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Effect of high pressure homogenization process on *Bacillus stearothermophilus* and *Clostridium sporogenes* spores in skim milk

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Abstract

High pressure homogenization (HPH) is an alternative food processing technique. As product heating is minimum, its characteristics are not affected in a large extend. This characteristic makes the HPH an interesting process to guarantee the safety of thermo-labile food. Inactivation of *Bacillus stearothermophilus* ATCC 7953 and *Clostridium sporogenes* PA 3679 spores in skim milk by HPH was studied. Results showed that pressures up to 300 MPa were not able to cause any reduction on spore counts or promote changes on its thermal resistance. The application of heat shock (100ºC/15 min) before HPH treatment and the homogenization process realized at mild inlet temperature (45ºC) – which results in homogenization temperature of around 84ºC at 300 MPa - also did not cause reduction on viable spores counts. A few spores reduction (0.67 logarithmic cycles) were only observed when the milk samples were subjected to homogenization treatment 16 times (multiple passes) at 300 MPa. Therefore, although HPH be recognized as an effective method for milk pasteurization, it was conclude that the HPH process is not able to guarantee the commercial sterility of milk, being necessary the association of the homogenization with another preservative method, as refrigeration.

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Keywords: Novel technologies; high pressure; spores; milk

1. Introduction

Milk plays a significant role for human nutrition and stands for one of the most frequently sold types of food worldwide. The nutritional composition, high water activity and neutral pH turn milk into an adequate media for microbial development which can lead growth of enterobacterias, lactic acid bacteria,

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Pseudomonas, *Staphylococcus* and *Listeria* [1] and also sporulated microorganisms as Bacilli and Clostridia, which are thermoresistant and important for milk deterioration. High pressure homogenization (HPH) is based upon common milk homogenization processes, though it uses 10-15 times higher pressures [2]. It is a non thermal process developed to fluids and has the appeal of preserving heat-labile nutrients [3] and was previously studied mainly to stabilization of juices [4] and milk [5, 6].

The effect of HPH on microbiological quality of milk was evaluated by some authors that studied the inactivation of native contaminants [7-10] and the inactivation of important genera intentionally added to the milk [6, 11-13]. Considering the native contaminants, the HPH presented results similar to thermal pasteurization. The inactivation of the specifics microorganisms intentionally added varied from 0.3 to 8 cycle reductions, which was attributed to different resistance of each one and also to different process condition (pressure, fat milk content, inlet temperature).

Few studies [14, 15] described the effect of HPH on spore inactivation and the effectiveness of this process when associated with pre or post thermal treatment. The present work aimed to evaluate the inactivation of *Bacillus stearothermophilus* ATCC 7953 and *Clostridium sporogenes* PA 3679 spores in skim milk by HPH.subheadings, images and formulae. The section headings are arranged by numbers, bold and 10 pt. Here follows further instructions for authors.

2. Materials and Methods

Commercial UHT skin milk was used to guaranty the absence of initial spore counts. *Bacillus stearothermophilus* ATCC 7953 was obtained in a culture collection from Food Technology Department of the School of Food Engineering of the University of Campinas (DTA- FEA- UNICAMP) and the *Clostridium sporogenes* PA 3679 was obtained from the Microbiology Center of the Institute of Food Technology (ITAL). Spore suspensions from both microorganisms were obtained following an procedure previously proposed [16]. The suspensions were stored at refrigeration temperature for one year.

The assays were performed in a high pressure homogenizer Stansted, model FPG 7400H:350 (STANSTED Fluid Power LTD®, EssexUK) at pressures from 100 to 300 MPa, with a flow rate of approximately 270 mL/min. T-type thermocouples (needle) were set at the outlet of intensifiers, outlet of the main homogenizer valve, and at the product cooler. The temperatures were registered (at intervals of 10 s) by a data logger (model 692-8010 Barnant Co.® Barrington, U.S.A.). A heat exchanger of shell and tube (SPIREC R®) for cooling was connected to the homogenizer to reduce the temperature of the fluid exiting the homogenizer valve. The heat exchanger outlet was connected to an aseptic collection system. After each assay the equipment was cleaned and sanitized with peracetic acid 0,1%.

For the tests, skin milk was inoculated with 10^5 spores ml^{-1} and than subjected to homogenization at pressures of 100, 200 and 300 MPa. The heat resistance of spores at 110, 115 and 121ºC were measure before and after HPH at 300 MPa using TDT method [17]. The effect of previous heat shock (110ºC / 15 min) on resistance to high pressure homogenization was also evaluated by heating of the inoculated milk prior to homogenization at 300 MPa. To measure the effect of inlet temperature in the spore inactivation, skin milk inoculated was pre heated to 45ºC and than subjected to 300MPa. Additionally, the effect of successive treatment of homogenization at 300 MPa was evaluated by skin milk recirculation on the homogenizer until it reached the equivalent to 16 times.

In all tests performed the count of spores suspension were determined before and after the treatment, in order to evaluate the spores reduction (NDR), through equation 1.

$$NDR = \log_{initial\_spores} - \log_{spores\_after\_treatment} \quad (1)$$

Data were statistically evaluated through variance analysis (ANOVA) and average test (Tuckey) using the software STATISTICA 5.0.

