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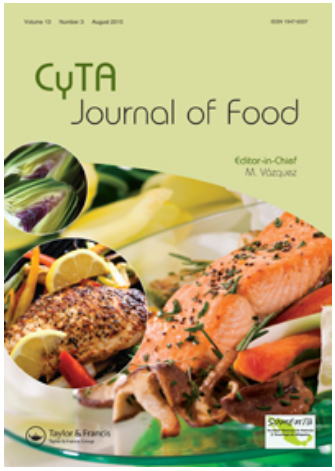

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Preservation under pressure (hyperbaric storage) at 25°C, 30°C and 37°C of a highly perishable dairy food and comparison with refrigeration

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Preservation under pressure (hyperbaric storage) at 25°C, 30°C and 37°C of a highly perishable dairy food and comparison with refrigeration

Conservación bajo presión (almacenamiento hiperbárico) a 25°C, 30°C y 37°C de un producto lácteo altamente perecedero y comparación con la refrigeración

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Hyperbaric storage (HS) under mild pressure of *requeijão*, a traditional Portuguese whey cheese, as a case study of a highly perishable dairy food, was evaluated as a possible energy costless alternative to refrigeration. Whey cheese was stored for 4 and 8 hours, at different pressure levels (0.1, 100 and 150 MPa) and temperatures (25°C, 30°C and 37°C), and the results were compared with refrigeration (4°C). Microbial analyses showed that storage for 4 hours at 100 MPa was able to maintain microbial counts similar to refrigeration and initial load, ≈3 Log_{10} CFU/g, at all tested temperatures. By increasing the pressure to 150 MPa and the storage time to 8 hours, microbial loads were reduced to undetectable counts, with the exception for total aerobic mesophiles that were reduced to about ≈1 Log unit. HS in general maintained pH, water activity and lipid oxidation values, at levels similar to that in refrigeration.

Keywords: food preservation; high pressure; hyperbaric storage; refrigeration; whey cheese

El almacenamiento hiperbárico bajo una presión suave del *requeijão*, un queso de suero típico en Portugal, como caso de estudio de un producto lácteo altamente perecedero, fue evaluado como una alternativa sin ningún coste energético a la refrigeración. El queso de suero fue almacenado durante 4 y 8 horas, a diferentes niveles de presión (0,1; 100; y 150 MPa) y temperaturas (25, 30, y 37 °C). Los resultados fueron comparados con la refrigeración (4 °C). Los análisis microbianos mostraron que el almacenamiento de 4 horas a 100 MPa era capaz de mantener los recuentos microbianos similares a los de la refrigeración y carga inicial, ≈3 Log_{10} CFU/g, para todas las temperaturas examinadas. Mediante el incremento de la presión a 150 MPa y el tiempo de almacenamiento a 8 horas, la carga microbiana fue reducida a recuentos indetectables, excepto el total de aerobios mesófilos que fue reducido alrededor de ≈1 unidad Log. El almacenamiento hiperbárico en general conservó pH, actividad del agua y valores de oxidación lipídica, a niveles similares a la refrigeración.

Palabras clave: conservación alimentos; alta presión; almacenamiento hiperbárico; refrigeración; queso de suero

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Introduction

High pressure processing (HPP) is a nonthermal processing technology that can inactivate microorganisms and enzymes, extend a product's shelf life, and cause negligible impairment in food sensory properties and nutritional quality, such as flavor, color and nutritional value (Matser, Krebbers, van den Berg, & Bartels, 2004). Nowadays, it is a commercially implemented technology, applied worldwide for cold pasteurization of a wide range of different foods, such as precooked meals, juices, beverages, fruits and vegetables (Norton & Sun, 2008). For HPP pasteurization, pressure ranging from 400 to 600 MPa is applied for some minutes to ensure the destruction of vegetative microbial cells (Huang, Lung, Yang, & Wang, 2014; Ramirez, Saraiva, Pérez Lamela, & Torres, 2009).

Pressure is also now viewed as an increasingly important thermodynamic parameter, with unique effects on biological systems, which have recently been explored for other new and promising applications in several areas, for example, the modulation of enzymes activity (Salvador, Santos, & Saraiva, 2010), controlling of physiological processes (Saraiva & Rodrigues, 2011) and the creation of novel features of microbial growth under pressure (Mota, Lopes, Delgadillo, & Saraiva, 2013). Another very recently reported possible application of HHP is food preservation under pressure by microbial growth inhibition, as a possible alternative to refrigeration. This possibility arose by chance, about 40 years ago, with the recovery of the sunken submersible Alvin. After 10 months at a 1540-m depth (≈15 MPa) and a temperature of approximately 4°C, food (bouillon, sandwiches and apples) was found in a consumable condition (Jannasch, Eimhjellen, Wirsen, & Farmanfarmalan, 1971). These authors hypothesized that low pressure in addition to temperature could have an additional inhibitory effect in the microbial cells' biochemical activity, resulting in an extended shelf life compared to refrigeration at atmospheric pressure.

