Food Preservation Under Pressure (Hyperbaric Storage) as a Possible Improvement/Alternative to Refrigeration

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Abstract
Food preservation is highly dependent on refrigeration, which is limited by its high energy costs. Among alternatives being developed, this review focused on hyperbaric storage (food preservation under pressure). This new preservation methodology has as main objective microbial growth inhibition similarly to freezing and refrigeration, showing a great potential to lower energy costs since refrigerated/room temperatures (RT) can be used. This, even at variable (uncontrolled) RT (up to 37 °C), has been shown to preserve foods and thus achieving significant energy savings. Covering the earliest up to the more recent studies, this review aimed to gather information about the state of art of hyperbaric storage at refrigerated and RTs, with the primary objective of showing it potential and possible future applications of this new preservation method based on microbial growth inhibition under pressure, using pressure as the main variable to slow down microbial growth.

Keywords Hyperbaric storage • High pressure • Food preservation • Refrigeration • Spoilage • Microbial growth inhibition

Introduction

Since the nineteenth century, thermal pasteurization and sterilization have been used to ensure food safety and achieve a significant shelf life extension [33]. However, these methodologies have negative impact on nutritional and sensorial levels (including nonenzymatic browning, protein denaturation and loss of vitamins and volatile flavour compounds) and do not meet the consumers demand for more natural and minimally processed food. These requirements led to the development of new food processing technologies, of which high pressure (HP) is now highlighted as a major trend [27, 43]. HP technology is a novel promising technology for gentle food preservation since it does not use heat, thus minimizing organoleptic and nutritional losses of food [24].

The first experiments regarding HP application for food preservation were developed in milk, fruits and vegetables [18, 19]. However, due to technological limitations, HP technology was mainly applied in the chemical, ceramic and metallurgic industries until the eighties. Nowadays, applications of HP in food and biotechnology have expended rapidly particularly for the commercial pasteurization of foods [4, 16, 36], inactivation of enzymes [17, 43] and modification of proteins [3, 48] and of food physicochemical properties [11, 12, 25]. Several reviews are already published regarding HP applied on food processing and its advantages [24, 34, 37, 42, 43], while several studies have investigated combining HP with other nonthermal technologies to explore the possibility of syn- ergy between processes [43, 46, 49].

Despite all the benefits of new food preservation tech- nologies, the storage and transportation of these products is generally handled at refrigerated temperatures to retard their spoilage. Moreover, about 30 % of the world food

Table 1 Studies of hyperbaric storage at sub-zero, refrigeration and RT

Product	Conditions	Results	References
Sub-zero			
Cod fish fillets	22.8 MPa for 36 days at −3 °C	Stable and consumable for at least 36 days. Similar quality to frozen samples at 0.1 MPa	[6]
Beef	200 MPa at −20 °C	Microbial load reduction and inactivation of yeasts and some bacteria	[8]
Strawberries and tomatoes	50–200 MPa at −5 to −20 °C	Stable for a few more days/weeks. Fresh flavour and colour preserved. Catalase, β-amylase, cathepsin and lactate dehydrogenase inhibition by pressure	[10]
Chicken and carp	170 MPa for 50 days at −8 and −15 °C	Stable for 50 days. Enzymatic activity associated to nucleic acids degradation reduced	[39]
Refrigeration			
Bouillon, sandwiches and apples	15 MPa for 10 months at 3–4 °C	All the products were stable and consumable after 10 months under pressure and for a few weeks at refrigeration and 0.1 MPa	[21]
Rice, wheat and soy beans	3.5 MPa for 1 year at 1 °C	Stable for 1 year. Lower changes in seed moisture, fatty acids and reducing sugars. Improved germinative capacity	[35]
Dressed cod	24.12 MPa for 21 days at 1 °C	Stable and consumable after 21 days contrarily to the samples stored at 0.1 MPa that were unacceptable	[6]
Pollock	24.12 MPa for 12 days at 1 °C	Stable and consumable after 12 days with higher quality than at 0.1 MPa	[6]
Mume fruit	0.5 MPa for 5 days at 5 °C (after high pressure treatment at 0.5–5 MPa for 10 min)	Stable for at least 5 days. Decreased weight loss and ethylene and CO_2 production	[1]
Mume fruit, sweet basil	0.5 MPa for 10 days at 4 °C	Stable for 10 days. Inhibition of discoloration and chilling injuries for mume fruit. Sweet basil exhibited browning injuries	[2]
Peach	0.414 MPa for 4 weeks at 4.4 °C	Decrease in total volatiles production	[51]
Tomato	0.1, 0.3, 0.5, 0.7 and 0.9 MPa for 5, 10 and 15 days at 13 °C	Decrease in respiration rate, weight loss and ripening	[14]
Room temperatures			
Pressures up to 1.0 MPa			
Sweet cherries	0.15 MPa for 4 h at 20 °C	Decrease in mould contamination (brown and total rots, grey and blue moulds)	[45]
Table grapes	0.15 MPa for 1 day at 20 °C	Reduction in infected berry and percentage of lesion diameter	[45]
Lettuce	0.1, 0.2, 0.4, 0.6 and 0.85 MPa for 3, 5 and 7 days at 20 °C	Weight loss and firmness similar to refrigeration for higher pressures. Faster lettuce decay (colour) under pressure than under refrigeration	[28]
Tomato	0.1, 0.3, 0.5, 0.7 and 0.9 MPa for 4 days at 20 °C	Lycopene synthesis inhibition during hyperbaric storage. No influence in total phenolic and ascorbic acid content	[29]
Tomato	0.1, 0.3, 0.5, 0.7 and 0.9 MPa for 4 days at 20 °C	Effective reduction in weight loss. Firmness conservation and retardment in ripening colour development	[31]
Pressures above 1.0 MPa			
Mushroom	3.5 MPa for 96 and 393 h at 20 °C	Reduction in moisture loss and browning. Larval forms growth inhibition	[44]
Tilapia fillets	203 MPa for 12 h at 25 °C	Improved freshness than those stored at 0.1 MPa. Microbial count reduction of about 2.0 Log CFU/g	[26]
Strawberry juice	25, 100 and 220 MPa for 15 days at 20 °C	Stable for 15 days under pressure and also more 15 days (0.1 MPa and 5 °C) after hyperbaric storage. Microbial load (yeast and moulds and total aerobic mesophiles) below the detection limits	[47]
Watermelon juice	100 MPa for 60 h at 18–21 °C	Inactivation plus inhibition of microbial growth up to 60 h. Extended shelf life at 0.1 MPa after hyperbaric storage	[13]
Melon juice	25, 50, 75, 100 and 150 MPa at 20, 30 and 37 °C for 8 h	Stable at all temperatures under pressures above 50 MPa. Microbial growth inhibition at 50/75 MPa and reduction plus inhibition at 100/150 MPa	[40]

