HYPERBARIC STORAGE AT ROOM TEMPERATURE FOR FOOD PRESERVATION: A STUDY IN STRAWBERRY JUICE

Segovia-Bravo, K. A.; Guignon, B.; Bermejo-Prada, A.; Sanz, P. D. and Otero, L.*

Malta Consolider Team.

Department of Processes, Institute of Food Science, Technology and Nutrition (ICTAN-CSIC)

c/ José Antonio Nováis, 10, 28040 Madrid, Spain

ABSTRACT

Hyperbaric storage at room temperature was evaluated as a new food preservation method. To do that, strawberry juices maintained at different pressure levels (0.1, 25, 100 or 220 MPa) and 20 °C for 15 days were compared to raw and thermally pasteurized samples stored at atmospheric pressure and 5 °C for the same period. Hyperbaric storage reduced the initial microbial load of the juices by more than 2 log units to levels below the limit of detection. Moreover, pressure was effective to attenuate viscosity and color losses in the samples stored at 20 °C. Stability of the samples after the hyperbaric storage was good and microbial load, viscosity and color remained stable when the samples were kept under refrigeration at atmospheric pressure for 15 additional days. All these results show that hyperbaric storage could represent an interesting technology for short-term preservation of food.

Keywords: high-pressure; food preservation; hyperbaric storage; strawberry juice

1. INTRODUCTION

The effectiveness of hyperbaric storage for food preservation has been known for more than four decades. Thus, the first clear evidence was the recovery, in 1968, of edible food from the research submarine Alvin sunk for ten months at a depth of 1540 m. Sandwiches, bouillon and apples, maintained at environmental conditions of 3-4 °C and 15 MPa, were practically untouched by decay, but when they were recovered and kept under refrigeration at atmospheric pressure, they were spoiled in a few weeks (Jannasch, Eimhjellen, Wirsén, & Farmanfarmaian, 1971). Successful experiments of underwater storage of cereal grains, at 30 m below the surface of Lake Biwa, had been previously made in Japan since May 1967 (Mitsuda, Kawai, & Yamamoto, 1972) with the main purpose of maintaining a constant low temperature during the storage. Ten years later, Charm et al. (1977) proposed the refrigerated storage of food under pressure as a new method for food preservation. The authors proved that pressure, at low temperatures, inhibited microbial growth and enzymatic activity and, in this way, it substantially extended the storage life of highly perishable food, like fish or meat. Moreover, pressure lowers

* Corresponding author. Tel.: +34 91 544 56 07; fax: +34 91 549 36 27.
E-mail address: l.otero@ictan.csic.es (L. Otero).

the freezing point of water and, therefore, it allowed storage temperatures below 0 °C without freezing. Nevertheless, the technical difficulties in building commercially feasible high-pressure storage units made that this preservation method had not been extensively studied. Thus, in the last three decades, only a few experiments of food storage under pressure have been reported at different pressure levels and temperatures both above and below 0 °C (Baba & Ikeda, 2003; Baba, Ito, Ikeda, & Manago, 2008; Kalichevsky, Knorr, & Lillford, 1995; Robitaille & Badenhop, 1981; Yang, Balandran-Quintana, Ruiz, Toledo, & Kays, 2009). The results obtained indicate that refrigerated storage of food under pressure could be an effective preservation technique with interesting advantages over freezing. Thus, damage in the product due to ice crystals formation is avoided and substantial energy savings can be achieved since the storage temperature can be set considerably higher and no latent heat needs to be removed (Charm et al., 1977). But, more important energy savings could be attained if food preservation under pressure were proved to be also efficient at room temperature.

It is well known that immediate refrigeration of raw food ingredients, just after harvest, fishing or butchery, and their storage and transportation in a low temperature environment are required to provide safe food products of high organoleptic and nutritional quality. Nevertheless, less than 10% of perishable foodstuffs are in fact currently refrigerated. This involves losses which account for 30% of worldwide production (Coulomb, 2008). It is estimated that if developing countries could acquire the same level of refrigerated equipment as that in industrialized countries, over 200 million tons of perishable foods would be preserved, but economic reasons hamper food refrigeration (James & James, 2010). In industrialized countries, cold chains are designed and maintained from the producer to the retailer to preserve the quality and safety of foods. Thus, refrigeration is used both for short-term preservation of raw food and also for long-term preservation of processed products. But, the effective management of these cold chains is expensive and energy consuming. As an example, about 50% of total energy in the food industry is consumed by refrigeration related facilities. Nowadays, this can jeopardize the sustainability of the food supply chains. For this reason, in the last years, many efforts have been made to look for new energy saving opportunities in the food cold chain.