The possibility of using pressure to slow down microbial growth has recently gained renewed interest. Two groups of authors reported the possibility of preserving foods under pressure in the range of room temperatures, with the potential to substitute refrigeration, but with no need to control the temperature (Fidalgo et al., 2013; Queirós et al., 2014; Segovia-Bravo, Guignon, Bermejo-Prada, Sanz, & Otero, 2012). This novel food preservation methodology is tentatively called hyperbaric storage (HS) to differentiate it from hyperbaric food processing by pasteurization. Segovia-Bravo et al. (2012) maintained strawberry juices at different pressures (0.1, 25, 100 and 220 MPa) and at 20°C for 15 days and verified a microbial load reduction,

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attenuated viscosity and color losses in the samples stored under pressure, compared to storage at atmospheric pressure. Later, raw watermelon juice was stored for a period of up to 60 hours at 100 MPa, in uncontrolled, naturally variable room temperature conditions (18–21°C) and above (30°C), (Fidalgo et al. 2013). It was verified that storage at 100 MPa for 60 hours was capable of reducing the juice's initial microbial counts and avoiding microbial growth, thus yielding better results than refrigeration. Recently, Queirós et al. (2014) studied the feasibility of HS at and above room temperature (25°C, 30°C and 37°C) for melon juice, under pressure (25, 50, 75 and 150 MPa) for up to 8 hours. HS at pressures between 75 and 150 MPa was capable of achieving a better microbial quality (total aerobic mesophiles (TAMs), *Enterobacteriaceae* (ENT), yeasts and molds (YMs)) for all tested temperatures compared to samples stored under refrigeration.

The possibility of storing foods under pressure in the range of room temperature without requiring refrigeration would potentially allow substantial energy savings throughout the storage period (Coulomb, 2008). This is possible since there is no need for temperature control during storage; and energy would only be required during the compression/decompression phases to reach the desired pressure/decompress, no energy is required to maintain the pressure.

The aim of this work was to study the feasibility of HS (100 and 150 MPa) on *requeijão* (a characteristic Portuguese whey cheese) as a case study of a highly perishable food with an almost neutral pH (6.49) and high water activity (0.987), at room temperature (25°C) and above it (30°C and 37°C). This array of temperatures was selected to simulate a rather broad range of room/environmental temperatures, considering the possible use of HS under naturally variable room temperature conditions, with no temperature control. Microbial load (TAMs, lactic acid bacteria (LAB), ENT, YMs), physicochemical parameters (pH, water activity and lipid oxidation) and color were analyzed. Results were compared with samples stored at atmospheric pressure (0.1 MPa), at the same temperatures and under refrigeration (4°C).

Materials and methods

Requeijão samples

The whey cheese (*Requeijão*) was kindly supplied by a local producer on the day it was produced. The product was divided into smaller samples (≈10 g) that were individually packed into low permeability polyamide/polyethylene bags (Albipack-Packaging Solutions, Águeda, Portugal) that had been previously sterilized by UV light (BioSafety Cabinet Telstar Bio II Advance, Terrassa, Spain) and manually heat-sealed under vacuum (Packman, Albipack – Packaging Solutions, Águeda, Portugal) to avoid air inside the bags. Samples were then frozen and stored at –80°C until used to ensure samples had the same characteristics at the beginning of each experiment. Samples were thawed at 4°C before each experiment.

Preservation experiments

Samples were stored under pressure using hydrostatic pressure equipment (High-pressure system U33, Institute of High Pressure Physics, Warsaw, Poland) with a pressure vessel of 35-mm inner diameter and 100-mm height, surrounded by an external jacket connected to a thermostatic bath (Huber Compatible Control CC1, Trenton, New Jersey, USA) for temperature control. A mixture of propyleneglycol and water (40:60) was used as a pressurizing fluid.

Table 1. Storage period, temperature and pressure conditions applied on the storage experiments of whey cheese.

Tabla 1. Periodo de almacenamiento, temperatura y presión aplicados en los experimentos de almacenamiento del queso de suero.