production is lost due to the high costs inherent to the cooling process that less developed countries cannot afford [7]. In the food industry, about 50 % of the energy consumed is used for cooling, raising concerns of sustainability and environmental footprint [20, 47]. Additionally, many foods, as is the case of raw foods, are stored frozen. This process has even higher energetic costs and causes undesirable changes particularly in the texture of solid foods. Consequently, the use of HP technology for the preservation of foods during storage has attracted interest due to its potential energy savings [23, 47, 51]. The possibility of using this new preservation method- ology arose by chance, about 40 years ago when the Sub- marine Alvin sank to a depth of *1,540 m (*15 MPa and 4 °C) and then 10 months later, well-preserved foods (bouillon, sandwiches and apples) were recovered (the apples recovered showed a tyrosinase activity and pH that were half and the same, respectively, than fresh apples) [21]. This opened the possibility to store foods and other biomaterials at above atmospheric pressure to increase their shelf life as a possible enhancement of conventional refrigeration.

High pressure decreases the water-freezing point to below zero, making possible to store food at sub-zero temperatures without the textural changes associated with freezing and thawing processes [23]. In Japan, sub-zero hyperbaric storage tests showed very promising results; however, no substantial energy savings are achieved throughout storage, when compared to the conventional freezing method, since only the latent heat removal is avoided using this methodology [10, 23].

Further studies developed by Mitsuda et al. [35] and Charm et al. [6] evidenced the possibility to maintain food quality parameters for significant periods of time, using HP and low positive temperature combination. These and other studies [2, 14, 51] have led to an emerging new food preservation methodology called ‘‘hyperbaric storage'', meaning food storage (for its preservation by microbial growth inhibition) under pressure. Further recent developments revealed the possibility of food storage under pressure at room temperature (RT) and up to 37 °C, with higher energy savings since the energy requirements are only for the compression and decompression processes [40, 45, 47].

This new preservation methodology is expected to be commercialized in the next years. In this context, this review aimed to gather the scientific knowledge about food hyperbaric storage at sub-zero, refrigerated and RT, as an alternative/enhancement to the traditional frozen and refrigerated storage of foods. Storage at sub-zero is also included in this work. Table 1 compiles the scientific works that were found in the literature on this emerging research topic. Emphasis is given to food hyperbaric storage under naturally RT variation since this is expected to be a major research trend in the coming years.

Hyperbaric Storage at Sub-zero Temperatures

Using different pressure and temperature combinations, it is possible to shift the water-freezing point, reaching a -22 °C minimum at 209 MPa [38]. Therefore, it is possible to preserve food bellow 0 °C without freezing and thawing. These advantages were taken into account and led to the first studies regarding sub-zero hyperbaric storage [6].

In order to evaluate the sub-zero hyperbaric storage potential on strawberries and tomatoes, Deuchi and Hayashi [10] showed that catalase, b-amylase, cathepsin and lactate dehydrogenase activity is reduced under sub-zero hyperbaric conditions (200 MPa and -20 °C), but not inactivated as observed in freezing. The same inhibitory effect of pressure (-8 or -15 °C at 180 MPa) on enzymes activity (associated to nucleic acids degradation) in chicken and carp muscle was also observed by Mitsuda et al. [35]. The same author showed that enzymes remained active, despite their activity reduction under pressure. Enzymes related to nucleic acids degradation did not show differences between refrigeration and hyperbaric storage conditions, whereas freezing significantly reduced their activity. The evaluation of sub-zero hyperbaric conditions on microbial load in fish (cod fish and pollock), beef and chicken storage at -20, -3 and 1 °C at 0.1 and 24 MPa showed that storage under pressure remained stable at *4 Log CFU/g during storage, whereas those stored at 0.1 MPa revealed an increase (*1-3 Log CFU/g) [6].

Deuchi and Hayashi [8] evaluated the microbial load of ground beef under hyperbaric storage at sub-zero temperatures and higher pressures (-20 °C and 200 MPa) observing a microbiological load reduction (coliform, enterobacteriaceae, psychrotrophs, enterococci, lactic acid bacteria and yeast), which in some experiments was significantly higher than for freezing. Due to sub-zero hyper- baric storage inhibition of enzyme activity and microbial growth, the food products shelf life may increase. Charm et al. [6] showed, by an expert panel evaluation, that cod fillets stored at 22.8 MPa and -3 °C for 36 days appeared to have similar, or even better quality than those stored at -20 and -3 °C (at 0.1 MPa), respectively. On the other hand, the cod fish fillets stored at 1 °C and 0.1 MPa were unacceptable after 9 days while those at 24.12 MPa remained acceptable. Deuchi and Hayashi [9] (as reported by Kali- chevsky et al. [23]) showed that agar gels properties can be preserved at sub-zero and hyperbaric conditions (-20 °C e 200 MPa). The same author also showed that strawberry and tomato storage at pressures between 50 and 200 MPa and temperatures ranging from -5 to -20 °C for several days or weeks kept their fresh flavour and colour [8, 10]. It has been demonstrated also that raw pork storage under pressure can be effective, preventing the characteristic dripping of the defrosting process. In work developed by Ooide et al. [39] regarding chicken and carp muscles stored at – 8 or -15 °C and 170 MPa for 50 days, it was showed that meat texture can be preserved without significant protein denaturation.