Hyperbaric storage, at room temperature, could be one of these opportunities since it only implies energy costs during compression and no additional energy is required to maintain the product under pressure for long times. Therefore, hyperbaric storage of food commodities in industrial size vessels, at room temperature, could involve important savings in storage and distribution. Capital costs of current high-pressure equipment are high, but it is important to note that pressure requirements and technical specifications implied in hyperbaric storage should be considerably less severe than those now employed in the food industry for high-pressure processing. Moreover, the exponential expansion that high-pressure food processing has experimented during the last decade should help to enhance innovations to reduce equipment costs. In fact, innovations performed during the last years in equipment design have made possible a decreasing trend in equipment costs from 1996 up to now (Balasubramaniam,

Farkas, & Turek, 2008). The identification of new high-pressure applications with proven advantages over current technologies will also contribute to this aim (Balasubramaniam et al., 2008; Hogan, Kelly, & Sun, 2005).

The objective of this paper is to compare the hyperbaric storage of food at room temperature, as a new preservation technique, to refrigeration, a well-established method. To do that, storage experiments were carried out at different pressures (0.1, 25, 100 and 220 MPa) and 20 °C in raw strawberry juice, a simple liquid matrix appropriate for these initial experiments and widely employed as a food ingredient. After 15 days of storage, some safety and quality attributes (microbial population, viscosity and color) were measured and compared to those of raw and pasteurized juice samples stored at atmospheric pressure and 5 °C for the same period. The stability of the samples after decompression was also studied to know if the juice should be immediately processed or consumed after the hyperbaric storage or if it remains stable, in refrigeration, for some time. All the results obtained in this paper can provide important information to evaluate the viability of hyperbaric storage at room temperature for food preservation.

2. MATERIALS AND METHODS

2.1. Sample

Strawberries (*Fragaria x ananassa* Duch., cv. Chandler) were purchased at commercial maturity from a local supplier. The fruits were washed with tap water and processed with a Moulinex Frutti Pro juicer (Moulinex, France). The liquid obtained was then centrifugated at 3500 g and 7 °C for 10 min in a Sorvall Evolution RC Superspeed centrifuge (Thermo Scientific, Madrid, Spain) using a Fiberlite F8-6x1000y rotor (Thermo Scientific, Madrid, Spain). The supernatant was subsequently collected, filtered through a 0.1 mm pore diameter sieve and bottled. This juice was frozen and stored at -80 °C until utilization.

2.2. Physicochemical analyses in the raw material

Before each experiment, a frozen batch of strawberry juice was thawed overnight at 5 °C. This juice was then characterized by measuring some of its physicochemical properties (see Table 1). Soluble solids concentration (°Brix) was approximated by using a digital refractometer (Leica AR200, Leica Microsystems Inc, New York, USA) with automatic temperature compensation. pH was measured with a pH-meter (pH-Burette 24 1S equipped with a pH 50 21 electrode and a C.A.T. 55 31 temperature sensor, Crison Instruments, Barcelona, Spain). Density was determined by the vibrating tube technique with a DMA 5000 density-meter (Anton-Paar GmbH, Graz, Austria). Viscosity and color were estimated as described in the next sections. All these measurements were performed in triplicate for each thawed batch of juice employed in each experiment. Data in Table 1 are mean and standard deviation values calculated from the results obtained in all the experiments.

2.3. Storage experiments under pressure

Experiments were carried out in a pilot-plant high-pressure storage system V1 (Institute of High Pressure Physics, Unipress Equipment Division, Poland). It was composed of two high-pressure stainless steel vessels with independent pressure control, two control terminals and a high-pressure pump BP3 (Institute of High Pressure Physics, Unipress Equipment Division, Poland). Each high-pressure vessel was located in an individual thermostatic chamber and was connected, via a feed-through in the chamber wall, to its control terminal by means of a high-pressure capillary tube. Both vessels had 100 mm internal diameter, 130 mm height and a working volume of 1 L. The high-pressure pump was able to reach a maximum pressure of 250 MPa and was commanded by a programmable controller with a control panel. A mixture of propylene glycol and water (44% v/v) was used as compressing fluid.

Temperature was measured in each pressure vessel by a metal sheathed thermocouple, type T, located at its geometric center. Pressure produced in the high-pressure intensifier was monitored by a pressure transducer (0-400 MPa, SH-1, WIKA, Germany). All sensor measurements were recorded every 30 s by a data acquisition system (MW100 Data Acquisition Unit, Yokogawa Electric Corporation, Tokyo, Japan).

Storage experiments for 15 days under pressure were performed at 20 °C and three different pressure levels (25, 100 and 220 MPa) to obtain samples labeled as SUP_25, SUP_100 and SUP_220, respectively. After compression, temperature in SUP samples increased by 1-4 °C, depending on the pressure level applied. In all the cases, the target temperature was subsequently achieved in no more than 15 minutes.

Control juices (C samples) were maintained at 0.1 MPa and 20 °C for the same period. All the samples were stored in 50 mL polypropylene Falcon tubes. The tubes were completely filled with the strawberry juice and closed with screw caps sealed by a nitrile rubber O-ring.

2.4. Refrigerated storage at atmospheric pressure

Raw (R_R samples) and heat pasteurized (R_P samples) strawberry juices were placed in 50 mL polypropylene Falcon tubes and stored at 5 °C and atmospheric pressure for 15 days. Samples to be pasteurized were thermally treated in a water bath at 90 °C until the temperature at the core was maintained at 85 °C for 90 s. Once processed, the juice was immediately cooled in an ice-water bath.

