Preservation of a highly perishable food, watermelon juice, at and above room temperature under mild pressure (hyperbaric storage) as an alternative to refrigeration

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Abstract

The feasibility of food preservation under pressure (hyperbaric storage) at and above room temperatures, as an alternative to refrigeration was evaluated. Preservation of a highly perishable food, watermelon juice, was studied at pressures of 25e150 MPa and temperatures ranging 20e37 °C, being compared to refrigeration and storage at atmospheric pressure at the same temperatures.

Hyperbaric storage at 75 MPa (20e37 °C) revealed an inhibitory effect on microbial growth, with at least an equal performance compared to refrigeration. An additional inactivation effect was verified for storage at 100 and 150 MPa, with reduction of the initial microbial counts to <1.00 Log CFU/mL for enterobacteriaceae and yeasts and moulds, and from 4.43 ± 0.04 to 3.31 ± 0.04 and 2.99 ± 0.07 Log CFU/ mL, respectively, for total aerobic mesophiles (25 °C).

In general, pH, titratable acidity and total soluble solids did not show a clear variation trend with pressure and no considerable differences among storage conditions were verified. Cloudiness decreased for samples stored under pressure and browning degree was in general lower in samples stored under pressure compared to refrigeration.

This work demonstrates the potentiality of hyperbaric storage as a new preservation methodology, at variable (uncontrolled) room temperature without energetic costs during storage, as an alternative to refrigeration.

1. Introduction

Food spoilage is a constant threat along the entire food chain, therefore suitable and efficient preservation methodologies are needed. For foods that cannot be kept at room temperature, the technologies currently applied are in general refrigeration and freezing that, while effective, are energy consuming.

Just like temperature, pressure is a significant thermodynamic parameter that may influence molecular systems and it is being explored for new and promising High Pressure (HP) applications (Aertsen, Meersman, Hendrickx, Vogel, & Michiels, 2009; Mota, Lopes, Delgadillo, & Saraiva, 2013). Currently, HP is increasingly applied as a non-thermal pasteurization process, with already a great variety of commercialized food products with an extended shelf life and fresh-like characteristics (Mújica-Paz, Valdez-Fragoso, Samson, Welti-Chanes, & Torres, 2011; Norton & Sun, 2008; Ram-irez, Saraiva, Lamela, & Torres, 2009; Sousa, Delgadillo, & Saraiva, 2013). Usually, to obtain HP pasteurized products, pressure ranging from 400 to 600 MPa must be applied to ensure the desired microbial inactivation.

Besides these applications, other processes based on HP are being developed, with the purpose of inhibiting/reducing the microbial load of foods, such as sub-zero storage under pressure, which consists in storing food products under low/mild pressure at temperatures below 0 ° C without them going through the freezing and thawing processes (Charm, Longmaid, & Carver, 1977; Kalichevsky, Knorr, & Lillford, 1995). This methodology allows the food storage at temperatures below 0 °C, without submitting it to the freezing and thawing processes, thus avoiding textural modifications due to substantial damages in cellular/tissue structures (Kalichevsky et al., 1995).

The submarine Alvin sank in 1968 and remained at a depth of 1540 m, at approximately 4 °C, during 10 months. Surprisingly, when it was recovered, food was found in consumable conditions, opening the possibility for food storage under pressure at refrigerated conditions (Jannasch, Eimhjellen, & Farmanfarmalan, 1971). A few years later Charm et al. (1977) demonstrated that microbial growth and enzyme activity were inhibited by pressure at refrigerated temperatures, resulting in fish and meat shelf life extension. Nevertheless, it is important to note that both methods (sub-zero and refrigerated hyperbaric storage) still require energy to maintain the low temperatures needed for food storage, throughout all the storage period, with the inherent energetic costs.

