High Hydrostatic Pressure Processing of Beetroot Juice: effects on Nutritional, Sensory and Microbiological quality

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ABBREVIATIONS: AAPH, 2,2'-azobis(2-methylpropionamidine) dihydrochloride; ABTS, 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid; AUC, area under the curve; CFU, colony-forming unit; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalents; HPP, high pressure processing; ORAC, oxygen radical absorbance capacity; TA, titratable acidity; TSS, total soluble solids; TPTZ, 2,4,6-tri2-pyridyl-s-triazine; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TE, Trolox equivalents.

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ABSTRACT

Beetroot juice is a source of polyphenols and betalains. Betalains serve both as colourants and bioactive compounds, and are known to be sensitive to temperature. Conventional thermal treatments can significantly affect the visual appearance and the nutritional quality of beetroot juice and, in consequence, the ultimate consumer acceptance. Our aim is therefore to evaluate the effects of an alternative high pressure processing on the physicochemical, nutritional, sensory and microbiological quality of a beetroot juice. The results showed that a high pressure-processed beetroot juice (600MPa, RT, 3min) retained the physicochemical and nutritional properties (bioactive compounds and antioxidant properties) of the freshly squeezed juice with no clearly differentiated sensory quality. The microbiological shelf-life of the juice was established at 21 days, under refrigerated conditions, observing only minor nutritional losses throughout storage. The promising results of high pressure processing for the preservation of betalain-rich juices therefore suggests that the technique represents a useful alternative to conventional thermal treatments.

Keywords: beetroot juice, high hydrostatic pressure processing, bioactive compounds, antioxidant properties; microbiological quality, sensory quality.

1. Introduction

Epidemiological research has established an inverse relationship between fruit and vegetable intake and the risk of cardiovascular disease, cancer, and other morbid conditions [1]. Although all the reasons may never be crystal clear, the anti-oxidant properties of fruit and vegetables are frequently cited as protective agents against oxidative stress, due to their high content of bioactive (phytochemical) compounds with antioxidant potential [2].

Commercial fruit and vegetable juices are marketed today as alternative and convenient products that contain those health promoter compounds [3]. Comparative studies have highlighted the high antioxidant potential of beetroot juice, even greater than pomegranate or cranberry juice [4,5].

The total antioxidant capacity of beetroot is mainly explained by two bioactive compounds: betalains and polyphenols [6]. Betalains are water-soluble pigments that include two sub-groups of compounds: purple-red betacyanins (mainly betanin) and yellow-orange betaxanthins (mainly vulgaxanthin) [7]. In addition to the antioxidant properties of betalains, these pigments have been variously described as having immunosuppressive, anti-inflammatory, hepatoprotective and antitumorous biological activity [7]. The powerful antioxidant potential of polyphenols is also widely recognised. Considerable and plausible biological evidence suggests that dietary polyphenols will influence human health. Investigations with human and animal cell lines show different forms of polyphenol biological activity in various different environments with proapoptotic effects on abnormal cellular proliferation, yet with a protective effect on normal cells. Their ability to reduce inflammatory stress in certain cells has also been established [2,8].

Thus, beetroot juice contains a promising mix of bioactive compounds. Nevertheless, the low acidity of this juice and undesirable traces of root vegetable soil microflora restricts its

availability as a freshly squeezed product in the market place. In this respect, thermal treatments are widely applied to improve the microbiological stability of many fruit juices. However, in the processing of beetroot juice, they can negatively affect juice quality due to organoleptic and nutritional losses [9]. The attractive colour is caused by the betalain pigment that is especially sensitive to heat treatment [10].

High-pressure processing (HPP) is an alternative to the thermal treatment of many foods and can inactivate microbial pathogens with minimal loss of food quality. Labile nutrient deterioration is lower in comparison with traditional methods, and natural flavor and food coloring is not significantly altered, as high pressures are not conducive to the Maillard reaction or non-enzymatic browning [11].