2.5. Stability of the strawberry juices after the hyperbaric storage

After 15 days of hyperbaric storage at 20 °C, SUP samples were decompressed and stored at 5 °C for 15 additional days to assess the stability of the product after the storage under pressure. R_R and R_P samples were also stored at 5 °C for fifteen more days to make comparisons.

2.6. Microbial analysis

Ten-fold serial dilutions of the strawberry juice samples were prepared in buffered peptone water. Total aerobic counts at 30 °C were determined, following the standard method AFNOR NF V 08-051, by the pour plate method in plate count agar (PCA). Plates were incubated at 30 ± 1 °C for 72 ± 3 h and the colonies formed were counted. Yeasts and moulds were enumerated on Sabouraud Chloramphenicol Agar (SCA) medium by the surface spread plate method, according to the standard method AFNOR XP V 08-059. SCA plates were incubated at 25 ± 1°C for 5 days and the colonies of yeasts and moulds were counted. Data were expressed as log₁₀ cfu/ml. The detection limits were 10 cfu/ml for aerobic total counts and 100 cfu/ml for yeasts and moulds.

2.7. Viscosity measurement

The kinematic viscosity of the samples was determined using a Cannon-Fenske reverse-flow glass capillary viscometer (N° 150, Fungilab S.A., Spain), immersed in a thermostatic water bath (Thermocap, Fungilab S.A., Spain) tempered at 40 ± 0.05 °C. The efflux time was measured manually using a digital stopwatch (Oregon Scientific TR118, Oregon Scientific, Spain). The kinematic viscosity (ν), expressed in centiStokes (1 cSt = 10⁻⁶ m²·s⁻¹), was calculated from the efflux time (t) and the viscometer calibration constant (C) at 40 °C, provided by the manufacturer, by using the following equation:

$$\nu = t \cdot C \quad (1)$$

2.8. Color measurement

Strawberry juice color was characterized objectively according to the L*, a*, and b* color parameters in the CIELab uniform color space defined by the Commission Internationale d'Eclairage. A CM-3500d spectrophotometer managed by the color data software CM-S100w SpectraMagic™ (Konica Minolta, Japan) was used for this purpose. The spectrophotometer operated in the reflectance specular included mode with an aperture size of 8 mm in diameter. Measurements were made with the D65 standard illuminant and the ultraviolet component of the illumination was included. Illuminating and viewing configurations complied with the CIE diffuse/8° geometry. The instrument was calibrated with black and white (No. 14671004) standards before each series of analysis. To make the measurements, a glass Petri-dish (42 mm internal diameter) was filled with 10 mL of juice, closed with its cap and covered by the black standard (a closed cylindrical tube with black walls). In each Petri-dish, five measurements were performed: one at its center and four at radial positions distributed 90 degrees apart and the obtained L*, a* and b* values were averaged. From these mean values, the total color change ΔE^* , hue angle h° and chroma C* parameters were also calculated. The formulas for their calculation are given below:

$$\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (2)$$

where L_0^* , a_0^* and b_0^* are values for the raw juice (Table 1).

$$h^{\circ} = \arctan\left(\frac{b^{*}}{a^{*}}\right) \quad (3)$$

$$C^{*} = \sqrt{a^{*2} + b^{*2}} \quad (4)$$

2.9. Statistical analysis

All the storage experiments were performed, at least, in triplicate and all the analyses in each sample obtained were also done in triplicate. From these data, means and standard deviations were calculated for each storage method.

The results were statistically analyzed using IBM SPSS Statistics v. 19.0.0 for Windows (SPSS Inc., Somers, NY, USA). After a one-way analysis of variance (ANOVA), significant differences among means ($P < 0.05$) were determined by Tukey's multiple range test.

3. RESULTS AND DISCUSSION

3.1. Stability of the strawberry juices during the hyperbaric storage

3.1.1. Microbial load

The mean initial populations of total aerobic mesophiles and yeasts and moulds in the raw strawberry juice were 2.9 and $< 2.6 \log_{10}$ cfu/ml, respectively (Table 1). Thermal pasteurization at 85 °C for 90 s reduced both population levels below the detection limits.

After 15 days of storage at 20 °C, microbial growth was evident in the control juices maintained at 0.1 MPa as expected: both total aerobic plate counts and yeasts and moulds increased by more than 3 $\log_{10}$ units. C samples were spoiled and off flavors, unpleasant odors and gas production were detected in them. On the contrary, all the samples stored under pressure (25, 100 or 220 MPa) at 20 °C reduced their natural microflora below the detection limits. The pressure level employed during the storage did not have a significant effect on the microbial load after 15 days.

On the other hand, refrigerated storage for 15 days at 5 °C was hardly efficient to slow down the microbial growth in the raw juices stored at atmospheric pressure. Thus, total aerobic plate counts increased by more than 2 $\log_{10}$ units in R_R samples as compared to 3 $\log_{10}$ units in C samples. Refrigeration, unlike hyperbaric storage, was not effective to avoid microbial growth in raw juices and thermal pasteurization was needed to obtain stable strawberry juices for 15 days at 5 °C (Table 2).

