



Impact of ultrasound treatment on viability of *Staphylococcus aureus* and the human milk antioxidant activity

Nayara Resende GOMES¹, Paola Machado PARREIRAS¹ , Camila Carvalho MENEZES^{1*} , Thaís Silva FALCO¹, Michele Cristina VIEIRA¹, Maria Cristina PASSOS², Luciana Rodrigues CUNHA¹

Abstract

This study aimed to evaluate the application of thermosonication for the viability of *Staphylococcus aureus* and the antioxidant activity of HM. The US effect on the *S. aureus* was measured by counting viable cells in discarded HM immediately before and after different treatments varying time (1, 5 and 10 minutes) and temperature (20, 30, 40, 50 and 60 °C) through surface plating on standard agar. The antioxidant activity evaluation was carried out by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging method. With Regarding microbiological quality, it can be concluded that treatment with US at 60 °C for 10 minutes was the most effective in terms of reducing the viability of *S. aureus*, in addition to presenting a significant increase in the content of antioxidants when compared to raw and pasteurized milk samples.

Keywords: human milk bank; food microbiology; antioxidant activity; thermosonication.

Practical Application: Thermosonication reduced the *S. aureus* and improved in antioxidant activity in HM.

1 Introduction

Human milk (HM) is especially suitable for infants, both with regard to its nutritional composition and bioactive compounds. Unlike infant formula, which is standardized within a very narrow range of components, HM is a dynamic biofluid, the composition of which varies according to the stage of lactation and between term and preterm babies (Ballard & Morrow, 2013). That is why it is considered by health professionals and breastfeeding defense forums as the “gold standard” for its numerous benefits, which include: savings, practicality, psycho-emotional health, immunological protection, in addition to being ecologically and nutritionally superior in relation to infant formula (Erick, 2018). However, some situations such as the presence of the human immunodeficiency virus (Neves & Marin, 2013), cases of mothers using drugs, undergoing chemotherapy, who have hepatitis or cytomegalovirus, may make breastfeeding unsafe (Meira et al., 2008).

Within the context described, human milk banks (HMB) play an essential role in providing milk to children who would otherwise not be able to receive HM (Haiden & Ziegler, 2016). It is important to consider that HM donations are primarily intended for premature and/or ill newborn (Agência Nacional de Vigilância Sanitária, 2008). In this sense, the microbiological quality of human milk distributed by HMB is a subject of wide public health concern, since the children who will consume this food have a low resistance to neonatal infections, and HM is an excellent culture medium for various types of microorganisms (Silveira et al., 2012).

The reasons of microbial growth in HM may be associated with improper collection techniques, the inadequate hygiene

conditions of the donor and utensils and the unrefrigerated storage of the HM. Among the contaminating microorganisms, *Staphylococcus aureus* is normally found in the oropharynx, mouth and saliva of human beings. However, its presence in HM can be interpreted as secondary contamination from the skin and nasal cavities, or unsatisfactory hygienic-sanitary conditions of the utensils used during milking.

The *S.aureus* strain can contaminate dairy products during processing and infect humans, so this bacterium plays a significant role in public health (Unlu et al., 2018). In addition, diseases caused by the presence of pathogens in dairy products due to inadequate hygiene practices are the greatest concerns of consumers, researchers and food regulatory agencies (Zavareh & Ardestani, 2020). Also, the greatest concern regarding its presence is the occurrence of strains that produce toxins resistant to pasteurization. Therefore, this microorganism species was selected for this study due to its potential to contaminate HM during milking and improper handling, in addition to the fact that it is a gram-positive bacterium, therefore, more resistant to thermal treatments (Serafini et al., 2003).

Normally, in HMB, HM withdraw, which is accepted by quality control tests, is pasteurized at 62.5 °C for 30 minutes in a water bath, a process that ensures the inactivation of pathogenic microorganisms that can contaminate it at the time of milking, in addition to the saprophytic microbiota (Agência Nacional de Vigilância Sanitária, 2008). It is known that slow pasteurization is a standard technique used to inactivate enzymes and microorganisms in human milk (Scudino et al., 2020; Agência Nacional de Vigilância Sanitária, 2008). However,

Received 23 Aug., 2020

Accepted 28 Oct., 2020

¹Departamento de Alimentos, Universidade Federal de Ouro Preto – UFOP, Ouro Preto, MG, Brasil

²Departamento de Nutrição Clínica e Social, Universidade Federal de Ouro Preto – UFOP, Ouro Preto, MG, Brasil

*Corresponding author: camilamenezes@ufop.edu.br

the effectiveness of this method requires prolonged exposure to high temperatures, which leads to changes in the functional properties, sensory characteristics and nutritional value of food products, such as protein denaturation, vitamin degradation, off-flavor formation and lactose degradation (Scudino et al., 2020).