HPP has been tested on several juices [11], though its effect on beetroot juice needs further research. Some authors have studied the effect of high pressure processing on the microbiological quality and pigment stability of a beetroot juice [12]. However, other quality attributes (acidity, pH, ºBrix, colour, phenolic compounds, total antioxidant capacity) and the sensory acceptance of the processed product have, to the best of our knowledge, not been studied in any evaluation of the impact of HPP on beetroot juice. In addition, a processed juice will be consumed after several days of storage and there is no available data concerning the evolution of these quality parameters throughout the shelf life of beetroot juice. Therefore, the main objectives of this study will be to investigate beetroot juice and the effects of HPP treatment on its physicochemical parameters, native micro flora, bioactive compounds, antioxidant activity, and sensory quality.

2. Material and methods

2.1. Beetroot juice extraction

Fresh beetroots (*Beta vulgaris, cv. Detroit 2*) were used in the experiment within twenty-four hours following their purchase from a local market. The beetroot was washed in running tap water, peeled, cut into pieces and crushed in a domestic juicer. The extracted juice was filtered through a cheesecloth to remove the remaining pomace, poured into 150 ml polyethylene bottles, and immediately processed.

2.2. High hydrostatic pressure processing (HPP)

The juice was pressurized in a commercial high pressure machine Hiperbaric 135 model, equipped with a 135 L vessel (Hiperbaric, Burgos, Spain). The high-pressure treatment was conducted in triplicate, at 600 MPa, 3 min, room temperature (RT), the most common conditions for juice preservation. Processed juice was stored at 4°C during 21 days, and analysed every 7 days (HPP-0, HPP-7, HPP-14, HPP-21).

2.3. Physicochemical analysis

2.3.1. pH, titratable acidity and total soluble solids

The pH value of the sample was determined at 25.0 ± 2°C with a pH meter, calibrated with pH 4.0 and 7.0 buffer.

Titratable acidity was determined by titrating 20 mL of beetroot juice, with standardized 0.1 M NaOH, to the phenolphthalein end point (pH 8.1). The results are expressed as mg NaOH/ml juice.

Total soluble solids (°Brix) were measured with a refractometer (Atago 3T) at 22.0 ± 2°C.

2.3.2. Colour analysis

L* (lightness), a* (green to red), and b* (yellow to blue) parameters were measured using a Hunter Lab colorimeter (Hunter Lab Colour Flex EZ 45/0° colour spectrophotometer, USA).

The results were expressed in accordance with the CIELAB system with a reference to illuminate D65 and with a visual angle of 10°. The total colour difference (ΔE*) was calculated using Eq. (1) and is a measure of the difference in colour between an HPP beetroot juice sample and a fresh (non-processed) sample [13].

Eq. (1) $\Delta E^* = \sqrt{(L^* - L_o^*)^2 + (a^* - a_o^*)^2 + (b^* - b_o^*)^2}$

2.4. Total betalain content

The content of betaxanthins and betacyanins in the extracts was determined spectrophotometrically at 480 nm and 538 nm, respectively, according to a previously described method [14]. Total betalains were the sum of both betacyanins and betaxhantins. The results were expressed as mg of total betalains (µg TB) per ml juice.

2.5. Total phenolic content

The quantification of total polyphenols was based on the Folin-Ciocalteu method [15]. Briefly, beetroot juice (50 µl) was added to 0.5 mL of freshly prepared Folin Ciocalteu reagent (1:10 v/v, with water). The mixture was allowed to equilibrate for 5 min and then mixed with 0.4 mL of sodium carbonate solution (7.5%). After incubation (RT, 2 h in the dark), the absorbance of the mixture was read (760 nm). The results were calculated based on a calibration curve and expressed as mg of gallic acid equivalents (µg GAE) per ml juice.

