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Hyperbaric storage at room temperature with several short intermittent interruption periods at atmospheric pressure results in similar microbial growth inhibition and inactivation as without interruption

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ABSTRACT

In order to study the possible effect of several compressions and decompressions (C/D) cycles performed during hyperbaric storage (HS) in microbial behaviour, raw milk was stored under 75 MPa at room temperature (RT) for 31 days, under three different total C/D cycles conditions, 5, 31 and 93.

The different C/D cycles achieved similar microbiological counts reduction for the same sampling period, independently of the number of C/D cycles, with D_p-values around 3.5 and 19 days for Enterobacteriaceae and total aerobic mesophiles, respectively, reaching similar microbial levels at the end of the storage period.

These results contribute to enhance HS research, allowing the use of a single vessel with several samples to be taken along time, since several C/D cycles have no effect on results, thus leading to faster research advances in the field of HS.

1. Introduction

Moderate pressure is employed in hyperbaric storage (HS), usually in a range between 25 and 150 MPa, during prolonged storage periods (Santos et al., 2020). These long storage periods noticeably restrict the number of experiments that can be carried out and so on the number of samples that can be studied, unless a large number of HS vessels are available, what is not currently the case at all. To reduce this limitation and to facilitate HS experiments, the same pressure vessel can be filled with several samples, with sampling taking place over time, with this requiring several compression and decompression (C/D) cycles, with each one taking usually about 5 min. During each sampling, samples are under atmospheric pressure (AP) and room temperature (RT) perishability, which should not be a problem due to the short time which samples are exposed to these conditions, even considering several C/D cycles. A rather analogous situation occurs when foods are taken in/out of the refrigerator to AP/RT conditions and go back into the refrigerator, but with a striking difference. While temperature changes are mass/time dependant, what is particularly important for bulky foods, meaning that it takes some time for foods to cool/heat in consecutive cycles of in/out of the refrigerator, while pressure changes are mass/time independent. This peculiar feature of pressure is advantageous when pressuring a food for HS, but it could be disadvantageous when decompressing for sampling.

Although we have tested the possible effect of compression/decompression cycles in several HS/RT works in our research group, this was never reported expressly in the literature, except for a PhD thesis (Lopes, 2018). In this work the effects of several C/D cycles during milk fermentation for yoghurt production under pressure was evaluated, with each C/D cycle taking about 5 min. For this, in the first set of experiments, sampling was performed every two hours (with 3 C/D cycles taking place), while in the second experiment, samples were only removed from the pressure vessel after 6 h (at the end of the fermentation). The results showed that the C/D cycles performed to collect samples during the fermentation under pressure had no effect on the fermentative process, with similar values of pH, titratable acidity and fermentation rate being observed, when compared to fermentation under pressure without interruptions.

To better evaluate the effect of several C/D cycles during HS/RT of foods, concerning microbial behaviour, a systematic study was carried out in the present work, with a large number of C/D cycles. For this, raw cow's milk samples were kept under 75 MPa (the lowest pressure level verified to cause microbial growth inactivation in raw milk in a previous work (Duarte, Pinto, Gomes, Delgadillo & Saraiva, 2022)) at RT for a total of 31 days in three different vessels, with three specific C/D cycles during storage, with one being only C/D on each sampling day for microbial evaluation, while the other two were intentionally C/D one time or three times every day, respectively.

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2. Materials and methods

Raw cow's milk was kindly supplied by a local dairy farm association being packed under aseptic conditions, inside a laminar flow cabinet (BioSafety Cabinet Telstar Bio II Advance, Terrassa, Spain) in previously UV-light sterilized, low permeability polyamide-polyethylene bags (90 μm, IdeiaPack, Comércio de Embalagens, LDA, Abraveses, Viseu, Portugal), 10 mL of raw milk per replica, and heat-sealed individually. The experiments were performed in a custom designed high pressure equipment SFP FPG13900 Model (Stansted Fluid Power, Stansted, UK), equipped with three pressure vessels of 30 mm inner diameter and 500 mm height, at variable uncontrolled room temperature (RT – 18–22 ˚C) under 75 MPa.

