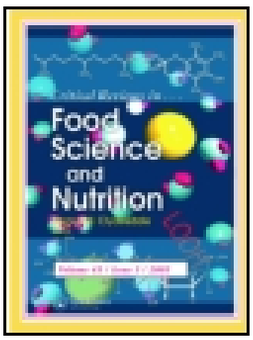

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REVIEW

Hyperbaric storage at room like temperatures as a possible alternative to refrigeration: evolution and recent advances

Mauro D. Santos, Liliana G. Fidalgo, Carlos A. Pinto, Ricardo V. Duarte, Álvaro T. Lemos, Ivonne Delgadillo, and Jorge A. Saraiva

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ABSTRACT

From 2012, the preservation of food products under pressure has been increasingly studied and the knowledge acquired has enlarged since several food products have been studied at different storage conditions. This new food preservation methodology concept called Hyperbaric Storage (HS) has gain relevance due to its potential as a replacement or an improvement to the conventional cold storage processes, such as the traditional refrigeration (RF), or even frosting, from the energetic savings to the reduction of the carbon foot-print. Briefly, HS is capable to inhibit the microbial proliferation or its inactivation which results in the extension of the shelf-life of several food products when compared to RF. Moreover, the overall quality parameters seem not to be affected by HS, being the differences detected on samples over storage similar to lower when compared to the ones stored at RF. This review paper aims to gather data from all studies carried out so far regarding HS performance, mainly at room temperature on fruit juices, meat and fisheries, as well on dairy products and ready-to-eat meals. The HS advantages as a new food preservation methodology are presented and explained, being also discussed the industrial viability and environmental impact of this methodology, as well its limitations.

KEYWORDS

Food preservation; pressure; food spoilage; microbial growth inhibition; shelf-life; environmental impact

Introduction

Nowadays, High Pressure Processing (HPP) is being widely applied in different food industries as a non-thermal pasteurization procedure (450–600 MPa, over 1–10 min, at ≈8–14 °C), allowing to obtain pathogenic-free (vegetative forms) food products with minimal impact on their nutritional and sensorial features, contrarily to thermal treatments that can induce changes on foods characteristics, including non-enzymatic browning, protein denaturation, loss of vitamins, among others (Considine et al. 2008). Moreover, HPP is also applied, for instance, in the texturization and removal of meat from shellfish/crustaceans, and for bacterial spores inactivation when applied at high temperatures (pressure-assisted thermal sterilization, PATS) (Campus 2010; Mújica-Paz et al. 2011). Enzymes inactivation (Hendrickx et al. 1998) and macromolecules modification (Tabilo-Munizaga and Barbosa-Cánovas 2004; Santos, Saraiva, and Gomes 2015) have also been accomplished by high pressure technology.

Recently, a new food preservation technique known as Hyperbaric Storage (HS) has emerged based on high pressure technology. In 1968, the "(un)fortunately" accident with the research submarine Alvin was the starting point, since when it was recovered after being sunk over 10 months at 1540 m (≈15 MPa) and ≈4 °C, some foods were found in consumable conditions (including apples, sandwiches, and bouillon) (Jannasch et al. 1971). This event opened the possibility to preserve food products under pressure.

Since then, several studies have been performed, such as the evaluation of organic matter degradation at ≈50 MPa/≈3 °C, where it was verified lower degradation rates in these samples compared to samples stored at the same temperature and atmospheric pressure (AP) (Jannasch et al. 1971). Moreover, Charm, Longmaid, and Carver (1977) also observed for ≥20 MPa a shelf-life extension due to microbial growth inhibition and enzymatic activity constraining on foods stored under pressure (≈1–40 MPa) at temperatures between -3 and 0 °C. However, it must be noted that these two experiments required temperature control, leading to substantial energetic costs, similarly to the conventional refrigeration (RF).

Although several HS experiments had been carried out at refrigerated temperatures, recently, some HS studies have been performed at room temperature (RT), or above it, making this methodology environmentally friendlier than the conventional RF process, with lower energy consumption, since it is only required in the compression and decompression phases, and no additional temperature control is needed along storage (Bermejo-Prada et al. 2017). Thus, according to the last published studies, HS can be defined as a food preservation methodology that consists on food storage under pressure, mainly between 25 and 220 MPa, at RT or above it, as an alternative/improvement

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to RF (Segovia-Bravo et al. 2012; Fernandes et al. 2019; Fidalgo et al. 2018). HS has become a novel conceptual food preservation methodology by microbial growth inhibition, where an additional microbial inactivation can also be achieved after storage at higher pressure levels, remaining the physicochemical parameters, in general, unchanged over that period (Freitas et al. 2016; Duarte et al. 2017; Fidalgo et al. 2014).

In fact, the promising results of HS as a food preservation methodology relies on the effect of pressure on biological structures (bacteria, yeasts and molds …), enzymes and other compounds. Hydrostatic pressure is reported to trigger several effects on vegetative microorganisms, such as the abolishment of biological and structural functions (e.g. motility, cell division, DNA replication/growth, and viability, when applied a pressure level from 10, 20–50, 50, and 200 MPa, respectively, for *E. coli*), as reported by Oger and Jebbar (2010). Moreover, the effect of low pressures (20–200 MPa) is reported for several *Bacillus* spp. and *Clostridium* spp endospores, mainly in buffer solutions and culture media, usually above RT and up to 24 h. For instance, pressures up to 200 MPa seems to trigger a nutrient-like physiological germination, in which endospores release dipicolinic acid from the core that activates the cortex lytic enzymes, with consequent cortex degradation (Black et al. 2007; Reineke, Ellinger, et al. 2013). Thus, pressure acts as an hurdle that does not allow further outgrowth of the endospores and the development of a vegetative microorganism (Reineke, Mathys, et al. 2013). Two patents regarding HS concept were already published: (1) "Method of pressure preservation of food products" US5593714, 1997 (Hirsch 1997) and (2) "Hydraulic pressure sterilization and preservation of foodstuff and feedstuff" US6033701, 2000 (Hirsch 2000), where is claimed that food products could be preserved at RT from a few hours to more than a month using a pressure range up to 250 MPa. However, these patents expired due to failure to pay maintenance fee.

Although food storage under pressure have been studied at pressures ranging from 0.1 to 220 MPa, this review paper will focus on HS studies performed at a pressure level above 25 MPa, mainly at RT and above it, since recent studies have demonstrated its feasibility as an alternative to RF for different food products (e.g., meat, fish, dairy, ready-to-eat (RTE) meals, fruit juices) confirmed by several microbial, physicochemical, biochemical and sensorial analyses, as it was already pointed a few years ago by Fernandes et al. (2015); Segovia-Bravo et al. (2012), among others.

