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

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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 ºC for 15 days were compared to raw and thermally pasteurized samples stored at atmospheric pressure and 5 ºC 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 ºC. 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 ºC 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, Wirsen, & 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

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the freezing point of water and, therefore, it allowed storage temperatures below 0 ºC 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 ºC (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 ºC 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 ºC 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 ºC 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 ºC until utilization.

2.2. Physicochemical analyses in the raw material

Before each experiment, a frozen batch of strawberry juice was thawed overnight at 5 ºC. This juice was then characterized by measuring some of its physicochemical properties (see Table 1). Soluble solids concentration (ºBrix) 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 ºC 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 ºC, 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 ºC 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 ºC and atmospheric pressure for 15 days. Samples to be pasteurized were thermally treated in a water bath at 90 ºC until the temperature at the core was maintained at 85 ºC 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 ºC, SUP samples were decompressed and stored at 5 ºC 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 ºC for fifteen more days to make comparisons.

2.6. Microbial analysis

158 Ten-fold serial dilutions of the strawberry juice samples were prepared in buffered
159 peptone water. Total aerobic counts at 30 ºC were determined, following the standard method
160 AFNOR NF V 08-051, by the pour plate method in plate count agar (PCA). Plates were
161 incubated at 30 ± 1 ºC for 72 ± 3 h and the colonies formed were counted. Yeasts and moulds
162 were enumerated on Sabouraud Chloramphenicol Agar (SCA) medium by the surface spread
163 plate method, according to the standard method AFNOR XP V 08-059. SCA plates were
164 incubated at 25 ± 1ºC for 5 days and the colonies of yeasts and moulds were counted. Data
165 were expressed as $\log_{10}$ cfu/ml. The detection limits were 10 cfu/ml for aerobic total counts and
166 100 cfu/ml for yeasts and moulds.
167
168 **2.7. Viscosity measurement**
169 The kinematic viscosity of the samples was determined using a Cannon-Fenske
170 reverse-flow glass capillary viscometer (Nº 150, Fungilab S.A., Spain), immersed in a
171 thermostatic water bath (Thermocap, Fungilab S.A., Spain) tempered at 40 ± 0.05 °C. The efflux
172 time was measured manually using a digital stopwatch (Oregon Scientific TR118, Oregon
173 Scientific, Spain). The kinematic viscosity (ν), expressed in centiStokes (1 cSt = 10^{-6} $m^2 \cdot s^{-1}$),
174 was calculated from the efflux time (t) and the viscometer calibration constant (C) at 40 ºC,
175 provided by the manufacturer, by using the following equation:
176 $$\nu = t \cdot C \quad (1)$$
177
178 **2.8. Color measurement**
179 Strawberry juice color was characterized objectively according to the L*, a*, and b* color
180 parameters in the CIELab uniform color space defined by the Commission Internationale
181 d'Eclairage. A CM-3500d spectrophotometer managed by the color data software CM-S100w
182 SpectraMagic™ (Konica Minolta, Japan) was used for this purpose. The spectrophotometer
183 operated in the reflectance specular included mode with an aperture size of 8 mm in diameter.
184 Measurements were made with the D65 standard illuminant and the ultraviolet component of
185 the illumination was included. Illuminating and viewing configurations complied with the CIE
186 diffuse/8º geometry. The instrument was calibrated with black and white (No. 14671004)
187 standards before each series of analysis. To make the measurements, a glass Petri-dish
188 (42 mm internal diameter) was filled with 10 mL of juice, closed with its cap and covered by the
189 black standard (a closed cylindrical tube with black walls). In each Petri-dish, five
190 measurements were performed: one at its center and four at radial positions distributed 90
191 degrees apart and the obtained L*, a* and b* values were averaged. From these mean values,
192 the total color change ΔE^*, hue angle h^o and chroma C* parameters were also calculated. The
193 formulas for their calculation are given below:
194 $$\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (2)$$
195 where L_0^*, a_0^* and b_0^* are values for the raw juice (Table 1).

