Advance Journal of Food Science and Technology 15(SPL): 179-183, 2018
DOI:10.19026/ajfst.15.5891
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: September 14, 2017 Accepted: December 9, 2017 Published: July 10, 2018

Research Article

Bactericidal Effect of Ultrasound on the Microbiota of Raw Milk Cream

[1]Pino Alexandra, [1]Caballero Luz A. and [1,2]Rivera María E.
[1]University of Pamplona. Master's degree in Food Science and Technology. Group Research GIBA, University Campus, The Buque, Pamplona, Norte of Santander, Colombia
[2]University of Pamplona. Master's degree in Food Science and Technology. Environmental Research Group Water, Air and Soil GIAAS, University Campus, The Buque, Pamplona, Norte of Santander, Colombia

Abstract: The objective of this research was to evaluate the effect of Ultrasound on the microbiota present in raw milk cream in order to decrease the initial microbial load. Ultrasound offers an alternative in the conservation and preservation of food. The raw milk cream as a by-product of industrial processing is susceptible to deterioration by high microbial load and the presence of enzymes, which develop unwanted defects. The analysis did to themselves for triplicate, taking randomly a quantity average of cream, packed, labeled in Polyethylene bags low thickness (300 g) and refrigerated (4±2°C). The samples were evaluated microbiologically (mesophyll's aerobic, total coliforms, fungi and yeasts), before and after treatment with ultrasound at a frequency of 37 KHz, with times of 2, 5 and 10 min, with temperatures of 30 and 40±2°C and stored in refrigeration (4±2°C) for 10 days (days 0, 3, 5, 7 and 10). The experimental data evaluated using a factorial design (time and temperature of exposure to US at 4 and 2 levels with three replicates for each treatment. An Analysis of Variance (ANOVA) was performed at a significance level of $p \leq 0.05$ and test POST HOC of Tukey using the statistical package SPSS v. 19.0 was shown that the treatment at 37 Hz for 10 min at a temperature of 40°C decreased the initial microbial load by 79%.

Keywords: Frequency, fungi, mesophylls aerobes, temperature, total coliforms, yeasts

INTRODUCTION

Consumers in the food industry are more concerned about food hygiene aspects as well as safety and transparency in production methods. Hence the importance of starting with a raw material (raw milk cream) of the highest quality. Freskaleche Collection Center isn´t no stranger to this situation, presenting relevant problems as: acidification of the milk cream before being ultra-pasteurized, high bacterial load of raw milk and prolonged waiting time at environmental temperature (35±2°C) in the process of skimmed raw milk, which leads to an increase in acidity, pH variation, protein instability and others.

The cream microbiological quality depends directly on the initial bacterial load of the raw milk. Freskaleche Collection Center, Aguachica report that an average is 2,150,000 cfu/mL established by the Colombian legislation in Resolution 017 of 2012 (200.000 to 800.000 cfu/mL of analyzed milk). For this reason the prolonged wait during the process of skimmed of the raw milk cause lost a great amount of kilos of product is without reaching a final thermal process such as ultra-pasteurization.

In the last years, emergent food conservation alternatives have arisen like the ultrasound, limiting logarithmic bacteria charges to a very low percentage without need to add chemical preservatives, this leads directly to improve straight the useful life of the completed product and to minimize the losses for acidification of the cream in the intermediate processes. Likewise, Chemat *et al*. (2017) considered that the ultrasound is an innovative skill of food processing and its role in the promotion of the sustainable food industry. As a whole with the microwave, electrical field of impulses, fall of instantaneous controlled pressure, prosecution of supercritical fluids, they shape an Eco technology used for 30 years and takes a lot of force today

Furthermore, the new food processes allow a significant reduction in processing times overall savings in energy consumption, while ensuring the safety of

Corresponding Author: Pino Alexandra, University of Pamplona. Master's degree in Food Science and Technology. Group Research GIBA, University Campus, The Buque, Pamplona, Norte of Santander, Colombia, Tel.: 3123291567

food and extensive benefits for the industry (Misra *et al*., 2017). The ultrasound power generates a series of effects that result from the collapse of cavitation bubbles, which are beneficial in food processing. Some of the most useful applications are the extraction assisted by ultrasound, emulsification, viscosity changes and the modification of proteins, crystallization and sterilization, drying and softening of the meat (Mason *et al*., 2015).