Storage period (hour)	Temperature (°C)	Pressure (MPa)
4	4	0.1
	25	0.1, 100 and 150
	30	0.1, 100 and 150
	37	0.1 and 100
8	4	0.1
	25	0.1, 100 and 150
	30	0.1 and 100

The experiments were performed storing the samples for 4 and 8 hours at 0.1, 100 and 150 MPa and at different temperatures, 25°C, 30°C and 37°C (Table 1 shows the preservation conditions studied). The control samples (0.1 MPa) were kept in the same conditions as the samples stored under pressure (immersed in the same fluid used for pressurization, during the same period of time and at the same temperature), except for the pressure conditions, to eliminate the influence of these variables.

Microbial analyses

All samples were analyzed for TAM microorganisms, ENT, LAB and YMs. Two grams of each sample, obtained aseptically, were homogenized with 18.0 ml of Ringer's solution. Decimal dilutions were made with the same diluent and triplicates of dilutions were plated on the appropriate media, according to the following procedures: TAM were enumerated on plate count agar incubated at 30°C ± 1°C for 72 ± 3 hours; ENT were determined on violet red bile glucose agar incubated at 37°C ± 1°C for 24 hours; YMs were determined using rose-Bengal chloramphenicol agar at 25°C ± 1°C for 5 days; LAB were determined on man rogosa sharpe agar after incubation at 30°C for 5 days. In all cases, Petri dishes containing 15–300 colony forming units (CFUs) were selected for counting, and the results were expressed as Log_{10} CFU per gram of whey cheese (Log_{10} CFU/g).

pH

After sample homogenization with distilled water using an Ultraturrax T25 homogeniser (Janke & Kunkel IKA-Labortechnik, Saufen, Germany) 1:10 (w/v), the pH value was measured at room temperature with a properly calibrated pH meter (Titromatic 1 S, Crison Instruments, S.A., Barcelona, Spain).

Water activity

Samples were measured with a water activity analyzer (Novasina Lab Swift-aw, Lachen, Switzerland) at room temperature.

Lipid oxidation

Lipid oxidation was determined by malondialdehyde (MDA) quantification, using 2-thiobarbituric acid reactive substances method adapted by King (1962). Triplicates were measured

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using a micro-plate spectrophotometer (Multiskan GO Microplate Spectrophotometer, Thermo Scientific, Thermo Fisher Scientific, Waltham, Massachusetts, USA) with a Brand plate of 96 wells, at 532 nm. Standard solutions of MDA in 7.5% trichloroacetic acid were prepared from 1,1,3,3-tetrametoxipropano. Calibration curves were prepared at a concentration ranging from 0.2 to 10 μM.

Color

Color was measured using a spectrophotometer (Konica MinoltaCM 2300d, Osaka, Japan). The color parameters were recorded at 25°C according to the *Commission internationale de l'éclairage* (CIE) system and directly computed through the original SpectraMagic™ NX software (Konica Minolta, Osaka, Japan), according to the International Commission on Illumination regulations: red/green color (a^*), yellow/blue color (b^*) components and luminosity (L^*). The a^*, b^* and L^* values were obtained from six measurements; three random measurements in samples duplicates. The samples color parameters L^*, a^*, and b^* were measured and the total color difference (ΔE) was calculated by Equation (1).

$$\Delta E^* = \left[\left(L^* - L_0^*\right)^2 + \left(a^* - a_0^*\right)^2 + \left(b^* - b_0^*\right)^2\right]^{1/2} \quad (1)$$

where ΔE^* is the total color difference between a sample and the control (initial values), L^* and L_0^* are the lightness, a^* and a_0^* are the redness and b^* and b_0^* are the yellowness of sample and control, respectively.

Statistical analysis

Experiments were carried out in duplicate and all analyses were done in triplicate. Results were compared using analyses of variance, followed by a multiple comparison post hoc test, Tukey's Honestly Significant Difference (HSD), at a 5% level of significance.