2.6. Antioxidant activity assays

2.6.1. ABTS method

The ABTS assay was conducted following a method described elsewhere [16]. Briefly, the $ABTS^{+\bullet}$ was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark 12-16 h before use. The $ABTS^{+\bullet}$ working solution was diluted with water to an absorbance of 0.75-0.80 (734 nm). The sample

(20 µl of diluted juice) was mixed with 980 µl of $ABTS^{+\bullet}$ reagent. The absorbance reading was taken after 30 min of incubation (RT in the dark). Trolox solutions were used to perform the calibration curve and the results were expressed as µmol equivalents of Trolox per ml of juice (µmol TE/ml).

2.6.2. DPPH method

The DPPH assay was performed as suggested elsewhere [17]. Stock solution of $DPPH^{\bullet}$ was prepared daily by dissolving 40 mg of $DPPH^{\bullet}$ in 100 mL of methanol. The working solution of $DPPH^{\bullet}$, having an absorbance value of 0.75–0.80 at 525 nm, was prepared by diluting the stock $DPPH^{\bullet}$ solution with methanol. The sample (20 µl of diluted juice) was mixed with 980 µl of $DPPH^{\bullet}$ reagent. The absorbance reading was taken after 120 min of incubation (RT in the dark). Trolox solutions were used to perform the calibration curve and the results were expressed as µmol equivalents of Trolox per ml of juice (µmol TE/ml).

2.6.3. FRAP (Ferric Reducing Antioxidant Power) method

The ferric reducing ability of the samples was determined using a modification of a previously described method [18]. Briefly, FRAP reagent was prepared from 300 mM acetate and glacial acetic acid buffer (pH 3.6), 20 mM ferric chloride and 10 mM 4,6-tripryridyl-s-triazine (TPTZ), and mixed together in a ratio of 10:1:1 (v:v:v). Diluted juice (20 µl) was mixed with 980 µl of FRAP reagent. The sample was incubated at 37°C in the dark and the absorbance readings were taken at 30 min (593 nm). The results were expressed as µmol equivalents of Trolox per ml of juice (µmol TE/ml).

2.6.4. ORAC (Oxygen Radical Absorbance Capacity) assay

This assay was conducted following a method described elsewhere [19]. The analysis was performed in a fluorimeter (Cary-Eclipse, Varian) at 37°C. Briefly, the samples (juice, Trolox

solution or phosphate buffer (pH=7.4) for the blank) were mixed with a freshly prepared fluorescein solution. The fluorescence reading (λexc = 493 nm and λem = 511 nm) was started, and AAPH solution was added after 5 min. The fluorescence decay curve was monitored for 120 min. The net area under the curve (AUC) was calculated for each sample (both juice and Trolox solution) by subtracting the blank AUC from the sample AUC. The AUC of the Trolox solution was used for the expression of the ORAC values as µmol equivalents of Trolox per ml of juice (µmol TE/ml).

2.7. Microbiological analysis

Microbiological analysis was performed to enumerate spoilage (aerobic mesophilic bacteria, yeasts and moulds and lactic acid bacteria) and hygiene indicators (coliforms). For each sample, an aliquot of 10 mL was diluted (1/10, v/v) with sterile Ringer's solution (Oxoid, UK). Further decimal dilutions were prepared with the same diluent. Undiluted beetroot juice was also sampled. Aerobic mesophilic bacteria were determined on Plate Count Agar (Oxoid, UK) after incubation at 30°C for 5 days. Yeasts and moulds were counted on Sabouraud agar (Sigma Aldrich, USA) after incubation at 25°C for 5 days. Coliform counts were determined on a VRBG agar plate (Condalab, Spain) after incubation at 37°C for 24-48 h. Lactic acid bacteria were cultivated in MRS agar (Oxoid, UK) and incubated at 6% in a CO_2 incubator, at 30°C for 48 h. All determinations were performed in triplicate and counts were expressed in log10 [colony-forming unit (CFU)/ml]. The detection limit was 1.0 CFU/ml for aerobic mesophilic bacteria and coliform determination with a detection limit of 10.0 CFU/ml for lactic acid bacteria and mould and yeast determination.