The processing of ultrasound covers a wide range of acoustic frequencies, starting with a frequency as low as 18 kHz up to several MHz. The applications are diverse as the spectrum of frequency is wide. At the end of lower frequency (18 kHz to approximately 100 kHz, also referred to as ultrasonic power, the effects are mainly caused by cavitation unstable (Knoerzer *et al*., 2015). Cavitation refers to the formation of gas bubbles and water vapor caused by the pressure wave traveling through the liquid; These bubbles grow over several cycles of pressure until they reach a size unstable and undergo a violent collapse, generating high pressures (up to 2000 bar), temperatures (up to 5000 K) and shear (Schössler *et al*., 2014; Knoerzer *et al*., 2015).

The ultrasounds are sonorous waves with a higher frequency higher than the perceived by the human ear (greater than 16 kHz). These waves as they pass through the food produce various phenomena that are responsible for their action against microorganisms: Extreme increases of temperature, changes of pressure and radicals' formation, provoking damages in the walls of the microorganisms after provoking an important physical stress. A major effect has in yeasts; bacteria gram positive and gram negative that in spore bacteria (Guerrero and Alzamora, 2004).

At present, investigated in liquid food as juices and milk, trying to reach the deadliness of a pasteurization and a sterilization without deteriorating organoleptic characteristics. This technique has advantage that can added other technologies during the treatment as the heat and the application of pressures changes, thus making more effective the process of inactivation. Another useis the inactivation of altering enzymes without applying heat (Zhao *et al*., 2007).

Get a safe food in time without losing its sensory characteristics in the line of research of food preservation technology. Among the new processes such as ionizing radiations or high hydrostatic pressures, we also find ultrasounds (Chemat *et al*., 2011).

Based on the above, is necessary to realized research to establish the effect of emerging non-thermal technologies such as ultrasound on the microbiological stability of products of nutritional importance, causing simultaneously a positive technological impact in the food industry of the region. This way, the food industry in Colombia needs to confront in competitively the technological challenges established for the new generations and for this requires a rethinking of the processing technologies currently used. For this reason, this research proposed to evaluate the effect of the application of the ultrasound in the bacterial micro flora present in the raw milk cream processed in the company Freskaleche S.A.S., in order to reduce high load microbial and to take in not very distant future at industrial levels.

MATERIALS AND METHODS

For the study, raw milk cream samples were taken directly from the raw milk processing plant at the Freskaleche S.A.S. Located in Aguachica, Cesar The percentage of fat of the samples of raw cream (300±2 g) will be 50%, packed in bags of polyethylene, wrapped in foil and refrigerated (4±2°C) for 24 h. The samples of raw milk cream obtained were microbiological tests of mesophylls aerobes, total coliforms, molds and yeasts (NTC 399, 2002).

The ultrasound used in the project was Elmasonic P: 500 W., with a frequency of 37 KHz and times used were 2, 5 and 10 min at a temperature of 30 and 40±2°C. All samples were packed in low density polyethylene with 95% vacuum in a labeled Henkovac packing machine, protected from light (with foil) and stored under cooling conditions (4±2°C) for 0, 3, 5, 7 and 10 days; Period in which they were evaluated microbiologically. The design of experiments realized to the raw cream milk samples presents the following:

$$N= (1TRUL \times 3TUL \times 2TMP \times 5D)\ 3R$$

where,

N = Number of packaged samples to be analyzed
TRUL = Number of ultrasound treatments
TUL = Application time ultrasound
TMP = Treatment Temperature
D = Number of days in refrigerated storage
R = Number of replicas

For execution realized the following actions:

Bibliographic search and analysis of information: A bibliographic review realized to determine the most important parameters in the evaluation of the ultrasound effect on the microbiological load according with the established objectives.

Microbiological characteristics evaluation: The microbiological inventory of the raw cream without treatment was realized of petrifilm methodology by 3M in the microbial groups following: total count of aerobic mesophylls microorganisms (AOAC 986.33, 2002a), total coliforms (AOAC 991.14, 2002b), fungus and yeasts (AOAC 997.02, 2002c), to different temperatures of the ultrasonic treatment in 0, 3, 5, 7 and 10 storage days under refrigeration conditions (4+/-2°C).

Experimental determination of the effect of the ultrasound on microbiological characteristics: To the results obtained of the different experimental tests to the milk cream was applied a factorial design at a significance level of 0,05 determining if exist or not statistically significant differences of the ultrasound effect on the microbiological characteristics of the milk cream during the storage refrigerated (4±2°C) for 0, 3, 5, 7 and 10 storage days.