In conclusion, combinations of sub-zero temperature and pressure can be used to extend food products shelf life by reducing the enzyme and microbial activity. In some cases, sub-zero hyperbaric storage is similar or more efficient than refrigeration and freezing since it allows avoiding the damages caused by freezing/thawing processes, and in some cases reduces microbial loads. How- ever, the enzyme activity observed under these hyperbaric conditions and after hyperbaric storage (indicate that no significant inactivation occurs) may be a limiting factor for shelf life extension, when compared to freezing [6].

Hyperbaric Storage at Refrigeration Temperatures

Charm et al. [6] studied the effect of two key enzymes related to food quality, trypsin and peroxidase, at temperatures of –3, 0, 4 and 23 °C, and pressures of 0, 27.6, 34.5 and 41.3 MPa. In general, the authors observed that the increase in temperature and pressure caused a decrease in the enzyme activity at constant pressure and temperature, respectively. For instance, peroxidase (POD) activity at 4 °C and 41.3 MPa decreased 25-30 % when compared to its activity at 0.1 MPa. However, trypsin activity increased or decreased when the pressure was increased at temperatures near 23 °C or below 4 °C, respectively. It was hypothesized that an enzyme has a critical temperature value below which the pressure reduces the reaction rate and above it increases the reaction rate [6].

In order to understand the feasibility of HP in food preservation, Jannasch et al. [21] studied organic matter degradation at a depth of 5,300 m (*53 MPa) using various carbon sources marked radioactively with ^{14}C, such as acetate, mannitol and amino acids. From this experiment, it was observed that their decomposition rates were 8-700 times slower at 5,300 m than in the laboratory at 3 °C and 0.1 MPa. In another experiment carried out by the same authors, the incubation of several carbon sources such as starch, galactose, peptone and albumin with mixed microbial populations and pure cultures placed at 5,300 m depth did not give rise to turbid cell suspensions (indicative of microbial growth), unlike the control samples at 3 °C and 0.1 MPa. Regarding the substrates, no significant bio- chemical changes were observed in the samples stored at 5,300 m. The authors stated that HP at low temperatures may have a higher inhibitory effect in the biochemical activity of microbial cells, when compared to low temperature alone [21]. Similar results were obtained by Charm et al. [6] who studied the effect of 24 MPa at 1 °C in cod fish fillets and whole pollock stability. They demonstrated that under pressure, the microbial load remained stable in pressurized samples, as previously mentioned. The samples stored under pressure were also classified by an expert panel with characteristics of fish stored for shorter periods than the real storage time (12 days stored pollock was classified as having been preserved only 6.7 and 21 days stored codfish as only 8.2 days). While pollock and codfish stored under pressure were considered acceptable for consumption after 12 and 21 days, respectively, they were unacceptable when stored at 0.1 MPa for the same periods. Mitsuda et al. [35] studied the storage of rice, wheat and soybeans at a depth of 30 m in a fresh water lake, for 1 year. With this experiment, the authors concluded that the biochemical changes on seed moisture, fatty acids, vitamin B_{12} and reducing sugars were less pronounced than those stored at 0.1 MPa.

Hyperbaric Storage of Fruit and Vegetables up to 1.0 MPa

Nowadays, there is a growing demand for fresh fruit and vegetables given their high concentration of functional compounds with benefits to human health [32, 50]. How- ever, these commodities provide a perfect environment for the survival and growth of spoilage microorganisms that when associated to their normal metabolic and respiration rate may jeopardize their shelf life [5, 22]. Well-known technologies such as cooling and modified/controlled atmosphere (CA) are efficient to lower the activity of these spoilage factors [41]. However, new technologies minimizing postharvest losses and deterioration inhibiting microbial growth are necessary.

Hyperbaric treatments applied to fruit/vegetable preservation consist of low pressure applications (ranging from 0.1 to 1.0 MPa) by compressing usually air to maintain cell structure, a very important feature of these products, to retain consumer acceptance to achieve market viability [15]. This subsection was included in this review even though this approach to the preservation of fruits and vegetables requires much lower pressures, when compared to those applied in the hyperbaric storage described in the next subsection.

To evaluate HP (0.1-0.5 MPa) at 4 °C on the postharvest life of fruit and vegetables, Baba et al. [2] studied the physical and physiological changes caused by these conditions in mume fruit (Prunus mume), sweet basil (Ocimum basilicum) and rocket-salad (Eruca sativa). The authors observed that storage at 0.025 MPa prevented fungal growth throughout the 2-month storage of rocket-salad leaves. A similar effect was observed in sweet cherries and table grapes stored under pressure at 0.15 MPa and 20 °C for 4 and 24 h, respectively, with a decrease in brown/total rots and grey/blue moulds for sweet cherries and a reduction in infected berries [45].

As stated before, fruits and vegetables are usually very perishable, reaching microbiological unacceptable levels in a matter of days. Liplap et al. [30] studied the effect of hyper-baric storage (0.1, 0.2, 0.4, 0.6 and 0.85 MPa) at 20 °C, for 7 days, on three bacterial species (Pseudomonas cichorri, Pectobacterium carotovorum and Pseudomonas marginalis).