196 $$h^{\circ} = \arctan\left(\frac{b^*}{a^*}\right) \quad (3)$$

197 $$C^* = \sqrt{a^{*2} + b^{*2}} \quad (4)$$

198

199 **2.9. Statistical analysis**

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

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

206

207 **3. RESULTS AND DISCUSSION**

208

209 **3.1. Stability of the strawberry juices during the hyperbaric storage**

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211 **3.1.1. Microbial load**

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

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

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

228 It is widely recognized that high hydrostatic pressures, between 10 and 100 MPa, are
229 generally nonlethal for those microorganisms adapted to atmospheric conditions, but they exert
230 adverse effects on them and reduce their growth (Abe, 2007; Abe & Horikoshi, 2000; Bartlett,
231 2002; Matsumura, Keller, & Marquis, 1974). The inhibitory effect of pressure on the growth of
232 the natural microflora present in different food products was already reported by Charm et al.
233 (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 ºC 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 ºC (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

274 releasing methanol and low-methoxyl pectin. This de-esterified pectin is the substrate for PG
275 which subsequently catalyzes its depolymerization and, in this way, drastic decreases in the
276 viscosity of juices can be produced during their storage.
277 It is well known that pressure can induce structural rearrangements in enzymes which
278 can cause their activation, especially at relatively low pressures (~ 100 MPa), or produce their
279 partial or total inactivation, in a reversible or irreversible manner. An apparent enzyme activation
280 can also be produced by pressure-induced disruption of intact tissues which enhances enzyme
281 extraction and enzyme-substrate interactions (Hendrickx, Ludikhuyze, Van den Broeck, &
282 Weemaes, 1998). The specific effect of pressure on a particular enzyme depends on several
283 factors such as the structure of the enzyme, its origin, the medium composition, pH, or the
284 temperature and pressure levels applied, among others. Different authors have shown that PME
285 from different plant sources like pepper (Castro, Loey, Saraiva, Smout, & Hendrickx, 2006),
286 tomato (Crelier, Robert, Claude, & Juillerat, 2001; Shook, Shellhammer, & Schwartz, 2001;
287 Tangwongchai, Ledward, & Ames, 2000), white grapefruit (Guiavarc'h, Segovia, Hendrickx, &
288 Van Loey, 2005), plum (Nunes et al., 2006) or carrot (Trejo Araya et al., 2007) can be regarded
289 as a pressure resistant enzyme since pressures higher than 700 MPa are usually required to
290 induce short-term inactivation at room temperature. In purified strawberry PME, pressures from
291 850 MPa, at 10ºC and pH = 7, were needed to produce some inactivation. Moreover, the
292 occurrence of a pressure-stable PME fraction, which contributes about 10% of the total activity,
293 was detected (Ly-Nguyen et al., 2002). Pressure effects on polygalacturonase are considerably
294 less studied and no data have been found on strawberry PG. Available data on tomato show
295 that tomato PG is much more pressure-labile than PME. Thus, an almost complete PG
296 inactivation has been described in cherry tomatoes after a pressure treatment at 500 MPa and
297 ambient temperature (Tangwongchai et al., 2000). Similar results were found by Crelier et al.
298 (2001) and Fachin et al. (2003) in tomato juice and by Shook et al. (2004) in tomato dices.
299 Nevertheless, no significant PG inactivation has been described at pressures lower than 350
300 MPa (Crelier et al., 2001; Fachin et al., 2003; Shook et al., 2001; Tangwongchai et al., 2000).
301 All these data show that, in principle, no significant PME and PG inactivation should be
302 expected at the pressure/temperature conditions employed in this paper, although the effect of
303 long times under pressure should not be neglected.
304 Anyway, in hyperbaric storage, it is important to evaluate not only the pressure stability
305 of enzymes, but also their catalytic activity under pressure. Pressure can induce changes in the
306 rate of enzyme-catalyzed reactions and they can be accelerated or decelerated under pressure.
307 These changes, as Eisenmenger and Reyes de Courcuera (2009) pointed out, can be produced
308 by pressure-induced changes in the structure of enzymes or in the reaction mechanisms; for
309 example, a change in the rate-limiting step. Moreover, pressure can also induce changes in the
310 physical properties (e.g. pH, density, viscosity, phase) of the substrate and/or solvent which
311 affect the enzyme structure or the rate-limiting step. Previous studies in the literature, most of
312 them in model systems, showed that PME and PG activities under pressure are highly
313 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 ºC 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 ºC and 65 ºC, 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 ºC 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 ºC and 65 ºC. 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 ºC and 50 ºC (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 ºC. 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 ºC 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 ± 0.11), but no changes were found in redness (a* = 7.99 ± 0.18) and yellowness (b* = 3.60 ± 0.13). Thus, the total color change was quite small (ΔE* = 0.43 ± 0.08) in these samples. Similar results were found by Gösinger et al. (2009) in strawberry nectar thermally treated at 85 ºC for 10 min. Aguiló-Aguayo et al. (2009) also found an increase in the lightness of strawberry juices thermally treated at 90 ºC 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 ºC 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 ΔE* = 4.5 ± 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 ºC. 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 ºC. 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 ºC, SUP samples were carried to atmospheric pressure and stored at 5 ºC 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 ºC 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 ºC 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 $\log_{10}$ 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 ºC for 15 days, were stable after decompression for, at least, two more weeks at 5 ºC. 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 ºC for 15 days. Pressure was also effective to attenuate viscosity and color losses in samples stored at

20 ºC, 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 ºC. 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.

