LWT - Food Science and Technology 101 (2019) 54–60

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

High hydrostatic pressure treatment improves physicochemical properties of calcium- and soybean protein-added peach juice

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ARTICLE INFO

Keywords:
Peach juice
Protein solubility
Colloidal stability
High hydrostatic pressure
Soybean proteins

ABSTRACT

Calcium- and soybean proteins-added peach beverages were prepared and subjected to high hydrostatic pressure (HHP, 600 MPa, 5 min) in order to evaluate their physicochemical properties (protein solubility, physical stability, viscosity and color). Calcium addition decreased protein solubility and destabilized the beverage. Solubilizing effect of HHP was detected only for non-calcium-added beverages. Nevertheless, HHP treatment stabilized calcium-added beverages, with a greater effect in samples with 10 mmol L^{-1} $CaCl_2$. HHP treatment increased viscosity of calcium-added beverages, which could contribute to their increase in physical stability. This work provides information that may be useful in the handling of functional foods, since physicochemical properties such as physical stability was improved by HHP in a drink with improved nutritional value (incorporation of proteins and calcium).

1. Introduction

In recent years consumers have become aware of the relationship between food and health. This induces a growing demand for products that preserve nutritional and sensory characteristics of fresh foods. In this sense, food industry seeks to satisfy this demand by diversifying its products by preparing new highly nutritious and healthy ones (Sakhale, Pawar, & Ranwwer, 2012). In this context, soy- and fruit-based beverages are an excellent source of macro- and micro-nutrients that constitute an opportunity to incorporate minerals, such as calcium, with important physiological functions (Sacco & Lábbé, 2016). In this sense, soybean is a non-expensive source of proteins with high biological value (compared with other vegetable proteins) and interesting functional properties and health-improving compounds (Delbyshire, Wright, & Boulter, 1976; Nishinari, Fang, Guo, & Phillips, 2014). Nevertheless, low solubility and aggregation processes, that occurs in liquid systems containing calcium and soybean proteins isolate (SPI), limit their use in foodstuff (Ono, Katho, & Mothizuki, 1993; Scilingo & Añón, 2004; Tang, Wang, Yang, & Li, 2009).

Thermal pasteurization is the most common technology employed to extend liquid foods shelf-life. However, this kind of treatment leads to losses in flavor and health-promoting compounds (Odriozola-Serrano, Aguiló-Aguayo, Soliva-Fortuny, & Martin-Belloso, 2013; Yeom, Streaker, & Zhang, 2000). Innovative non-thermal processes for food preservation have attracted the attention of many food manufacturers. In this sense, when applying high hydrostatic pressure (HHP), viable microorganisms and enzymes are inactivated without the use of heat and, therefore, the effect on flavor, color and vitamin content is minimized (Swientek, 1992; Wang, Huang, Hsu, & Yang, 2016). For this reason, the application of HHP is considered a "cold" pasteurization process (Crawford, Murano, Olson, & Shenoy, 1996). Moreover, in previous works by our group, it was found that HHP induced an increment in protein solubility (PS) and improvement in colloidal stability in calcium-added SPI dispersions (Manassero, David-Briand, Vaudagna, Anton, & Speroni, 2018; Manassero, Vaudagna, Añón, & Speroni, 2015) and in more complex foodstuff such as soymilk (Manassero, Vaudagna, Añón, & Speroni, 2016) without the addition of chemical stabilizers such as hydrocolloids.

Abbreviations: HHP, high hydrostatic pressure; SPI, soybean proteins isolate; PS, protein solubility; η_{app}, apparent viscosity; %T, transmission percentage; PME, pectinmethylesterase; PG, polygalacturonase; PPO, polyphenoloxidase

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https://doi.org/10.1016/j.lwt.2018.11.021
Received 5 September 2018; Received in revised form 4 November 2018; Accepted 5 November 2018
Available online 09 November 2018
0023-6438/

Fruit juices are complex and heterogeneous multicomponent systems formed by tissue fragments, insoluble large particles, supra-colloidal and colloidal materials dispersed throughout a continuous medium rich in soluble compounds, including sugars, organic acids, soluble pectins, phenolic compounds and salts (Filippi, Genovese, & Lozano, 2008). Fruit juices are unstable dispersions that settle quickly, which depreciates the visual appearance of the product (Beveridge, 2002). In such dispersions of solid particles in sugar solution, particle-particle, particle-water and particle-sugar interactions govern the stability and the rheological behavior of the suspension (Benítez, Genovese, & Lozano, 2009). In order to avoid sedimentation, homogenization treatments and the addition of hydrocolloids have been suggested as ways to stabilize cloudy liquid products (Neidhart et al., 2002). In HHP-treated fruit juices, it was found that this treatment kept its characteristics similar to the fresh product with a better retention of bioactive compounds (Carbonell-Capella, Barba, Esteve, & Frígola, 2013).