METHODOLOGY

The raw milk cream sampling realized randomly once the skimming process stabilized inside the equipment, this occurred to 30 min of the start of production.

The samples of raw cream were subjected to ultrasound treatment with a 37 KHz frequency, with the Elmasonic P equipment: 500 W; to 30 and 40±2°C, in 2, 5 and 10 min of exhibition, in storage times of 0, 3, 5, 7 and 10 days. Control Samples were left in three times interval without application of ultrasound, only evaluating the characteristics presented with the samples of raw cream with three time intervals.Las treated samples to themselves they took to the laboratories of microbiology of Freskaleche S.A.S. in the Aguachica plant-Cesar, to do the respective test and analysis depending on the exposure times stipulated for each test.

The experimental results were analyzed with Variance Analysis (ANOVA) at a significance level of $p \leq 0.05$, to establish there is or not statistically significant differences between the data obtained by each treatment with respect to the characteristics evaluated, finally applied the multiple comparisons test or POST HOC test of DMS y Tukey in the Software SPSS v. 19.0.

RESULTS AND DISCUSSION

According to Fig. 1, was observed that there is a statistically significant difference in the aerobic mesophylls count, after the application of ultrasound compared to the standard sample: Milk cream without treatment, demonstrating that the ultrasound treatment 40°C during the storage days reduced the number of bacteria from 2 to 4 logarithms.

From Fig. 2, is observed that the aerobic mesophylls count is reduced from 2 to 3 logarithms with respect the blank sample (raw milk cream without treatment) and treatments (30 and 40°C), during the time of exposure from 2 to 10 min, which is corroborated with Ahmed Ansari *et al*. (2017), who affirms that ultrasound in combination with heat is an alternative technology for the reduction of microbial spores, which are characteristic of the aerobic mesophyll´s group.

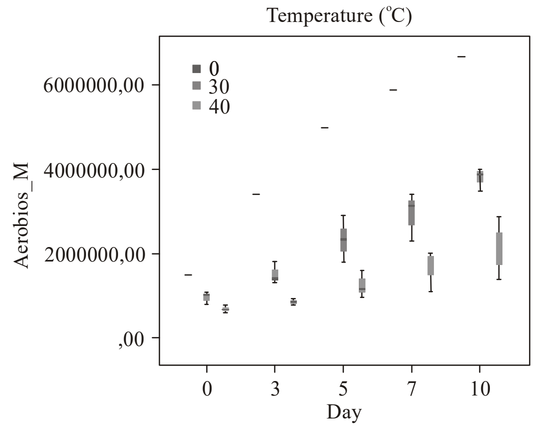

Fig. 1: Box plot of mesophylls aerobic influenced by the storage days and temperature Front: Authors, 2017

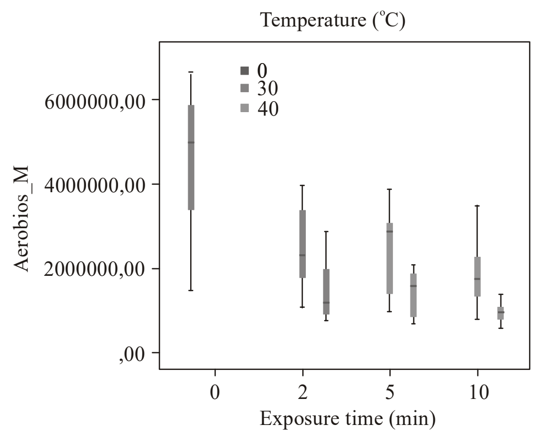

Fig. 2: Box plot of aerobes mesophylls influenced by exposure time and temperature Front: Authors, 2017

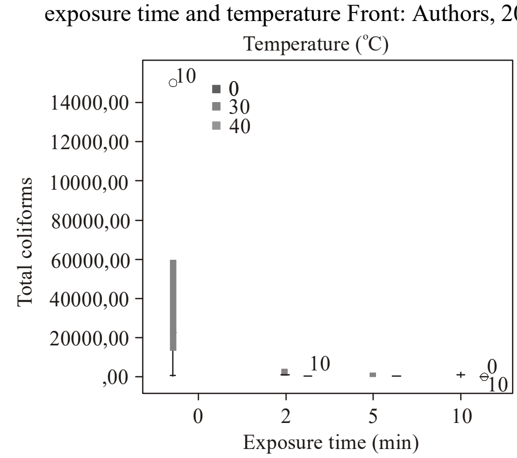

Fig. 3: Box plot of Total Coliforms influenced by exposure time ultrasound and temperature Front: Authors, 2017

Figure 3 shows a significant reduction lower than 2.000 cfu/mL in the total coliforms count during the storage days at temperatures of 30-40°C and the control sample (raw milk cream without treatment) coinciding with the results found by Wu *et al*. (2015) microbial inactivation is due to ultrasound.