Food storage under pressure up to ≈1.0 MPa

It must be noted that although HS experiments can be in general described as studies applying a pressure level considerable higher than 1.0 MPa (usually between 25 and 220 MPa), using for that a pressurization fluid (for instance, water), there are another kind of experiments regarding food storage under pressure using milder pressure levels of 0.1-1.0 MPa, reached by the flow of compressed gases. This latter case has been mostly used for fruit and vegetables preservation, such as mushrooms, in which ≈3.6 MPa at 20 °C with an atmosphere composed by O_2, N_2 and CO_2 allowed to obtain a lower moisture loss, a lower browning extent and no larval flies development up to 16.4 days when compared to control samples (Robitaille and Badenhop 1981).

Moreover, Baba et al. (2008) verified that 0.5 MPa for 10 days at 4 °C prevented discoloration of mume fruit and reduced its chilling injuries such as skin pitting and browning, as well 0.025 MPa was effective in inhibiting chilling injuries on sweet basil leaves and prevented yellowing and fungal growth on rocket-salad leaves over two months of storage.

Sweet cherries and table grapes were also preserved by this kind of technology, in which a decrease of brown/total rots and grey/blue molds in sweet cherries was observed at 0.15 MPa and 20 °C up to 24 h (Romanazzi, Nigro, and Ippolito 2008).

Hyperbaric storage performed at different temperatures

It is known that pressure decreases the water-freezing point, making possible to store foods at sub-zero temperatures without the textural changes associated to the freezing/thawing process (Kalichevsky, Knorr, and Lillford 1995). Given this fact, food storage under pressure at sub-zero temperatures was carried out successfully (Deuchi and Hayashi 1992), as summarized in Table 1.

In 1977, a study performed by Charm, Longmaid, and Carver (1977) proved that fish and meat products (cod fish, pollock, beef and chicken) could be preserved under pressure (≥20 MPa) at temperatures between −3 and 0 °C by microbial growth inhibition and enzymatic activity constraining (peroxidase and trypsin), showing that cod fillets stored at 22.8 MPa/−3 °C during 36 days appeared to have similar, or even better quality (organoleptically tested by an expert panel) than those stored at AP and −20 and −3 °C, respectively.

Later on, Deuchi and Hayashi (1992) also demonstrated that catalase, β-amylase, cathepsin and lactate dehydrogenase activities were only reduced at 200 MPa/−20 °C, contrarily to frozen storage at AP. These authors also observed that strawberry and tomato colors, as well as its fresh flavors were maintained over weeks under pressures of 50–200 MPa and sub-zero temperatures (−5 to −20 °C) (Deuchi and Hayashi (1992), cited by Fernandes et al. (2015)). Additionally, the feasibility of this methodology for microbial load reduction was also revealed for coliforms (COL), *Enterobacteriaceae* (ENT), psychrotrophs, *enterococci*, lactic acid bacteria (LAB), and yeasts on ground beef preserved at 200 MPa/−20 °C (Deuchi and Hayashi 1990). For chicken and carp muscles, the meat texture was preserved without significant protein denaturation over 50 days (170 MPa and −8 or −15 °C), and an inhibitory effect by pressure on the enzymatic degradation of nucleic acid-related substances was observed (Ooide et al. 1994). As it was previously mentioned, the "(un)fortunate" accident, that occurred with the sinking of the research submarine Alvin in 1968, opened the possibility to store food products under pressure using low

Table 1. Hyperbaric storage studies at sub-zero temperatures. Adapted from Fernandes et al. (2015).

Product	Conditions	Storage period (up to)	Outcomes	Author (year)
Cod fish fillets	22.8 MPa at −3 °C	36 days	Preserved at least up to 36 days by microbial inhibition and enzymatic activity constraining (peroxidase and trypsin). Similar to better organoleptic quality when compared to frozen samples at 0.1 MPa	Charm, Longmaid, and Carver (1977)
Beef	200 MPa at −20 °C	(*)	Microbial load reduction by inactivation of several microorganisms (e.g., coliforms, *Enterobacteriaceae*, lactic acid bacteria, yeasts)	Deuchi and Hayashi (1990)
Strawberry and tomatoes	50–200 MPa at −5 to −20 °C	(*)	Well preserved for a longer time period, presenting fresh flavor and typical color. Catalase, β-amylase, cathepsin and lactate dehydrogenase activities were reduced by pressure	Deuchi and Hayashi (1992)
Chicken and carp	170 MPa at −8 and −15 °C	50 days	Meat texture preservation over 50 days without significant protein denaturation. Inhibitory effect by pressure on the enzymatic degradation of nucleic acid-related substances	Ooide et al. (1994)

(*) The information was collected from Fernandes et al. (2015) not being available the accurate period of storage time.

Table 2. Hyperbaric storage studies performed at refrigerated temperatures. Adapted from Fernandes et al. (2015).

Product	Conditions	Storage period (up to)	Outcomes	Author (year)
Apples, bouillon and sandwiches	15 MPa at 3–4 °C	10 months	Well preserved food was recovered from a sunk research submarine after 10 months at deep sea	Jannasch et al. (1971)
Rice, wheat and soy beans	3.5 MPa at 1 °C	1 year	Lower changes in seed moisture, fatty acids and reducing sugars for 1 year under pressure than at 0.1 MPa	Mitsuda, Kawai, and Yamamoto (1972)
Dressed cod	24.12 MPa at 1 °C	21 days	Preserved during 21 days and classified as having 8.2 days, while samples stored at 0.1 MPa were unacceptable	Charm, Longmaid, and Carver (1977)
Pollock		12 days	Preserved over 12 days with higher quality (evaluated as having 6.7 days) than those at 0.1 MPa	
Hake loins	50 MPa at 4 °C	7 days	Microbial proliferation was hindered and and the total volatile basic nitrogen remained stable, contrarily to RF. Increased shear resistance and higher whiteness for samples kept at HS. Moderated differences between samples kept at HS and RF were detected by the expert panel	Otero, Pérez-Mateos, and López-Caballero (2017)

temperatures of ≈4 °C (water temperature in that place at a depth of 1540 m). As far the authors are aware, all studies regarding the use of HS at refrigerated temperatures are cited in Table 2.