Recently, the possibility of strawberry juice preservation under pressure (25,100 and 200 MPa) at room temperature (20 °C) was studied (Segovia-Bravo, Guignon, Bermejo-Prada, Sanz, & Otero, 2012). These authors observed that hyperbaric storage at these conditions was an effective preservation method, while minimizing viscosity and colour losses in strawberry juice. However, it is note- worthy that strawberry juice has

a pH of approximately 3.3, which provides some intrinsic microbiological stability. Fidalgo et al.(2013) studied the feasibility of hyperbaric storage at naturally variable room temperature (18e21 °C) and above (30 °C) on watermelon juice, which is a highly perishable food, due to a pH of approximately 5.9 and a high water activity. The watermelon juice was successfully preserved underHP(100 MPa) over60 hat room temperature, while at atmospheric pressure the food was intensely spoiled already after 8 h. Another recent study also supported the viability of this preservation method for melon juice (also a highly perishable food) stored for 8 h at 25, 30 and 37 °C, under a pressure range of 25e150 MPa (Queiros et al., 2014). In this case, the hyperbaric storage of melon juice resulted in microbial growth inhibition at 50/75 MPa, with maintenance of the microbial load during all the storage period studied, while a microbial inactivation was verified for higher pressures between 100 and 150 MPa, resulting in the reduction of the initial microbial loads (Queiros et al., 2014). In both works, storage under pressure at 100 and 150 MPa resulted in similar to better microbial preservation, when compared to refrigeration.

The study of hyperbaric storage e storage under pressure e to preserve food products, with no need for temperature control (at and above room temperature), and thus with a considerable reduction of energetic costs compared to refrigeration, is very recent. This is possible since energy would only be required for some minutes to reach the necessary pressure used in hyperbaric storage, being the pressure maintained throughout storage, not requiring further energy. Due to the great potential that this methodology presents, more studies about this subject are needed. Thereby, the aim of this work was to test the use of hyperbaric storage as a preservation method for watermelon juice, using broader conditions of the binomial pressure/temperature (25e150 MPa/20e37 °C). For this purpose, microbial load (total aerobic mesophiles, enterobacteriaceae and yeasts and moulds) and physicochemical parameters (pH, titratable acidity, total soluble solids, browning degree and cloudiness) were quantified, and the results compared with storage at 0.1 MPa, maintaining all other storage conditions. Samples were also stored under refrigeration (4 °C), to compare hyperbaric storage with refrigeration.

2. Materials and methods

2.1. Preparation of watermelon juice

Seeded red watermelon (Citrullus lanatus) was purchased at commercial maturity from a local supermarket and kept at 4 °C. Watermelon juice was prepared, being the watermelon washed, peeled and crushed with a blender (Braun MR 6500/500, Kronberg, Germany). After filtration the juice was frozen and stored at -80 °C. For each experiment, the juice was thawed at 4 °C and the samples aseptically packed into low permeability polyamide-polyethylene bags (PA/PE-90, Albipack - Packaging Solutions, Portugal), which were heat sealed avoiding the presence of air inside the bags. These bags were double packed into a second bag sealed under vacuum. The packaging film was previously sterilized by UV light for 15 min (BioSafety Cabinet Telstar Bio II Advance, Terrassa, Spain).

2.2. Preservation experiments

A hydrostatic press (High pressure system U33, Institute ofHigh Pressure Physics, Warsaw, Poland) was used for preservation experiments. It is equipped with a pressure vessel of35 mm diameter and 100 mm height surrounded by an external jacket, connected to a thermostatic bath (Huber Compatible Control CC1, New Jersey, USA) to control the temperature, and a mixture of propylene glycol and water (40:60) was used as pressurizing fluid and to control the temperature in the external jacket.

Different preservation experiments, at pressures up to 150 MPa (25, 50, 75, 100 and 150 MPa) over 8 h of storage, at different temperature conditions (20, 25, 30 and 37 °C), were carried out, being always kept samples at the same temperature and at 4 °C under atmospheric pressure conditions. These control experiments were performed exactly in the same conditions (in the dark and immersed in the same fluid used for compression).

2.3. Microbial analysis

Homogenization of 1.0 mL of each sample with 9.0 mL of Ringer's solution was performed. Then, decimal dilutions were made with Ringer's solution and triplicates of dilutions were plated on the appropriate media: Total aerobic mesophilic (TAM) counts at 30 °C were determined in plate count agar (PCA), incubated at 30 ± 1 °C for 72 ± 3 h (ISO 4833:2003). Enterobacteriaceae (ENT) counts were quantified in violet red bile dextrose agar (VRBDA), incubated at 37 °C for 24 h (ISO 8523:1991). Yeasts and moulds (YM) were enumerated on rose-bengal chloramphenicol agar (RBCA) medium (ISO 7954:1987), incubated at 25 ± 1 °C for 5 days.

The results were expressed as logarithmic of Colony Forming Unit (CFU) units per millilitre of watermelon juice (Log CFU/mL).