It is widely recognized that high hydrostatic pressures, between 10 and 100 MPa, are generally nonlethal for those microorganisms adapted to atmospheric conditions, but they exert adverse effects on them and reduce their growth (Abe, 2007; Abe & Horikoshi, 2000; Bartlett, 2002; Matsumura, Keller, & Marquis, 1974). The inhibitory effect of pressure on the growth of the natural microflora present in different food products was already reported by Charm et al. (1977). These authors did not find any increase of the total bacteria counts in cod fillets, beef

and chicken samples stored at 27 MPa and temperatures close to 0 °C for 10 to 60 days. Similar results were found by Deuchi & Hayashi (1992) in unfrozen ground beef stored under pressure (50-200 MPa) at subzero temperatures for a few days or weeks. Thus, coliforms, *Enterobacteriaceae*, Gram (-) and Gram (+) psychrophiles, *Enterococci* and lactic acid bacteria counts in beef decreased after the hyperbaric storage.

The microbial inactivation detected, in this paper, in SUP samples could be related not only with the pressure applied during the storage, but also with the acidic nature of the strawberry juice which could be enhanced by the reversible pH shift that aqueous solutions undergo under pressure (Neuman, Kauzmann, & Zipp, 1973). It is well known that pressure effects on microorganisms depend not only on the magnitude and duration of the pressure applied, but also on other physicochemical factors like temperature, pH, or the composition of the culture media. In this sense, Matsumura et al. (1974) showed that pressure markedly narrows the pH ranges for growth of a variety of bacteria and, therefore, minimum pH values for microbial growth are higher under pressure than at atmospheric conditions. Moreover, different authors in the literature have shown that as pH is lowered, most microbes become more susceptible to high-pressure inactivation (Farkas & Hoover, 2000; Garcia-Graells, Hauben, & Michiels, 1998; Linton, McClements, & Patterson, 1999; Smelt, 1998).

3.1.2. Viscosity

Viscosity is an important quality parameter in fruit juices which mainly affects their mouthfeel and the ability to hold their solid portion in suspension for all the product shelf life.

The initial kinematic viscosity measured in the raw strawberry juice was 5.0 ± 0.3 cSt (see Table 1). The thermal treatment applied to pasteurize samples severely reduced their viscosity by more than 50% (Figure 1) probably due to the thermal degradation of pectic substances. Similar viscosity reductions were found by other authors in tomato and guava juices after thermal processing (Thakur, Singh, & Nelson, 1997; Yen & Lin, 1998).

After 15 days of storage, viscosity fell down in all the raw samples stored at 20 °C (Figure 1). The reduction was significantly more pronounced ($P < 0.05$) in those samples maintained at atmospheric pressure (C samples), where the viscosity values were less than 1 cSt. In these samples, an evident phase separation was also observed. On the contrary, no significant cloud losses were detected in SUP samples, although a small amount of solid sediments was observed at the bottom of the Falcon tubes. Moreover, hyperbaric storage was effective to attenuate, to a certain extent, the viscosity decay. Thus, viscosity reduced by 79.2%, 71.1% and 63.7% in SUP_25, SUP_100 and SUP_220 samples, respectively, as compared to 83.6% in C samples. Pressure level applied during the storage had a significant effect ($P < 0.05$) and the higher the pressure, the lower was the decrease in viscosity observed.

Viscosity losses, during the storage of fruit juices, are mainly related to the depolymerization of pectin caused by the combined action of different endogenous pectinases (Duvetter et al., 2009). Among them, pectin methylesterase (PME) and polygalacturonase (PG) are the most widely studied in the literature. PME catalyzes the de-esterification of pectin

releasing methanol and low-methoxyl pectin. This de-esterified pectin is the substrate for PG which subsequently catalyzes its depolymerization and, in this way, drastic decreases in the viscosity of juices can be produced during their storage.