In addition, studies have shown that slow pasteurization leads to reduced levels of some of the antioxidant components present in HM such as glutathione peroxidase (Silvestre et al., 2008), vitamin C (Moltó-Puigmartí et al., 2011), total antioxidant capacity (Nogueira et al., 2018; Silvestre et al., 2008)

In this context, innovative methods of food preservation such as ultrasound (US), high pressure, ionizing radiation, pulsed electric field, microfiltration and ultraviolet radiation, have been tested as possible alternatives to conventional thermal treatments in order to maintain microbiological safety with less impact on nutritional, functional and sensory aspects (Monteiro et al., 2020; Shabbir et al., 2020; Jasmi et al., 2020; Awad et al., 2012). In this context, the treatment of food with ultrasound (US) can offer an alternative to traditional methods (Piñon et al., 2019; Fernández-Barbero et al., 2019). This technology is considered a non-thermal emerging technology and has been extensively investigated for food processing in order to avoid the negative effects of conventional heat treatment (Bastos et al., 2019; Guimarães et al., 2019).

The treatment of food with US induces the phenomenon of acoustic cavitation, US waves promote rapid localized changes in pressure and temperature causing disruption by shearing, implosion of bubbles (cavitation), reducing the thickness of the cell membrane, localized heating and the production of free radicals that have a lethal effect on microorganisms. Also, the energy released as well as the mechanical shock associated with the cavitation affect the structure of the cells in the microenvironment (Carrillo-Lopez et al., 2019).

As studies on the processing of HM with US on the nutritional and microbiological aspects are still scarce, this study aimed to collaborate with the development of new technologies for processing HM with effectiveness in inactivating pathogenic microorganisms and in reducing the loss of antioxidant activity of this food compared to conventional thermal treatment with slow pasteurization, in view of the importance of antioxidants and microbiological safety for the health of infants. Given the above, the objectives of this study were to evaluate different time and temperature binomials associated with the application of US on the viability of *S. aureus* and to compare the effect of US on the best time and temperature binomials found for the inactivation of *S. aureus* on the total antioxidant activity of HM.

2 Materials and methods

The experimental study was conducted at the Experimental Nutrition (LABNEX) and Food Microbiology laboratories of the School of Nutrition and Immunoparasitology of the Research Center in Biological Sciences (NUPEB) of the Federal University of Ouro Preto (UFOP). The experimental part was divided into two stages: i) microbiological analysis; and ii) evaluation of in vitro antioxidant activity.

The first step consisted of checking the effect of US by assessing the binomial time and temperature on the viability of *S. aureus* in HM. Fifteen treatments (20, 30, 40, 50 and 60 °C for 1, 5 and 15 minutes respectively) were tested, in duplicate.

HM used in the first stage of the experiment was the disposal HM obtained from the HMB of Santa Casa da Misericórdia of Ouro Preto. The material was frozen in the HMB and was transported in transparent glass flasks in an isothermal box with ice to maintain a temperature close to 0 °C, according to Agência Nacional de Vigilância Sanitária (2008), and sent immediately to the laboratories where the analyses were carried out, remaining under refrigeration until the time of the experiments.

S. aureus (ATCC6538P) was thawed at room temperature, activated in BHI (Brain Heart Infusion) broth and incubated at 37 °C for 24h. This process was carried out twice to guarantee microbial growth, as described by Viazis et al. (2008).