2.4. Physicochemical analysis

The samples pH value was measured with a properly calibrated glass electrode at 25 °C (pH electrode 50 14, Crison Instruments, S. A., Spain). The titratable acidity was determined with an automatic titrator (Titromatic 1S, Crison Instruments, S. A., Spain) by titrating 10 mL of diluted watermelon juice (3:7, watermelon juice:distilled water) to pH = 8.1 with a standardized 0.02 M sodium hydroxide solution, being the results expressed as g citric acid/L of water- melon juice (Liu, Hu, Zhao, & Song, 2012).

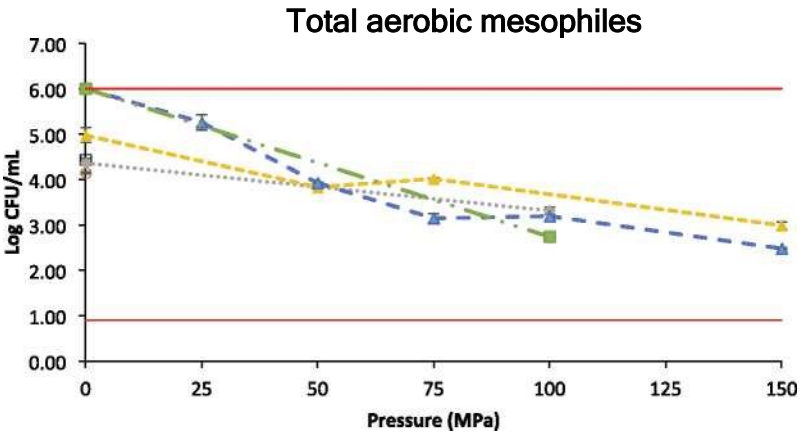
The browning degree value was determined by centrifugation of the juice samples at 9000 x g at 4 ° C for 20 min and measurement of the absorbance at 420 nm in a UV-VIS microplate spectropho- tometer (Multiskan GO Microplate Spectrophotometer, Thermo Scientific, Thermo Fisher Scientific Inc., USA) (Zhang et al., 2011).

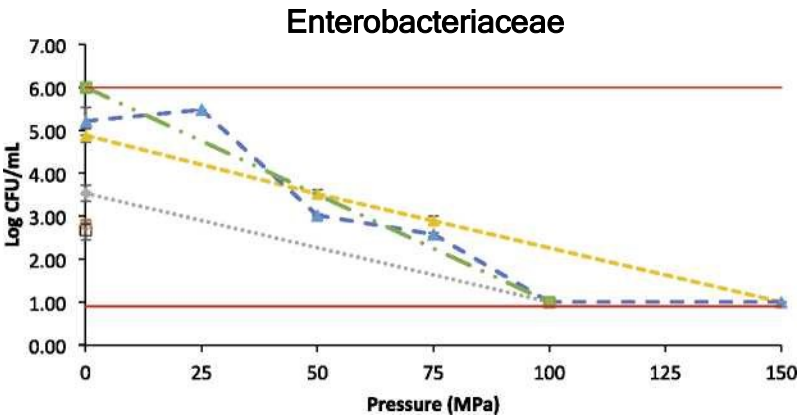
Cloudiness was evaluated by direct measurement of absorbance at 700 nm using a UV-VIS microplate spectrophotometer described above.

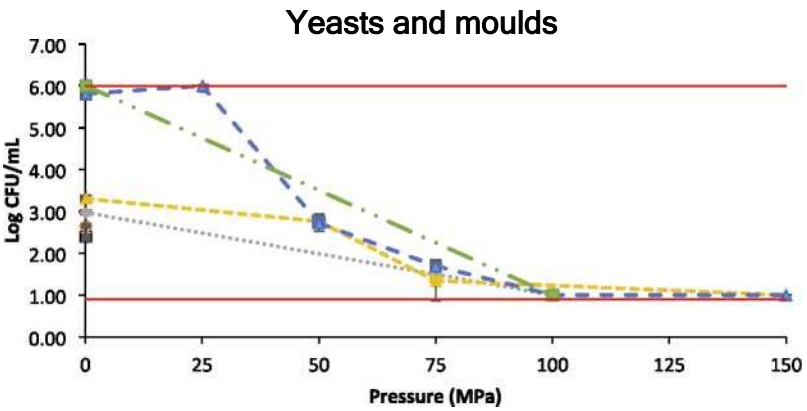
The total soluble solids content was determined by measuring ° Brix (Handheld Refractometer Atago, ATC-1E) at 20 ° C(Wang et al., 2006).