The aim of this work was to study the influence of HHP treatment on protein solubility, physical stability, viscosity and chromatic parameters of calcium- and soybean proteins-added peach juice. This knowledge will be useful to obtain liquid foodstuff with interesting nutritional properties.

2. Materials and methods

2.1. Preparation of peach juice

Peaches (*Prunus persica* (L.) Batsch) cv. Summerset were harvested in Alto Valle, Río Negro, Argentina (Latitude 38°30′S-39°5′S, Longitude 66°45′W-68°30′W). Prior to processing, the fruits were washed in running tap water and cut into quarters. Thereafter, peach pieces were processed with a juice extractor Yelmo model JG 1700 (Yelmo, Argentina) and the juice was vacuum-packed in double bag Cryovac BB2800 (O_2 transmission rate: 6–14 cm^3/m^2/24 h at 23 °C, 101.3 kPa, Sealed Air, Argentina) using a double chamber vacuum packaging machine (Rapivac, model Maximax 800, Argentina). The juice was kept at – 40 °C until use. Prior to storage, pH (range between 4.33 and 4.37) and °Brix (mean value of 10.4 ± 0.3) of peach juice were determined at 20 °C.

2.2. Preparation of SPI

SPI was prepared from defatted soybean flour manufactured by The Solae Company (Brazil). Alkaline extraction (pH 8.0–90 min – 20 °C) was followed by isoelectric precipitation (pH 4.5–15 min), as described by Speroni, Milesi, and Añón (2010). The precipitate was dispersed in distilled water and the pH was adjusted to 7.0 with 2 mol L^{-1} NaOH. Then, the dispersion was freeze-dried. The same batch of SPI was used for the whole study.

2.3. Juice formulation

To obtain 1 L of beverage, 200 g of filtered peach juice was mixed with 500 g of 50 g protein L^{-1} SPI dispersion and 300 g of 214 g sucrose L^{-1} solution (Cicarelli, Santa Fe, Argentina) (Table 1). Then, 0, 10 or 20 mmol $CaCl_2$ L^{-1} was added from a stock solution of 1 mol L^{-1} $CaCl_2$ prepared from $CaCl_2{\cdot}2H_2O$ (Sigma, St Louis, USA). Finally, pH of beverage was adjusted with 2 mol L^{-1} NaOH at 6.0. This pH value was chosen according to previous work (Manassero et al., 2015) in which the effect of HHP on PS was substantial.

Table 1
Formulation of calcium- and soybean protein-added peach beverage.

Amount per 1 L of beverage	
Soybean proteins	25 g
Filtered peach juice	200 g
Sucrose	214 g
Calcium chloride	0, 10 or 20 mmol

2.4. HHP treatment

Juices were placed in plastic bottles (110 mL) and then processed in the HHP system (Stansted Fluid Power Ltd. model FPG 9400:922, Stansted, UK). Samples were treated at 600 ± 5 MPa for 5 min. Processing conditions were chosen according to data from previous works in which the maximum solubilizing effect was detected in such conditions (Manassero et al., 2015, 2016). Moreover, from the microbiological point of view, treatments at 600 MPa have been effective in inactivating a variety of pathogenic and spoilage vegetative bacterial cells, yeasts, molds and viruses (Balasubramaniam, Martínez-Monteagudo, & Gupta, 2015; Fernandez, Denoya, Agüero, Jagus, & Vaudagna, 2018). Thus, that condition could satisfy aspects related to functional properties and food safety. The compression fluid used was a mixture of propylene glycol and distilled water (30:70). The working pressure was reached at 300 MPa min-1 and released at 200 MPa s-1. Conditioning temperature of vessel and initial temperature of samples were 20 °C. The compression heating produced an increase of temperature up to 33.5 °C at the end of compression stage.

2.5. Experimental design

Beverages were characterized by evaluating the effect of calcium concentration (0, 10 or 20 mmol Ca L^{-1}) and HHP level (0.1 or 600 MPa) on PS, physical stability, viscosity and chromatic parameters. Each treatment was performed in triplicate. Analyses of variance (one way ANOVA) of PS, viscosity and chromatic parameters were conducted. Differences between means were analyzed by Tukey's test at a-α level of 0.05. The statistical analysis was carried out using the Origin software (OriginLab Corporation, Northampton, MA, USA).