ACKNOWLEDGEMENTS

This work has been supported by the R+D+I National Plan of the Spanish Ministry of Education and Science (MEC), through the projects AGL2007-63314/ALI and CSD2007-00045 MALTA CONSOLIDER-INGENIO 2010, by CSIC through the project 201070I033 and by the Madrid Community through the project QUIMAPRES S2009/PPQ-1551. K. Segovia has a contract from CSIC (JAE-DOC program) and A. Bermejo is supported by a predoctoral grant also from CSIC (JAE-PreDoc program).

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692

693 **TABLE 1**
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696 Main analytical characteristics of the raw strawberry juice employed in the experiments
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Parameter	Mean ± Standard Deviation
Soluble solids (º Brix)	7.8 ± 0.1
pH	3.33 ± 0.04
Density ($g \cdot cm^{-3}$)	1.0294 ± 0.0003
Total aerobic mesophiles (log_{10} cfu/ml)	2.9 ± 0.2
Yeasts and moulds (log_{10} cfu/ml)	< 2.6
Kinematic viscosity (cSt)	5.0 ± 0.3
L_0*	27.43 ± 0.05
a_0*	8.23 ± 0.22
b_0*	3.68 ± 0.16

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Table 2

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 significant differences ($P < 0.05$) between means.

	Storage at 20 ºC				Storage at 5 ºC	
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

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 ºC				Storage at 5 ºC	
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 ºC; (■): R_P samples stored at 0.1 MPa/5 ºC; (○): C samples stored at 0.1 MPa/20 ºC; (△): SUP_25 samples stored at 25 MPa/20 ºC for 15 days; (△): SUP_100 samples stored at 100 MPa/20 ºC for 15 days and (▲): SUP_220 samples stored at 220 MPa/20 ºC for 15 days. After 15 days of hyperbaric storage, SUP samples were carried to atmospheric pressure and stored at 5 ºC 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 ºC; (■): R_P samples stored at 0.1 MPa/5 ºC; (○): C samples stored at 0.1 MPa/20 ºC; (△): SUP_25 samples stored at 25 MPa/20 ºC for 15 days; (△): SUP_100 samples stored at 100 MPa/20 ºC for 15 days and (▲): SUP_220 samples stored at 220 MPa/20 ºC for 15 days. After 15 days of hyperbaric storage, SUP samples were carried to atmospheric pressure and stored at 5 ºC for 15 additional days. Note that chroma values corresponding to SUP_25 and SUP_220 samples are almost overlapped.

739 FIGURE 1

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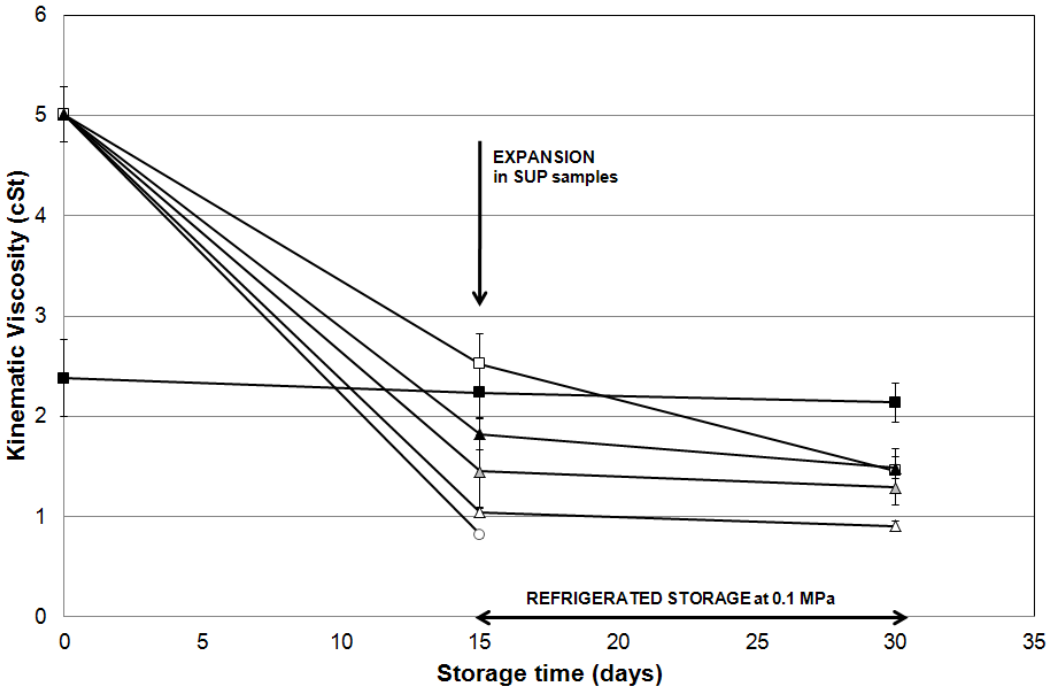

