after inoculation was 5.63 log CFU/g (Tables 2 and 3), which is very close to the findings of other authors shortly after inoculating this starter culture into fermented meat products (Najjari et al., 2020; Guo et al., 2000). In contrast, significantly lower counts ($p < 0.01$) were observed for the treatment TUS130N50A sonicated for 30 min (5.01 log CFU/g) and 45 min (4.96 log CFU/g), TUS130N80A batch sonicated for 15 min (4.89 log CFU/g), and the treatment TUS130D50A sonicated for 45 min (5.10 log CFU/g), when compared to the counts immediately after *S. xylosus* inoculation. Concerning the treatment TUS130D80A, the values did not differ from the Control (Table 2) for the three sonication times employed. Thus, the three sonication times (15, 30, and 45 min) at the frequency of 130 kHz and amplitudes of 50 and 80% did not provide a stimulant effect on the growth of *S. xylosus* soon after sonication (0 h).

However, after 24 h of incubation at 37 °C, the treatment TUS130N50A sonicated for 30 min showed a higher *S. xylosus* count ($p < 0.01$) (8.25 log CFU/g) when compared with the Control and the treatment TUS130N80A (Table 2). In addition, the treatment TUS130N80A subjected to the sonication times of 15 min (6.99 log CFU/g) and 45 min (6.27 log CFU/g), and 24 h of incubation, presented lower *S. xylosus* counts ($p < 0.01$) than the Control (7.77 log CFU/g) and the other treatments (Table 2). The treatment TUS130D80A (7.65 log CFU/g) had a lower count ($p < 0.01$) when compared with the Control and the treatment TUS130N50A (8.05 log CFU/g) only for the sonication time of 15 min (Table 2). These results demonstrate that the operation mode, the sonication time, and the amplitude affected the development of *S. xylosus*.

Within 48 h of incubation at 37 °C (Table 2), only the treatment TUS130D80A sonicated for 45 min showed lower *S. xylosus* count (6.33 log CFU/g) ($p < 0.01$) when compared with the Control (7.99 log CFU/g). Concerning the other treatments, no differences were observed for *S. xylosus* counts for this incubation time (Table 2). In the 72 h incubation time (Table 2), only the treatment TUS130N80A sonicated for 15 min presented higher *S. xylosus* count ($p < 0.01$) (8.47 log CFU/g) than the Control (7.35 log CFU/g), while the other treatments displayed counts close to the Control (Table 2), even when exposed to different sonication times.

At the frequency of 130 kHz, in degas operating mode, and under the conditions employed in this study, it was not possible to observe a stimulant effect of US on the growth of *S. xylosus*, when compared with to Control and the treatments subjected to the normal operating mode (Table 2). This result is due to different behavior of the ultrasonic waves within the bath, which varied with the operating modes. In degas mode, the ultrasonic waves operate at intervals, while in the normal mode they are constant, providing an optimized liquid flow within the US bath (Elma, 2015). This behavior of the ultrasonic waves in the normal operating mode provided a stimulant effect on the growth of *S. xylosus* in the treatment TUS130N50A sonicated for 30 min and incubated for 24 h when compared to the Control.

3.1.2. Influence of different incubation times at 37 °C of sonicated samples at 130 kHz on the growth of S. xylosus

In the Control sample, the initial *S. xylosus* count was 5.63 log CFU/g, which increased ($p < 0.01$) to 7.77 log CFU/g after 24 h of incubation at 37 °C, remaining ($p > 0.01$) at 7.99 CFU/g and 7.35 log CFU/g after 48 and 72 h, respectively (Table 2).

In contrast, after 24, 48, and 72 h of incubation of the samples at 37 °C, the sonication times showed different effects on the growth of *S. xylosus*. The lowest counts ($p < 0.01$) of *S. xylosus* were observed for the treatment TUS130N80A in 24 h of incubaction, for two times of sonication (15 and 45 min), and when incubated for 72 h and sonicated for 30 min (7.06 log CFU/g) (Table 2). Similar behavior was observed for the treatment TUS130D80A (Table 2) sonicated for 45 min, with incubation of 48 (6.33 log CFU/g) and 72 h (7.16 log CFU/g). The treatment TUS130N50A sonicated for 30 and 45 min and incubated for 48 h presented the lowest *S. xylosus* counts ($p < 0.01$) when compared to all samples incubated for 24 h (Table 2). Concerning the treatment TUS130D50A, the different sonication and incubation times did not

affect the *S. xylosus* counts (Table 2).