2.8. Sensory analysis

A consumer triangular test was used in this study. Sixty-six untrained consumers, who were unaware of the experimental conditions, analysed the beetroot juice. The group comprised 44

women and 22 men aged between 18 and 65 years old. Twenty millilitre-samples from fresh and high pressure-processed juice were prepared for sensory evaluation. Three cups (two of which were known to be alike -from the same treatment condition- and the third different) were presented to each person on a plate with a code, as per Spanish guidelines [20]. Panelists received these samples and were invited to discriminate between them in individual cabins under controlled environmental conditions and white light. Panel members were provided with room-temperature water to cleanse their palates between samples. Data were analysed according to the Spanish guidelines [20].

2.9.Statistical analysis

All analysis was carried out in triplicate (n=3) and the results expressed as mean values ± standard deviation. One-way analysis of variance (ANOVA) and LSD test ($p<0.05$) were computed with the Statgraphics Centurion XVI software package (StatPoint Technologies, Inc., USA).

3. Results and Discussion

3.1. Effect of HPP and storage on pH, titratable acidity and total soluble solids

The pH and titratable acidity (TA) values of the untreated juice are presented in Table 1.

After HPP treatment, a slight decrease in the pH of the beetroot juice was observed, which might be considered negligible. The treatment applied had no significant effect on the TA of beetroot juice. No significant changes were observed in either the pH or the TA during refrigerated storage at 4ºC (Table 1). These results agree with the results of other studies on pomegranate [21], watermelon juice [22], orange juice [23], cashew apple juice [24], blueberry juice [25], and sugarcane juice [26], where HPP retained the physicochemical properties of the untreated juices. In contrast, the previously reported increase in the pH of thermally pasteurized beetroot juice is perhaps related to ascorbic acid degradation under high temperatures [27].

The total soluble solids (TSS) value of fresh beetroot juice is presented in Table 1. There was no change in TSS, immediately after HPP treatment, in comparison with the untreated juice. Neither were significant changes observed throughout storage (Table 1). Similar findings in different juices have previously been reported [21–26,28]. Some authors argued that high hydrostatic pressures could inactivate enzymes related to converting soluble and insoluble solids, and therefore the maintenance of TSS observed after this processing. Kathiravan et al. (2014) [27] showed an increase in the TSS of thermally pasteurized beetroot juice, which might be due to water evaporation during thermal pasteurizing in the steam jacketed kettle .

3.2. Effect of HPP and storage on beetroot juice colour

The influence of HPP treatment on the instrumental colour parameters (L*, a* and b*) of beetroot juice is presented in Table 2. The fresh beetroot juice had redness (a*), yellowness (b*) and luminosity (L*) values that highlights the characteristic red colour of beetroot and is in line with previously reported data [27].

A significant increase in the three colour parameters was observed after HPP treatment, the most remarkable of which was redness. Based on these findings, high HPP treatment would result in a redder beetroot juice. However, according to previous authors, a noticeable difference between two colours can be visualized when they differ by $\Delta E>2–3.5$ [13]. Our results showed that ΔE just after processing was 3.49±0.04, which indicates that HPP could maintain the original colour of beetroot juice, which is in agreement with previous research [29].

Some authors have tried different permutation and combinations to explain the colour of beetroot in terms of ‘L’, ‘a’ and ‘b’ values, and the ratio ‘a/b’ was found to be the best parameter to express the colour degradation correctly and quantitatively [30]. Our results showed no significant change in the “a/b” value immediately after the treatment; from 6.54±0.59 to 6.27±0.29 for the control and the HPP juices, respectively, corresponding to a colour

degradation of 4%, much lower than the degradation (27%) observed in conventional pasteurized beetroot juice [27].