The disposal HM samples were thawed at room temperature and after making the pool, the pH was measured (6.56), and the pool was subdivided into 40 mL aliquots, which were sterilized at 121 °C for 15 minutes. Then, the samples were heated to different temperatures (20, 30, 40, 50 and 60 °C) and inoculated with 1% *S. aureus*, representing 6.48 log CHU/mL HM. After inoculation, the samples were treated with US at different times and temperatures (1, 5 and 10 minutes at 20, 30, 40, and 60 °C) under a frequency of 40 kHz and power of 110 W. The equipment used was the bath US BRANSONIC, Emerson, model CPX3800H.

For pasteurization, samples were subjected to conventional heat treatment used in HMB, as recommended by Brazil, which consists of warming the milk in a water bath at 62.5 °C for 30 minutes with manual shaking of the flasks every five minutes, without taking them out of the water bath. After 30 minutes, the flasks were cooled in an ice bath until the milk reached a temperature of 5 °C or less.

The effect of US on the test microorganism was assessed by counting viable cells in HM immediately before and after treatments. The samples treated with US were subjected to serial dilutions in peptone water (0.1%) added with NaCl (0.85%) and the counting was performed by plating (surface) on PCA agar (Plate Count Agar). The dishes were incubated at 37 °C for 24h. The results of the counts were expressed in log Colony Forming Units (CFU) per mL (Czank et al., 2010; Viazis et al., 2008).

The second stage of the study consisted of evaluating the effect of US on the in vitro antioxidant activity of HM by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging method. For that, the treatments that showed the best efficiency in inactivating *S. aureus* were applied and the following treatments were used with controls: raw HM and HM pasteurized by the conventional method (62.5 °C for 30 minutes). In order to determine the effect of US on the in vitro antioxidant activity of HM, participants in the study, by donating 40 mL HM, were all woman who donate HM on a continuous basis to the HMB of Santa Casa da Misericórdia of Ouro Preto (n = 10), which were registered in the period from July to October. As an exclusion criterion, age below 20 years was adopted.

The evaluation of DPPH free radical scavenging activity was carried out according to the method presented by Brand-Williams et al. (1995), with modifications by Zarban et al. (2009). Initially, 50 µL of each HM sample was added with 1 mL DPPH in ethanol solution (0.06 mM). Subsequently, the mixture was homogenized and left to stand for 30 minutes in a water bath at 37 °C. Then, 0.5 mL chloroform was added, at 30 second intervals and then centrifuged at 8,000 rpm for 5 minutes. The reading was done on a FEMTO 700 S spectrophotometer at a wavelength of 517 nm.

The DPPH solution in ethanol (0.06 mM) was used as a control and the percentage of activity that scavenges the DPPH radical was calculated according to Equation 1:

$$\text{Scavenging activity \%} = \left[\frac{\text{absorbance of the control} - \text{absorbance of the sample}}{\text{absorbance of the control}} \right] \times 100 \quad (1)$$

Mean values, standard deviations and coefficients of variation of two repetitions were calculated for *S. aureus* counts found at different times (1, 5 and 10 minutes) and temperatures (20, 30, 40, 50 and 60 °C) (Table 1) and the results were expressed in log CFU/mL. The microbial reduction in relation to the initial count was also calculated.

The Kolmogorov-Smirnov and Shapiro-Wilk tests were applied to test data normality. The antioxidant results were analyzed using analysis of variance (ANOVA) followed by Tukey's test using a 5% significance level. Statistical analyses were run with the aid of the Statistical Package for the Social Science (SPSS) version 17.0.

The project was approved on February 19, 2018 by the Ethics Committee of the Federal University of Ouro Preto under the number CAAE 824118181.0000.5150.

3 Results

According to Table 1, there was a decrease in the count of *S. aureus* in samples treated with US at 50 °C for 5 minutes under a frequency of 40 kHz and Power of 110 W; this reduction was more accentuated in samples treated at 60 °C for 10 minutes (4.58 log CFU/mL). No significant reduction of *S. aureus* was verified in samples treated at 20, 30 and 40 °C. This emphasizes the importance of ensuring the microbiological quality of HM distributed by HMB, since the children who will consume this food have a low resistance to neonatal infections.