Jannasch et al. (1971) had studied the impact of pressure on organic matter degradation over storage at a depth of 5300 m (≈53 MPa) and ≈3 °C. In this study, the decomposition rate of several carbon sources (acetate, mannitol and amino acids) marked radioactively with $_{14}C$ were 8–700 times slower under pressure than at the same temperature at AP. On the other hand, the same authors verified that the incubation of several carbon sources (e.g. starch, galactose, albumin) with mixed microbial populations and pure cultures at the same conditions did not allow the microbial growth, contrarily to samples at the same temperature and AP (Jannasch et al. 1971). Moreover, when some food products, as rice, wheat and soybeans were kept at a depth of 30 m in a fresh water lake for one year, biochemical changes on seed moisture, fatty acids, vitamin B_{12} and reducing sugars were minor than those stored at AP (Mitsuda, Kawai, and Yamamoto 1972).

Afterwards, Charm, Longmaid, and Carver (1977) verified that a pressure increase (up to 41.3 MPa) at a constant temperature (tested at four temperature levels, between ≈−3 and ≈23 °C) led to a decrease on enzymes activity, i.e., peroxidase activity was reduce 25–30% under 41.3 MPa/≈4 °C, when compared to AP. In the same study, trypsin activity did not present the same behavior, since an increase and a decrease were detected when pressure increased (to 41.3 MPa) at temperatures near ≈23 °C or below ≈4 °C, respectively. Thus, leading to a general conclusion by the authors that at these tested conditions (between ≈-3 and ≈23 °C, and 0.1 and 41.3 MPa), trypsin should have a critical temperature value below which pressure reduces the reaction rate and above it, it increases the reaction rate (Charm, Longmaid, and Carver 1977). More temperature and pressure values would have needed to be studied to increase the accuracy of the temperature/pressure value below which the reaction rate decreases and above it increases.

Charm, Longmaid, and Carver (1977) also studied the effect of ≈24 MPa/1 °C over 12 days in cod fish fillets, demonstrating that under pressure the microbial load nearly did not change (total bacterial count of ≈4.5 log units), contrarily to samples stored at AP. Additionally, an expert panel evaluated the characteristics of fish stored under pressure, indicating that pollock with 12 days was classified as having 6.7 days, and codfish with 21 days as having 8.2.

More recently, Otero, Pérez-Mateos, and López-Caballero (2017) studied the shelf-life extension of hake loins in about 7 days using HS at 50 MPa/5 °C, where it was observed a better performance when compared to the traditional RF. After 7 days under HS at low temperature it was possible to perceive a slight reduction of total aerobic mesophiles

(TAM) and ENT counts (4.51 ± 0.34 and <1 log CFU/g, respectively) when compared to the initial ones (4.76 ± 0.43 and 1.87 ± 0.34 log CFU/g, respectively), while samples under RF presented higher values (7.70 ± 0.21 and 6.48 ± 0.24 log CFU/g, respectively) at the 7th day of storage. Regarding physicochemical parameters, although without statistical differences in pH between samples preserved by HS and RF, the total volatile basic-nitrogen content of refrigerated samples increased (38.65 ± 4.52 mg/100g), contrarily to HS samples (9.96 ± 1.12 mg/100g), which showed values close to the initial one (11.08 ± 1.02 mg/100g). On the other hand, although water content, water holding capacity, shear resistance and whiteness of samples preserved by HS were reported to be statistically different from the initial ones, after cooking, weight losses were less than half for control samples, as well as whiteness differences disappeared, leading to a sensorial analysis where only moderate differences were found between cooked samples (Otero, Pérez-Mateos, and López-Caballero 2017).

Additionally, a study performed by Lemos et al. (2017) proved the HS feasibility on extending watermelon juice shelf-life (up to at least 58 days) by combining pressure with lower temperatures (up to 10 °C), pointing out that at lower pressures, temperature is a limiting factor on the microbial stability along storage. In this work, a pressure level of 50 MPa and 10 °C allowed to obtain a slower microbial growth throughout storage when compared to HS (50 MPa) at 15 and ≈25 °C, wherein TAM and total aerobic psychrophiles (TAP) grew above 6.0 log CFU/mL after 7 days of storage at HS/15 °C, and after 3 days at HS/≈25 °C (Lemos et al. 2017). The authors also carried out HS experiments at 75 and 62.5 MPa (both at 15 °C), where it was observed a slower microbial load reduction on the latter for ENT and YM and similar microbial counts were obtained in both pressure conditions on the last days of storage (21st and 58th day) for TAM and TAP (Lemos et al. 2017). Regarding physicochemical parameters, the behavior detected for color and pH was similar to other HS studies (Fidalgo et al. 2014; Santos et al. 2015; Pinto et al. 2017; Pinto et al. 2016), since pH remained stable along HS conditions (50, 62.5 and 75 MPa, and 15 °C) when compared to control samples, as well as total color variation, ΔE^* were from less to equally affected by HS (50, 62.5 and 75 MPa, and 15 °C) when compared to AP storage (Lemos et al. 2017). Briefly, this work proved the HS feasibility at low temperature to control microbial and physicochemical degradation of watermelon juice, promoting the shelf-life extension up to, at least, 58 days compared with only about 3 days by RF, which represent a huge potential of HS to future replacement of RF, either on food industries or even on our houses.

Hyperbaric storage at and above room temperature (HS/RT)

From 2012, HS application at and above RT has become a trend, and several studies have been performed since then, being pointed in all of them possible energy savings and lower carbon footprint when compared to RF (Fernandes et al. 2015; Bermejo-Prada et al. 2017). It must be noted that several studies described on this review (Fernandes et al. 2015; Fidalgo et al. 2018; Freitas et al. 2016; Moreira, Duarte, et al. 2015) were carried out using the current existing high pressure equipment, capable to reach 600 MPa (industrial scale), or even more as 900 MPa (in some laboratorial equipment), in seconds to minutes, and for short periods of time. HS does not require so powerful equipment since a pressure level range between 25 and 200 MPa and lower pressurization rates are capable to preserve food products with an equal to better performance than RF (Bermejo-Prada et al. 2017).