Both the a* and the b* colour parameters increased throughout storage with the highest increase shown for yellowness. Consistent with previous research [27], this increase may be related to the degradation of violet-red pigments into yellow-brownish ones while in storage. Our results showed that during the storage, the "a/b" value decreased to 4.20±0.22 after 21 days at 4ºC, corresponding to 36% colour degradation. A similar degradation was found in a conventional pasteurized beetroot juice after the same storage period (32%) [27], which suggests that the colour stability of beetroot juice throughout the storage period may not depend on the initial food processing technique. Some authors have also observed that the colour changes of orange juice during storage do not correlate with the type of processing [31,32]. In contrast, colour changes of high pressure-processed tomato and carrot juices after refrigerated storage were lower than those observed in the thermal treated samples [33]. Therefore, the interaction processing-storage would appear to depend on the food matrix.

Other available data concerning the impact of high hydrostatic pressures on beetroot colour were obtained from beetroot slices, where the values of both a* and b* were significantly lower in HPP samples [34], indicating a blue-shift in colour (from red to red-violet). Although not consistent with our findings, these authors discussed their results in relation to the higher content of betanin (absorbing at 536 nm) found in the pressurized samples. Whether there is an effect on the betalain pigments in our samples will be discussed in the next section.

3.3. Effect of HPP and storage on betalains of beetroot juice

The total betalain content of the beetroot juice analysed in this study was 1231±63 µg/ml, in which betacyanins and betaxanthins accounted for 61% and 39% of the total amount, respectively (Table 3). These results are within the wide range reported by other authors (610-

1630 µg/ml and 310-950 µg/ml for red and yellow pigments, respectively) taking into account that the betalain content is variety-specific [35].

There were no significant changes in betalain content following HPP treatment (Table 3). Some authors have also reported the stability of betalains under 400 MPa processing [36]. However, higher pressures (500-550 MPa) and longer treatments (4-10 min) caused higher betalain decreases (8-12%) in prickly pear beverage and beetroot juice [12,36]. The temperature increase of 26-to-33ºC during processing, due to adiabatic heating under compression [12,36], might suggest that pigment degradation under these conditions is merely a consequence of pressure mechanisms. The high sensitivity of betanin to the effect of oxygen, temperature, pH, light and enzymatic activity is well known. Some authors have even speculated that the baro-induced increase of oxygen partial pressure in high pressure-processed samples could account for the betalain reduction observed over more extended treatment periods [34].

In contrast, there is a significant decrease in betalains after conventional heat treatment, a long way off from the levels of betalain stability under the processing conditions applied in our study. Both UHT and pasteurization treatments are known to cause decreases in the betalain content of juices of around 26% (UHT) and 40-42% (pasteurization) for betacyanins; and 45% (UHT) and 43-48% (pasteurization) for betaxanthins [12,27,36], which is consistent with betalain degradation under high temperatures [27].

Our results showed a reduction in the betacyanin fraction throughout the shelf life of the high pressure-processed juice (36% decrease after 21 days of storage) (Table 3) with a concomitant increase in the betaxanthins. Betacyanin pigments degrade and form yellow pigments, which is why the increase observed in the betaxanthin fraction may be mostly due to the betacyanin derivatives. These derivatives have been identified by several authors [10,37] and our results confirm the partial degradation of betacyanins throughout storage [10]. Irreversible inactivation

of the enzymes that affects pigment degradation while in storage might be a possible explanation. So, residual enzymatic activity plus dissolved oxygen in small concentrations could be the cause of betacyanin degradation throughout the storage life of the HPP treated juice, as widely reported in the literature [38].

A higher degradation of betacyanins (50% reduction) has been reported in thermally treated beetroot juice after the same storage period [27], although the storage conditions in that study, were at "room temperature". Based on these data, it is difficult to conclude whether the higher reduction in the betacyanin content of the thermally treated samples was due to a residual effect of the treatment itself or to the higher temperatures throughout storage. In any case, these findings confirm that although betalain stability is influenced by different factors such as pH, temperature, light, and oxygen [10], temperature may be the most important factor in betalain stability during food processing and storage [7].