3. Results and Discussion

The effect of HPH at 100, 200 and 300MPa in the *B. stearothermophilus* spores and 300MPa in the *C. sporogenes* spores were measured. For these three conditions, no inactivation of spores was observed and the NDR determined for *B. stearothermophilus* were negative, indicating a possible activation of the spores, as previously described [18] using high hydrostatic pressure. Table 1 shows the effect of HPH against the spores.

Table 1. Temperatures reached during homogenization process

Pressure (MPa)	NDR	
	B. stearothermophilus	*C. sporogenes*
100	-0.41±0.12	---
200	-0.29±0.18	---
300	-0.23±0.19	0.12±0.05

* NDR was determined as the average of the triplicate results

Considering that HPH was not enough to inactivate the spores in milk, it was tested if the HPH could be able to sensitize the spores heat, due to its activation. This process was previously observed by using high hydrostatic pressure [19]. The results obtained for *B. stearothermophilus* spores are shown in the Table 2.

Table 2. Heat resistance of *B. stearothermophilus* spores before and after HPH at 300 MPa.

Parameters	Before HPH			After treatment at 300 MPa		
D-value* (min)	$30.33^{a(**)}$	8.24^{b}	1.10^{c}	31.37^{a}	7.71^{b}	1.04^{c}
SD	0.59	1.21	0.13	0.7500	0.05	0.07
CV (%)	1.94	14.72	11.51	2.39	0.66	6.72
z (°C)		7.41			7.60	
R^2		0.9976			0.9949	

*D-value was determined as the average of the triplicate results. ** Different letters means significant difference at $p<0.05$.

The D and z-value determined to *B. stearothermophilus* were equal before and after the homogenization, indicating that the process was not able to cause germination or sensitize the spores to a thermal treatment. In addition, results determined to *C. sporogenes* also did not presented any change on thermal resistance after treatment at 300 MPa. Consequently, it was concluded that the HPH can not sensitize spores to post treatment, which is in accordance with others authors [11,13] but in discordance with recent publication [4,14], which may be attributed to different species of microorganisms.

Another alternative to combine heat and HPH was the previous thermal treatment to promote the spores germination and posterior homogenization to inactivate the germinated spores. Again, no significant reductions of *B. stearothermophilus* and *C. sporogenes* were observed, indicating that HPH up to 300 MPa was also not able to inactivate previous germinated spores. Considering that the combination of heat and homogenization did not presented satisfactory results, it was evaluated the effect of homogenization at high temperature (around 84ºC) in the spores. The results again did not indicated efficacy on spores inactivation. The last alternative to makes viable the use of HPH to inactivate *B. stearothermophilus* and *C. sporogenes* spores was the multiple treatments at 300 MPa (multiple passes). The results of this test for *B. stearothermophilus* spores inactivation are shown in figure1.

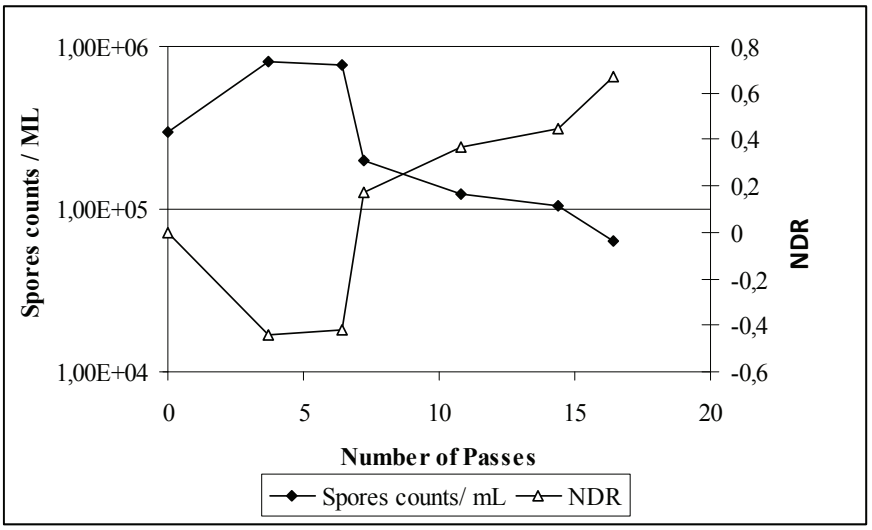

Fig. 1. Effect of multiple passes at 300 MPa on the inactivation of *B. stearothermophilus* spores

The evaluation of figure 1 indicates low efficacy of the HPH even when the spores are subjected to 300 MPa for 16 passes through the homogenizing valve. Therefore, this process is not suitable for commercial purposes aiming to inactivate spores in milk.

4. Conclusion

It was concluded that HPH process was not able to reduce or sensitize *Bacillus stearothermophilus* ATCC 7953 and *Clostridium sporogenes* PA 3679 spores in skim milk at the pressure range tested (100 to 300 MPa), even when applied multiple treatment at high pressure of 300 MPa, high temperature during homogenization (high inlet temperature) and a heat shock before homogenization. Therefore, the efficacy of HPH in skin milk is similar to heat pasteurization and the HPH must to be associated to other preservation technique, as refrigeration, to guarantee the milk safety and stability.

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