2.6. Determination of PS

Samples were centrifuged at 10,000 × g for 20 min at 4 °C in an air-cooled benchtop centrifuge Hermle Z 326 K (Wehingen, Tuttlingen, Germany). Protein concentration was determined in the supernatants using the Bicinchoninic Acid Protein Assay (Smith et al., 1985) (BCA Sigma Kit, Sigma Chemical Co., St. Louis, MO, USA). Bovine serum albumin was used as standard (Sigma Chemical Co., St. Louis, MO, USA). Absorbance was measured at 562 nm in a Synergy HT™ Multimode Microplate Reader (BIO TEK Instruments, Winooski, VT, USA). PS was determined in triplicate for each sample. Results were expressed as:

Soluble protein: protein concentration in the supernatant (g L^{-1}).

2.7. Physical stability

The velocity of sedimentation of particles was analyzed through the use of a vertical dynamic light scan analyzer, Quick Scan (Beckman–Coulter inc., Miami, USA). Beverages were loaded into a cylindrical glass cell and the transmission percentage of light (λ = 850 nm) at different heights along the tube (25, 30, 40 and 50 mm from the bottom) was monitored every 5 min during 24 h as an indirect measure of stability. The velocity of sedimentation was determined in triplicate for each sample.

2.8. Viscosity

Viscosity of beverages was determined at 20 °C using a rheometer (HAAKE RheoStress 6000, Thermo Electron Corporation, Germany) equipped with a cylindrical sensor (Z34 DIN, 34 mm diameter).

Samples were thermostated in the sensor for 1 min. Then, the apparent viscosity (η_{app}) was registered by increasing the shear rate from 20 to 500 s^{-1} for 60 s, holding at 500 s^{-1} for 90 s, and decreasing the shear rate from 500 to 20 s^{-1} for 60 s. Viscosity was determined in triplicate. The values of η_{app} at 400 s^{-1} were reported.

2.9. Chromatic parameters

Chromatic parameters of beverages were measured in a sample holder (CR A505) and a glass cell 20 mm (CM-A99) with a Minolta model CR-400 chromameter (Konica Minolta Sensing Inc., Osaka, Japan) using the CIE L*a*b* color space. The measures were done using standard illuminant D_{65} (Ohta & Robertson, 2005) and 2° observed angle and calibrated using a standard white plate. Three samples were evaluated per each treatment.

3. Results and discussion

3.1. Protein solubility

PS of unpressurized (0.1 MPa) beverages without calcium addition was 15.1 ± 0.4 g L^{-1} (Fig. 1), which represented the 60% of protein added. At pH 6.0, the percentage expected was ca. 45% for 10 g L^{-1} SPI aqueous dispersions (Manassero et al., 2015); thus, beverage features made that a higher amount of protein remained in the supernatant. As it was expected, the addition of $CaCl_2$ decreased PS at both salt concentrations. The magnitude of the insolubilization was equal for both calcium concentrations, PS was 3.7 and 3.3 g L^{-1} for 10 and 20 mmol $CaCl_2$ L^{-1}, respectively, the relative decreased of solubility was ca. 75%.

HHP treatment produced a relative increase of PS of 48% in beverages without calcium addition. Nevertheless, HHP treatment produced no change in PS in calcium-added beverages (Fig. 1). This behavior was different to that observed in calcium-added SPI aqueous dispersions (Manassero et al., 2015, 2018). This lack of effect in calcium-added beverages could be due to the presence of some components of beverage that would interfere with the solubilizing effect of HHP. Furthermore, the calcium/protein ratio in these samples could also originate this lack of effect, since it may correspond to a ratio in which the solubilizing effect of HHP treatment is no longer verified. In the first case, high sucrose concentration could induce a decrease in water activity, the amount of water available to interact with proteins would be decreased, and thus protein-protein interactions would be promoted. Moreover, other compounds from peach such as fiber, protein, zinc and copper (Matei, Popescu, Munteanu, & Lucian Radu, 2013) could also interact with soy proteins and/or calcium modifying PS. In the second case, calcium/protein ratio (0.4 and 0.8 mmol calcium/g of protein) and pH value (6.0) corresponded to experimental conditions in which no effect of HHP treatment were observed in calcium-added SPI dispersions (pH 5.9 and calcium/protein ratio: 0.5 mmol calcium/g of protein) (Manassero et al., 2015).