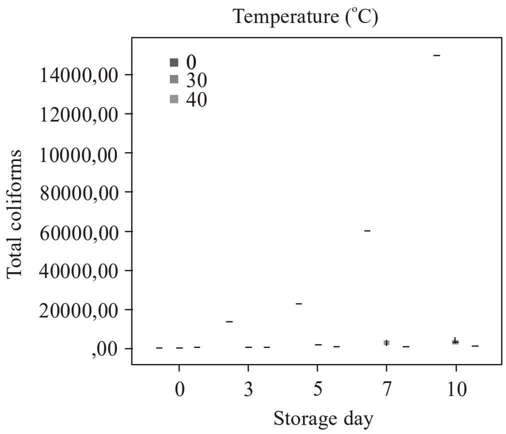

Fig. 4: Total Coliforms influenced by storage day and temperature; Front: Authors, 2017

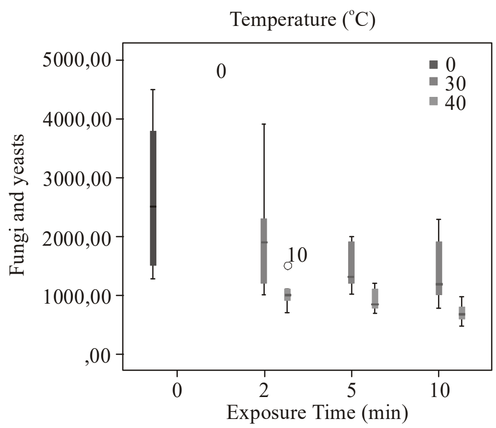

Fig. 5: Fungi and yeasts influenced by exposition times and temperature; Front: Authors, 2017

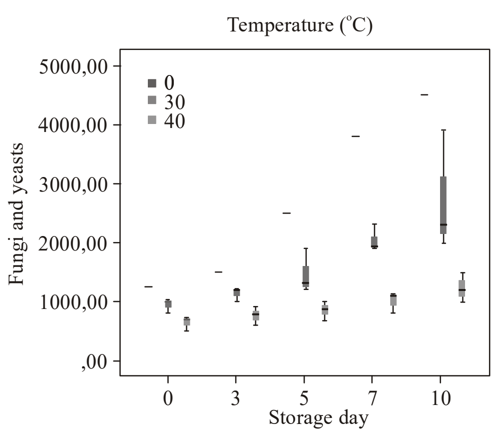

Fig. 6: Fungi and yeasts influenced by storage days and temperature; Front: Authors, 2017

Figure 4 showed that the total coliform count remained constant in the 10 days of storage of the treated milk cream, coinciding with Chandrapala *et al*. (2012), who showed that the microbial growth curves are maintained in other alternative thermal processes, as well as in storage at refrigeration temperatures.

The decrease in fungi and yeast counts ranges between 500 and 990 cfu/mL for 10 exposure time and 40°C of temperature with ultrasound treatment (Fig. 5). While the treatment at 30°C showed a decrease from 800 to 2300 cfu/mL compared to the control sample (1.250 to 4.500 cfu/mL).

In Fig. 6 was found that the decrease in the counts of fungi and yeasts is similar in the times and temperatures of exposure with the ultrasound treatment, that is, the load of microorganisms is not significantly reduced, keeping a similar count to the milk cream without treatment

Although, Bermúdez-Aguirre *et al*. (2008) shows that bacteria adhere to the fat globules contained in the medium and that ultrasonic treatments can alter the lipid membrane of the cells, generating smaller size leaving gaps and different surface roughness. Apparently, these new conditions allow the microorganisms adhere with greater force and hide inside the globules, which represents a protective effect against heat and cavitation. This leads low destruction of the bacteria that help to acidify the cream during storage.

CONCLUSION

The raw milk cream with ultrasound treatment (37 Hz) at temperatures of 30 and 40°C obtained a significant reduction of the group of microorganisms: aerobic mesophylls and total coliforms.