Fruit juices

Segovia-Bravo et al. (2012) tested the HS concept (25/100/220 MPa) at controlled RT (20 °C) for 15 days using strawberry juice as a case-study. In this work, it was recognized the HS feasibility at RT as a novel food preservation technique for strawberry juice, since not only a microbial growth inhibition was observed (for TAM and yeasts and molds, YM), but also a microbial growth inactivation was detected by the reduction of the initial microbial loads (from >2 log units to levels below of 10 CFU/ml for TAM and below 100 CFU/ml for YM), being these results better than the ones obtained when RF was applied to the same sample where TAM and YM increased more than 3 log units after 15 days of storage. Some evidences also showed that HS is effective on viscosity and color losses attenuation when compared to control samples (AP/20 °C), since a viscosity reduction between 79.2% and 63.7% was observed for HS samples being these values lower than in control sample (83.6%), as well lower ΔE^* values were obtained for HS (4.5 ± 0.7 *vs.* 1.3 ± 0.1 for control sample and HS, respectively). An additional storage at refrigerated temperature after a HS period (called "post-HS" experiment) was also evaluated, and from that, the stability of the microbial load, viscosity and color were confirmed over those days (Segovia-Bravo et al. 2012).

At this point, it must be highlighted the usefulness of a post-HS study as well the existence of these studies in some published works, e.g. Fernandes et al. (2019); Freitas et al. (2016). From those, it was possible to analyze the behavior of the samples when stored at refrigerated conditions after have been preserved by HS over days to weeks, leading to several conclusions on the microorganism inhibition/inactivation and their ability to proliferate at RF conditions after HS. This could be of great importance for a HS application at a practical/industrial level since the microbial load in post-HS studies is lower than on samples stored only at RF conditions (without any HS period), due to microbial inactivation by HS, leading to higher microbial shelf-lives. All HS studies regarding fruit juices are briefly compiled in Table 3.

Further studies performed by the same research group regarding HS/RT effect on strawberry juice quality parameters allowed to assign to pressure some color degradation, where a lower percent of polymeric color and a significant peroxidase inactivation (15%) in samples stored at 200 MPa were detected when compared to RF (and AP) samples (Bermejo-Prada and Otero 2016). The same authors

Table 3. Published studies on the last few years regarding hyperbaric storage of fruit juices. Adapted from Fernandes et al. (2015).

Fruit juice	Conditions	Storage period (up to)	Main outcomes	Author (year)
Watermelon juice	100 MPa at 18–21 °C	60 h	Microbial growth inhibition and microorganism's inactivation at the tested conditions. Further shelf-life extension at RF conditions after a HS period	Fidalgo et al. (2014)
	25–150 MPa at 20–37 °C	8 h	Microbial growth inhibition at 75 MPa and inactivation at 100 and 150 MPa. No significant changes on the physicochemical parameters	Santos et al. (2015)
	100 MPa at 18–21 °C	7 days	Shelf-life extension when compared to the juice kept at 4 °C and 0.1 MPa	Pinto et al. (2016)
	50–75 MPa at 10–25 °C	58 days	Microbial slowdown at 50 MPa/10 °C and microbial inactivation at 62.5 and 75 MPa. Additionally, 62.5 MPa/25 °C promoted a shelf-life extension up to at least 58 days. Color parameters and pH less affected by HS than AP (both 4 and 15 °C) storage.	Lemos et al. (2017)
	50–100 MPa at 18–23 °C	10 days	At 50 MPa was verified a microbial growth similar to RF, while at 75/100 MPa were observed microbial load reductions on endogenous and inoculated microorganisms, resulting in a shelf-life extension compared to RF. Physicochemical parameters remained stable at 75 MPa when compared to the initial raw juice.	Pinto et al. (2017)
Melon juice	25–150 MPa at 20–37 °C	8 h	Microbial growth inhibition achieved at 50/75 MPa and microorganism's inactivation at 100/150 MPa. The overall physicochemical characteristics remained unchanged	Queiros et al. (2014)
Strawberry juice	25–220 MPa at 20 °C	15 days	Microorganism's inactivation at HS over 15 days, e.g., yeasts and molds and total aerobic mesophiles below the detection limits at the tested conditions	Segovia-Bravo et al. (2012)
	50 and 200 MPa at 20 °C		Pressure avoided spoilage of samples stored at 20 °C for 15 days and kept the volatile profile of the strawberry juice similar to the initial samples	Bermejo-Prada, Vega, et al. (2015)
			Neither pectin-methylesterase (PME) catalytic activity was affected by pressure on strawberry extract, nor PME inactivation was found up to 200 MPa	
			Significant peroxidase inactivation on longer storage periods (5, 7 and 15 days) and lower percent of polymeric color at the 5th, 7th and 10th days at 200 MPa, compared to samples stored at 0.1 MPa	Bermejo-Prada and Otero (2016)
	25–200 MPa at 20 °C		At 25 MPa the microbial growth was retarded, HS at 50 MPa yielded microbial load reductions. Higher pressures resulted in higher microbial loads reductions	Bermejo-Prada, López-Caballero, and Otero (2016)
	25 MPa at 20 °C		Reduced energetic costs and lower carbon foot-print than RF (4 °C), despite the higher total storage costs for HS. No significant differences were detected by the expert panel on samples at HS and RF	Bermejo-Prada et al. (2017)
Carrot juice	25–100 MPa at 18–23 °C	60 days	Inoculated *Bacillus subtilis* endospores inactivation along storage (≈6 log CFU/mL) at 50 and 100 MPa. Endospore germination triggered at 25 MPa, resulting in juice spoilage	Pinto et al. (2018)
Apple juice	25–100 MPa at 18–23 °C	30 days	Inoculated *Alicyclobacillus acidoterrestris* endospores inactivation below the detection limit at 50 and 100 MPa after 24 and 48 h, respectively. Slight endospore inactivation at 25 MPa along storage	Pinto et al. (2019)

highlighted that pressure did not significantly affect total phenolic and total monomeric anthocyanin contents during storage and only storage time had a significant effect on them. Nonetheless, although color differences were instrumentally perceptible, they were very slight to be easily perceived by the naked eye since differences between ΔE^* values did not exceed 1 (the threshold value frequently assumed as a basis for a color perceptible difference) (Bermejo-Prada and Otero 2016). Bermejo-Prada, Vega, et al. (2015) also studied the effect of this preservation methodology on the volatile profile of strawberry juice and found that samples preserved by HS were more similar to the juice at day 0 than to the samples stored under RF. Moreover, HS was also efficient to avoid changes in all key aroma compounds detected on strawberry juice (Bermejo-Prada, Vega, et al. 2015).