The study was carried out for 31 days, and sampling took place at days 2, 7, 9, 14 and 31 of storage. The three different C/D conditions studied were in detail: condition 1 (Cond 1), where samples were only C/D when a sample was removed from the vessel for microbiological evaluation at the specific sampling period (at days 2, 7, 9, 14 and 31, thus resulting in 5 C/D cycles in total); condition 2 (Cond 2), where samples were intentionally C/D once a day (hence resulting in a total of 31 C/D cycles); condition 3 (Cond 3), where samples were also intentionally C/D three times a day, to simulate a situation of a great number of C/D cycles (therefore resulting in a total of 93 C/D cycles). For the three conditions, in each C/D cycle profile, samples remained at AP/RT about 5 min per cycle, in a total time of 25/0.42, 155/2.58, and 465/7.75 min/h, respectively for condition 1, 2, and 3.

Quantification of total aerobic mesophiles (TAM) and Enterobacteriaceae (ENT) counts were evaluated after 2, 7, 9, 14 and 31 days of storage. At each sampling period, raw milk samples were serially diluted in Ringer's solution and plated on the appropriate media for microbiological evaluation. TAM were enumerated on plate count agar, incubated at 30 ˚C for 3 daysy (ISO 4833:2013), while ENT counts were determined on violet red bile glucose agar and incubated at 37 ˚C for 1 day (ISO 21,528:2017).

TAM and ENT inactivation along HS/RT was verified to follow a first order inactivation kinetics and D_p-values determination was carried, in cases where measurable values were obtained (values below the quantification and detection limits were not considered). A D_p-value is the time needed at a constant pressure, to reach a decimal reduction in the microbial load (expressed here in days) and was calculated based on the negative inverse of the log linear slope of Eq. (1).

$$Log\ (N) = Log\ (N_0) - \frac{t}{D_p} \quad (1)$$

where N is the microbial load (CFU/ml) under a certain pressure (MPa) for certain time (t) in days, and N_0 is the initial microbial load (CFU/mL).

All experiments were carried out in triplicate and all analyses were done in triplicate. The results for the different storage conditions were compared using Analysis of Variance (ANOVA), followed by a multiple comparison post hoc test, Tukey's HSD test, at a 5% level of significance.

3. Results and discussion

At each sampling period TAM and ENT counts were evaluated, with the results shown in Figs. 1 and 2. Raw milk presented initial TAM counts of 6.66 ± 0.09 log CFU/mL, which were gradually reduced over time, reaching after 31 days of storage, no statistically ($p > 0.05$) different values, 4.85 ± 0.07, 4.76 ± 0.06, 4.90 ± 0.20 log CFU/mL, for condition 1, 2 and 3, respectivly. These results indicate that the number of C/D cycles does not cause changes in TAM response behaviour during HS/RT, at least up to 31 days at 75 MPa/RT and for up to at least 93 C/D cycles.

Comparable results were obtained regarding ENT, although the inactivation rate was higher compared to TAM. As it can be seen in Fig. 2, inactivation of ENT occoured gradually over time, without a significant ($p > 0.05$) effect regarding the number of times each vessel was