Mesophylls aerobic microorganisms acidify the raw milk cream to unacceptable levels within the production process. The reduction in the aerobic mesophylls counts in milk cream treated with ultrasound offers an alternative to the company Freskaleche SAS to store the cream of milk for 3 to 10 days of storage before finishing the UHT treatment and optimize the costs in the production line.

Regarding the effectiveness of exposure time and the temperatures application (30 and 40°C) during storage day of the milk cream, is evident that the best treatment was at 40°C for 10 days with a reduction of 79% in total coliforms with respect the aerobic mesophylls count.

ACKNOWLEDGMENT

To the company and personnel of Freskaleche S.A.S and University of Pamplona for theirs collaborating.

CONFLICT OF INTEREST

This study does not provide conflicts of interest.

REFERENCES

Ahmed Ansari, J., M. Ismail and M. Farid, 2017. Investigation of the use of ultrasonication followed by heat for spore inactivation. Food Bioprod. Process, 104: 32-39.

AOAC, 2002a. Bacterial and Coliform Counts in Milk. Dry Rehydratable Film Methods. Sec. 17. 7. 03. Method 986.33, In: Official Methods of Analysis of AOAC International, AOAC International, Gaithersburg, MD.

AOAC, 2002b. Coliform and Escherichia coli Counts in Foods: Dry Rehydratable Film Methods. Sec. 17.3.04, Method 991.14. In: Cunniff, P.A. (Ed.), Official Methods of Analysis of AOAC International, 16th Edn., AOAC International, Gaithersburg, MD, pp: 13-15.

AOAC, 2002c. Yeast and Mold Counts in Foods. Sec. 17.2.09, Method 997.02, In: Official Methods of Analysis of AOAC International. AOAC International, Gaithersburg, MD.

Bermúdez-Aguirre, D., R. Mawson and G.V. Barbosa-Cánovas, 2008. Microstructure of fat globules in whole milk after thermosonication treatment. J. Food. Sci., 73(7): E325-E332.

Chandrapala, J., C. Oliver, S. Kentish and M. Ashokkumar, 2012. Ultrasonics in food processing-food quality assurance and food safety. Trends Food Sci. Technol., 26(2): 88-98.

Chemat, F., Z.E. Huma and M. Kamran Khan, 2011. Applications of ultrasound in food technology: Processing, preservation and extraction. Ultrason. Sonochem., 18(4): 813-835.

Chemat, F., N. Rombaut, A. Meullemiestre, M. Turk, S. Perino, A.S. Fabiano-Tixier and M. Abert-Vian, 2017. Review of green food processing techniques. Preservation, transformation, and extraction. Innov. Food Sci. Emerg. Technol., 41: 357-377.

Guerrero, S. and S. Alzamora, 2004. The paper of ultrasonic in foods preservation. Latin Am. Food., 244: 18-21.

Knoerzer, K., R. Buckow, F.J. Trujillo and P. Juliano, 2015. Multiphysics simulation of innovative food processing technologies. Food Eng. Rev., 7(2): 64-81.

Mason, T., F. Chemat and M. Ashokkumar, 2015. Power ultrasonic for food processing. Appl. High-Intensity Ultras., 27: 815-843.

Misra, N.N., M. Koubaa, S. Roohinejad, P. Juliano, H. Alpas, R.S. Inacio, J.A. Saraiva and F.J. Barba, 2017. Landmarks in the historical development of twenty first century food processing technologies. Food Res. Int., 97: 318-339.

NTC 399, 2002. Raw Milk. 4thEdn., National Institute of Technical Standards and Certification. ICONTEC.

Schössler, K., H. Jäger, C. Büchner, S. Struck and D. Knorr, 2014. Non-Thermal Processing Ultrasonication. 2nd Edn., Reference Module in Food Science: From Encyclopedia of Food Microbiology, pp: 985-989.

Wu, T., X. Yu, A. Hu, L. Zhang, Y. Jin and M. Abid, 2015. Ultrasonic disruption of yeast cells: Underlying mechanism and effects of processing parameters. Innov. Food Sci. Emerg., 28: 59-65.

Zhao, B., O. Basir and G. Mittal, 2007. Innovation in food engineering: New techniques and products: Maximum components integration for image processing: An application of ultrasound for detection of small objects in containers. J. Food Pro. Eng., 30: 393-405.