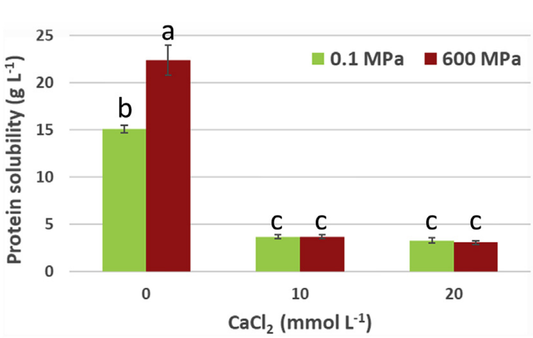

Fig. 1. Protein solubility of beverages with different $CaCl_2$ concentrations added, unpressurized (0.1 MPa) or HHP-treated (600 MPa). Values are expressed as means ± standard deviation. Different letters indicate significant differences ($p < 0.05$).

3.2. Physical stability

Physical stability influences markedly the final quality of foodstuff and is affected by several factors such as total solid content, particle size distribution, charge and shape of particles (Silva et al., 2010).

Physical stability of beverages was evaluated by analysis of transmission percentage (%T) at 25 (middle of the tube), 30, 40 and 50 (top of the tube) mm (Fig. 2). In unpressurized samples without calcium addition %T remained constant and barely higher than 0% in all analyzed zones, which indicates that beverages were cloudy but stable. The opacity of these systems has been associated with the presence of dispersed insoluble particles such as pectins, proteins, lipids, cellulose, hemicellulose and other minor components (Benítez, Genovese, & Lozano, 2007; Yamasaki, Yasui, & Arima, 1964). HHP treatment produced no change in stability of samples without calcium addition (Fig. 2). However, all along the tube the %T was higher (*ca.* 13%) than that of unpressurized samples. This result seemed to be due to the increase in PS detected in these samples.

Unpressurized 10 mmol L^{-1} $CaCl_2$-added beverages started with a zero %T but showed an increase of %T due to phase separation after *ca.* 115 min in the upper part of the tube (40–50 mm), extending to the middle part of the tube (25 mm) after 300 min (Fig. 3). These changes in %T were due to sedimentation of insoluble aggregates and particles. After 1440 min, %T reached values between 34% (25 mm) and 43% (50 mm) (Fig. 3, panels a and d, respectively). This behavior indicates that calcium addition destabilized the dispersion, and a part of the protein aggregates and/or tissue fragments settled. Worth noting, no change in %T (*ca.* 0%) all along the tube was detected in pressurized 10 mmol L^{-1} $CaCl_2$-added beverages (Fig. 3). These data indicate that HHP treatment stabilized these samples at least for 24 h.

Increasing calcium concentration to 20 mmol L^{-1} worsened physical stability in both pressurized and unpressurized beverages, in relation to 10 mmol L^{-1} $CaCl_2$ (Fig. 4). Unpressurized beverages showed a rapid increase in %T all along the tube. In the upper part of the tube (40 and 50 mm) there was an increase in %T after 100 and 50 min respectively, reaching a constant value close to 95% (Fig. 4, panels c and d). In the middle of the tube (25 mm), the destabilization process could be evidenced after 300 min (Fig. 4, panel a). This phenomenon was also observed in beverages with 10 mmol L^{-1} $CaCl_2$ after 300 min (Fig. 3, panel a); however, the rate of increase of %T and the final value reached (*ca.* 95%) were higher for 20 mmol L^{-1} $CaCl_2$ samples. This value of transmission suggests that practically all the protein aggregates and tissue fragments precipitated after 1440 min. HHP treatment improved physical stability of beverages with 20 mmol L^{-1} $CaCl_2$ compared with unpressurized samples. The increase in transmission occurred only at the highest zone of the tube (40 and 50 mm). Besides, the clarification was slower and reached lower final values (42% *vs.* 95%, for pressurized and unpressurized samples, respectively) (Fig. 4, panels c and d). This suggests that HHP treatment caused part of the aggregates to remain in suspension.

Our results indicate that HHP treatment was effective to stabilize beverages based on peach juice that contained soybean proteins and calcium. Calcium concentration was a critical variable for the magnitude of the stabilizing effect.