3.2. Staphylococcus xylosus counts (log CFU/g) in the samples by-product sonicated at 35 kHz

3.2.1. Influence of the sonication time of the amplitude and mode of operation of the US device on the growth of S. xylosus

The initial count of *S. xylosus* in the treatment TUS35N50A sonicated for 15 min (5.13 log CFU/g), and the treatment TUS35D50A (Table 3) sonicated for 30 and 45 min (both with 5.14 log CFU/g) were lower ($p < 0.01$) when compared with the Control (5.63 log CFU/g). Thus, right after the US application at a frequency of 35 kHz, using different operation modes, and sonication times, there was no stimulant effect on the growth of *S. xylosus* (Table 3). After 24 h of incubation, the treatment TUS35N80 sonicated for 15 (8.21 log CFU/g) and 45 min (8.23 log CFU/g) showed higher ($p < 0.01$) *S. xylosus* counts when compared with the Control (7.77 log CFU/g) and the other treatments (Table 3).

After 48 h of incubation, the treatment TUS35N50A sonicated for 15 min (6.28 log CFU/g) had lower *S. xylosus* count ($p < 0.01$) when compared with the Control (7.99 log CFU/g) and the other treatments. For the sonication time of 45 min (6.66 log CFU/g), lower counts were observed ($p < 0.01$) (Table 3). Also, after 48 h of incubation, the treatment TUS35D50A (7.12 log CFU/g) sonicated for 45 min showed the lowest *S. xylosus* count ($p < 0.01$) when compared to the Control. At the sonication time of 30 min, and 48 h of incubation, no significant differences ($p > 0.01$) were observed for the *S. xylosus* counts between treatments and the Control (Table 3).

At the frequency of 35 kHz, a stimulant effect of US on the growth of *S. xylosus* was observed only for the sonication times of 15 and 45 min, in normal operating mode, 80% amplitude, and 24 h incubation time at 37 °C (TUS35N80A), which presented the highest *S. xylosus* counts ($p < 0.01$) (Table 3).

3.2.2. Influence of different incubation times at 37 °C of sonicated samples at 35 kHz on the growth of S. xylosus

For the Control and the treatments TUS35N80A and TUS35D80A, the *S. xylosus* counts did not differ from each other ($p > 0.01$) in the different incubation times (24, 48, and 72 h). Concerning the treatment TUS35N50A, lower *S. xylosus* counts were observed ($p < 0.01$) at the incubation time of 48 h and sonication for 15 min (6.28 CFU/g) and 45 min (6.66 CFU/g) when compared to the 24-h incubation, and similar sonication times (Table 3). Furthermore, this treatment presented a lower ($p < 0.01$) *S. xylosus* count (7.19 log CFU/g) at the 72-h incubation time and 45 min of sonication, when compared to the 24 h incubation time and similar exposure time to US (7.51 log CFU/g).

The lowest *S. xylosus* count ($p < 0.01$) was observed for the treatment TUS35D50A only at the 72 h incubation time, and sonication for 15 min (6.33 log CFU/g). Thus, at 35 kHz, the sonication time of 15 min and the incubation time of 24 h (TUS35N80A) can be considered as optimum processing conditions, as they promoted a stimulant effect on the growth of *S. xylosus*, which was not observed for the other treatments subjected to this frequency (Table 3).

3.3. Discussion of results obtained at frequencies of 130 kHz and 35 kHz

The present results suggest that the US conditions used in the treatment TUS130N50A (31 W) sonicated for 30 min, and the treatment TUS35N80A (103 W) sonicated for 15 and 45 min, and incubation times of 24 h, provided stimulant changes in the permeability of the cell membrane of *S. xylosus*, which favored bacterial growth (Tables 2 and 3). Alarcon-Rojo et al. (2015) reported that low and high-frequency waves promote physical, mechanical, and chemical effects, as well as changes in the cell structure and permeability. The effect of US on the cell membrane is called sonoporation, which can lead to a progressive membrane opening, due to the cavitation of microbubbles (Maciulevičius et al., 2016; Lentacker et al., 2014). These microbubbles generate a strong shear force, which breaks the chemical bonds between the components of the cell membrane, leading to membrane opening, which can be permanent or transient, and with varying sizes (Mortazavi and Tabatabaei, 2008).

A low sonoporation level improves the permeability of the cell membrane, and favors the entry of essential substrates into the bacterial cell, and the exit of products of cellular metabolism, thus favoring bacterial growth. To reach this condition and prevent cell death, the US process parameters (exposure time, amplitude, frequency, and process temperature) must be controlled to prevent a high sonoporation level, which can lead to cell death (Ojha et al., 2017).