In view of the benefits of antioxidants present in HM for newborns and the importance of innovating the processing techniques of this food to preserve more these compounds in relation to conventional treatments, in the present study, the in vitro antioxidant activity of HM was assessed by the DPPH free radical scavenging. The results are shown in Figure 1. The effect of US treatment on the antioxidant activity of HM at 60 °C in the three times (1, 5 and 10 minutes) was evaluated to analyze the interference of the treatment time in relation to the content of these compounds. It was observed that pasteurization did not significantly reduce ($p > 0.05$) the antioxidant activity of HM, when evaluated by this method. However, thermosonication at a frequency of 40 khz and power of 110 W increased its antioxidant activity, especially when applied in the times of 1 (56.43%) and 5 minutes (57.27%).

4 Discussion

D'Amico et al. (2006) despite evaluating the effect of US on different parameters (frequency of 20 kHz, power of 150 W), reported a reduction of 3.47 log CFU/mL after 6 minutes at 57 °C in the UHT cow's milk inoculated with *Listeria monocytogenes*. Similar results were found in samples treated at 60 °C for 5 minutes,

Table 1. Count of *Staphylococcus aureus* in human milk treated with bath ultrasound at different times and temperatures under the frequency of 40 kHz and power of 110 W.

Temperature/ time (minutes)	Count (log CFU/mL)		
	Mean*/standard deviation	Coefficient of variation	Reduction***
T0** (control)	6.48 ± 0.04	0.63	–
20 °C/1'	6.51 ± 0.18	2.74	0.03
20 °C/5'	6.34 ± 0.06	0.88	0.14
20 °C/10'	6.63 ± 0.39	5.91	0.15
30 °C/1'	6.54 ± 0.11	1.63	0.06
30 °C/5'	6.43 ± 0.16	2.54	0.05
30 °C/10'	6.41 ± 0.19	2.91	0.07
40 °C/1'	6.60 ± 0.10	1.55	0.12
40 °C/5'	6.35 ± 0.04	0.65	0.13
40 °C/10'	6.35 ± 0.21	3.36	0.13
50 °C/1'	6.16 ± 0.06	1.04	0.32
50 °C/5'	5.92 ± 0.13	2.24	0.56
50 °C/10'	5.74 ± 0.00	0.00	0.74
60 °C/1'	4.86 ± 1.02	23.14	1.62
60 °C/5'	3.43 ± 0.02	0.66	3.05
60 °C/10'	1.9 ± 0.08	4.06	4.58

*Mean of two repetitions; **Data refer to the count of *S. aureus* at time zero (immediately after inoculation of the microorganism in HM); ***Modulus of the difference between the count of *S. aureus* at time zero and after treatment.

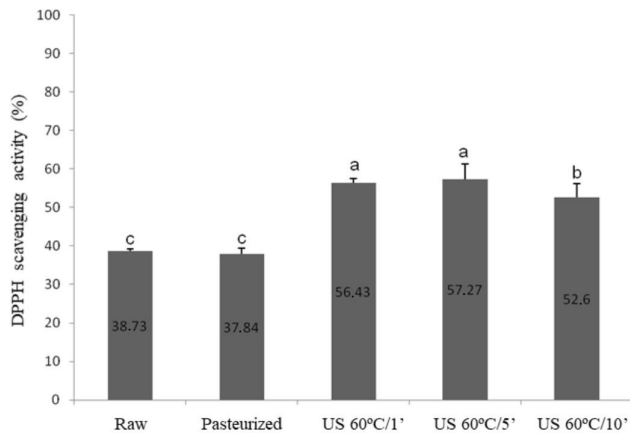


Figure 1. Mean in vitro antioxidant activity and standard deviation of human milk, raw, pasteurized and processed by thermosonication at 60 °C and different times. Different letters above the bars indicate significant differences by Tukey's test ($p < 0.05$).

where a reduction of 3.05 log CFU/mL was observed. According to these authors, ultrasonic liquid processing is more effective when combined with moderate heating due to a synergistic relationship, which has already been reported by several other (Baumann et al., 2005; Villamiel & De Jong, 2000; Sala et al., 1995; Ordoñez et al., 1987).