Furthermore, pectin methylesterase (PME) activity and serum viscosity of strawberry juice stored under pressure were also evaluated (Bermejo-Prada, Segovia-Bravo, et al. 2015). The PME activity decreased (≈55%) throughout 15 days under different storage conditions (AP included), being not detected a HS effect (up to 200 MPa and 20 °C, during 15 days) on PME activity. Nonetheless, the methanol release was also quantified to check the enzymatic behavior of PME under pressure, and its release was twice higher at 200 MPa and 20 °C than at RF and 50 MPa, what might be

related with the enhanced-activity of other endogenous pectinases (others than PME) that could facilitate the access of PME to the methyl ester bonds of pectin, thus affecting the characteristics of pectin and serum viscosity. In this study of Bermejo-Prada, Segovia-Bravo, et al. (2015), contrarily to the first one published (Segovia-Bravo et al. 2012) regarding viscosity decay on strawberry juice (where lower values of viscosity reduction were observed for HS samples than in control samples), it was found that HS enhanced it occurring in a greater manner in the first days: 42.5%, 55.5%, and 74.5%, for AP, 50 and 200 MPa, respectively, at first day of storage.

Since strawberry juice is an acidic food product and, in consequence, already presents some intrinsic microbial barriers at RT and particularly under RF, experiments regarding watermelon juice, a highly perishable food product, with low acidity and high water activity (a_w) were carried out by a different research group. These authors, Santos et al. (2015); Fidalgo et al. (2014) had also tried to prove for watermelon juice the HS feasibility at temperatures up to 37 °C, controlled and uncontrolled, since the previous studies performed with strawberry juice were carried out only at controlled RT.

In Fidalgo et al. (2014) and Santos et al. (2015) studies, watermelon juice was preserved by HS (25–150 MPa/20–37 °C) from 8 up to 60 h, being observed, for TAM, ENT and YM, an equal to better growth inhibition than in RF. In summary, 75 MPa presented an inhibitory effect on microbial growth over 8 h, and an additional inactivation effect was verified for storages at 100 and 150 MPa with the reduction of the initial microbial loads below to the detection limit (1.00 log CFU/mL) for ENT and YM, and from 4.43 ± 0.04 to 3.31 ± 0.04 and 2.99 ± 0.07 log CFU/mL, respectively, for TAM (25 °C) (Santos et al. 2015). When performed at 30 °C/100 MPa up to 60 h, it was verified that after the initial microbial load decrease (≈1/≈2/≈1 log units for TAM/ENT/YM, respectively, reaching ≈3 log units for TAM and <1.00 log CFU/mL for ENT and YM) over the first 8 h, the values remained unchanged until the end of storage (Fidalgo et al. 2014). The physicochemical analyses performed in these two studies (pH, titratable acidity, total soluble solids, browning degree and cloudiness) did not show a clear variation trend with pressure and no considerable differences among storage conditions were verified (Santos et al. 2015; Fidalgo et al. 2014).

A post-HS experiment for watermelon juice (7 days of storage at 5 °C, after a HS period of 60 h at 100 MPa/≈21 °C) confirmed a similar behavior previously observed for strawberry juice, where the microbial loads were found at a level of 2.27 ± 0.38 and 3.57 ± 0.86 log CFU/ml for TAM and YM, respectively, and <1.00 log CFU/mL for ENT at the 7th day, becoming these results clearly better than the ones observed on juice preserved only under RF (≥4, ≈3, ≈3 log units, for TAM, ENT and YM, respectively, in the first 2 days of RF) (Fidalgo et al. 2014).

Similar microbial and physicochemical results were observed for a HS (25–150 MPa and 25/30/37 °C, over 8 h) of melon juice in the publication of Queiros et al. (2014), in which 50/75 MPa resulted in similar or lower microbial counts while at 100/150 MPa an additional inactivation effect was observed (for TAM, ENT and YM). The authors also concluded that for the pressure levels and temperatures studied, the microbial load reduction was temperature independent, being higher as the storage pressure increased, since a linear behavior was observed with slopes of −0.011 and −0.020 log CFU/mL/MPa (R^2 = 0.968 and 0.985) for TAM and YM, respectively.

The studies regarding watermelon juice were furtherly expanded, with a very recent publication (Pinto et al. 2016) showing the possibility of shelf-life extension (compared to RF) for this highly perishable food product using HS at 100 MPa and ≈21 °C. These HS conditions allowed to preserve watermelon juice up to at least 7 days, being obtained lower microbial loads (≈2 log CFU/ml for TAM and TAP, and below the detection limit, 1.00 log CFU/mL, for YM) when compared to samples stored at AP (≈21 °C and RF) that presented values above the acceptable limit (>6.00 log CFU/mL) for the same microorganisms. The physicochemical parameters evaluated after storage (pH, total soluble solids, browning degree, cloudiness and color) presented levels very close to those found initially for this sample (Pinto et al. 2016).

Moreover, Pinto et al. (2017) tested three pressure levels for watermelon juice storage (50, 75, and 100 MPa) and compared to AP/RT (0.1 MPa/18–23 °C) and AP/RF (0.1 MPa/4 °C) samples inoculated with two specific microorganisms, *Listeria innocua* (ATCC 33090) and *Escherichia coli* (ATCC 25992), being also determined along storage the enzymatic activities of polyphenol oxidase (PPO), peroxidase (POD), and pectin methylesterase (PME). In this study, the overall microbial analyses performed at the natural juice microflora were in accordance to previous studies (Fidalgo et al. 2014; Santos et al. 2015). Regarding inoculated samples, AP/RT and AP/RF resulted in microbial loads increments to values above 6.00 log CFU/mL for both microorganisms, whilst 50 MPa condition reduced ($p < 0.05$) *E. coli* loads in about 1.00 log CFU/mL after 3 days, and to below the detection limit at the 6th day of storage and onwards, contrarily to *L. innocua*, whose counts increased to above 6.00 log CFU/mL after 10 days of storage, similarly to juice stored at AP/RF. Nonetheless, on HS/RT at 75 MPa, *E. coli* and *L. innocua* were reduced ($p < 0.05$) to below the detection limit at the 10th day, indicating that this pressure was effective on *E. coli* and *L. innocua* inactivation over storage, as occurred for HS/RT at 100 MPa (Pinto et al. 2017). Concerning enzymatic activities, PPO activity during HS was less affected along storage when compared to AP/RF, showing a less pronounced activity reduction (Pinto et al. 2017). For POD, HS/RT at 50 and 75 MPa led to an activity decrease ($p < 0.05$) similar to AP/RF, presenting residual activities of 40.6% (50 MPa) and 54.6% (75 MPa) at the end of storage. Lastly, the pressure level increase tended to decrease PME activity, since after 10 days of HS/RT at 100 MPa it was verified a residual activity of 42.8%. Pinto et al. (2017) also reported on the same study that all physicochemical parameters studied remained stable at 75 MPa

Table 4. Hyperbaric storage studies at and above room temperature regarding dairy and ready-to-eat (RTE) meals.