3.3. Viscosity

Fig. 5 shows the shear stress vs. share rate curves of beverages. All samples presented a common pattern: an inflection point was observed in the shear stress curves. This point appeared at 200 s^{-1} for unpressurized and/or non-calcium-added samples, whereas it appeared at 400 s^{-1} for calcium-added and pressurized ones. This phenomenon could be related to wall slip, a discontinuity in shear rate near the wall

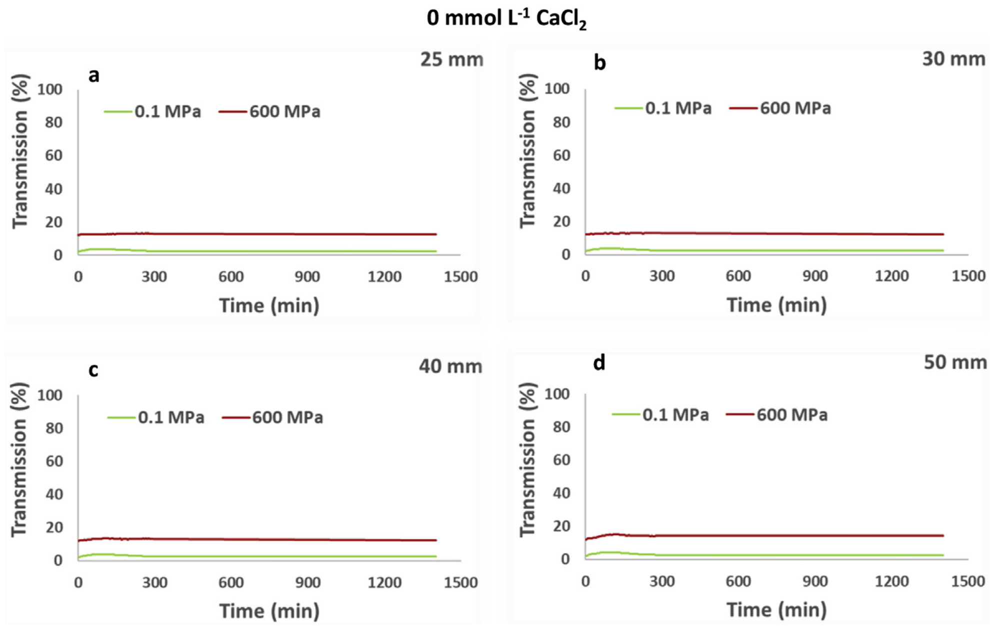

Fig. 2. Sedimentation of insoluble material of non-calcium added beverages as a function of time, unpressurized (0.1 MPa) or HHP-treated (600 MPa). Analysis of transmission percentage (%T) were conducted at 25 mm (middle of the tube, panel a), 30 mm (panel b), 40 mm (panel c) and 50 mm (top of the tube, panel d).

that occurs in particulate systems (Bertola, Bertrand, Tabuteau, Bonn, & Coussot, 2003). According to Saravacos (1970) the flow behavior of fruit pulps is strongly influenced by the amount of suspended particles. In this sense, Barbieri et al. (2018) demonstrated that fiber presence in gabiroba (*Campomanesia xanthocarpa*) pulp promote a slip effect when was subjected to shear. In the range of share rate tested, the shear stress values of pressurized calcium-added (10 or 20 mmol L^{-1}) were higher than those of unpressurized ones. Table 2 shows apparent viscosity (η_{app}) obtained at 400 s^{-1}. Calcium addition at 10 mmol L^{-1} increased ($p < 0.05$) η_{app} in unpressurized samples probably due to the formation of soluble and insoluble aggregates. This effect was also observed in unpressurized calcium-added soymilk (Manassero et al., 2016). However, 20 mmol L^{-1} $CaCl_2$ produced no effect on unpressurized beverage viscosity. Probably, the combination of structure, size, and surface charge of the aggregates formed at the highest calcium concentration resulted in a lower interaction with water or other components of the continuous phase, giving a balance that produced no change in apparent viscosity.

HHP treatment produced no changes in viscosity of beverages without calcium addition, whereas HHP treatment significantly increased viscosity in calcium-added beverages. This behavior could be due to interactions established between soybean proteins, pectin and calcium inducing a “sol-like” state that would be responsible for the highest viscosity values registered (Fig. 5 and Table 2). HHP treatment can enhance the activity of pectinmethylesterase (PME) and decrease the polygalacturonase (PG) one (Oey, Lille, van Loey, & Hendrickx, 2008). PME catalyzes de-esterification reaction of methyl groups of pectin producing methanol, galacturonic acid and pectin molecules with a lower degree of methylation. On the other hand, PG subsequently catalyzes the hydrolysis of glycosidic bond of de-esterified pectic molecules. In this sense, since HHP would cause an increase in PME activity accompanied by a reduction in PG activity, pectin