Results and discussion

Microbial load

The initial load of the whey cheese for TAM, ENT, YM and LAB was 3.38 ± 0.06, 2.97 ± 0.15, 3.04 ± 0.13 and 2.22 ± 0.05 Log_{10} CFU/g, respectively. Samples stored at 4°C maintained the initial counts with no significant differences ($p > 0.05$), with the exception of TAM (4.03 ± 0.04 and 4.01 ± 0.01 Log_{10} CFU/g) for 4 and 8 hours of storage, and LAB during 4 hours of storage (3.76 ± 0.23 Log_{10} CFU/g), for which a small increase was observed. Even under refrigeration, whey cheeses are reported to achieve microbial count increments after a few days of storage. For LAB, an increase of 4 Log units was observed after 2 days of storage, while after 6 days of storage an increment of ≈3 Log units for ENT and YM was verified (Pintado, Macedo, & Malcata, 2001). This behavior is related to the high water activity (0.987) and pH (6.49) values of the whey cheese that pose no considerable restraints to microbial proliferation. In the present case, samples stored at or above room temperature at 0.1 MPa quickly reached high microbial count values during the 4 and 8 hours of study, showing the perishable nature of this dairy food. The maximum microbial load was observed for 8 hours at 30°C, reaching 6.97 ± 0.01, 7.04 ± 0.10, 4.68 ± 0.18 and 5.39 ± 0.20 Log_{10} CFU/g for TAM, ENT, YM and LAB, respectively. Independent of the temperature, storage period and pressure applied, HS was able to at least inhibit the microbial growth and in some cases inactivate microorganisms, resulting in equal to lower microbial loads (mostly lower) compared to the initial values, at the same temperature and time period at atmospheric pressure (Figure 1). Compared to refrigeration, HS also resulted in equal to lower microbial loads for the four microorganisms under study.

TAMs were the least affected microorganisms by HS conditions. For the three temperatures studied, during 4 and 8 hours at both 100 and 150 MPa, HS was able to inhibit TAM growth and to cause a small TAM inactivation, resulting in lower microbial counts compared to the initial load. A greater TAM growth inhibition was observed for all temperatures at hyperbaric conditions when compared to refrigeration ($p < 0.05$), resulting in lower TAM values than preservation under refrigeration in all cases (Figure 1).

ENT under HS at 25°C at 100 MPa, for both studied periods, suffered microbial growth inhibition when compared to the initial load ($p > 0.05$), being significantly lower ($p < 0.05$) compared to refrigeration. Similar results were verified at 150 MPa at 25°C, and at 100 MPa at 30°C and 37°C all for the 4 hours periods. At 150 MPa at 25°C for 4 hours, at 100 MPa at 30°C for 8 hours and at 150 MPa at 30°C for 4 hours, ENT inactivation was observed, resulting in values below the detection limit. Globally, and compared to refrigeration, HS at all tested conditions performed significantly better ($p < 0.05$), resulting in lower microbial counts that reached in some cases values below the detection limit.

YM showed a similar behavior to ENT under hyperbaric conditions. At 25°C, under HS conditions, microbial growth inhibition occurred for all conditions, resulting in similar values ($p > 0.05$) when compared to the initial load and refrigeration counts. For samples stored for 8 hours at 150 MPa, also at 25°C, YM counts were reduced ($p < 0.05$) below the detection limits, showing an inactivation effect at these storage conditions. At 30°C and 37°C and at 0.1 MPa, YM counts increase, reaching values of at least 4 Log_{10} CFU/g. Contrarily, at these temperatures, HS resulted in YM inactivation ($p < 0.05$) below the detection limits at all conditions, with the exception of storage at 100 MPa for 4 hours at 30°C, where YM growth inhibition was observed, yielding values similar to the initial load and to storage under refrigeration.

The results obtained for storage at 0.1 MPa showed considerable LAB growth, reaching a maximum value higher than 5 Log units at 30°C. On the contrary, LAB were found to be more susceptible to HS conditions, being reduced to undetectable counts at all HS conditions, with the exception of 25°C for 4 hours at both pressures studied (100 and 150 MPa), where LAB microbial growth were inhibited, resulting in counts similar ($p > 0.05$) to the initial load. Considering these results, HS at all tested conditions resulted in lower to equal microbial LAB counts, compared to samples stored at refrigeration. These results are according to Molina-Höppner, Sato, Kato, Gänzle, and Vogel (2003), who studied the pressure effect from 0.1 to 100 MPa, for 20 and 48 hours at 30°C on two mesophile LAB, *Lactococcus lactis* and *Lactobacillus sanfranciscensis* growth rates, concluding that 50 MPa were able to inhibit *Lb. sanfranciscensis* growth, and reduce *Lc. lactis* growth rate to less than 30%, compared to its growth rate at atmospheric pressure.

HPP has been successfully applied in dairy products to inactivate microorganisms, while causing minimal effects on physicochemical characteristics (Arqués, Garde, Gaya, Medina, & Nuñez, 2006; Capellas, Mor-Mur, Sendra, & Guamis, 2001).