Dairy product/ RTE meal	Conditions	Storage period (up to)	Main outcomes	Author (year)
Requeijão (Portuguese whey cheese)	100 and 150 MPa at 25–37 °C	8 h	Microbial load reduction after HS. Pressure retained the color, pH and water activity of the whey cheese. Lipid oxidation levels stable when compared to RF	Duarte et al. (2015)
	100 MPa at ≈17 and 21 °C	10 days	Microbial inactivation observed for all microorganisms in the 3rd day, with physicochemical parameters unaffected under HS comparatively to RF.	Duarte et al. (2017)
Carrot soup	100 and 150 MPa at 25 and 30 °C	8 h	Microbial growth inhibition at 100 MPa and inactivation at 150 MPa after HS. General physicochemical parameter similar to RF.	Moreira, Fernandes, et al. (2015)
Caldo verde and *bacalhau com natas*	50–150 MPa at ≈21 °C	12 h	Microbial growth inhibition at 100 MPa and inactivation at 150 MPa. No significant changes on the physicochemical parameters evaluated.	Moreira, Duarte, et al. (2015)

when compared to the initial raw juice, except for browning degree that increased 1.72-fold, whilst at 100 MPa were observed higher color variations attributed to a lycopene content decrease (25%), as well as reductions on POD residual activity (16.8%) after 10 days, while both PPO and PME residual activities were similar to RF.

Very recently the germination and outgrowth control of endospores by HS/RT (18–23 °C) was accessed by Pinto et al. (2018), who reported the possibility of controlling *Bacillus subtilis* endospore germination and outgrowth in carrot juice (pH 6.00). The results showed that, at pressures of 50 and 100 MPa there were endospore loads inactivation to below the quantification limit after 60 days of storage, in contrast with conventional RF, whose loads increased ≈1 log unit. At 25 MPa, the endospore germination and outgrowth was quickly triggered, leading to juice spoilage (Pinto et al. 2018).

In another study Pinto et al. (2019) performed with an atypical case of an endospore able to germinate and outgrowth under very acidic conditions, *Alicyclobacillus acidoterrestris* in commercial apple juice (pH 3.50), it was observed that pressure levels of 50 and 100 MPa were able to inactivate *A. acidoterrestris* ascospores to below the detection limit (10 CFU/mL) right after 48 and 24 h of storage, respectively. At 25 MPa, an endospore load reduction was also observed but at lower rates than at 50 and 100 MPa (Pinto et al. 2019).

Dairy products and ready-to-eat meals

The studies performed by Duarte et al. (2015) and Moreira, Fernandes, et al. (2015) aimed to study a highly perishable dairy food (a traditional Portuguese whey cheese, *requeijão*) and a RTE carrot soup at HS conditions, as presented in Table 4.

In Duarte et al. (2015) work, *requeijão* samples were preserved over 4 and 8 h at 100 and 150 MPa in a temperature range from 25 to 37 °C, wherein the authors observed that 4 h at 100 MPa yielded the maintenance of the microbial counts similarly to RF and the initial load (≈3 log CFU/g at all tested temperatures for TAM, ENT, and LAB), whereas 150 MPa during 8 h allowed a microbial load reduction to undetectable levels, except for TAM (reduction of ≈1 log unit). Generally, HS retained whey cheese color, pH and water activity parameters, however for lipid oxidation, it was verified a similar behavior to RF storage (for instance, lipid oxidation values slightly increased from 0.022 ± 0.004 to 0.035 ± 0.006 and 0.037 ± 0.002 mg MDA/g for 150 MPa/25 °C and RF, respectively) (Duarte et al. 2015).

A second study was conducted by the same authors for longer storage periods, up to 10 days, using the same food product (*requeijão*) under 100 MPa and variable RT. As predicted, YM and LAB counts were inactivated to values below the detection limit under HS, just in the first 12 h. For TAM and ENT, HS was capable to avoid microbial growth in the first 24 h, while after 3 days of storage it was observed a microbial inactivation to values below the detection limit, which were maintained up to 10 days (Duarte et al. 2017). Similar results were found by Moreira, Fernandes, et al. (2015), in which a RTE carrot soup was kept at 100 and 150 MPa, over 4 and 8 h and at 25 and 30 °C. In this study, the authors concluded that, globally, despite the microbial growth inhibition, the microbial inactivation effect observed was more evident when soup was stored at 150 MPa over 8 h, being TAM less susceptible to HS, which confirmed previous studies (Santos et al. 2015; Duarte et al. 2015). Regarding physicochemical analyses (pH, titratable acidity, reducing sugars, and color) of samples preserved by HS a similar performance to RF was observed (Moreira, Fernandes, et al. 2015).

A possible application of this methodology using the current available industrial high pressure equipment (in this case an equipment with 55 litters capacity, Hiperbaric model 55, Burgos, Spain) was already performed by Moreira, Duarte, et al. (2015), wherein two RTE meals (*bacalhau com natas* and *caldo verde* soup, traditional Portuguese RTE meals) were stored under pressure over 12 h at 50, 100 and 150 MPa, at variable RT (≈21 °C). In this experiment (Table 4), the authors observed a microbial growth inhibition at 100 MPa for all microorganisms studied and an additional inactivation effect at 150 MPa resulted in values below the detection limit for ENT and YM, leading to an equal to better storage performance when compared to RF, without detectable changes on the evaluated physicochemical parameters (pH, titratable acidity, color and fatty acid content) (Moreira, Duarte, et al. 2015).

Table 5. Hyperbaric storage studies performed with raw/processed meat and fish products.