The authors observed, by analysing the patterns in carbons source utilization, that under pressure, growth reached a lower maximum microbial load when compared to the con- trol. After the 7 days ofstorage, P. cichorrii was able to grow at 0.1 MPa and 20 °C following a pattern similar to that at ambient conditions while P. carotovorum and P. marginalis did not. Baba and Ikeda [1] studied the hyperbaric storage effect at 0.5 MPa for 5 days on previously HP-treated (0.5, 1, 3, 4 and 5 MPa for 10 min) mume fruit. After storage, when compared to controls, the fruit showed less of the damage typically associated with chilling such as skin pitting and browning. Hyperbaric storage also caused a decrease in ethylene and CO_2 production, probably due to the inhibition suffered by aminocyclopropanecarboxylate oxidase (ACC oxidase), an enzyme responsible for the conversion of ACC to ethylene. In the case of mume fruit stored for 10 days under pressure, chilling injuries such as skin pit- ting and browning were inhibited. In addition, no damage signals were observed after transferring to 25 °C and 0.1 MPa. In contrast, the sweet basil leaves exhibited browning injuries at 0.5 and 0.1 MPa, whereas a pressure of 0.025 MPa protected the leaves against chilling injuries [2]. Romanazzi et al. [45] also observed a reduction in the lesion diameter (from 8.7 to 7.2 mm) and in the percentage of infected berries (from 49.0 to 30.8 %) for table grapes stored under pressure (0.15 MPa). Lettuce was stored at 0.1, 0.2, 0.4, 0.6 and 0.85 MPa for 3, 5 and 7 days at 20 °C, and its quality was evaluated in terms of consumer's acceptance by an expert panel. It was observed that lettuce quality was mainly affected by storage temperature, since the refrigerated lettuce showed few quality changes during the 7 days of storage, while the pressurized one only remained in acceptable conditions for the first 3 days (with a maximum time of 5 days). The sensorial tests also showed that refrigeration was more effective in preserving the lettuce attributes, followed by storage at 0.85 MPa and 20 °C [28].

Yang et al. [51] studied hyperbaric storage (0.414 MPa) of peach (Prunus persica L.) volatiles with and without (CA, 3 kPa O_2 and 7 kPa CO_2) and ultraviolet (UV) pre- treatment (1.5 min on each side) for 4 weeks at 4.4 °C identifying a total of 21 and 59 compounds before and after storage, respectively. When HP was combined with CA (HP ? CA), the total volatiles and ester concentration were significantly reduced. The author speculated that hyper- baric storage appears to reduce ester biosynthesis, possibly
by decreasing alcohol acyltransferase activity, an enzyme regulated by ethylene. These preliminary results indicate a possible undesirable effect of HP storage on the total volatiles concentration of peach, when applied after harvest, since odour is an important characteristic to consumers. A study of poststorage potential for recovery of normal synthesis and concentration is needed in order to evaluate this new concept application for fruit storage.

To explore the feasibility of tomato storage under pressure, Goyette et al. [14] determined the pressure effect (0.1, 0.3, 0.5, 0.7 and 0.9 MPa) at 13 °C (relative humidity 95 %) for 5, 10 and 15 days. They observed that the increase in pressure led to a respiration rate decrease, with a maximum reduction at 0.9 MPa (22 %). In addition, the weight loss was less pronounced in the pressurized samples than in control samples at 0.1 MPa. In another study of tomato hyperbaric storage (0.3, 0.5, 0.7 and 0.9 MPa) at 20 °C for 4 days, the same pattern was observed with respect to the respiration rate reduction with the pressure level increase. However, the respiration rate of the pressurized samples at 20 °C was higher than the one stored at 13 °C and 0.1 MPa and, in some cases, higher than at 20 °C and 0.1 MPa [31]. Tomatoes stored under pressure at 20 °C also showed higher respiration rates than those studied by Goyette et al. [14], stored under the same conditions at 13 °C. These results show that contrarily to pressure, the temperature increase leads to higher respiration rates, being this an important factor regarding fruit and vegetable storage under hyperbaric conditions. Pressure also reduced the weight loss during the storage period, when compared to controls. In other studies with lettuce and mume fruit, hyperbaric storage led to a reduction in the respiration rate of the former and a reduction in the weight loss for both commodities with the increase in pressure [1, 28].

The hyperbaric storage of tomato at 13 °C reduced the ripening process, and this hypothesis was supported by higher hue values (a high hue of 180° represents a pure green, and a low hue value of 0° represents a pure red), i.e., hyperbaric storage (0.3, 0.5, 0.7 and 0.9 MPa) was most efficient in retarding the red colour development when compared to control [14]. Liplap et al. [31] also studied the evolution of tomato colour parameters with results similar to those obtained by Goyette et al. [14]. The same effects were observed for refrigerated tomatoes. The hyperbaric storage of lettuce and mume fruit was also more efficient in retaining the colour quality, when compared to controls [1, 2, 28]. The hyperbaric storage of lettuce was also efficient in retarding the chlorophyll loss, however, with less effi- ciency than refrigeration [28].

Regarding tomato firmness, it was observed that the HP conditions (0.3, 0.5, 0.7 and 0.9 MPa) allowed to maintain its initial firmness for a longer period of time, i.e., the decrease in firmness was less accentuated in hyperbaric condition than at 0.1 MPa [14, 31]. However, after ripening (posthyperbaric storage period), no significant differences were observed between the different conditions [31].