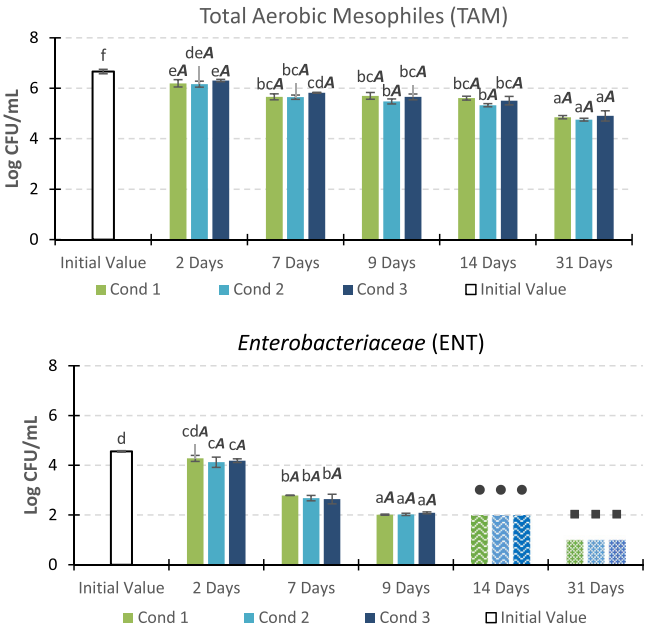

Fig. 1. – TAM and ENT microbial evolution in raw milk stored by HS under 75 MPa at room temperature (RT), at the different compression/decompression (C/D) conditions: represented by Cond 1 (total of 5 C/D cycles), where samples were only C/D when a sample was removed for microbiological evaluation, Cond 2, and Cond 3, where samples were intentionally C/D once (total of 31 C/D cycles) or three times (total of 93 C/D cycles) a day, respectively. The symbols • and ■ represent microbial counts below the quantification (2 log CFU/mL) and detection limit (1 log CFU/mL), respectively. Different letters denote statistically significant differences ($p < 0.05$) between all the different storage conditions and storage times (a-f) and only within each storage times between the 3 storage conditions (A).

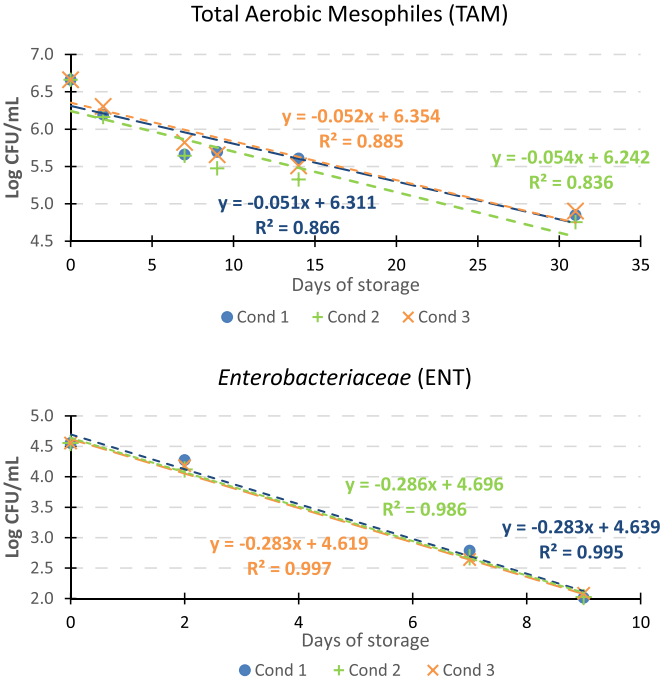

Fig. 2. - Log linear decrease of TAM and ENT present in raw milk (expressed in log CFU/mL), throughout HS storage under 75 MPa/RT at the different decompression/compression (C/D) conditions, represented by Cond 1 (total of 5 C/D cycles), where samples were only C/D when a sample was removed for microbiological evaluation, Cond 2, and Cond 3, where samples were intentionally C/D once (total of 31 C/D cycles) or three times (total of 93 C/D cycles) a day, respectively.

Table 1
D_p-values (days) determined for total aerobic mesophiles (TAM) and Enterobacteriaceae (ENT) loads in raw milk, stored by HS under 75 MPa/RT at the different compression/decompression (C/D) conditions, represented by Cond 1 (total of 5 C/D cycles), where samples were only C/D when a sample was removed for microbiological evaluation, Cond 2, and Cond 3, where samples were intentionally C/D once (total of 31 C/D cycles) or three times (total of 93 C/D cycles) a day, respectively.