Meat/fish product	Conditions	Storage period (up to)	Main outcomes	Author (year)
Tilapia fillets	203 MPa at 25 °C	12 h	Microorganisms inactivation about 2.0 log CFU/g at HS, presenting an improved freshness when compared to samples stored at 0.1 MPa	Ko and Hsu (2001)
Sea cucumber guts	60 MPa at 30 °C	24 h	Reduction of the psychotrophic counts of about 0.9 log CFU/g	Okazaki, Shigeta, and Aoyama (2007)
Sliced cooked ham	25–150 MPa at 23–37 °C	8 h	HS was efficient to inhibit microbial growth at pressures above 50 MPa for similar levels of RF. Microbial inactivation at 100 and 150 MPa	Fernandes et al. (2015)
Raw bovine meat	50–150 MPa at ≈21 °C	12 h	At 50 MPa it was faced a similar microbial development inhibition when compared to the refrigerated samples, while at 100 and 150 MPa it was verified an additional microbial inactivation effect	Freitas et al. (2016)
	100 MPa at ≈21 °C	10 days	Shelf-life extension by HS over RF and no significant differences were found on the quality parameters of the meat	
Atlantic salmon fillets	50–60 MPa at 25–37 °C	10 days	HS at 50 MPa promoted the increase of the microbial load. When the pressure was increased to 60 MPa a microbial growth slowdown was observed, increasing the microbial shelf-life up to at least 6 days.	Fidalgo et al. (2018)
	75 MPa at 25 °C	25 days	HS at 75 MPa caused a reduction of about 3.5 log units of initial microbial counts, leading to an increase of the microbial shelf-life of at least 25 days, compared to RF (3 days). Additionally, no changes in color was detected during the storage period.	

Raw and processed meat/fish products

As far the authors are aware, only two studies performed by Ko and Hsu (2001) and Fidalgo et al. (2018) regarding HS at RT of fresh fish were carried out (Table 5). In Ko and Hsu (2001) work, it is reported that raw tilapia fillets stored at ≈101 and ≈203 MPa and 25 °C over 12 h presented a microbial growth inhibition at ≈101 MPa and an inactivation at ≈203 MPa with reductions from 4.7 to ≈2.0 log CFU/g. It was also observed by the freshness quality index (*K*-value) that a higher freshness was obtained in samples stored under pressure (Ko and Hsu 2001). In fact, the values of 51, 44, 33, and 28% that were obtained for ≈51, ≈101, ≈203, and ≈304 MPa, respectively, over 12 h demonstrated that K-value increased slowly at higher pressures, compared to that obtained at AP over 12 h (92%) (*K*-value above 60% indicates putrefaction) (Ko and Hsu 2001). Recently, Fidalgo et al. (2018) evaluated the quality parameters of Atlantic salmon over 10 days when stored under pressure at RT (25 and 37 °C), at different storage conditions (50, 60 and 75 MPa at 25 °C, and 75 MPa at 37 °C). The authors reported that 60 MPa allowed a microbial growth slowdown promoting a possible shelf-life extension up to 6 days (at least). Moreover, 75 MPa/25 °C led to a reduction of ≈3.5 log units, fact that could increase this product shelf-life at least up to 25 days (when compared to only 3 days of shelf-life at RF). Additionally, the physicochemical analyses performed were similar to the ones obtained on previous studies and no changes on color, a_w and pH of salmon fillets were detected over storage, possibly due to microbial load reduction. Nonetheless, HS/RT enhanced an increase on the primary and secondary lipid oxidation products compared to RF (more pronounced on higher storage pressure), while for AP/RT the tertiary lipid oxidation had increased.

Later on, Okazaki, Shigeta, and Aoyama (2007) found out while developing an autolysis process for sea cucumber guts, that psychrotrophic bacteria counts were reduced of about ≈0.9 log CFU/g after being under pressure at 60 MPa/30 °C during 24 h, although mesophilic bacteria count presented an increase of ≈1.2 log units under these conditions.

Another work performed by Fernandes et al. (2015), which aimed the use of HS at RT (25–150 MPa and 25–37 °C along 4 and 8 h) on sliced cooked ham, yielded similar results to the ones cited above. Here, 50 MPa/30 °C has led to a microbial growth inhibition, resulting in TAM and LAB loads similar to RF, being observed that when the storage pressure increased to 100–150 MPa, it resulted in an additional microbial inactivation. Globally, in this study, the quality attributes of sliced cooked ham were similarly affected by HS and RF.

The HS feasibility was also proved for raw bovine meat by Freitas et al. (2016), during 12 h at 50, 100 and 150 MPa and RT (≈21 °C). The microbial results obtained agreed with the aforementioned studies, since it was observed for a 12 h storage at 50 MPa a similar microbial growth inhibition when compared to RF, and at 100 and 150 MPa an additional microbial inactivation effect. For instance, TAM decrease from 2.86 ± 0.08 log CFU/g to 2.06 ± 0.03 log CFU/g and <2.0 log CFU/g for 100 and 150 MPa, respectively, being verified a similar behavior for TAP. Although, the initial counts of ENT, YM and COL were below 2.0 log CFU/g, these microorganisms showed some susceptibility under HS, being gradually more affected by the increase of pressure, with counts below the detection limit (<1.0 log CFU/g) under HS. Moreover, these authors found log-linear curves to describe the microbial inactivation of TAM and TAP during 12 h of HS of fresh beefs, as a function of

pressure, being noticed a similar susceptibility for both microorganisms, −0.013 log CFU/MPa/g as the specific inactivation rate. A post-HS during 6 days was also evaluated for raw bovine meat, where it was verified that microorganisms were able to grow under RF after a HS period (150 MPa, RT, 12 h), indicating that pressure inhibited/inactivated the microbial growth during HS but microorganisms were still able to proliferate after it under RF and AP (Freitas et al. 2016). In a second experiment, the same authors, studied for a longer storage period (10 days at 50 MPa/RT) raw bovine beef. The outcomes hint for a possible raw bovine meat shelf-life extension by HS when compared to samples stored at RF conditions. HS was effective in TAM and TAP loads maintenance with values below 6.0 log CFU/g up to the 7th day of storage, contrarily to samples stored at AP that presented values of >6.0 log CFU/g right after 3 days at RT and after 7 days at RF temperatures. As regard to the physicochemical analyses performed in this study (pH, color, fatty acid determination), the authors did not find significant differences in pH for RF and HS samples (10 days) relatively to the initial value, as well as for color parameters wherein no significant differences in L^*, a^* and b^* were observed between samples stored under RF and by HS up to 10 days (Freitas et al. 2016). In what concerns to fatty acid content, the monounsaturated fatty acids and the several polyunsaturated fatty acids class proportions were not significantly affected by HS, whereas saturated fatty acid presented some significant differences, although no consistent pattern relating HS and fatty acid composition arose from the results obtained, thus indicating no particular detrimental effect caused by HS (Freitas et al. 2016).