It is well known that pressure can induce structural rearrangements in enzymes which can cause their activation, especially at relatively low pressures (~ 100 MPa), or produce their partial or total inactivation, in a reversible or irreversible manner. An apparent enzyme activation can also be produced by pressure-induced disruption of intact tissues which enhances enzyme extraction and enzyme-substrate interactions (Hendrickx, Ludikhuyze, Van den Broeck, & Weemaes, 1998). The specific effect of pressure on a particular enzyme depends on several factors such as the structure of the enzyme, its origin, the medium composition, pH, or the temperature and pressure levels applied, among others. Different authors have shown that PME from different plant sources like pepper (Castro, Loey, Saraiva, Smout, & Hendrickx, 2006), tomato (Crelie, Robert, Claude, & Juillerat, 2001; Shook, Shellhammer, & Schwartz, 2001; Tangwongchai, Ledward, & Ames, 2000), white grapefruit (Guiavarc'h, Segovia, Hendrickx, & Van Loey, 2005), plum (Nunes et al., 2006) or carrot (Trejo Araya et al., 2007) can be regarded as a pressure resistant enzyme since pressures higher than 700 MPa are usually required to induce short-term inactivation at room temperature. In purified strawberry PME, pressures from 850 MPa, at 10°C and pH = 7, were needed to produce some inactivation. Moreover, the occurrence of a pressure-stable PME fraction, which contributes about 10% of the total activity, was detected (Ly-Nguyen et al., 2002). Pressure effects on polygalacturonase are considerably less studied and no data have been found on strawberry PG. Available data on tomato show that tomato PG is much more pressure-labile than PME. Thus, an almost complete PG inactivation has been described in cherry tomatoes after a pressure treatment at 500 MPa and ambient temperature (Tangwongchai et al., 2000). Similar results were found by Crelie et al. (2001) and Fachin et al. (2003) in tomato juice and by Shook et al. (2004) in tomato dices. Nevertheless, no significant PG inactivation has been described at pressures lower than 350 MPa (Crelie et al., 2001; Fachin et al., 2003; Shook et al., 2001; Tangwongchai et al., 2000). All these data show that, in principle, no significant PME and PG inactivation should be expected at the pressure/temperature conditions employed in this paper, although the effect of long times under pressure should not be neglected.

Anyway, in hyperbaric storage, it is important to evaluate not only the pressure stability of enzymes, but also their catalytic activity under pressure. Pressure can induce changes in the rate of enzyme-catalyzed reactions and they can be accelerated or decelerated under pressure. These changes, as Eisenmenger and Reyes de Courcuera (2009) pointed out, can be produced by pressure-induced changes in the structure of enzymes or in the reaction mechanisms; for example, a change in the rate-limiting step. Moreover, pressure can also induce changes in the physical properties (e.g. pH, density, viscosity, phase) of the substrate and/or solvent which affect the enzyme structure or the rate-limiting step. Previous studies in the literature, most of them in model systems, showed that PME and PG activities under pressure are highly dependent on their origin, the substrate employed, the ionic environment and the temperature

and pressure levels applied (Duvetter et al., 2006; Sila et al., 2007; Van Den Broeck, Ludikhuyze, Van Loey, & Hendrickx, 2000; Verlent, Van Loey, Smout, Duvetter, & Hendrickx, 2004b). Unfortunately, studies at 20 °C and at the low pressures levels employed in this paper are very scarce and no conclusive data can be extracted from them. In general terms, PME activity, at temperatures between 30 °C and 65 °C, increases with pressure up to an optimal pressure level and then decreases with increasing pressure (Duvetter et al., 2006; Sila et al., 2007; Van Den Broeck et al., 2000). But, Van den Broeck et al. (2000) found that purified tomato PME activity, at 20 °C and neutral pH, was slightly lower at pressures up to 300 MPa than at atmospheric conditions. A subsequent study by the same research group (Verlent et al., 2004a) in purified tomato PME, at pH = 4.4 and pH = 8, showed that pressure up to 450 MPa accelerates the PME catalyzed de-esterification of pectin at temperatures between 30 °C and 65 °C. This effect was clearly dependent on temperature and pH with the least response at the lowest pH and temperature conditions assayed. As regards polygalacturonase, different studies in purified tomato PG showed a reduced activity under pressure (100-400 MPa) at temperatures between 30 °C and 50 °C (Verlent, Smout, Duvetter, Hendrickx, & Van Loey, 2005; Verlent et al., 2004b). This reduced PG activity under pressure could justify the results obtained in this paper which prove that pressures up to 220 MPa are effective to slow down viscosity losses in raw strawberry juice during its storage at 20 °C. But, it is important to note that all the previous results, obtained with purified PME and PG in buffer solutions, may be not representative of real tomato or strawberry products and more research work on the activity of pectolytic enzymes under pressure is needed to convincingly explain the results obtained in this paper.

Refrigeration was significantly more efficient than hyperbaric storage to slow down viscosity decay in raw strawberry juices. No significant cloud losses were observed in refrigerated samples (raw or pasteurized), although a small amount of sediments, similar to that found in SUP samples, was distinguished at the bottom of the Falcon tubes.

Figure 1 shows how viscosity in R_R samples reduced by 49.7% after 15 days of storage. Storage at low temperature is widely recognized as an effective method to reduce the activity of pectin-hydrolyzing enzymes (Imsabai, Ketsa, & Van Doorn, 2002), but Figure 1 shows that thermal pasteurization is needed if viscosity decay must be delayed for long times. Thermal pasteurization is able to reduce PME and PG activities and, in this way, it allows long-term preservation of refrigerated samples. In general terms, PME can be considered a rather heat-labile enzyme while PG is very heat-resistant (Duvetter et al., 2009). Thus, in strawberry juices, Aguiló-Aguayo et al. (2009) found PME and PG residual activities of 22.2% and 76.2%, respectively, after thermal processing at 90 °C for 60 s. Therefore, the thermal treatment applied to R_P samples in this paper is expected to cause an important decrease on PME activity, although it should hardly affect PG activity. This decrease on PME activity altogether with the refrigerated storage applied should strongly slow down the depolymerization of pectin and it can explain the high stability found in the viscosity of R_P samples.