2.5. Statistical analysis

All experiments were carried out in duplicate and all analysis were done in triplicate. Statistical data analysis of the results was







	Initial value	4 °C	20 °C		25 °C				30 °C						37 °C	
		Pressure (MPa)														
		0.1	0.1	100	0.1	50	75	150	0.1	25	50	75	100	150	0.1	100
Total aerobic mesophiles	b	bc	b	e	a	d	cd	fg	*	a	cd	ef	ef	h	*	gh
Enterobacteriaceae	e	e	d	#	c	d	e	#	bc	b	e	e	#	#	*	#
Yeast and moulds	b	b	ab	#	a	ab	c	#	*	*	ab	c	#	#	*	#

Fig. 1. Total aerobic mesophiles, *Enterobacteriaceae* and yeasts and moulds counts (expressed in Log CFU/mL) in watermelon juice initially (▫) and after 8 h of storage at different pressure and temperature (—○— 4 °C, ···◆·· 20 °C, – –★– – 25 °C, – –▲– – 30 °C, —■— 37 °C) conditions. Values superimposing the horizontal upper and lower lines (—), indicate values above 6.00 and below 1.00 Log units, respectively. Different letters between pressure/temperature conditions (a–h) indicate significant differences ($p < 0.05$), * and # are indicative of higher than 6 and lower than 1 Log CFU/mL, respectively.

performed using Analysis of Variance (ANOVA) and Tukey's HSD Test, at a 5% level of significance.

3. Results and discussion

3.1. Microbial analysis

The results obtained in Fig. 1 showed that the initial microbial load of watermelon juice was 4.43 ± 0.04, 2.68 ± 0.23 and 2.40 ± 0.07 Log CFU/mL, for TAM, ENT and YM, respectively.

Observing Fig. 1, it is possible to verify the pressure influence on microbial loads after 8 h of storage. For TAM counts, at 25 and 30 °C, a minimum of 50 MPa was required to obtain an inhibitory effect on microbial growth, being the results statistically lower to equal (3.83 ± 0.02 and 3.92 ± 0.02 Log CFU/mL, respectively) than the control samples maintained at 4 °C (4.15 ± 0.02 Log CFU/mL) and the initial microbial load. Storage at 100 and 150 MPa, at the same temperatures, showed a microbial inactivation effect in addition to the microbial growth inhibition observed for the lower pressure levels. It was possible to observe a reduction of the initial microbial load of about 1.44 and 1.95 Log CFU/mL under 150 MPa at 25 and 30 °C, respectively. At 25 MPa storage at 30 °C (the only temperature studied at this pressure), no effect was observed in TAM counts, which indicates that a pressure level above 25 MPa is necessary to achieve an effect on TAM growth inhibition.

Regarding ENT counts, it was verified that for 25 and 30 °C conditions, a pressure of 75 MPa (2.90 ± 0.11 and 2.57 ± 0.04 Log CFU/mL, respectively) was necessary to observe a microbial growth inhibitory effect, compared to the results at atmospheric pressure over 8 h at 4 °C (2.82 ± 0.02 Log CFU/mL). At these temperatures (25 and 30 ° C), increasing the pressure level led to an inactivation effect, causing the decrease of ENT to values below the detection limit (<1.00 log CFU/mL).

Concerning to YM, it was verified that, at 25 and 30 °C, a pressure of 50 MPa resulted in counts (2.77 ± 0.14 and 2.73 ± 0.21 Log CFU/mL, respectively) similar to the samples stored under refrigeration at atmospheric pressure (2.65 ± 0.04 Log CFU/mL), thus being observed an inhibitory effect on YM growth. The increase of pressure level (100 and 150 MPa) also reduced the YM counts below the detection limit (<1.00 Log CFU/mL), at all tested temperatures, showing these pressure levels, an inactivation effect additionally to the inhibitory effect.