Herceg et al. (2012) evaluated the effect of US treatment on the count of *S. aureus* and *E. coli* in cow milk containing 4% fat with different parameters of temperature, amplitude and time of treatment. These authors observed that gram-negative bacteria were more susceptible to ultrasonic treatment compared to gram-positive bacteria. In addition, it was clear that the effect of combining US with heat treatment resulted in better inactivation of both bacteria, when applied in isolation. The authors concluded that the three parameters evaluated substantially affected the inactivation of *E. coli* and *S. aureus*, since the results indicated an increase in the inactivation of these microorganisms in longer exposure times, higher temperatures and broader amplitudes. Despite not evaluating the amplitude, the present study showed more pronounced reductions in treatments with higher temperatures and longer exposure times, where samples treated at 60 °C for 1, 5 and 10 minutes showed reductions of 1.62, 3.05, 4.58 log CFU/mL, respectively.

The mechanisms by which sonication leads to microbial reduction have been elucidated by Tiwari & Mason (2012). According to these authors, US promotes damage to the bacterial cell wall due to mechanical effects induced by pressure gradients generated during the collapse of cavitation bubbles, by shear force and also by chemical attack due to the formation of free radicals during cavitation that lead to disintegration of the cell wall. In addition, during the treatment, a small amount of hydrogen peroxide is formed via sonication, which is bactericidal, further contributing to the reduction of the microbial population as observed in this study.

Serafini et al. (2003) evaluated the microbiological quality of HM withdraw samples collected in HMB from a maternal

and child hospital in Goiânia, State of Goiás. Of the 194 samples of unpasteurized HM, 136 strains (70.4%) of indicator and/or potentially pathogenic microorganisms were isolated, and of 144 samples of pasteurized milk, 73 (50.7%) presented contamination. In the samples of raw HM, 10 strains (7.35%) of *S. aureus* were isolated and of the samples of pasteurized HM, five strains (6.9%) of *S. aureus* were found. According to these authors, the higher the microbial load of the product, the less effective pasteurization, which can be applied to any processing, considering that if the initial contamination is high, the more difficult it will be to control it. Thus, it emphasizes the importance of proper hygiene practices during milking and handling HM for better treatment efficiency with US, as well as other types of processing that can be applied to contribute to microbiological quality.

Regarding antioxidant activity, our results corroborate the hypothesis that treatment with US improves this parameter in comparison to the conventional pasteurization process, as demonstrated in other studies (Nadeem et al., 2018; Aadil et al., 2013; Cameron et al., 2009). Aadil et al. (2013) observed a significant increase in DPPH free radical scavenging activity and total antioxidant activity in sonicated grapefruit juice. This finding was explained by the increased exposure to phenolic free radicals as a result of the cavitation produced during sonication (Patist & Bates, 2008). Nadeem et al. (2018) observed an increase in the antioxidant activity in sonicated grape and carrot juice and attributed this increase to the release of phenolic compounds and ascorbic acid by sonication. The cavitation generated by sonication can cause the release of these compounds from the collapse of the cell wall and, thus, cause an increase in antioxidant activity as observed in this study. Türken & Erge (2017) evaluated the effect of sonication in cherry juice using the ABTS method, and attributed the increased antioxidant activity in these samples to increased exposure to free radicals of total phenolics, anthocyanins and other antioxidant molecules that can be extracted by sonication from food materials (Ashokkumar et al., 2008).

5 Conclusion

With Regarding microbiological quality, it can be concluded that treatment with US at 60 °C for 10 minutes was the most effective in terms of reducing the viability of *S. aureus*, in addition to presenting a significant increase in the content of antioxidants when compared to raw and pasteurized milk samples. In summary, thermosonication is an effective process to reduce the viability of the microorganism evaluated in this study, in addition to promoting an improvement in antioxidant activity compared to the slow pasteurization process.

References

- Aadil, R. M., Zeng, X. A., Han, Z., & Sun, D. W. (2013). Effects of ultrasound treatments on quality of grapefruit juice. *Food Chemistry*, 141(3), 3201-3206. <http://dx.doi.org/10.1016/j.foodchem.2013.06.008>. PMID:23871078.
- Agência Nacional de Vigilância Sanitária – ANVISA. (2008). *Banco de leche humana, funcionamiento, prevención y control de riesgos* (160 p.). Brasília. Retrieved from <http://www.redeblh.fiocruz.br/media/blhanv2008.pdf>