It is only ascorbic acid according to several researchers that is better preserved as a bioactive compound by HPP than by thermal treatment [39]. However, our results confirmed that betalains should also be highlighted and that HPP is a promising treatment for betalain-rich juices.

3.4. Effect of HPP and storage on the total polyphenol content of beetroot juice

The total polyphenol content of the beetroot juice analysed in the present study (Table 4) is within the range reported for this type of juice (0.9 to 1.3 g/l) [5,35]. Most phenolic compounds in beetroot form part of the betalain family, while all other compounds amount to only a very small fraction. Among these substances, the most abundant group are hydroxicinnamic acids among which gallic acid shows higher levels, followed by caffeic acid, syringic acid and ferulic acid [35].

There was no significant change in the total polyphenol content of beetroot juice immediately after HPP treatment (Table 4). The literature on the effects of HPP on total polyphenols for beetroot juice is scarce. An increase was observed in the total polyphenol content of beetroot slices treated with HPP [34]. However, some studies have shown this influence in several kind of juices and different trends in the behaviour of polyphenols in response to pressure have been reported. In agreement with our findings, no significant changes after HPP were reported for blueberry juice (600 MPa, 5 min) [25], sugar cane juice (300-600 MPa, 10-25 min) [26], papaya beverage (350-650 MPa, 5-10 min) [40], mango nectar (600 MPa, 1 min), [41] and watermelon juice (200-600 MPa, 5-60 min) [22]. In contrast, an increase in the total polyphenol content was observed in pomegranate juice (400 MPa, 10 min) [38], (450-550, 30-150 s) [21] and cashew apple juice (250-400 MPa, 3-5 min) [24]. These increases in total polyphenol content could be due to the fact that the high pressure has the capacity to increase the permeability and can even rupture plant cell membranes, resulting in the release of these phytochemicals [42]. Some authors even reported a decline in total polyphenol content after the high pressure processing for green asparagus juice (600 MPa) [43] and aronia juice (200, 400, 600 MPa, 15 min) [44].

These varying effects could be related to either the type of matrix or the pressure and time conditions of the treatment. Some authors compared two prickly pear varieties with different contents of vitamin C, observing that the effect of HPP treatment depended on the fruit variety (no change *vs.* a decrease in the total polyphenol content); vitamin C might therefore exert a protective effect on the oxidative reactions of phenolic compounds [36]. Matrix composition is therefore a key factor for any prediction of the effect of HPP, hence the need to evaluate the effect of the treatment on each specific product. The variations in the results among processing conditions may be attributed to a balance between higher extractability after plant cell disruption and oxidation degradation of phenolic compounds [26].

The specific type of polyphenol is an additional factor to take into account. Indeed, some authors proved that treatment differed depending on the type of polyphenol under study [45,46]. Caffeic acid, narirutin and hesperidin showed no significant differences among the different types of processing (550-650 MPa), whereas p-coumaric acid, chlorogenic acid, daidzin and genistein increased significantly after HPP [45]. In other reports, higher pressures increased the concentration of hesperidin (34-22%, depending on the treatment conditions applied), while no change was reported for flavanones in orange juice [46].

While some fluctuations in the total polyphenol content of the high pressure-processed juice were observed during the first week of storage (maybe due to some reducing compounds formed throughout storage that react with the unspecific Folin-Ciocalteu reagent and enhance the phenolic content [47]), our results showed a progressive decline in the total polyphenol content from day 14 of storage. Similar results were observed in other high pressure-processed juices throughout the shelf life [21,25,40,41,44,48]. This decrease could be due to both enzymatic and no enzymatic degradations. Polyphenol oxidase and peroxidase were considered the main enzymes responsible for the decay of phenolic compounds [48]. Even though HPP enzyme inactivation was reported in several juices [49,50], the inactivation may be reversible. Since there is no data concerning such inactivation in beetroot juice, residual activities of the enzymes together with a small concentration of dissolved oxygen could be hypothesized for the decrease observed throughout storage. Decreased amounts of polyphenol compounds might also be the consequence of their oxidative degradation and the polymerization of phenolic compounds with proteins [51].