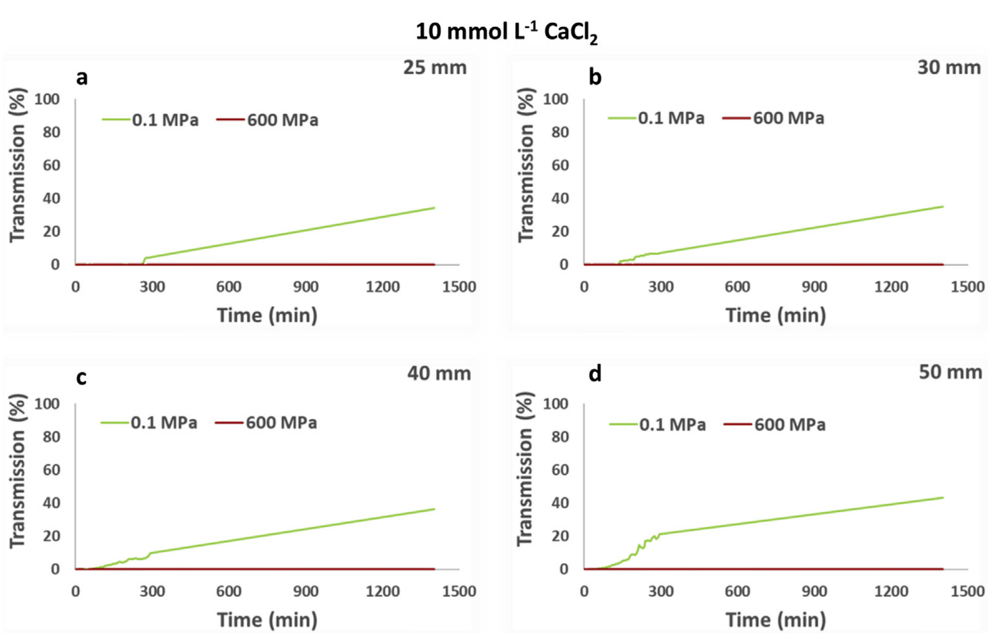

Fig. 3. Sedimentation of insoluble material of 10 mmol L^{-1} $CaCl_2$-added beverages as a function of time, unpressurized (0.1 MPa) or HHP-treated (600 MPa). Analysis of transmission percentage (%T) were conducted at 25 mm (middle of the tube, panel a), 30 mm (panel b), 40 mm (panel c) and 50 mm (top of the tube, panel d).

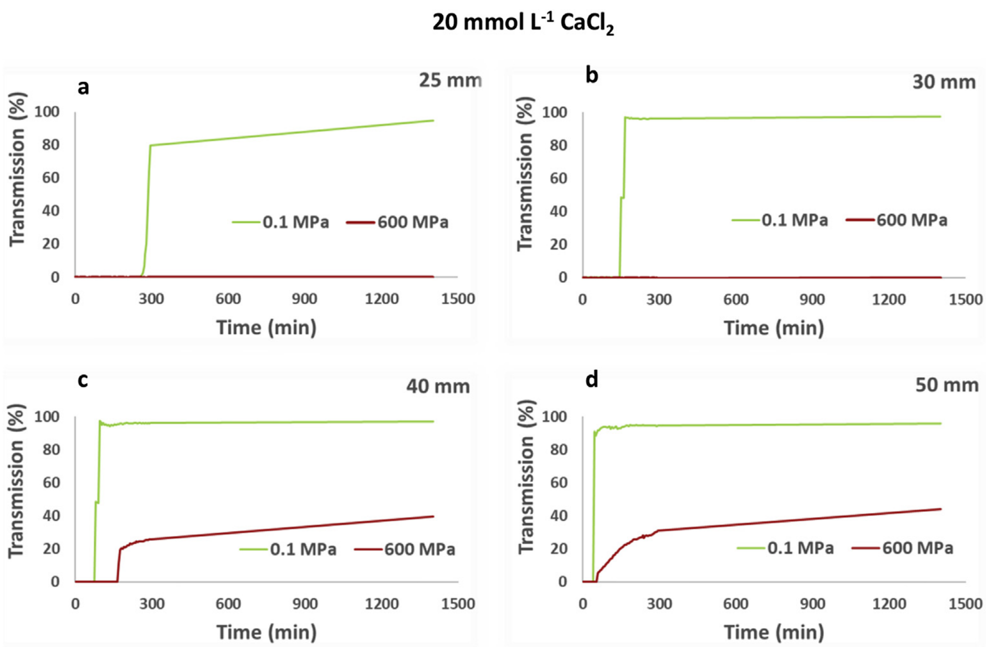

Fig. 4. Sedimentation of insoluble material of 20 mmol L^{-1} $CaCl_2$-added beverages as a function of time, unpressurized (0.1 MPa) or HHP-treated (600 MPa). Analysis of transmission percentage (%T) were conducted at 25 mm (middle of the tube, panel a), 30 mm (panel b), 40 mm (panel c) and 50 mm (top of the tube, panel d).