Titratable acidity (TA) reduction is related to the respi- ration process and thus associated to a faster ripening of a commodity. Goyette et al. [14] observed that tomatoes kept under pressure (0.3, 0.5, 0.7 and 0.9 MPa) for 10 and 15 days of storage had a lower TA value than the control (0.1 MPa, 13 °C). Similar results were observed by Liplap et al. [31] showing that refrigeration is more efficient in retarding the tomato-ripening process. Relatively to total soluble solids (TSS), the tomatoes samples stored under pressure showed a lower value than the control [14, 31] . The ratio TA/TSS (used to describe the tartness and taste of fruit and vegetables) was not significantly different between the pressurized and unpressurized tomatoes [14]. Different results were reported by Liplap et al. [31] for TA/TSS, with a lower value being observed for refrigerated samples. However, all conditions yielded a ratio over 12.5, which make them marketable.

Liplap et al. [29] studied the effect of the hyperbaric storage of tomato at 0.1, 0.3, 0.5, 0.7 and 0.9 MPa and 20 °C for 4 days on the lycopene, phenolic and ascorbic acid contents, and on the antioxidant activity, and com- pared the results with the samples at 0.1 MPa and at 13 and 20 °C. Also assessed were the effects of these conditions on the poststorage for 10 days (summing up 14 days). In general, the authors found that the hyperbaric storage and refrigerated conditions inhibited the synthesis of lycopene when compared to control obtaining results similar to those obtained by Goyette et al. [14]. However, this process causes an increase in lycopene content in poststorage period, reaching a maximum at 0.9 MPa and a minimum at 13 °C and 0.1 MPa (27.17 and 19.12 mg of lycopene/ 100 g fresh weight, respectively). Nonetheless, none of these conditions showed significant effects on overall phenolic and ascorbic acid contents after the storage and ripening period, except for the tomatoes stored at 13 °C that presented a lower content than samples stored at 20 °C and 0.9 MPa.

These results showed that a complex diversity of behaviours can be observed when hyperbaric storage is applied to different fruits and vegetables commodities and the optimal conditions to preserve them might be different depending on the commodity. This preservation technology appears efficient in retarding fruit and vegetables ripening and deterioration processes in order to maintain its marketability for equal or longer periods than refrigeration.

Hyperbaric Storage at RT Above 1.0 MPa

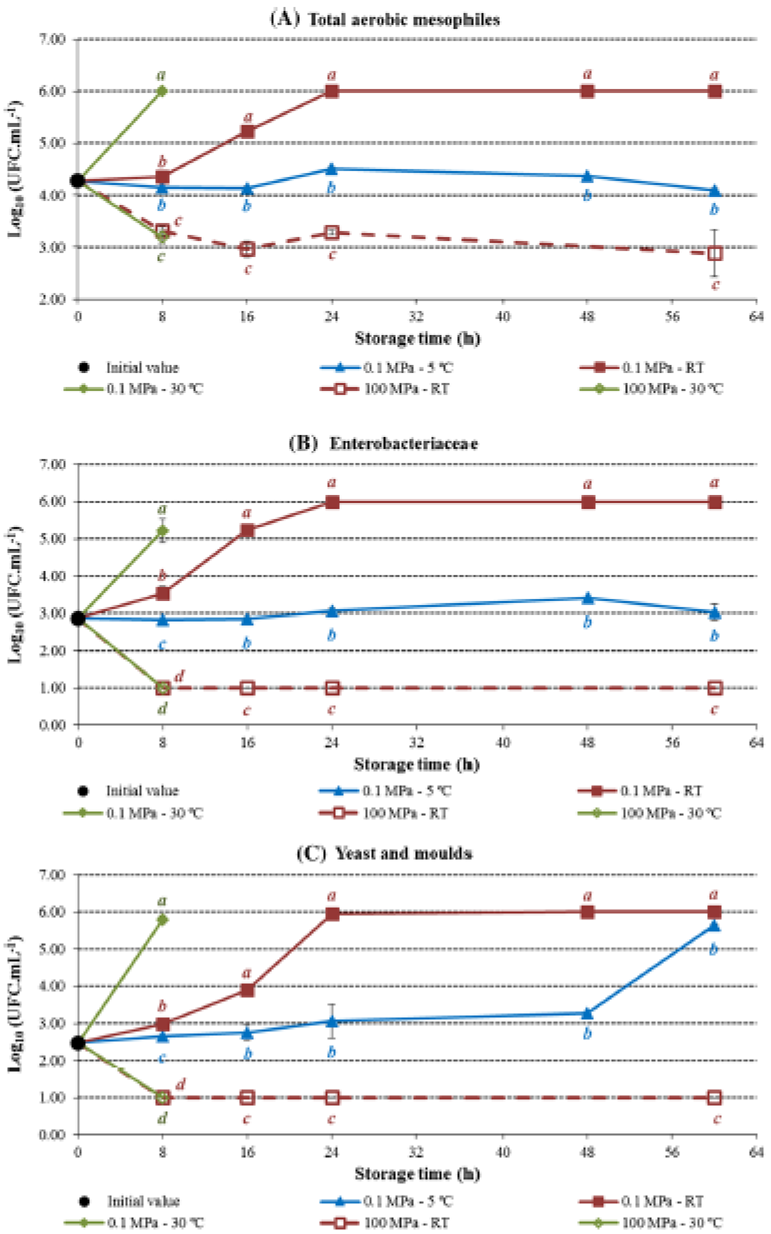
Recent studies on the hyperbaric storage at and above RT reflect an interest in its possible energy savings [13, 40, 47].

The first hyperbaric storage study at RT achieved by com- pressing air was carried out by Robitaille and Badenhop [44]. These authors studied the hyperbaric storage effect, at 35 atm (*3.6 MPa) and 20 °C with an atmosphere com- posedbyO_2,N_2andCO, on the moistureloss, respiration rate and quality of mushrooms. In general, the authors observed that mushrooms stored at 3.6 MPa and 20 °C for 96 and 393 h showed a lower moisture loss and browning extent when compared to those stored at 0.1 MPa. However, pressure was found to have no effect on respiration rate. Larval growth occurred during the storage of control samples while in mushrooms stored under pressure larval development was observed only 1 week after depressurization and subsequent storage at 0.1 MPa.