With the results obtained in this work, it was possible to obtain an equal to better preservation of watermelon juice under pressure at 75 MPa, regardless of temperature (4.01 ± 0.02, 2.90 ± 0.11, 1.35 ± 0.49 Log CFU/mL and 3.15 ± 0.10, 2.57 ± 0.04, 1.70 ± 0.05 Log CFU/mL, for TAM, ENT, YM, at 25 and 30 °C, respectively), than with the refrigeration methodology (4.15 ± 0.02, 2.82 ± 0.02, 2.65 ± 0.04, for TAM, ENT and YM, respectively).

Even more, storage at 100 MPa, revealed that regardless of temperature (20e37 °C), the ENT and YM were <1.00 Log CFU/mL and the TAM counts <3.34 ± 0.03 Log CFU/mL (lower than the initial microbial load). Additionally, it is possible to observe that, at the pressure levels studied, no significant effect of the storage temperatures was observed. This opens the possibility for food storage under pressure at variable (uncontrolled) temperature, at room temperature conditions. At all temperatures tested in the present work (20, 25, 30 and 37 °C), pressure levels of 75e150 MPa revealed a significant microbial growth inhibition, with additional microbial inactivation for pressure levels of 100e150 MPa.

3.2. Physicochemical analyses

3.2.1. Total soluble solids

The initial value of total soluble solids (°Brix) was 7.00 ± 0.07 °Brix (Table 1). No statistical differences ($p > 0.05$) were verified among the samples stored at the following conditions: 4 °C (0.1 MPa), 25 °C (0.1, 50 and 150 MPa) and in all samples stored at 30 °C, with values ranging between 7.10 and 7.85 °Brix (Table 2). In contrast, °Brix values below the watermelon juice initial value were observed for samples stored at 20 ° C (0.1

and 100 MPa), 25 °C (75 MPa) and 37 °C (0.1 and 100 MPa). There were no statistically significant ($p > 0.05$) effects among these samples, which ranged between 6.35 and 6.85 °Brix (Table 2).

3.2.2. pH

The watermelon juice initial pH was 5.78 ± 0.19 (Table 1), similarly to values reported in literature (Fidalgo et al., 2013; Liu et al., 2012). The lowest pH value was observed for the sample stored at 37 °C and 0.1 MPa (5.46 ± 0.01), while the higher pH value was observed for the sample stored at 30 °C and 0.1 MPa (Table 2). With the exception of 100 MPa at 37 °C, the pH value of the samples under pressure was lower, for all temperatures, compared to the samples stored at 0.1 MPa at the same temperatures.

3.2.3. Titratable acidity

The initial titratable acidity was 260 ± 10 g citric acid/L (Table 1). All the samples subjected to hyperbaric storage showed a similar titratable acidity value ($p > 0.05$), regardless ofthe temperature and pressure studied. The sample stored at 37 °C and 0.1 MPa, presented a higher acidity value (424 ± 10 g citric acid/L), consistent with the lower pH value verified. Thus, an 8 h hyperbaric storage period does not significantly influence the titratable acidity, while atmospheric pressure storage at 37 °C increased it (Table 2).

3.2.4. Cloudiness

Watermelon juice samples stored at atmospheric pressure at 4, 20, 25 and 37 °C showed cloudiness values ranging between 0.479 and 0.625 (Table 2), being the initial value of 0.468 ± 0.024 (Table 1). In general, hyperbaric storage caused a decrease in juice cloudiness, compared to storage at 0.1 MPa (values between 0.412 and 0.447), being the samples stored at 30 °C (25 and 50 MPa) an exception (Table 2). This behaviour might be related with pectin methylesterase and polygalacturonase activity, as previously pro- posed by Fidalgo et al. (2013) and Segovia-Bravo et al. (2012).

3.2.5. Browning degree

The watermelon juice initial browning degree was 0.110 ± 0.007 (Table 1), a value higher than the one obtained by Zhang et al. (2011) for the same product (z0.75). This difference might be due to different cultivars being used in the two works and also by to freezing and frozen storage of the juice, prior to the measurements in the current work. The browning degree increased in watermelon juice stored at 4 °C(0.1 MPa), 20 °C(0.1 and 100 MPa) and 30 ° C(0.1, 50 and 75 MPa). On the other hand, lower values were registered on

Table 1
Raw watermelon juice physicochemical properties.