3.1.3. Color

The bright red color of strawberry juice is one of the most important quality parameters to which consumers are sensitive, but it easily degrades during processing and storage. Instrumental color parameters (L^* , a^* and b^*) initially measured in the raw strawberry juice are shown in Table 1. The thermal treatment applied to the pasteurized samples caused a slight, but significant ($P < 0.05$), increase in L^* values ($L^* = 27.71 \pm 0.11$), but no changes were found in redness ($a^* = 7.99 \pm 0.18$) and yellowness ($b^* = 3.60 \pm 0.13$). Thus, the total color change was quite small ($\Delta E^* = 0.43 \pm 0.08$) in these samples. Similar results were found by Gösinger et al. (2009) in strawberry nectar thermally treated at 85 °C for 10 min. Aguiló-Aguayo et al. (2009) also found an increase in the lightness of strawberry juices thermally treated at 90 °C for either 30 s or 60 s, but they reported a significant decrease in a^*/b^* probably due to the more severe thermal conditions applied.

Storage at 20 °C for 15 days produced color losses in all the samples. The color decay was considerably more pronounced in those samples stored at atmospheric pressure (C samples) as expected. In these samples, significant alterations ($P < 0.05$) in L^* , a^* and b^* were found: lightness increased by 9.6% and redness and yellowness decreased by 33.5% and 64.1%, respectively. Thus, the color of C samples became less intense and more violet, which was indicated by a significant decrease ($P < 0.05$) in chroma and hue values (Figure 2). These color changes produced the highest $\Delta E^* = 4.5 \pm 0.7$ value. Hyperbaric storage was effective to substantially attenuate the color degradation in SUP samples. Thus, maximum L^* increases of 0.8% were detected in SUP_25 samples and maximum a^* and b^* decreases of 13.6% and 19.7% were found in SUP_25 and SUP_100 samples, respectively. This resulted in a reduced degradation of hue and chroma values as compared to C samples (Figure 2). Pressure level applied during the storage had not a significant effect ($P < 0.05$) on the color decay in the pressure range studied and ΔE^* was 1.3 ± 0.1 in all the samples stored under pressure.

It is well known that the attractive color of strawberry juice mainly comes from the anthocyanins present in the fruit. Pelargonidin-3-glucoside is the major anthocyanin found in cultivated strawberries, although other compounds such as pelargonidin-3-rutinoside and cyanidin-3-glucoside are also found in smaller concentrations (Aaby, Mazur, Nes, & Skrede, 2012; Garcia-Viguera, Zafrilla, & Tomás-Barberán, 1998). Anthocyanins may degrade during the storage of juices and several factors such as light, temperature, pH and presence of oxygen, certain metal ions or L-ascorbic acid, among others, are implicated. All these parameters affect the condensation of anthocyanins (self-association) and copigmentation phenomena (interaction of anthocyanin with polyphenols) which produce color changes in the juice. Nevertheless, the key role in color degradation during juice storage is commonly attributed to the presence of some enzymes such as polyphenoloxidase (PPO), peroxidase (POD) and β -glucosidase which can be responsible for anthocyanin degradation (Chisari, Barbagallo, & Spagna, 2007; López-Serrano & Ros Barceló, 2002; Zabetakis, Leclerc, & Kajda, 2000b).

Figure 2 and Table 3 clearly show that hyperbaric storage was efficient to attenuate, to a great extent, the color decay in samples stored at 20 °C. Different authors have shown that

high-pressure processing (200-800 MPa) for some minutes, at low and moderate temperatures, has a limited effect on the color and anthocyanin content of different fruits (Cao et al., 2011; Kouniaki, Kajda, & Zabetakis, 2004; Oey, Lille, Van Loey, & Hendrickx, 2008; Patras, Brunton, Da Pieve, & Butler, 2009; Terefe, Matthies, Simons, & Versteeg, 2009; Zabetakis et al., 2000b) but, the effect of long-term storage under pressure has not been studied yet. The reduced color decay found in SUP samples as compared to C samples can be indicative of a slowdown in anthocyanin degradation under pressure. It could be related to some partial PPO, POD or β -glucosidase inactivation since different authors have shown that high-pressure processing for some minutes can produce a partial inactivation of these enzymes in strawberry products (Cano, Hernandez, & De Ancos, 1997; Cao et al., 2011; Dalmadi, Rapeanu, Van Loey, Smout, & Hendrickx, 2006; Garcia-Palazon, Suthanthangjai, Kajda, & Zabetakis, 2004; Terefe et al., 2009; Terefe, Yang, Knoerzer, Buckow, & Versteeg, 2010; Zabetakis, Koulentianos, Orruño, & Boyes, 2000a). On the other hand, no studies have been found in the literature about polyphenoloxidase, peroxidase or β -glucosidase activities under pressure but, as previously commented, pressure can also induce changes in the rate of enzyme-catalyzed reactions (Eisenmenger & Reyes de Corcuera, 2009). In this sense, the aforementioned reversible pH shift that aqueous solutions undergo under pressure (Neuman et al., 1973) should affect the rate of PPO and POD catalyzed reactions since Chisari et al. (2007) and Dalmadi et al. (2006) have proved that strawberry PPO and POD activities are strongly affected by pH.