It is important to highlight that our results showed that more than 90% of these bioactive compounds were retained after 21 days of refrigerated storage. Therefore, high pressure-processed beetroot juice has a similar nutritional value as a freshly squeezed juice, in terms of the total polyphenol content. In a comparison of the effects of HPP and thermal treatments on

the total polyphenol content of juices, certain authors concluded that high pressure processing preserved notably higher concentrations of total phenolics than thermal treatments [40,52]. Other authors stated no significant changes in polyphenols in HPP (550, 650 MPa) of soy smoothies [45] after 45 days of storage.

3.5. Effect of HPP and storage on the total antioxidant capacity of beetroot juice

The antioxidant capacity of fresh beetroot juice (Table 5) was consistent with previously reported data [5,35] and differed from others [35,53], due to the fact that the total antioxidant capacity was reported to depend on the beetroot variety [35].

While the total antioxidant capacity of beetroot juice, determined by ABTS and DPPH assays, decreased immediately following HPP (8 and 10%, respectively), no statistical differences between fresh and processed juices were observed when FRAP and ORAC assays were applied (Table 5). In relation to those results, both the FRAP and the ORAC assays followed similar trends to those observed for betacyanins and total polyphenols, as no changes were detected for those bioactives after the HPP treatment. The decrease in the total antioxidant capacity determined by ABTS and DPPH could perhaps be due to a decline in other antioxidant compounds not assayed in the present study, but which also accounted for the total antioxidant capacity of beetroot. No data can be found in the literature describing the changes in the total antioxidant capacity of beetroot juice following HPP treatment, again suggesting these changes are reported here for the first time. A decrease of around 13%, measured by DPPH, was reported for pasteurized beetroot juice [27]. Varying results can be found in literature concerning the effects of HPP on the total antioxidant capacity of other types of juices, with no changes, increases, or decreases [11,26,36,40,44,52]. Both the food matrix and its inherent composition, and the processing conditions together with the analytical method might explain the

inconsistent results that were found. Almost all the points in above discussion concerning the bioactive compounds may equally be applied to the antioxidant capacity.

Our results showed a decline in the total antioxidant capacity throughout the shelf life of the juice regardless of the method that was applied. The drop in the total antioxidant capacity of the pressurized juice throughout storage may be directly linked to the bioactive compound degradation, because of either enzymatic or non-enzymatic mechanisms, as discussed in the previous section.

Nevertheless, it is important to highlight that retentions of between 68-91% of the total antioxidant capacity of the fresh juice after 21 days of storage were observed. Therefore, the HPP treatments applied in our study will guarantee a beetroot juice with a high antioxidant potential throughout the shelf life.

3.6. Effect of HPP and storage on the microbiological quality of beetroot juice

The mean initial populations of coliforms, moulds and yeast, lactic bacteria and aerobic bacteria of fresh beetroot juice (Table 6) were within the range reported by other authors [12,27].

Immediately after the HPP treatment, the microbial population counts under study were significantly reduced (Table 6). HPP treatment decreased coliforms and lactic bacteria below their detection levels and resulted in a 2.5 and a 3.2 log reduction in moulds and yeast and aerobic bacteria, respectively. Higher reductions in the total count of spoilage microorganisms were found after treatment (500 MPa) of the beetroot juice for 10 min [12,29], showing that the total reduction of microorganisms was pressure and time dependent. However, it is important to highlight that the juice analysed by these authors had a lower pH; and a synergistic effect between pH and the high pressure processing can occur, as discussed below.