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744 FIGURE 2

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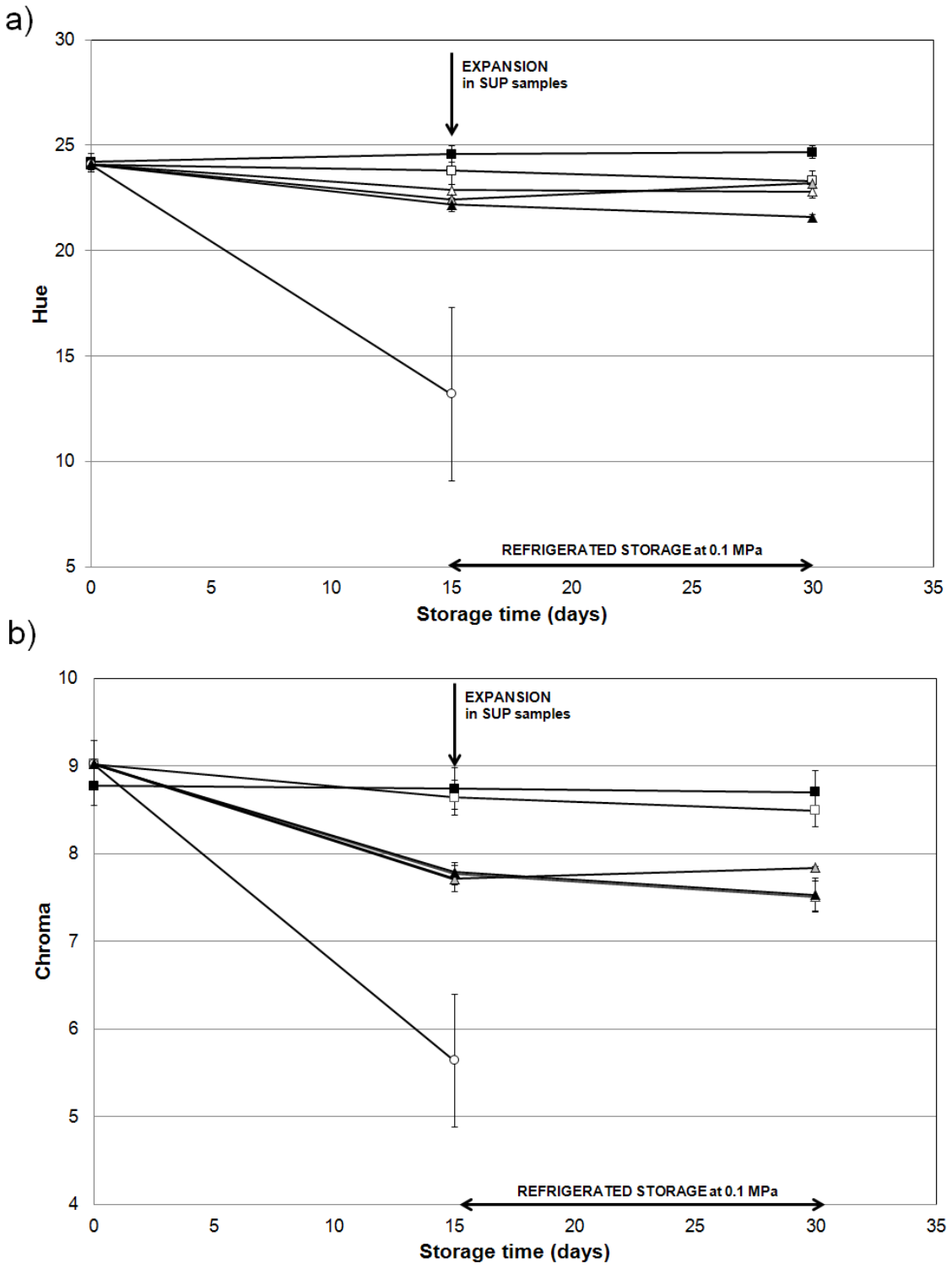

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