Therefore, the improved stability of color in SUP samples could be related to either some partial PPO, POD or β -glucosidase inactivation or some reduction in their catalytic activity under pressure, but specific experiments should be designed to probe the hypothesis presented. Moreover, other mechanisms implied in anthocyanin degradation, apart from enzymatic browning, should not be neglected.

On the other hand, Figure 2 and Table 3 show that refrigeration was slightly more efficient than hyperbaric storage to preserve color in strawberry juice. Raw and pasteurized samples only suffered minor changes in the color parameters measured after 15 days of refrigerated storage at 5 °C. Thus, only some significant differences ($P < 0.05$) appeared through the statistical analysis: a significant increase of 1.3% in L^* values was detected in R_P samples while a significant decrease of 3.9% in a^* values was perceived in R_R samples. These color degradations were, in any case, small and they involved limited total color changes: $\Delta E^* = 0.7 \pm 0.1$ and $\Delta E^* = 0.4 \pm 0.2$ in R_P and R_R samples, respectively. These ΔE^* values were rather close to those produced in SUP samples and, therefore, no sensorial differences should be noticed between refrigerated and pressure stored samples after 15 days of storage as a threshold value of $\Delta E^* = 1$ is frequently assumed as a basis for a color perceptible difference (Gonnet, 1998; Rein & Heinonen, 2004).

The results obtained confirm previous findings in the literature which proved that storage at low temperature is an efficient method to slow down the degradation of color components and anthocyanins (García-Viguera et al., 1999; Gössinger et al., 2009; Wang & Xu, 2007) and the activity of polyphenoloxidase and peroxidase (Chisari et al., 2007).

3.2. Stability of the strawberry juices after the hyperbaric storage

After 15 days of hyperbaric storage at 20 °C, SUP samples were carried to atmospheric pressure and stored at 5 °C for two weeks. Then, microbial analyses, viscosity and color measurements were made to evaluate the stability of the product, at 0.1 MPa and refrigerated conditions, after the storage under pressure. As previously commented, C samples, stored at 0.1 MPa and 20 °C for 15 days, were spoiled and, therefore, they were discarded in this phase of the work. Raw and thermally pasteurized juices, stored in refrigeration for 15 days, were maintained at 5 °C for two more weeks to make comparisons.

No microbial growth was detected in SUP samples two weeks after the hyperbaric storage. In all these samples, the microbial population remained below the detection limits. The same result was obtained in the thermally pasteurized juices after 30 days of refrigerated storage. On the contrary, total aerobic plate counts and yeasts and moulds in R_R samples increased by more than 1 and 2 log₁₀ units, respectively, during the last two weeks of refrigerated storage.

In regard to viscosity, all the SUP samples underwent, after the hyperbaric storage, a slight, but significant ($P < 0.05$) reduction in their viscosity values. Nevertheless, this viscosity decay was considerably lower than the decline detected in R_R samples during the last 15 days of storage (see Figure 1). By contrast, viscosity of pasteurized juices remained stable for the complete storage period.

Concerning color stability, Figure 2 clearly shows that chroma and hue values were very stable in all the samples considered, whichever the preservation technique employed in the first 15 days of storage. This confirms the major role played by temperature in slowing color degradation in strawberry juices.

Strawberry juices, stored under pressure at 20 °C for 15 days, were stable after decompression for, at least, two more weeks at 5 °C. But, pressure applied could be only partly responsible for the lack of microbial growth observed in SUP samples. Many evidences in the literature show that pressure processing, for some minutes, do not efficiently inactivate bacterial spores and ascospores of molds, even at pressure levels substantially higher than those employed in this paper (Farkas & Hoover, 2000; Smelt, 1998). The low pH of the strawberry juice and the refrigerated conditions applied after decompression must also be implied in the results obtained because it is well known that both factors (low pH and low temperature) make difficult the damage reparation in sublethally injured cells (Farkas & Hoover, 2000; Garcia-Graells et al., 1998; Linton et al., 1999; Smelt, 1998), the growth of many microorganisms and the germination of spores.

4. CONCLUSIONS

Hyperbaric storage has been found to be an efficient method to reduce the microbial load and avoid the growth of microorganisms in raw strawberry juices stored at 20 °C for 15 days. Pressure was also effective to attenuate viscosity and color losses in samples stored at

20 °C, although refrigerated storage was significantly more efficacious to delay viscosity and color decay. Nevertheless, low temperature by itself failed in avoiding microbial growth in raw juices and thermal pasteurization was needed to obtain stable strawberry juices for 15 days at 5 °C. All these results show that hyperbaric storage at room temperature could represent an interesting technology for short-term preservation of raw food commodities. Long-term preservation should involve the previous enzymatic inactivation of the product.