Hyperbaric storage – industrial viability and environmental impact

The conventional cold storage processes (both freezing and RF) are very well established at both domestic and industrial environments. In fact, the food cold-chain market is thought to be worth more than 167.4$billion United-States dollars (USD) and it is forecasted to be worth more than 271.3$billion USD by 2022, with an estimated annual growth of 7.0% (Marketandmarkets 2017), showing that these processes are widely consolidated. Nevertheless, the cold-chain industry is responsible for considerable emissions of greenhouse effect gases, such as the chlorofluorocarbons (also known as CFC's), as well by elevated energetic spends due to the almost constant power supply to keep these units operating, which results on significant emissions of CO_2 to the atmosphere.

On the perspective of a HS acceptance on both domestic and industrial contexts to mitigate the environmental impacts of RF, it is of upmost importance to evaluate the economic and environmental impacts of HS to ensure if it is economically advantageous and an environmental friendlier alternative to the traditional RF processes. For so, the next sections will focus an economic and environmental evaluation of HS.

Storage costs estimation

The potential energetic savings related to a HS application as a new food preservation methodology was referred by several authors, such as Fernandes et al. (2015), Freitas et al. (2016), Fidalgo et al. (2014), Segovia-Bravo et al. (2012), among others, with inherent economic and environmental gains, stating that energy would be only needed to compress and decompress the pressure vessel, since when the desire pressure is achieved, energy would not be needed to keep it along storage, and thus, virtually, no energetic costs. Though, until now, this statement was only investigated by Bermejo-Prada et al. (2017), which estimated that the energetic costs inherent to HS of 800 Kg of strawberry juice at 25 MPa and 20 °C for 15 days was 0.001 €/Kg against 0.026 €/Kg of RF.

Although a great reduction of the energetic costs is associated to this new food preservation methodology, the equipment price can overlap that potential, mainly due to the costs of the pressure vessel, intensifiers and hydraulic pumps, which are considerably higher than the conventional RF facilities, which resulted, as estimated by Bermejo-Prada et al. (2017), in a total storage cost for HS of about 0.291 €/Kg of strawberry juice, against 0.081 €/Kg for conventional RF. These costs include equipment maintenance and amortization (a measure of the initial investment depreciation), as well the inherent energetic costs (this last parameter seems to be the best of HS against RF). In addition, this study estimated HS/RT costs considering a completely loaded vessel (maximum mass of 2000 kg) so it could be moved to a warehouse with a forklift, thus, heavier pressure vessels are to be moved with more expensive and complex structures and equipment that are, by induction, more expensive than a forklift, which can increase even more HS costs. Moreover, as the storage pressure increases, the pressure vessel thickness required to keep it for long periods of time also increases, thus increasing the storage costs. As mentioned, despite the HPP equipment high initial cost, it did not stop its implementation in the food industry as a non-thermal pasteurization method, and as result, a decreasing trend in equipment costs was observed from 1996 until now. Innovations related to the HP technology, such as HS, might lead to the arising of new manufacturers, which could also lower the price of these units (Mújica-Paz et al. 2011), even if specifically designed for HS, that would require less resistant units since the pressure levels employed on HS are considerably lower than in HPP.

Carbon foot-print assessment

According to Gilbert (2012), RF is the third major source of CO_2 of all food industry (with 490 megatons of CO_2 released to the atmosphere in 2008), being even estimated that 35–50% of the energetic consumptions in super and hypermarkets is due to RF and freezing facilities, contributing for approximately 1% of the CO_2 emissions worldwide (James and James 2010). Thus, environmentally friendlier food preservation methodologies need to be considered, in order to reduce the carbon foot-print related with RF, being

HS a possible solution for this issue. Besides CO_2, RF facilities are also responsible for considerable emissions of greenhouse effect gases, which are used as refrigerant on these facilities, belonging to a class of compounds known as CFC's and hydrochlorofluorcarbons, which are responsible for ozone degradation (James and James 2010).

The carbon foot-print associated with HS of 1 Kg of strawberry juice for 15 days was assessed by Bermejo-Prada et al. (2017) and compared with RF storage. The outcomes revealed that RF had a 26-fold higher carbon footprint when compared to HS (0.1085 Kg CO_2/Kg of strawberry juice against 0.0042 Kg CO_2/Kg of strawberry juice, respectively). In what concerns to RF, the two main sources of CO_2 were the energetic consumption and the refrigerant leakage, while for HS the main source of CO_2 emission was attributed to the hyperbaric chamber material, with an estimated emission of 0.0041 Kg CO_2/Kg of strawberry juice, while the CO_2 released by the energetic consumption was negligible (3×10^{-5} Kg CO_2/Kg of strawberry juice), proving that HS is considerably less pollutant than the conventional RF processes.

From the social point of view, Bermejo-Prada et al. (2017) concludes that HS/RT could also be preferred over RF, since the pressure vessels could be shipped to foreign geographies where electricity is less available, thus providing safer food products, while contributing for a more sustainable food-chain industry.

Conclusion

The results obtained in the several published studies allowed to conclude that HS presents a great potential to substitute RF since an equal to better microbial quality of the food products can be attained, being the physicochemical parameters variations generally minor than the ones observed in RF. Moreover, in this review paper it was also reported the HS feasibility for possible shelf-life extensions of several food products, from fruit juices until ready-to-eat foods, including fresh fish, meat and dairy products by a better microbial inhibition or inactivation.

Despite the economic evaluation of HS revealed to be less competitive than the conventional RF processes, until now, it can be surpassed by the reduced energetic costs, reduced carbon foot-print, as well by the arose of new equipment manufacturers that could lower the price of industrial units specifically designed for HS (since the current industrial equipment are designed to support pressures up to 600 MPa, 3 to 6-fold higher than those employed on HS). Thus, it is expected for the next years a wide research regarding HS as a new food preservation, its impacts on specific microorganisms (e.g., spores and pathogenic), foods constituents, as well as the implementation on food industries and/or consumers' homes.

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Disclosure statement

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Abbreviations

HS	Hyperbaric Storage
RF	Refrigeration
HPP	High Pressure Processing
PATS	Pressure-assisted thermal sterilization
RT	Room temperature
COL	Coliforms
ENT	Enterobacteriaceae
LAB	Lactic acid bacteria
TAM	Total aerobic mesophiles
TAP	Total aerobic psychrophiles
PME	Pectin methylesterase
AP	Atmospheric pressure
PPO	Polyphenol oxidase
POD	Peroxidase
RTE	Ready-to-eat
USD	United-States dollars

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