A similar inhibitory effect on rotting agent growth was observed by Ko and Hsu [26]. Tilapia fillets stored at 101 MPa for 12 h at 25 °C, maintained the total plate counts at 4.7 Log CFU/g of meat, a value similar to the initial, while fillets stored at 203 MPa showed a reduction to 2.0 Log CFU/g of meat (the same effect was also observed for psychrophilic bacteria). The same authors also evaluated the K value (a freshness quality index that indicates putrefaction when its value is above 60 %). Tilapia fillets stored at 203 MPa showed a higher freshness than controls (K value below 40 % and up to 92 %, respec- tively). When the same authors studied the effect of a posthyperbaric storage period of 12 h at 25 °C, they observed that the inhibitory effect caused by pressure on enzyme activity and microbial growth was not caused by microbial inactivation, since during posthyperbaric storage, enzymes were active and microorganisms could grow. This reveals a pressure reduction in the enzyme activity and microbial growth, but no inactivation.

There are only three recent studies regarding hyperbaric storage at RT and above on fruit products (fruit juices). Segovia-Bravo et al. [47] compared the hyperbaric storage of raw strawberry juice at RT (20 °C) at 25, 100 and 220 MPa for a period of 15 days, with raw juice at 20 and 5 °C as well as pasteurized juice at 5 °C. One year later, Fidalgo et al. [13] evaluated hyperbaric storage (100 MPa) at naturally uncontrolled RT conditions (18-21 °C) and above (30 °C), for 60 and 8 h, respectively, of watermelon juice. Queirós et al. [40] studied the hyperbaric storage (25-150 MPa) at and above RT (25, 30 and 37 °C) during 8 h on melon juice. In the case of the strawberry juice, the control kept at 20 °C and 0.1 MPa showed that the microbial load increased by more than 3 Log units for total aerobic mesophiles and yeasts/moulds, presenting unpleasant smell and gas production after 15 days. In contrast, the juice stored at 5 °C showed a 2 Log units increase in the total aerobic mesophile counts while the pasteurized juice and the juice stored under pressure showed a microbiological load with values below the detection limits, at all the pressures

Fig. 1 Total mesophiles (**a**), *enterobacteriaceae* (**b**) and yeast and moulds counts (**c**) (expressed in Log CFU/mL of watermelon juice). Juice stored during 8, 16, 24, 48, and 60 h at atmospheric pressure and refrigerated temperature (0.1 MP/5 °C), atmospheric pressure and RT (0.1 MPa/ 18–21 °C), atmospheric pressure and above RT (0.1 MPa/30 °C), hyperbaric storage at RT (100 MPa/ 18–21 °C), and hyperbaric storage above RT (100 MPa/ 30 °C). Values, shown as 6 and 1 Log units, are meant to be higher than 6 and lower than 1 Log units, respectively. Used from Fidalgo et al. [13] with permission



tested [47]. Similar results were observed by Fidalgo et al. [13]. As seen in Fig. 1, watermelon juice stored at 0.1 MPa at variable RT (18-21 °C) and above (30 °C) presented a high microbial load increase in the first 24 and 8 h, respectively (from 4.28, 3.00 and 2.50 to above 6.00 Log CFU/mL of juice for total aerobic mesophiles, enterobac teriaceae and yeast/moulds, respectively), which resulted in the development of an unpleasant odour and strong off- flavours. On the other hand, the microbial load of juice stored under refrigeration conditions (0.1 MPa and 5 °C) remained similarto the initial value, except for yeast/moulds (increased from 2.5 to around 5.0 Log CFU/mL ofjuice after the storage period) showing its inhibitory effect on micro- bial growth. Nonetheless, it is noteworthy that the juice stored under pressure, at variable RT or above (30 °C), showed a microbial load decrease in the first 8 h of storage.