Physicochemical analysis	Mean ± STD
Total soluble solids (°Brix)	7.00 ± 0.07
pH	5.78 ± 0.19
Titratable acidity (g citric acid/L)	260 ± 10
Cloudiness	0.468 ± 0.024
Browning degree	0.110 ± 0.007

Table 2
Physicochemical properties of watermelon juice stored for 8 h at different pressure levels and temperature conditions. Different letters between (a–h) indicate significant differences ($p < 0.05$). Mean values were obtained from duplicated samples, each analysed in triplicate.

Temperature	Pressure	Total soluble solids °Brix	pH	Titratable acidity (g citric acid/L)	Cloudiness	Browning degree
4 °C	0.1 MPa	7.45 ± 0.07 abc	5.93 ± 0.01 bc	256 ± 5 b	0.625 ± 0.001 a	0.187 ± 0.004 b
20 °C	0.1 MPa	6.70 ± 0.14 ef	5.88 ± 0.01 d	268 ± 9 b	0.553 ± 0.002 b	0.137 ± 0.007 c
	100 MPa	6.35 ± 0.28 f	5.85 ± 0.01 d	265 ± 9 b	0.412 ± 0.013 f	0.216 ± 0.009 a
25 °C	0.1 MPa	7.10 ± 0.14 cde	5.95 ± 0.01 bc	266 ± 1 b	0.549 ± 0.007 b	0.087 ± 0.001 f
	50 MPa	7.25 ± 0.07 bcd	5.87 ± 0.01 d	280 ± 5 b	0.415 ± 0.012 f	0.092 ± 0.002 ef
	75 MPa	6.65 ± 0.07 ef	5.67 ± 0.01 f	264 ± 1 b	0.427 ± 0.002 f	0.041 ± 0.003 gh
	150 MPa	7.25 ± 0.07 bcd	5.76 ± 0.01 e	267 ± 1 b	0.447 ± 0.001 def	0.108 ± 0.003 de
30 °C	0.1 MPa	7.45 ± 0.07 abc	5.99 ± 0.01 a	239 ± 2 b	0.479 ± 0.010 cde	0.113 ± 0.001 d
	25 MPa	7.70 ± 0.14 ab	5.95 ± 0.01 b	266 ± 5 b	0.507 ± 0.006 c	0.057 ± 0.010 g
	50 MPa	7.85 ± 0.07 a	5.87 ± 0.01 d	270 ± 7 b	0.484 ± 0.004 cd	0.113 ± 0.004 d
	75 MPa	7.25 ± 0.07 bcd	5.89 ± 0.01 cd	252 ± 7 b	0.437 ± 0.010 ef	0.119 ± 0.004 cd
	150 MPa	7.45 ± 0.07 abc	5.86 ± 0.01 d	275 ± 3 b	0.446 ± 0.015 def	0.091 ± 0.002 ef
37 °C	0.1 MPa	6.70 ± 0.14 ef	5.46 ± 0.01 g	424 ± 10 a	0.598 ± 0.023 a	0.037 ± 0.001 h
	100 MPa	6.85 ± 0.07 def	5.93 ± 0.02 bc	288 ± 49 b	0.435 ± 0.002 f	0.035 ± 0.001 h

watermelon juice stored at 25 °C (75 MPa) and 37 °C (0.1 and 100 MPa), ranging between 0.035 and 0.041

(Table 2).

4. Conclusion

The feasibility of food preservation (8 h) under mild pressure (25e150 MPa), at different temperatures (20e37 °C), was evaluated for watermelon juice, as a case study of a highly perishable food. The microbiological analyses (total aerobic mesophiles, enterobacteriaceae, and yeast and moulds) showed a clear inhibitory effect for a pressure of 75 MPa, being the microbial load equal to lower than samples stored for the same time at 4 °C and 0.1 MPa. The increase of pressure level (100 and 150 MPa) resulted in an additional inactivation effect of microorganisms, with the samples stored at these pressures exhibiting a lower microbial load than the initial value and the samples kept under refrigeration.

Hyperbaric storage had minor impact in the studied physicochemical parameters (pH, titratable acidity and total soluble solids), having a more pronounced difference in cloudiness value (decreased for samples stored under pressure) and in browning degree analysis (lower in samples stored under pressure). The results obtained in this work open the possibility of using hyperbaric storage as an alternative to refrigeration, but more studies have to be carried out using other foods and longer storage periods. At the same time, additional analyses are needed (for instance, effect on enzymes) to ensure the feasibility of food storage under pressure (hyperbaric storage) in the studied temperatures range, and so under variable (uncontrolled) temperature.

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