- Ashokkumar, M., Sunartio, D., Kentish, S., Mawson, R., Simons, L., Vilku, K., & Versteeg, C. (2008). Modification of food ingredients by ultrasound to improve functionality: a preliminary study on a model system. *Innovative Food Science & Emerging Technologies*, 9(2), 155-160. <http://dx.doi.org/10.1016/j.ifset.2007.05.005>.
- Awad, T. S., Moharram, H. A., Shaltout, O. E., Asker, D., & Youssef, M. M. (2012). Applications of ultrasound in analysis, processing and quality control of food: a review. *Food Research International*, 48(2), 410-427. <http://dx.doi.org/10.1016/j.foodres.2012.05.004>.
- Ballard, O., & Morrow, A. L. (2013). Human milk composition: nutrients and bioactive factors. *Pediatric Clinics of North America*, 60(1), 49-74. <http://dx.doi.org/10.1016/j.pcl.2012.10.002>. PMID:23178060.
- Bastos, R. G., Oliver, J. C., Germano, J. L., Fernandes, G. R., & Veiga, S. (2019). Effectiveness evaluation of alternative sanitizers in microbiological quality of strawberry (*Fragaria ananassa* Duch Var. Oso Grande) after artificial contamination by *Escherichia coli*. *Food Science and Technology*, 39(Suppl. 2), 470-474. <http://dx.doi.org/10.1590/fst.21118>.
- Baumann, A. R., Martin, S. E., & Feng, H. (2005). Power ultrasound treatment of *Listeria monocytogenes* in apple cider. *Journal of Food Protection*, 68(11), 2333-2340. <http://dx.doi.org/10.4315/0362-028X-68.11.2333>. PMID:16300070.
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology*, 28(1), 25-30. [http://dx.doi.org/10.1016/S0023-6438\(95\)80008-5](http://dx.doi.org/10.1016/S0023-6438(95)80008-5).
- Cameron, M., McMaster, L. D., & Britz, T. J. (2009). Impact of ultrasound on dairy spoilage microbes and milk components. *Dairy Science & Technology*, 89(1), 83-98. <http://dx.doi.org/10.1051/dst/2008037>.
- Carrillo-Lopez, L. M., Luna-Rodriguez, L., Alarcon-Rojo, A. D., & Huerta-Jimenez, M. (2019). High intensity ultrasound homogenizes and improves quality of beef longissimus dorsi. *Food Science and Technology*, 39(Suppl. 1), 332-340. <http://dx.doi.org/10.1590/fst.05218>.
- Czank, C., Simmer, K., & Hartmann, P. E. (2010). Simultaneous pasteurization and homogenization of human milk by combining heat and ultrasound: effect on milk quality. *The Journal of Dairy Research*, 77(2), 183-189. <http://dx.doi.org/10.1017/S0022029909990483>. PMID:20053316.
- D'Amico, D. J., Silk, T. M., Wu, J., & Guo, M. (2006). Inactivation of microorganisms in milk and apple cider treated with ultrasound. *Journal of Food Protection*, 69(3), 556-563. <http://dx.doi.org/10.4315/0362-028X-69.3.556>. PMID:16541685.
- Erick, M. (2018). Breast milk is conditionally perfect. *Medical Hypotheses*, 111, 82-89. <http://dx.doi.org/10.1016/j.mehy.2017.12.020>. PMID:29407004.
- Fernández-Barbero, G., Pinedo, C., Espada-Bellido, E., Ferreiro-González, M., Carrera, C., Palma, M., & García-Barroso, C. (2019). Optimization of ultrasound-assisted extraction of bioactive compounds from jaboticaba (*Myrciaria cauliflora*) fruit through a box-behnken experimental design. *Food Science and Technology*, 39(4), 1018-1029. <http://dx.doi.org/10.1590/fst.16918>.
- Guimarães, J. T., Silva, E. K., Ranadheera, C. S., Moraes, J., Raices, R. S. L., Silva, M. C., Ferreira, M. S., Freitas, M. Q., Meireles, M. A. A., & Cruz, A. G. (2019). Effect of high-intensity ultrasound on the nutritional profile and volatile compounds of a prebiotic soursoy whey beverage. *Ultrasonics Sonochemistry*, 55, 157-164. <http://dx.doi.org/10.1016/j.ultsonch.2019.02.025>. PMID:30853535.
- Haiden, N., & Ziegler, E. E. (2016). Human milk banking. *Annals of Nutrition & Metabolism*, 69(2, Suppl. 2), 8-15. <http://dx.doi.org/10.1159/000452821>. PMID:28103607.