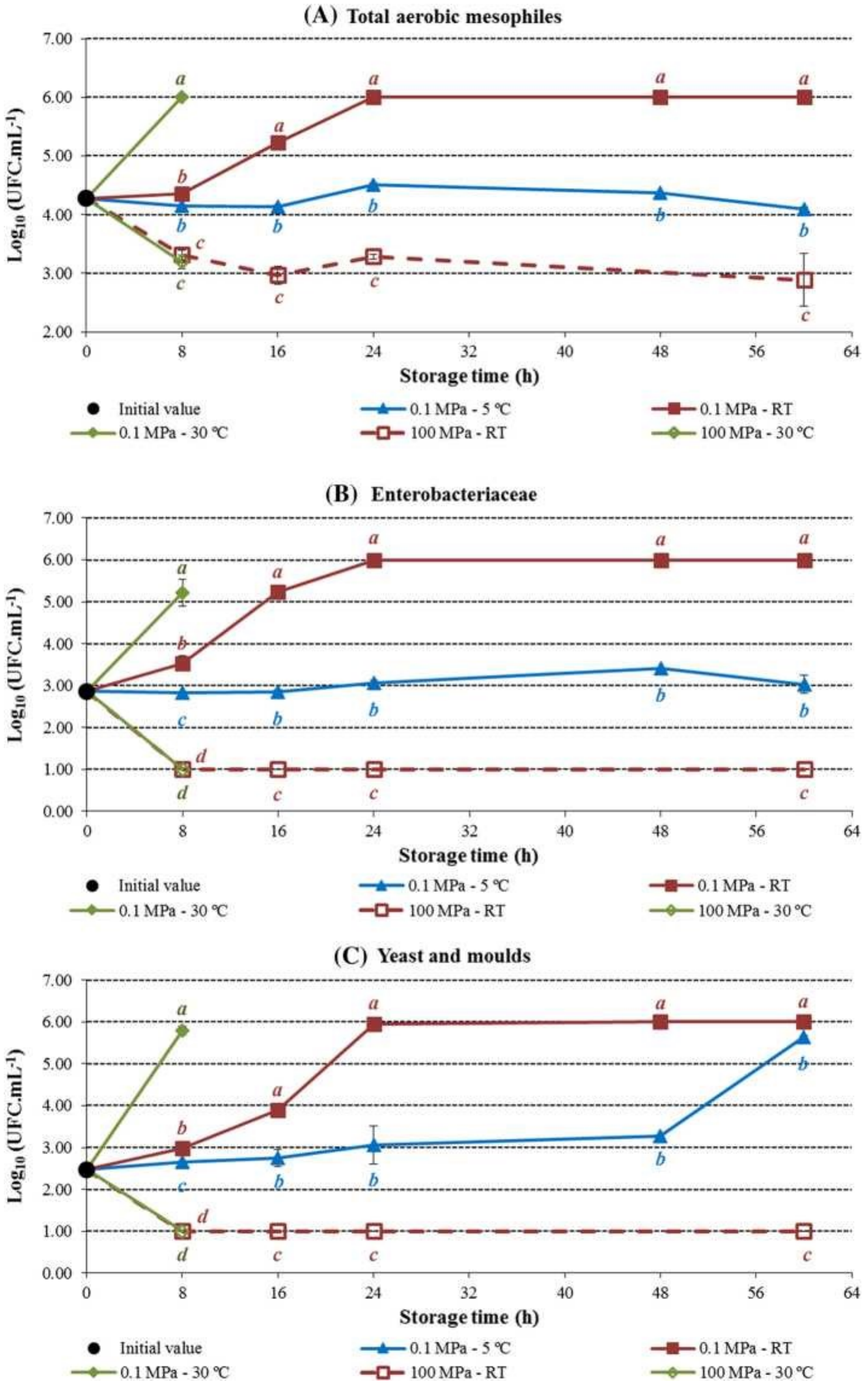
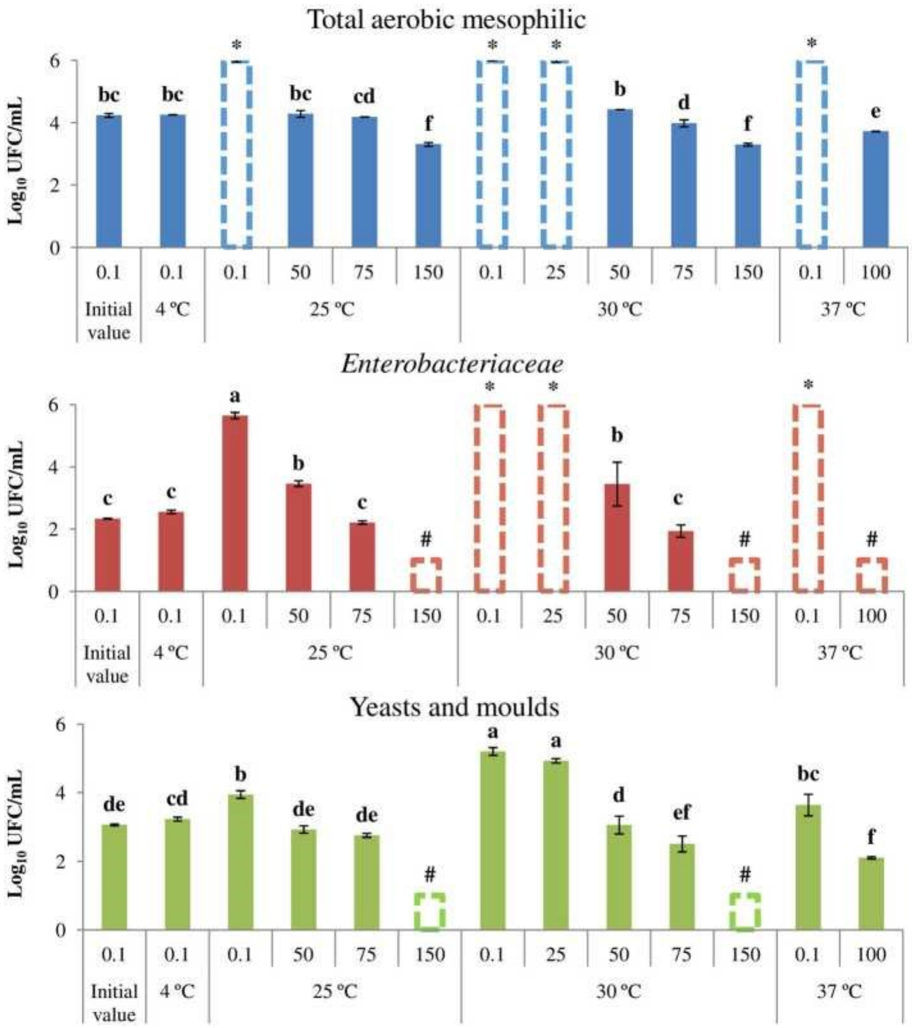


Fig. 2 Total aerobic mesophiles, enterobacteriaceae and yeasts/moulds counts (expressed in Log_{10} CFU/mL of melon juice) at different pressure and temperature storage conditions. Bars with asterisk and hash (unfilled and with discontinuous borders) are indicative of higher than 6 and lower than 1 Log_{10} CFU/mL, respectively. Different letters between (a-f) indicate significant differences (p \ 0.05). Used from Queiros et al. [40] with permission



(down to *3.0 and \1.0 Log CFU/mL of juice for total aerobic mesophiles and both enterobacteriaceae and yeast/ moulds, respectively) which remained stable along the remaining storage period, resulting on an odour of fresh raw watermelon juice.