As it can be seen in Table 6, residual mould and yeast were no longer detected after 7 days of storage. However, the aerobic bacteria count increased throughout storage, reaching counts of 5.12±0.06 log after 21 days. Some authors established the shelf-life of juices when the samples reached microbial counts of 6 log CFU/ml [54]. Therefore, the estimated shelf-life for a high pressure-processed beetroot juice (600 MPa, RT, 3 min) stored at 4ºC was over 21 days. Other researchers previously showed the benefits of high pressure processing on extending the microbiological shelf-life of fruit and vegetable juices [21,25,26,28,38,40,44,48,52]. Longer shelf lives were described in the literature for a variety of high pressure-processed juices: papaya 40 days/4ºC (pH=3.46; 550 MPa, 5 min) [40]; mango 16 weeks/4ºC (pH=3.96; 600 MPa, 1 min) [41]; pomegranate juice 35 days/4ºC (pH=3.2; 350-550 MPa, 30-150 s) [21]; aronia juice 80 days/4ºC (pH=3.5; 400-600 MPa, 15 min) [44]; and, blueberry juice 56 days/4ºC (pH=3.3; 600 MPa, 5 min) [25]. Longer shelf lives might be related to the harsh conditions created by the low pH acidity of the samples, which can increase the lethality of high pressure processing [21]. Some authors have reported that when the pH of a juice is lower than 4.5, the effect of high hydrostatic pressures on microorganism inactivation is enhanced, since low pH levels reduce the tolerance of the microorganisms to high pressure [40]. Decreasing the pH of the beetroot juice before the HPP is therefore an alternative to an increased shelf-life of this juice over 21 days, although further research should be performed to evaluate whether the nutritional quality of this product is still maintained over the suggested period.

3.7. Effect of HPP on the sensory quality of beetroot juice

High pressure-processed beetroot juice was compared with the fresh untreated juice using a consumer triangular test. Data analysis of the triangular tests determined whether the assessor was capable of identifying the different sample.

Our results showed that the tasters correctly selected the odd sample in 29 out of the 66 possible identifications, sufficient to satisfy the required significance level of $P<0.05$ [20]. The sensory panel was therefore able to discriminate between high pressure-processed and fresh-like beetroot juice, although the number of correct identifications were just on the border to decline the distinction between samples. Out of the possible total of 66 correct identifications, 32 would be required to show statistical significance between the samples at a significance level of $P<0.01$, [20]. Based on this level of significance, the sensory panel was unable to distinguish between samples, suggesting that the sensory quality of fresh beetroot juice was maintained in samples pressurized at 600 MPa, 3 min.

Varying results on this point can be found in the literature, with either no alteration or modification of the sensory quality of juices after HPP treatments. Such inconsistent results may be related to the treatment conditions, since more extreme pressures and holding times are prone to induce higher modifications in flavour [55]. Some authors have reported that the HPP treatment preserves sensory qualities better than thermal treatment [40,49], although others found no clear differences in the flavour profile or in the levels of related volatiles between treatments [56].

4. Conclusion

It has been shown that an HPP treated beetroot juice (600 MPa, RT, 3 min) retained the physicochemical and nutritional properties of the freshly squeezed juice with no clear differences in sensory quality. The processing conditions were sufficient to extend the microbiological shelf-life of the juice over more than 21 days under refrigerated storage (4ºC). Throughout the storage period, the physicochemical parameters were maintained and only minimal losses on the nutritional profile (bioactive compounds and antioxidant properties) were observed. On average, 80% of the total antioxidant capacity and 90% of the total polyphenol

content of the fresh juice were preserved after 21 days. Although lower retention levels of the betacyanin content (64%) and concomitant colour changes were observed, they were still higher than those reported for the conventional thermal treatments commonly applied for this juice. Varying the processing conditions and pH of the juice could even guarantee a higher retention of these pigments and a longer shelf-life of the juice, although it is an area that needs further research. Our results have highlighted the application of high hydrostatic pressures as a promising technique that provides a useful alternative to conventional thermal treatments for the preservation of betalain-rich juices.

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