Conditions	D_p-values *(days)*	
	TAM	ENT
Cond 1	19.81 ± 0.85[a]	3.50 ± 0.11[a]
Cond 2	18.81 ± 0.48[a]	3.55 ± 0.10[a]
Cond 3	19.63 ± 1.33[a]	3.56 ± 0.13[a]

C/D, with similar counts being observed for the same sampling period at each diferent storage conditon. For instance, after 7 days at HS/RT, ENT counts were 2.79 ± 0.01, 2.68 ± 0.11 and 2.64 ± 0.19 log CFU/mL for conditions 1, 2 and 3, respectively ($p > 0.05$), with all the conditions reaching quantification and detection limit levels (2 and 1 log CFU/mL, respectively), at the 14th and 31st days of storage, respetively.

As for the D_p-values (Table 1), both TAM and ENT presented similar values for the three different storage conditions (around 19 days for TAM and 3.5 days for ENT), confirming no effect on microbial inactivation rate regarding the number of C/D cycles.

As a preservation methodology, since food products are stored under HS inside a vessel/container, they could be subjected to several C/D cycles when the vessel is opened and closed for product removal and then recompressed to achieve HS conditions. Based on the results presented in this work, no significant changes regarding microbial behaviour were observed, even when comparing condition 1 with condition 3, being the first one subjected to a few number of cycles (total of 5 C/D) and the other one, subjected to a larger number of cycles (total of 93 C/D), resulting in a total time at AP/RT perishability conditions of 0.42 and 7.75 h, respectively. In what concerns physicochemical and nutritional parameters, based on previous results for raw milk (Duarte et al., 2022) stored under 75 and 100 MPa at RT for 60 days (total of 5 C/D cycles, resulting in 25/0.42 min/h at AP/RT perishability conditions), again no considerable changes were observed along storage, regarding pH, titratable acidity, density, total solids content, density, colour, lipid oxidation, viscosity, fatty acids and volatile organic profile, reaching similar values to those of the initial raw milk, prior to storage (Duarte et al., 2022).

The results of this work indicate so that HS/RT can be used to preserve foods in a practical situation, where several C/D cycles can occur, what is very important for instance for industrial applications. Also, these results show that experimental HS/RT work can be accelerated by using a single vessel to study several samples, since C/D the vessel several times do not affect the results.

4. Conclusion

The results obtained in this focused evaluation, indicate that several compression/decompression (C/D) cycles of raw milk do not change the microbial behaviour observed during HS/RT, even when comparing 5 C/D cycles with 93 C/D after 31 days. This is particularly important, since for practical applications, several C/D cycles could have to be done, with foods remaining at atmospheric pressure and room temperature perishability conditions for some minutes at each C/D cycle. Moreover, these results show also that experimental HS/RT work can be accelerated/improved using a single vessel to study several samples along time, wich is partircurly advantageous for long storage duration studies.

Ethical statement

The research of this MS involves no studies in humans or animals.

Conflict of interest

The authors have no conflict of interest to declare.

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References

Duarte, R. V., Casal, S., da Silva, J. A. L., Gomes, A., Delgadillo, I., & Saraiva, J. A. (2022a). Nutritional, physicochemical, and endogenous enzyme assessment of raw milk preserved under hyperbaric storage at variable room temperature. *ACS Food Science & Technology*. 10.1021/acsfoodscitech.2c00027.

Duarte, R. V., Pinto, C. A., Gomes, A. M., Delgadillo, I., & Saraiva, J. A. (2022b). A microbiological perspective of raw milk preserved at room temperature using hyperbaric storage compared to refrigerated storage. *Innovative Food Science & Emerging Technologies*, Article 103019. 10.1016/j.ifset.2022.103019.

Lopes, R. (2018). *Production of Yogurt Under High Pressure: Effect on Fermentative Process and Yogurt Characteristic* (Doctoral dissertation), Universidade de Aveiro, RIA - Repositório Institucional. Retrieved from http://hdl.handle.net/10773/25577 (10773/25577).

Santos, M., Fidalgo, L., Pinto, C., Duarte, R., Lemos, A., Delgadillo, & Saraiva, J. A. (2020). Hyperbaric storage at room like temperatures as a possible alternative to refrigeration: Evolution and recent advances. *Critical Reviews in Food Science and Nutrition*, 12. 10.1080/10408398.2020.1770687.