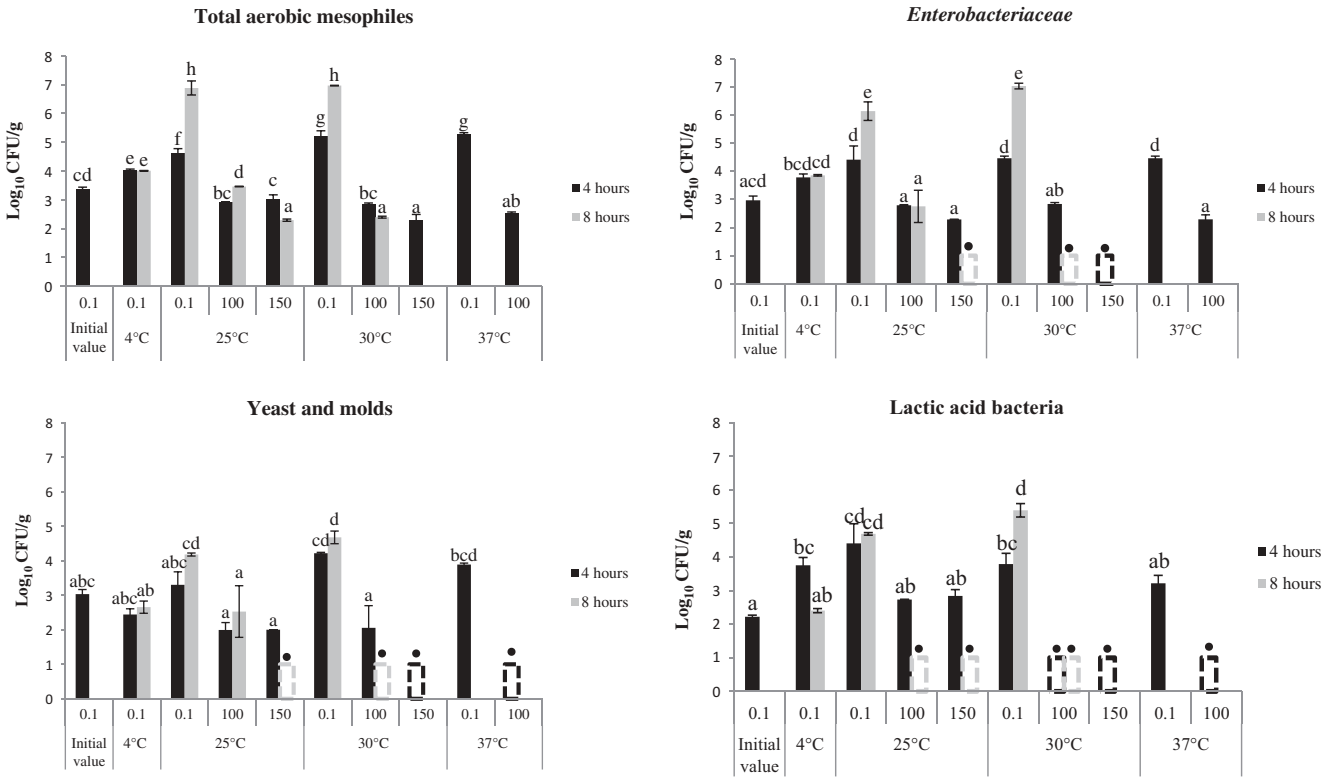

Figure 1. Total Aerobic Mesophiles, *Enterobacteriaceae*, yeast and molds and lactic acid bacteria counts (expressed in Log CFU/g) initially presented in whey cheese and stored during 4 and 8 hours at different pressure (0.1, 100 and 150 MPa) and temperature (4°C, 25°C, 30°C and 37°C) conditions. Bars with ● represent microbial loads below the detection limit (lower than 1 Log unit). Different letters (a–h) indicate significant differences ($p < 0.05$) between the different conditions.

Figura 1. Total de aerobios mesófilos, *Enterobacteriaceae*, Levadura y moho, recuento de bacterias ácido-lácticas (expresadas en Log CFU/g) que inicialmente se encuentran en los quesos de suero y se almacenaron durante 4 y 8 horas a diferente presión (0,1; 100 y 150 MPa) y temperatura (4, 25, 30 y 37 °C). Las barras con ● representan la carga microbiana por debajo del límite de detección (inferior a 1 unidad Log). Las letras (a–h) indican diferencias significativas ($p < 0,05$) entre las distintas condiciones.