Waves of frequency of 35 kHz (103 W) are classified as low frequency and high-intensity power, while the frequency of 130 kHz (31 W) is considered as high frequency and high-intensity ultrasonic waves (Verruck and Prudencio, 2018; Picó, 2015). Thus, at 35 kHz, a small number of large bubbles is formed, which releases a large amount of energy when imploding, with low penetration power. In contrast, at 130 kHz, a large amount of small bubbles is formed, which when imploding release a moderate amount of energy, with high penetration power (Alarcon-Rojo et al., 2019). Thus, the different US conditions of this study led to similar results in the cell membrane, which stimulated the growth of *S. xylosus* (Tables 2 and 3). The results showed that, for this situation, the effects of the US are dependent on the frequency, operation mode, intensity, and exposure time (Marchesini et al., 2015).

The application of US in spiced beef (Zou et al., 2018) and beef cattle (Kang et al., 2017) promoted rupture of muscle myofibrils, releasing proteins and amino acids (releasing substrates). The US altered the permeability of the bacterial cell membrane the *Escherichia coli* (Yang et al., 2021). These US effects were related to the frequency, time and potency used (Yang et al., 2021; Zou et al., 2018; Kang et al., 2017).

In treatments TUS35N80A (103 W) and TUS130N50A (31 W), US can provided beneficial changes in the permeability of the cell membrane of *S. xylosus*, which allowed the release of proteases. Thus, these released enzymes, together with the breakdown of muscle fibers promoted by cavitation favored the rupture of peptide bonds in the proteins present in the by-product stimulating the growth of *S. xylosus* in by-product (Tables 2 and 3).

The cocci shape of *S. xylosus* give greater resistance to US when compared to bacilli shaped gram-positive and gram-negative bacteria (Alarcon-Rojo et al., 2019; Pitt and Ross, 2003). These factors may have influenced the treatments submitted to the degas operating mode since there was no stimulus for the growth of *S. xylosus* in these treatments, which presented lower *S. xylosus* counts ($p < 0.01$) when compared to the Control, with no significant differences (Tables 2 and 3). Although similar sonication times, frequencies, amplitudes, and incubation times were used for the treatments TUS35N80A and TUS130N50A, this behavior may be due frequencies, amplitudes and mainly to the type of wave produced in the degas operating mode. The wave generated in this operation mode is not continuous, thus it impairs the permeability of the cell membrane of *S. xylosus*, interfering with the entry of substrates and exit of by-products (Piyasena et al., 2003), and not stimulating the growth of *S. xylosus*.

Jayasooya et al. (2007) evaluated the effect of ultrasound in improving the tenderness of steer's meat and concluded that there is an ideal cavitation time to obtain the desired effect. Thus, the sonication time may also have influenced the results obtained in the present study. The conditions used in the treatments TUS35N80A and TUS130N50A promoted a stimulant effect on the growth of *S. xylosus*, although this effect was observed at different sonication times (15 and 30 min for TUS35N80A and TUS130N50A, respectively) (Tables 2 and 3), which were affected by the different frequencies (35 and 130 kHz) and intensities (103 and 31 W, respectively) used in the US process.

The treatments TUS35N50A (US for 15 min), TUS35D50A (30 and 45 min), TUS130N50A (30 and 45 min), TUS130N80A (15 min) and TUS130D50A (US for 45 min) presented lower *S. xylosus* counts when compared with the Control (Tables 2 and 3) at different times, probably

Table 4
S. xylosus counts (log CFU/g) in the samples sonicated at 130 and 35 kHz and incubated for 24, 48 and 72 h at 37 °C.

Sonication time (min)	Incubation time (h) at 37 °C	Control	TUS130N50A	TUS35N80A	SEM	Sig.
15	0	5.63^{a}	5.07^{b}	5.18^{ab}	0.08	*
30	0	5.63^{a}	5.01^{b}	5.30^{ab}	0.08	*
45	0	5.63^{a}	4.96^{b}	5.28^{ab}	0.09	*
15	24	7.77^{b}	8.05^{a}	8.21^{a}	0.05	*
30	24	7.77^{b}	8.25^{a}	7.96^{ab}	0.07	*
45	24	7.77^{b}	7.82^{ab}	8.23^{a}	0.07	*
15	48	7.99	8.19	8.24	0.09	n.s.
30	48	7.99	7.50	7.74	0.12	n.s.
45	48	7.99	7.41	7.78	0.10	n.s.
15	72	7.35	8.25	7.68	0.14	n.s.
30	72	7.35	7.98	8.04	0.12	n.s.
45	72	7.35	7.55	8.13	0.14	n.s.