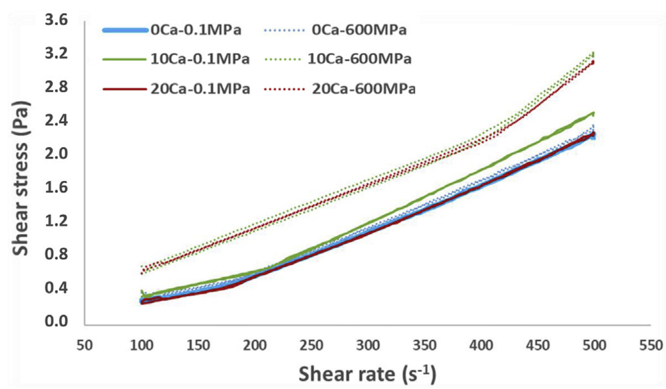

Fig. 5. Shear stress as a function of shear rate for unpressurized (0.1 MPa) or HHP-treated (600 MPa) beverages. Sample names correspond to concentration of $CaCl_2$-added (0, 10 or 20 mmol L^{-1}) and pressure level (0.1 or 600 MPa).

Table 2
Apparent viscosity (η_{app}) of beverages, unpressurized (0.1 MPa) or HHP-treated (600 MPa).

Beverage	η_{app} (cP)
0 Ca – 0.1 MPa	4.2 ± 0.1[c]
0 Ca – 600 MPa	4.3 ± 0.2[b,c]
10 Ca – 0.1 MPa	4.6 ± 0.1[b]
10 Ca – 600 MPa	5.6 ± 0.3[a]
20 Ca – 0.1 MPa	4.1 ± 0.1[c]
20 Ca – 600 MPa	5.4 ± 0.2[a]

Sample names of beverages correspond to concentration of $CaCl_2$-added (0, 10 or 20 mmol L^{-1}) and pressure level (0.1 or 600 MPa). Different letters indicate significant differences ($p < 0.05$).

molecules with a lower degree of methylation formed by PME will not be further degraded by PG. This would cause a higher crosslinking degree between low methoxyl pectin chains and divalent ions, such as calcium, with the consequent formation of a three-dimensional gel structure (Oey et al., 2008). This effect was observed in some preliminary formulations, in which SPI and filtered peach juice contents were higher (35 g L^{-1} and 300 g L^{-1}, respectively, data not shown). On the other hand, Min, Jin, and Zhang (2003) found no change in tomato juice viscosity treated by thermal or pulsed electric field processing and stated that this fact may be due to the effective inactivation of PG and PME. At the same time, HHP treatment could produce an increase of soluble pectin content throughout cell wall disruption, increasing sample viscosity. HHP treatment can disturb the cell permeability of fruits, allowing the movement of water and metabolites from or to the cell (Oey et al., 2008).

Our results suggest that the HHP-induced increase in viscosity of calcium-added samples could be due to at least two phenomena: on the one hand extracted components from fruit tissues would interact with calcium to form a matrix with increased viscosity. On the other hand, soybean proteins denatured by HHP in the presence of calcium were reported as having a higher ability to interact with themselves (Manassero et al., 2018). The present soybean protein concentration (25 g L^{-1}) was not enough high to form a gel, but the viscoelastic matrix could be reinforced due to protein-protein interactions resulting in a higher viscosity.

The increase in η_{app} produced by HHP treatment could contribute to the increase in physical stability shown by these samples. Sinchaipanit and Kerr (2007) reported that homogenized carrot juice showed an important increase of physical stability due to, among other factors, an increase in apparent viscosity.

3.4. Chromatic parameters

In peaches, red color is usually associated with a high content of anthocyanins, while yellow-orange colors are associated with carotenoid content. HHP treatment (at low and moderate temperatures) has a limited effect on pigments (carotenoids, chlorophyll, anthocyanins, etc.) responsible for the color of fruits and vegetables (Oey et al., 2008). García-Palazón, Suthanthangjai, Kajda, and Zabetakis (2004) determined polyphenoloxidase (PPO) activity in red raspberry (*Rubus idaeus*) and strawberry (*Fragaria X ananassa*) after HHP treatment between 400 and 800 MPa at 18–22 °C for 5, 10 or 15 min. The authors concluded that PPO resistance to HHP would be responsible for color changes due to anthocyanin degradation. Moreover, numerous studies reported the stability of carotenoids against HHP treatments (Qiu, Jiang, Wang, & Gao, 2006; Rodrigo, van Loey, & Hendrickx, 2007). Thus, changes in the color of fruit and vegetable derived products could be related to changes in enzymatic activities or in other food

Table 3
Chromatic parameters L*, a*, b*, C* and h° of beverages, unpressurized (0.1 MPa) or HHP-treated (600 MPa).