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However this is the first study, to the authors' knowledge, that uses pressure as a preservation technique at and above room temperatures for dairy food storage. Queirós et al. (2014) for melon juice (pH near to 6) obtained a microbial growth inhibition at pressures near 100 MPa at room temperature, reaching microbial inactivation at a high pressure 150 MPa at 30°C for all tested microorganisms, results that are similar to those obtained for HS of whey cheese.

Overall, for this product and for the studied conditions, it was possible to achieve a similar to better microbial stability compared to refrigeration with HS at and above room temperatures (25–37°C), independent of the temperature used. These results indicate the feasibility of this promising food preservation methodology, by storing food at variable room temperatures, as a promising lower energetic cost alternative to refrigeration. This fact is of great importance, for possible application to all foods preserved by refrigeration, since HS would allow food storage at uncontrolled, naturally variable room temperature, with no energy costs associated to temperature control, with energy being only required to reach the desired pressure and decompress at the end of the storage period. It should be highlighted however that more studies are needed to evaluate the full potential and practical conditions of this novel conceptual food preservation methodology, for example, the study of longer storage times and the study of other microorganisms and determination of shelf lives. These experiments will require longer term experiments, possibly over weeks and months, and therefore careful preparation.

pH

The initial whey cheese pH value was 6.49 ± 0.17 (Table 2). This value is similar to the values found in literature for this product (Pintado et al., 2001). It was reported that under refrigeration, the whey cheese slowly decreased in pH value on the first 10 days, reaching a significant lower value after 15 days of storage (Pintado & Malcata, 2000). For HS, the pH value did not change significantly ($p > 0.05$) compared to refrigeration, with the exception of the samples stored for 4 hours at 100 MPa and 30°C that showed a significantly ($p < 0.05$) lower value (6.22 ± 0.03).

Water activity

The initial water activity for whey cheese was 0.987 ± 0.002, as can be seen in Table 2. The main differences observed for HS, compared to refrigeration (0.986 ± 0.001), were a slightly higher ($p < 0.05$) water activity for samples stored for 4 hours at 150 MPa and 30°C (0.989 ± 0.001) and at 37°C at atmospheric pressure (0.988 ± 0.001) and at 100 MPa (0.988 ± 0.001), with no significant changes ($p > 0.05$), for all conditions stored for 8 hours.

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Table 2. Physicochemical properties of whey cheese stored at different pressure (MPa) levels and temperature (°C) conditions. Mean values were obtained from duplicated samples, each analyzed in triplicate.

Tabla 2. Propiedades fisicoquímicas del queso de suero almacenado a diferentes niveles de presión (MPa) y temperatura (°C). Las diferentes letras entre las condiciones (a–d) y tiempos de almacenamiento (A–B) indican diferencias significativas ($p < 0,05$). Los valores en negrita son estadísticamente distintos a los valores iniciales. Los valores promedio fueron obtenidos de muestras duplicadas, cada una de ellas analizadas por triplicado.

		pH		Water activity		Lipid oxidation (mg/g)	
Conditions		4 hours	8 hours	4 hours	8 hours	4 hours	8 hours
Initial		6.49 ± 0.17 b	6.49 ± 0.17 a	0.987 ± 0.002 abc	0.987 ± 0.002 a	0.022 ± 0.004 a	0.022 ± 0.004 bc
4°C	**0.1**	6.60 ± 0.16 bcdA	6.52 ± 0.11 aA	0.986 ± 0.001 aA	0.986 ± 0.002 aA	0.013 ± 0.002 aA	0.037 ± 0.002 abB
25°C	**0.1**	6.53 ± 0.31 bcA	6.60 ± 0.04 aA	0.986 ± 0.001 abA	0.986 ± 0.002 aA	**0.041 ± 0.002** bA	0.034 ± 0.004 abA
	100	6.42 ± 0.04 abA	6.46 ± 0.11 aA	0.987 ± 0.001 abA	0.985 ± 0.001 aA	0.014 ± 0.007 aA	**0.040 ± 0.003** aB
	150	**6.76 ± 0.04** dA	6.25 ± 0.22 aA	0.986 ± 0.001 abA	0.985 ± 0.001 aA	0.020 ± 0.010 aA	0.035 ± 0.006 abA
30°C	**0.1**	6.54 ± 0.07 bcA	6.63 ± 0.02 aA	0.987 ± 0.001 abA	0.986 ± 0.001 aA	**0.045 ± 0.001** bA	**0.037 ± 0.002** aB
	100	**6.22 ± 0.03** aA	6.64 ± 0.02 aB	0.986 ± 0.001 aA	0.989 ± 0.001 aA	0.030 ± 0.002 abA	**0.013 ± 0.001** cB
	150	6.43 ± 0.08 b	*	0.989 ± 0.001 c	*	0.030 ± 0.004 ab	*
37°C	**0.1**	**6.72 ± 0.01** cd	*	0.988 ± 0.001 bc	*	**0.047 ± 0.001** b	*
	100	6.62 ± 0.03 bcd	*	0.988 ± 0.001 bc	*	0.030 ± 0.001 ab	*