Hyperbaric storage at room temperature could imply important energy savings in different sections of the cold chain since, as already mentioned, energy costs of this technology are only produced during compression and maintaining the product under pressure does not involve any additional energy requirement. Nevertheless, more research is needed before giving categorical conclusions about the potential of this preservation method: it is necessary to test hyperbaric storage in other food products with different characteristics (vegetal and animal origin, liquids and solids, different textures, structures or pH, among others), to investigate the behavior of selected microorganisms for long periods under pressure, to study the activity of different enzymes implied in food spoilage during hyperbaric storage, to prove the stability of different bioactive compounds under pressure and, finally, to evaluate both the capital and the operating costs involved. Next studies should be focused in all these points.

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TABLE 1

Main analytical characteristics of the raw strawberry juice employed in the experiments

Parameter	Mean $\pm$ Standard Deviation
Soluble solids ($^{\circ}$ Brix)	7.8 $\pm$ 0.1
pH	3.33 $\pm$ 0.04
Density (g·cm ⁻³)	1.0294 $\pm$ 0.0003
Total aerobic mesophiles (log ₁₀ cfu/ml)	2.9 $\pm$ 0.2
Yeasts and moulds (log ₁₀ cfu/ml)	< 2.6
Kinematic viscosity (cSt)	5.0 $\pm$ 0.3
L ₀ [*]	27.43 $\pm$ 0.05
a ₀ [*]	8.23 $\pm$ 0.22
b ₀ [*]	3.68 $\pm$ 0.16

701 **Table 2**

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703 Microbial counts ($\log_{10}$ cfu/ml) in raw and pasteurized strawberry juices stored for 15 days at different conditions. Different letters within a row indicate
704 significant differences ($P < 0.05$) between means.

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	Storage at 20 °C				Storage at 5 °C	
Pressure	0.1 MPa	25 MPa	100 MPa	220 MPa	0.1 MPa	0.1 MPa
Type of juice	Raw C	Raw SUP_25	Raw SUP_100	Raw SUP_220	Raw R_R	Pasteurized R_P
Total aerobic mesophiles	6.0 ± 0.4 a	< 1 c	< 1 c	< 1 c	5.1 ± 0.4 b	<1 c
Yeasts and moulds	5.8 ± 0.5 a	< 2 c	< 2 c	<2 c	2.6 ± 0.4 b	<2 c

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Table 3
Instrumental color parameters (L*: lightness; a*: redness and b*: yellowness) in raw and pasteurized strawberry juices stored for 15 days at different conditions. Different letters within a row indicate significant differences (P < 0.05) between means.

	Storage at 20 °C				Storage at 5 °C	
Pressure	0.1 MPa	25 MPa	100 MPa	220 MPa	0.1 MPa	0.1 MPa
Type of juice	Raw C	Raw SUP_25	Raw SUP_100	Raw SUP_220	Raw R_R	Pasteurized R_P
L*	30.05 ± 0.39 c	27.62 ± 0.09 a	27.48 ± 0.11 a	27.45 ± 0.08 a	27.45 ± 0.10 a	28.06 ± 0.10 b
a*	5.47 ± 0.63 c	7.16 ± 0.11 b	7.13 ± 0.13 b	7.21 ± 0.06 b	7.91 ± 0.14 a	7.95 ± 0.19 a
b*	1.32 ± 0.59 c	3.02 ± 0.08 b	2.94 ± 0.08 b	2.94 ± 0.05 b	3.49 ± 0.17 ab	3.64 ± 0.15 a

FIGURE CAPTIONS

- Figure 1** Kinematic viscosity (cSt) in strawberry juices stored at different conditions.
- ( ): R_R samples stored at 0.1 MPa/5 °C; ( ): R_P samples stored at 0.1 MPa/5 °C; ( ): C samples stored at 0.1 MPa/20 °C; ( ): SUP_25 samples stored at 25 MPa/20 °C for 15 days; ( ): SUP_100 samples stored at 100 MPa/20 °C for 15 days and ( ): SUP_220 samples stored at 220 MPa/20 °C for 15 days. After 15 days of hyperbaric storage, SUP samples were carried to atmospheric pressure and stored at 5 °C for 15 additional days.
- Figure 2** (a) Hue and (b) Chroma evolution in strawberry juices stored at different conditions.
- ( ): R_R samples stored at 0.1 MPa/5 °C; ( ): R_P samples stored at 0.1 MPa/5 °C; ( ): C samples stored at 0.1 MPa/20 °C; ( ): SUP_25 samples stored at 25 MPa/20 °C for 15 days; ( ): SUP_100 samples stored at 100 MPa/20 °C for 15 days and ( ): SUP_220 samples stored at 220 MPa/20 °C for 15 days. After 15 days of hyperbaric storage, SUP samples were carried to atmospheric pressure and stored at 5 °C for 15 additional days. Note that chroma values corresponding to SUP_25 and SUP_220 samples are almost overlapped.

FIGURE 1

FIGURE 2