Beverage	L*	a*	b*	C*	h°
0 Ca – 0.1 MPa	68.0 ± 0.9^{e}	−3.8 ± 0.2^{b}	1.0 ± 0.5^{d}	4.0 ± 0.2^{e}	164.8 ± 6.1^{b}
0 Ca – 600 MPa	71.2 ± 3.6^{d}	−4.1 ± 0.1^{a}	−0.2 ± 1.5^{e}	4.3 ± 0.1^{e}	182.5 ± 19.8^{a}
10 Ca – 0.1 MPa	83.1 ± 0.3^{a}	−1.1 ± 0.1^{d}	10.5 ± 0.3^{b}	10.6 ± 0.3^{c}	96.0 ± 0.6^{c,d}
10 Ca – 600 MPa	77.1 ± 1.4^{c}	−1.8 ± 0.3^{c}	7.8 ± 1.2^{c}	8.0 ± 1.1^{d}	103.5 ± 3.5^{c}
20 Ca – 0.1 MPa	83.0 ± 0.3^{a}	−0.3 ± 0.1^{e}	12.9 ± 0.2^{a}	12.9 ± 0.2^{a}	91.2 ± 0.5^{d}
20 Ca – 600 MPa	79.4 ± 0.2^{b}	−0.5 ± 0.1^{e}	11.4 ± 0.2^{b}	11.4 ± 0.2^{b}	92.3 ± 0.4^{c,d}

Sample names of beverages correspond to concentration of $CaCl_2$-added (0, 10 or 20 mmol L^{-1}) and pressure level (0.1 or 600 MPa). Different letters in the same column indicate significant differences ($p < 0.05$).

component structure.

Chromatic parameters of beverages are shown in Table 3. L* value of pressurized beverage without calcium addition was higher than that of unpressurized one. Moreover, HHP treatment also increased h° and decreased a* values in non-calcium added beverages which indicates an increase in greenish hue.

Calcium incorporation significantly increased ($p < 0.05$) L*, C* and a* values of unpressurized beverages with 10 and 20 mmol L^{-1} $CaCl_2$ (Table 3). Calcium addition induces soy proteins insolubilization. Thus, whitish-ivory color of these aggregates could be responsible for L* increase. Moreover, calcium addition significantly increased b* and decreased h° values ($p < 0.05$) both at 10 and 20 mmol L^{-1} $CaCl_2$, which produced that beverages acquire a more yellowish color.

HHP treatment of calcium-added beverages (10 and 20 mmol L^{-1} $CaCl_2$) produced a decrease in L* and C* values in relation to unpressurized ones, obtaining darker and more opaque beverages. The decrease in L* values could be associated with a turbidity sample reduction because of the formation of smaller aggregates, as previously reported by Manassero et al. (2018), and/or with different structure due to HHP treatment with unaffected PS. This effect (turbidity decrease with no changes in PS) was also observed by Manassero et al. (2018) in 10 g protein L^{-1} SPI dispersions at pH 5.9 added with 2.5 mmol L^{-1} of $CaCl_2$. However, pressurized calcium-added beverages (10 and 20 mmol L^{-1} $CaCl_2$) showed higher values of L* and C* ($p < 0.05$) with lower values of h° (close to 90°) ($p < 0.05$) than those obtained from unpressurized and non-calcium added beverages (Table 3).

4. Conclusions

HHP treatment improved physical stability of calcium- and soybean proteins-added peach juice. Nevertheless, HHP treatment produced no change in protein solubility of these beverages. Factors depending on beverage composition (peach components and/or pH/calcium/protein ratio) could be the cause of the lack of the solubilizing effect observed in other systems. These results indicate that the stabilizing effect of HHP occurred independently of an increase in protein solubility.

This work provides information that may be useful in the handling of fruit-based beverages in order to improve physicochemical characteristics (e.g. increase of physical stability) and nutritional value (incorporation of proteins and calcium) by means of HHP treatments.

Further studies of the effect of HHP on the food safety would be needed to deepen the knowledge and provide new functional foods.

Acknowledgments

The authors wish to thank Claudio Sanow from the Instituto Tecnología de Alimentos INTA, for his kind assistance during the use of the HHP equipment. This research was supported by the Instituto Nacional de Tecnología Agropecuaria (INTA, grant PE PNAIyAV 1130033), and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, grant 11220110101109), Argentina.

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