Note: Control samples were not sonicated, but were kept in water (37 °C) for 15, 30 and 45 min.
Means followed by the same lowercase letter (in the same line) not differ significantly.
Treatments: see Table 1.
SEM: Standard error of the mean.
Sig: Significance level: *$p < 0.01$, n.s. (not significant).

due to the damaging effects of US on *S. xylosus*. Ultrasonic frequencies between 20 and 500 kHz offer an alternative method of food preservation. The antimicrobial efficacy of ultrasound depends on factors such as contact time with the microorganism, type of microorganism, amount of food, composition and treatment temperature. The antimicrobial power of ultrasound is enhanced at 60 and 70 °C. However, several researchers report that temperatures from 20 °C intensify the phenomenon of cavitation, allowing the formation of reactive oxygen species and shock forces, which act against microorganisms (Alarcon-Rojo et al., 2019). The samples from all treatments were sonicated and incubated at 37 °C, as this temperature can favor the growth of *S. xylosus* (Stahnke, 1995; McMeekin et al., 1987). For the treatments TUS35N50A and TUS130N80A subjected to the normal operating mode, and the treatments sonicated in degas operating mode, an optimum sonication time to provide a stimulant effect on the growth of *S. xylosus* was not observed even when an optimal temperature was used to favor the growth of *S. xylosus* (Tables 2 and 3).

These results reinforce that 24 h of incubation may be the optimum time to promote a stimulant effect on the growth of *S. xylosus* under the US conditions applied in this study.

3.4. Comparative study between treatments that stimulated the growth of *S. xylosus*

Whereas the treatments TUS130N50A and TUS35N80A had stimulant effects on the growth of *S. xylosus*, they were compared with the Control (Table 4). The *S. xylosus* counts right after sonication at the three different times were lower ($p < 0.01$) for the treatment TUS130N50A (4.96–5.07 log CFU/g) when compared to the Control (5.63 log CFU/g), which was not observed for the treatment TUS35N80A (5.18–5.30 log CFU/g) (Table 4). In the incubation times of 48 and 72 h, regardless of sonication times, the *S. xylosus* counts of the treatments TUS130N50A and TUS35N80A did not differ significantly ($p > 0.01$) between each other and the Control.

Thus, significant differences ($p < 0.01$) were observed only in the 24-h incubation time when comparing the *S. xylosus* counts between the treatments and the Control (Table 4). At 15 min of sonication time, the treatments TUS130N50A (8.05 log CFU/g) and TUS35N80A (8.21 log CFU/g) differed significantly ($p < 0.01$) from the Control (7.77 log CFU/g). This result observed for the TUS130N50A treatment within 15 min of sonication is important, as according to Table 2, the stimulant effect on the growth of *S. xylosus* when compared to the Control was not evidenced. In the sonication time of 30 min, only the TUS130N50A treatment (8.25 log CFU/g) had a higher *S. xylosus* count, which differed significantly ($P < 0.01$) from the Control. In the 45 min sonication time, only the TUS35N80A treatment (8.23 log CFU/g) presented higher ($p < 0.01$) *S. xylosus* count, which differed significantly ($p < 0.01$) from the Control (Table 4). These results demonstrated again that under the conditions of this study, the best incubation time for the samples was 24 h. Thus, to stimulate the growth of *S. xylosus* and save on processing time, these two treatments could be applied under conditions of 15 min of exposure to the US, and 24 h of incubation for the samples. These US conditions provided modifications at the bacterial cell membrane level, with stimulant effects on the development of *S. xylosus*. Furthermore, the activation of immobilized enzymes may also have occurred, which increased the amount of substrates in the chicken meat that was added to the medium, where *S. xylosus* was inoculated (Alarcon-Rojo et al., 2019). These results prove that the US process can be used to stimulate the growth of *S. xylosus* in by-products from the poultry industry.

4. Conclusions

The results of this study proved that US can stimulate the growth of *S. xylosus* inoculated in by-products from poultry industry. The best results were obtained in the "normal" application mode and under the following conditions: 130 kHz, 50% amplitude, 30 min sonication, and 35 kHz, 80% amplitude, and 15 min sonication in incubation time 24 h. The results obtained in this study are very important, as they open the perspective for the use of US and *S. xylosus* to obtain new value-added products from poultry by-products.

CRediT authorship contribution statement

Priscila Nehring: Conceptualization, Investigation, Writing – original draft. **José Manuel Lorenzo:** Investigation, Writing – review & editing. **Suelen Priscila Santos:** Investigation, Writing – review & editing. **Roger Wagner:** Investigation, Validation, Visualization. **Cristiano Ragagnin de Menezes:** Investigation, Validation, Visualization. **Bibiana Alves dos Santos:** Investigation, Writing – review & editing. **Juliano Smanioto Barin:** Investigation, Validation, Visualization. **Paulo Cezar Bastianello Campagnol:** Investigation, Writing – review & editing. **Alexandre José Cichoski:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing – original draft.

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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