Notes: *Experiments were not carried out in these conditions. Values in bold are statistically different from the initial value. Different letters between storage conditions (a–d) and storage times (A–B) indicate significant differences ($p < 0.05$).

Notas: *Los experimentos no se llevaron a cabo en estas condiciones. Los valores en negrita son estadísticamente diferente del valor inicial. Letras diferentes entre las condiciones de almacenamiento (a–d) y tiempos de almacenamiento (A–B) indican diferencias significativas ($p < 0,05$).

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Table 3. Color parameters of whey cheese stored at different pressure (MPa) levels and temperature (°C) conditions. Mean values were obtained from duplicated samples, each analyzed in triplicate.

Tabla 3. Los parámetros del color del queso de suero almacenado a diferentes niveles de presión (MPa) y temperatura (°C). Las diferentes letras entre las condiciones (a–d) y tiempos de almacenamiento (A–B) indican diferencias significativas ($p < 0,05$). Los valores en negrita son estadísticamente distintos a los valores iniciales. Los valores promedio fueron obtenidos de muestras duplicadas, cada una de ellas analizadas por triplicado.

		Color							
		L^*		a^*		b^*		ΔE^*	
Conditions		4 hours	8 hours	4 hours	8 hours	4 hours	8 hours	4 hours	8 hours
Initial		92.07 ± 0.06 c	92.07 ± 0.06 ab	−2.40 ± 0.02 c	−2.40 ± 0.02a	9.16 ± 0.10 ab	9.16 ± 0.10 a	*	*
4°C	**0.1**	91.54 ± 0.63 abcA	91.62 ± 0.41 aA	−2.39 ± 0.09 cA	−2.39 ± 0.05 aA	8.98 ± 0.02 abA	9.17 ± 0.30 aA	0.61 ± 0.05 abA	0.51 ± 0.36 aA
25°C	**0.1**	91.93 ± 0.20 bcA	92.74 ± 0.16 bB	−2.41 ± 0.04 bcA	−2.30 ± 0.20 aA	8.90 ± 0.36 abA	9.35 ± 0.43 aA	0.40 ± 0.16 abA	0.77 ± 0.28 aA
	100	91.72 ± 0.15 abcA	91.78 ± 0.48 abA	−2.51 ± 0.04 abcA	−2.49 ± 0.05 aA	9.56 ± 0.27 aA	9.22 ± 0.25 aA	0.60 ± 0.09 abA	0.45 ± 0.39 aA
	150	92.02 ± 0.18 bcA	91.98 ± 0.29 abA	**−2.66 ± 0.03** aA	−2.39 ± 0.05 aB	9.02 ± 0.19 abA	9.98 ± 0.12 aB	0.30 ± 0.01 bA	0.83 ± 0.01 aB
30°C	**0.1**	**90.92 ± 0.10** aA	92.31 ± 0.12 abB	−2.52 ± 0.04 abcA	−2.37 ± 0.02 aB	9.20 ± 0.24 aA	8.99 ± 0.30 aA	1.17 ± 0.11 aA	0.34 ± 0.23 aB
	100	**91.11 ± 0.23** abA	91.64 ± 0.34 abA	−2.53 ± 0.06 abcA	−2.59 ± 0.09 aA	8.43 ± 0.05 bA	9.05 ± 0.14 aB	1.21 ± 0.15 aA	0.51 ± 0.28 aA
	150	91.78 ± 0.10 abc	*	**−2.61 ± 0.01** ab	*	9.00 ± 0.01 ab	*	0.39 ± 0.07 ab	*
37°C	**0.1**	**93.17 ± 0.20** d	*	**−2.65 ± 0.09** a	*	8.99 ± 0.30 a	*	1.17 ± 0.14 a	*
	100	92.06 ± 0.10 bc	*	−2.46 ± 0.02 abc	*	9.05 ± 0.14 a	*	0.29 ± 0.11 b	*

Notes: *Experiments were not carried out in these conditions. Values in bold are statistically different from the initial value. Different letters between storage conditions (a–d) and storage times (A–B) indicate significant differences ($p < 0.05$).