- Herceg, Z., Jambrak, A. R., Lelas, V., & Thagard, S. M. (2012). The effect of high intensity ultrasound treatment on the amount of *Staphylococcus aureus* and *Escherichia coli* in milk. *Food Technology and Biotechnology*, 50(1), 46-52.
- Jasmi, N., Mansor, N., Lim, E. J., Yusof, N. L., Hajar-Azhari, S., & Rahim, M. H. A. (2020). The effect of sonication and heat treatment on the physicochemical, nutritional and microbiological properties of different sugarcane variants. *Food Science and Technology*, 40(3), 551-556. <http://dx.doi.org/10.1590/fst.12619>.
- Meira, M. M., Ibarra, S. D. A., & Santos, E. K. A. (2008). Inibição da lactação: (re) visitando a literatura. *Revista Eletrônica de Enfermagem*, 10(3). <http://dx.doi.org/10.5216/ree.v10.46699>.
- Monteiro, S. H. M. C., Silva, E. K., Guimarães, J. T., Freitas, M. Q., Meireles, M. A. A., & Cruz, A. G. (2020). High-intensity ultrasound energy density: how different modes of application influence the quality parameters of a dairy beverage. *Ultrasonics Sonochemistry*, 63, 104928. <http://dx.doi.org/10.1016/j.ultsonch.2019.104928>. PMID:31952002.
- Nadeem, M., Ubaid, N., Qureshi, T. M., Munir, M., & Mehmood, A. (2018). Effect of ultrasound and chemical treatment on total phenol, flavonoids and antioxidant properties on carrot-grape juice blend during storage. *Ultrasonics Sonochemistry*, 45, 1-6. <http://dx.doi.org/10.1016/j.ultsonch.2018.02.034>. PMID:29705302.
- Neves, C. V., & Marin, A. H. (2013). Breastfeeding's inability in different contexts. *BarBaroi*, (38), 198-214. Retrieved from <http://online.unisc.br/seer/index.php/barbaroi/article/download/2037/2988>
- Nogueira, J. A. V., Santos, M. M. O., Passos, M. C., Cunha, L. R. C., Parreiras, P. M., & Menezes, C. C. (2018). Stability of Antioxidant Capacity of Human Milk after Freezing and Pasteurization. *Journal of Food and Nutrition Research*, 6(7), 450-455. <http://dx.doi.org/10.12691/jfnr-6-7-5>.
- Ordoñez, J. A., Aguilera, M. A., Garcia, M. L., & Sanz, B. (1987). Effect of combined ultrasonic and heat treatment (thermoultrasonication) on the survival of a strain of *Staphylococcus aureus*. *The Journal of Dairy Research*, 54(1), 61-67. <http://dx.doi.org/10.1017/S0022029900025206>. PMID:3819154.
- Patist, A., & Bates, D. (2008). Ultrasonic innovations in the food industry: from the laboratory to commercial production. *Innovative Food Science & Emerging Technologies*, 9(2), 147-154. <http://dx.doi.org/10.1016/j.ifset.2007.07.004>.
- Piñon, M., Alarcon-Rojo, A., Paniwnyk, L., Mason, T., Luna, L., & Renteria, A. (2019). Ultrasound for improving the preservation of chicken meat. *Food Science and Technology*, 39(Suppl. 1), 129-135. <http://dx.doi.org/10.1590/fst.39017>.
- Sala, F. J., Burgos, J., Condón, S., Lopez, P., & Raso, J. (1995). Effect of heat and ultrasound on microorganisms and enzymes. In G. W. Gould (Ed.), *New methods of food preservation* (pp. 176-204). New York: Springer. http://dx.doi.org/10.1007/978-1-4615-2105-1_9.
- Scudino, H., Silva, E. K., Gomes, A., Guimarães, J. T., Cunha, R. L., Sant'Ana, A. S., Meireles, M. A. A., & Cruz, A. G. (2020). Ultrasound stabilization of raw milk: Microbial and enzymatic inactivation, physicochemical properties and kinetic stability. *Ultrasonics Sonochemistry*, 67, 105185. <http://dx.doi.org/10.1016/j.ultsonch.2020.105185>. PMID:32474185.
- Serafini, Á. B., André, M. C. D. P. B., Rodrigues, M. A. V., Kipnis, A., Carvalho, C. O., Campos, M. R. H., Monteiro, E. C., Martins, F., & Jubé, T. F. (2003). Microbiological quality of human milk from a Brazilian milk bank. *Revista de Saude Publica*, 37(6), 775-779. <http://dx.doi.org/10.1590/S0034-89102003000600013>. PMID:14666308.
- Shabbir, M. A., Ahmed, H., Maan, A. A., Rehman, A., Afraz, M. T., Iqbal, M. W., Khan, I. M., Amir, R. M., Ashraf, W., Khan, M. R., &