In another study, melon juice storage under pressure was also more stable when compared to samples kept at 0.1 MPa. Figure 2 shows that pressures of at least 50 MPa are necessary to have an efficient microbial growth inhibition of total aerobic mesophiles, enterobacteriaceae and yeast/moulds. Pressures of 50 and 75 MPa resulted in similar microbiological counts when compared to refrigeration (although some inactivation was observed for enterobacteriaceae and yeast/moulds), while juice at 100 and 150 MPa showed a reduction in the initial microbiological load (of about 0.5-2.0 Log CFU/mL of juice for total aerobic mesophiles, enterobacteriaceae and yeast/ moulds) resulting in better results when compared to samples stored under refrigeration [40].

Relatively to posthyperbaric storage for 15 days (0.1 MPa, 5 °C), strawberry juice showed no microbial growth in any pressurized or pasteurized juices, while the microbial load in the juice kept only under refrigeration increased [47]. Similarresults were observed for watermelon juice, since after 7 days (0.1 MPa, 5 °C) of posthyperbaric storage the total mesophiles and enterobacteriaceae loads remained stable, while yeast and moulds grown from\1.0 to 3.57 Log CFU/mL of juice. Contrarily, the juice stored only under refrigeration presented higher microbial loads that tended to increase along storage time [13]. In the case of strawberryjuice, Segovia-Bravo et al. [47]also observed that the pasteurized juice maintained its viscosity stable, while the pressurized ones had less pronounced viscosity decay than the refrigerated.

Viscosity and cloudiness changes in fruit juices are associated to pectin methylesterase (PME) and polygalac- turonase (PG) activity, which can be affected either by HP and/or temperature. Segovia-Bravo et al. [47] showed that viscosity of pasteurized strawberry juice had an initial value 50 % lower than the other juices due to pectin degradation by heat. Along the 15 days of storage, it was possible to observe a faster decay in the juice stored at RT (with evident phase separation), followed by samples kept under pressurized and refrigerated conditions. For all samples kept under pressure, an attenuation of the viscosity decay was verified when the pressure was increased, which indicates that the enzymes responsible for pectin degradation might have lower activity under pressure. Refrigeration was considered more efficient than hyperbaric storage to slow down viscosity decay but only for previously pasteurized samples. Similar results were observed for watermelon and melon juices, where cloudiness of samples stored under pressure was closer to the juice initial value. Concerning TA and browning degree evolution, it was concluded that pressure attenuated the increase in the for- mer and the decrease in latter [13, 40].

Juice colour was also studied by Segovia-Bravo et al. [47] and Fidalgo et al. [13] for strawberry and watermelon juice, respectively, since is one of the most important quality parameters to consumers reflecting mainly the anthocyanins present in the fruit. It was observed that the pasteurized strawberry juice colour remained unchanged over the 15 days of storage and for the remaining samples stored at 0.1 MPa, the colour decay was faster than in the pressurized juice, as expected. No significant colour degradation was observed in samples stored under pressure, which indicates a slower anthocyanin degradation that could be related to polyphenoloxidase, POD and b-glucosidase inactivation or lower activity under pressure [47]. Contrarily, Fidalgo et al. [13] demonstrated that pressure caused higher colour changes for pressurized samples of watermelon juice than in Controls kept at 0.1 MPa (5 °C and RT). These divergences could be related to the different pH values between straw- berry and watermelon juices.

These results show the potential of hyperbaric storage, at and above RT, to extend the shelf life of highly perishable fruit juices, causing microbial inactivation and microbial growth inhibition. In addition, it is noteworthy that this is achieved without significant energy costs, when compared to refrigeration, since energy is required only during the compression/decompression phases to generate the pressure and for decompression with no need for temperature control.

Conclusions

Although the first hyperbaric storage experiments were carried out about 40 years ago, only recently economic and technological obstacles were overcome to allow for more extensive and deeper studies. As often mentioned in this review, hyperbaric storage is an emerging and distinctive HP technology application with the potential to promote sustainability and reduce environmental footprints. Low temperature and sub-zero hyperbaric storage should also be taken into account, especially the latter given the disadvantages associated with their freezing and thawing of fruits and vegetables, particularly their impact on the products texture. Hyperbaric storage at RT has significant potential, not only due to its efficiency in food preservation, but also due to the energy savings that could be possibly achieved. Hyperbaric storage at RT can inhibit the microbial growth and, in some conditions, even reduce the microbial load. This new preservation methodology opens a whole new range of opportunities being the development of industrial and domestic hyperbaric storers, one of the mostinteresting. However, it is necessary toknowthe effect that these conditions mighthave on different food matrices and its advantages over the traditional preservation techniques requiring a very significant increase of research in this area. If shown to be effective and commercially viable, hyperbaric food preservation at variable (uncontrolled) RT may become a disruptive evolution.

Acknowledgments Thanks are due to Fundação para a Ciencia e a Tecnologia (FCT, Portugal), European Union, QREN, FEDER, COMPETE for funding the Organic Chemistry Research Unit (QOPNA) (projectPEst-C/QUI/UI0062/2013;FCOMP-01-0124-FEDER-037296).

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This is a post-peer-review version of an article published in Food Engineering Reviews, 2015 following peer review. The version of record Fernandes, P., Moreira, S., Fidalgo, L., Santos, M., Queirós, R., Delgadillo, I. & Saraiva, J. (2015). Food preservation under pressure (Hyperbaric Storage) as a possible improvement/alternative to refrigeration. Food Engineering Reviews, 7(1), 1-10. https://dx.doi.org/10.1007/s12393-014-9083-x