Notas: *Los experimentos no se llevaron a cabo en estas condiciones. Los valores en negrita son estadísticamente diferente del valor inicial. Letras diferentes entre las condiciones de almacenamiento (a–d) y tiempos de almacenamiento (A–B) indican diferencias significativas (p <0,05).

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Lipid oxidation

The whey cheese studied in this work is a product with a high-lipid content (≈30%) that can oxidize during storage and create unpleasant odors and flavors in the product. The initial MDA content of whey cheese was 0.022 ± 0.004 mg/g (Table 2). No significant ($p > 0.05$) changes were observed for HS for 4 hours of storage compared to refrigeration (0.013 ± 0.002 mg/g). On the other hand, samples stored for 4 hours at atmospheric pressure at 25°C, 30°C and 37°C reached significantly ($p < 0.05$) higher levels of MDA (0.41 ± 0.002, 0.045 ± 0.001 and 0.047 ± 0.001 mg/g, respectively). Hyperbaric samples stored for 8 hours showed higher levels of MDA ($p < 0.05$) compared to the initial value, but not different ($p > 0.05$) when compared to refrigeration, with the exception of samples stored at 30°C under 100 MPa (0.013 ± 0.001 mg/g), which was significantly ($p < 0.05$) lower. Some studies reported that short-time HPP (200–600 MPa) of raw fish may increase, during subsequent storage, the primary oxidation compounds (peroxides) (Ohshima, Ushio, & Koizumi, 1993), and increase secondary and tertiary lipid oxidation compounds at lower pressures (170–200 MPa) (Aubourg, Tabilo-Munizaga, Reyes, Rodríguez, & Pérez-Won, 2010). An increase of secondary lipid oxidation compounds was also observed by Delgado, González-Crespo, Cava, and Ramírez (2012) when 400 and 600 MPa where applied to goats cheese at three different times during ripening.

Color

The initial color parameter values of whey cheese, L^*, a^* and b^*, were 92.07 ± 0.06, −2.40 ± 0.02 and 9.16 ± 0.10, respectively. In general, for L^*, a significant variation ($p < 0.05$) was observed for storage above room temperature conditions (30°C and 37°C) at atmospheric pressure for 4 hours, when compared to the initial L^* value, as seen in Table 3. When compared to refrigeration, hyperbaric conditions at both 4 and 8 hours did not significantly change ($p > 0.05$) the L^* value. For a^*, significantly higher values ($p < 0.05$) were observed at atmospheric pressure storage for 4 hours at 37°C and at 150 MPa at 25°C and 30°C. No significant ($p > 0.05$) differences were found for b^*, in HS samples, compared to the initial and refrigeration value. Considering the total color change (ΔE^*), the main variations (increments) occurred at atmospheric conditions for 4 hours and at 30°C (1.17 ± 0.11), at 30°C for 4 hours at 100 MPa (1.21 ± 0.15) and at 37°C for 4 hours (1.17 ± 0.14), what resulted mainly from a higher increase in the L^* value. According to Drlange (1994), the color differences observed for hyperbaric treatments can be classified as "small difference", for ΔE^* values between 0.5 and 1.5. Moreover, these main variations of ΔE^* were not statistically different from those observed for refrigeration ($p > 0.05$). Capellas et al. (2001) and Evert-Arriagada, Hernández-Herrero, Juan, Guamis, and Trujillo (2012) studied the effect of higher pressures, 300 and 400 MPa for 5 min at 6°C, and 500 MPa for 5, 15 and 30 min at 25°C, on fresh cheese, and reported no significant changes on ΔE^*.

Conclusions

This study showed that storage up to 4 hours, using 100 MPa at room temperatures, was able to inhibit whey cheese microbial load, and in some cases microbial inactivation also occurred, which was more evident when pressure and the storage period were increased. Overall, pressure retained whey cheese color, pH and water activity, and maintained stable lipid oxidation levels throughout HS conditions when compared to refrigeration.

HS at variable temperatures seems to be a good and very promising food preservation methodology that can allow food safety, while reducing the energetic costs due to refrigeration. However, the information regarding HS at and above room temperature is scarce, and more studies need to be conducted to understand, for example, the impact of longer storage periods, enzymatic behavior under low pressures and its effect in pathogenic microorganisms.

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