- Aadil, R. M. (2020). Effect of non-thermal processing techniques on pathogenic and spoilage microorganisms of milk and milk products. *Food Science and Technology*. <http://dx.doi.org/10.1590/fst.05820>.
- Silveira, L. A. M., D'Amorim, M. F. G., Silva, V. R. D., & Terra, A. P. S. (2012). Controle microbiológico do leite humano de um hospital universitário. *Revista Baiana de Saúde Pública*, 36(3), 844-850. <http://dx.doi.org/10.22278/2318-2660.2012.v36.n3.a560>.
- Silvestre, D., Miranda, M., Muriach, M., Almansa, I., Jareo, E., & Romero, F. J. (2008). Antioxidant capacity of human milk: effect of thermal conditions for the pasteurization. *Acta Paediatrica*, 97(8), 1070-1074. <http://dx.doi.org/10.1111/j.1651-2227.2008.00870.x>. PMID:18477059.
- Tiwari, B. K., & Mason, T. J. (2012). Ultrasound processing of fluid foods. In P. J. Cullen, B. K. Tiwari & V. P. Valdramidis (Eds.), *Novel thermal and non-thermal technologies for fluid foods* (pp. 135-165). New York: Elsevier. <http://dx.doi.org/10.1016/B978-0-12-381470-8.00006-2>.
- Türken, T., & Erge, H. S. (2017). Effect of ultrasound on some chemical and microbiological properties of sour cherry juice by response surface methodology. *Food Science & Technology International*, 23(6), 540-549. <http://dx.doi.org/10.1177/1082013217708077>. PMID:28474971.
- Unlu, A., Sar, T., Seker, G., Erman, A. G., Kalpar, E., & Akbas, M. Y. (2018). Biofilm formation by *Staphylococcus aureus* strains and their control by selected phytochemicals. *International Journal of Dairy Technology*, 71(3), 637-646. <http://dx.doi.org/10.1111/1471-0307.12520>.
- Viazis, S., Farkas, B. E., & Jaykus, L. A. (2008). Inactivation of bacterial pathogens in human milk by high-pressure processing. *Journal of Food Protection*, 71(1), 109-118. <http://dx.doi.org/10.4315/0362-028X-71.1.109>. PMID:18236670.
- Villamiel, M., & De Jong, P. (2000). Inactivation of *Pseudomonas fluorescens* and *Streptococcus thermophilus* in Trypticase Soy Broth and total bacteria in milk by continuous-flow ultrasonic treatment and conventional heating. *Journal of Food Engineering*, 45(3), 171-179. [http://dx.doi.org/10.1016/S0260-8774\(00\)00059-5](http://dx.doi.org/10.1016/S0260-8774(00)00059-5).
- Zarban, A., Taheri, F., Chahkandi, T., Sharifzadeh, G., & Khorashadizadeh, M. (2009). Antioxidant and radical scavenging activity of human colostrum, transitional and mature milk. *Journal of Clinical Biochemistry and Nutrition*, 45(2), 150-154. <http://dx.doi.org/10.3164/jcbn.08-233>. PMID:19794922.
- Zavareh, H. T. S. A., & Ardestani, F. (2020). Antibacterial effects of chitosan coating containing *Mentha aquatica* L. essence against *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* in Iranian white cheese. *International Journal of Dairy Technology*, 73(3), 585-593. <http://dx.doi.org/10.1111/1471-0307.12678>.