

Original article

Effect of high hydrostatic pressure on the quality of four edible flowers: *Viola × wittrockiana*, *Centaurea cyanus*, *Borago officinalis* and *Camellia japonica*Luana Fernandes,^{1,2,3} Susana Casal,² José Alberto Pereira,¹ Ermelinda L. Pereira,¹ Elsa Ramalhosa^{1*} & Jorge A. Saraiva^{3*}

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Summary The aim of the study was to evaluate the effect of high hydrostatic pressure (HHP) on the appearance, bioactivity and microbial content of four edible flowers along storage. Several treatments at 75–450 MPa and holding times (1, 5 and 10 min) were applied. Borage and camellia were unacceptable after all treatments, while centaurea showed good appearance at 100/5 MPa min⁻¹; however, the shelf life did not increase. Pansies treated at 75/5 and 75/10 MPa min⁻¹ also retained the appearance of fresh flowers. Furthermore, pansies submitted at 75/5 MPa min⁻¹ maintained good appearance over 20 days of storage at 4 °C, while the untreated remained satisfactory only until 6 days. Even though no significant differences on microbial load were observed between untreated and HHP-treated pansies in day 0, HHP induced the production of bioactive compounds, increasing the shelf life of pansies. So, the HHP treatment is a promising technology for *Viola × wittrockiana*.

Keywords Appearance, bioactivity, edible flowers, high hydrostatic pressure, microbial load, storage.

Introduction

Edible flowers are becoming more popular, as evidenced by the increase in number of cookbooks, culinary magazine articles and television segments dedicated to edible flowers (Mlcek & Rop, 2011). On the other hand, despite still being regarded as a niche market, attention to this kind of product begins to increase due to their interesting potential as a source of nutrients and bioactive compounds (Patel & Naik, 2010; Benvenuti *et al.*, 2016; Loizzo *et al.*, 2016; Lu *et al.*, 2016) in line with the actual healthy food trends.

Nevertheless, edible flowers are quite perishable and have a very short shelf life. Until this moment, the unique technologies used by the industry are cold storage (Landi *et al.*, 2015), hot air convective drying,

freeze-drying and other drying methods (Oberoi *et al.*, 2007; Ding *et al.*, 2012; Zheng *et al.*, 2015). However, all these methods have drawbacks: cold storage is a short-term food preservation method, hot air convective drying may cause undesirable biochemical and nutritional changes in the processed product that may affect its overall quality, and freeze-drying has high productive costs. Therefore, the food industry is very interested in improving the marketability of edible flowers, not only as fresh but also as processed products. Furthermore, many health conscious people prefer unprocessed (e.g. lotus or marigold flowers) or minimally processed forms (e.g. dried rose petals or saffron powder) rather than supplements (Chen & Wei, 2017).

In this sense, finding new food technologies able to increase the shelf life of this kind of product will bring important economic benefits. Hence, high hydrostatic pressure (HHP) treatments appear as good alternatives to extend shelf life and keep the original

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freshness, taste and odour of products (Corbo *et al.*, 2009). HHP is an innovative and emerging technology, already in use by the food industry to preserve a wide range of products (Chawla *et al.*, 2011; Huang *et al.*, 2017). Recently, Fernandes *et al.* (2017) performed a review on the effect of HHP on edible flowers' properties, stating that broccoli and cauliflower, which are inflorescences usually not considered by consumers as flowers, have been the most studied. Much less information exists for other edible flowers. Thus, the aim of this work was to evaluate the potential of HHP to preserve four edible flowers, namely, pansies, borage, centaurea and camellia, the most sold and known by consumers. So, in this study several combinations of high pressure (between 75 and 450 MPa) and time (5 and 10 min) were tested. The quality of edible flowers was evaluated in relation to some physicochemical characteristics, namely: visual appearance, colour, water activity (a_w) and weight loss. Afterwards, the best binominal was selected for each flower according to the properties mentioned above, being studied the flowers' behaviour during normal storage conditions (4 °C). Previously, visual scales were developed in order to be used in the future to easily evaluate the appearance of the mentioned flowers.

Materials and methods

Samples

White/violet fresh pansies (*Viola × wittrockiana*), blue centaurea (*Centaurea cyanus*), blue borage (*Borago officinalis*) and rose camellia (*Camellia japonica*) in full ripening state were collected at the greenhouse of School of Agriculture, Polytechnic Institute of Bragança, Portugal. After harvest, the fresh flowers were immediately transported to the laboratory under refrigeration.

High hydrostatic pressure treatments

For each HHP treatment, fresh flowers were placed into polyethylene bags (one flower per bag) and sealed after eliminating the contained air. The bags were placed into a hydrostatic pressure vessel (55 L volume) of a Hiperbaric equipment (Burgos, Spain). Different pressures and holding times have been tested in each flower, as each flower had different behaviour when subjected to HHP. Pansies were treated at 75, 150 and 450 MPa for 5 and 10 min, centaurea at 75, 100, 200 and 300 MPa during 5 min, borage at 75 MPa for 1 and 5 min, and camellia at 75 MPa for 1 and 5 min and 100 MPa for 5 min. All assays have been done at room temperature, and each pressure/time combination was performed in triplicate.

Physicochemical characterisation

Visual appearance of the edible flowers

Visual scales were firstly established for the fresh flowers by evaluating their appearance along 8 days after harvest and storage at 4 °C. Every day, at the same time and conditions, pictures of the flowers were taken, being determined the a_w and weight loss (WL). For pansies, the colour and dimensions were also measured. For each flower, a scale with different classes was established. In Fig. 1, three levels of appearance, namely, excellent, satisfactory and unsatisfactory are represented. In supplement material, the scale used to establish the classes for each flower is presented (Table S1), as well as the pictures associated to these classes (Figure S1). These scales were also used to classify the samples subjected to HHP treatments in order to evaluate their effect on the visual appearance of the edible flowers.

Colour, dimensions, water activity (a_w) and weight loss

The colour of pansies and camellias was evaluated with a colorimeter Minolta CR-400 (Osaka, Japan), using the CIELab scale. L^* , a^* and b^* coordinates, as well as, Chroma (C^*) and Hue Angle (h^*) values, were determined. In order to analyse the colour changes due to HHP treatment, the total colour difference (ΔE^*) was also calculated according to the following equation: $\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$, where Δ was the difference of the parameters' values after the HHP treatment and before it (fresh sample, day 0).

The width and length of pansies were measured with a digital caliper (Powerfix, Berlin, Germany). To evaluate the width and length changes due to the HHP treatments, the differences were calculated according to eqns 1 and 2, respectively:

$$\Delta \text{Length} (\%) = \frac{\text{Length}_{\text{Flower after HHP}} - \text{Length}_{\text{Fresh flower}}}{\text{Length}_{\text{Fresh Flower}}} \times 100 \quad (1)$$

$$\Delta \text{Width} (\%) = \frac{\text{Width}_{\text{Flower after HHP}} - \text{Width}_{\text{Fresh flower}}}{\text{Width}_{\text{Fresh Flower}}} \times 100 \quad (2)$$

The colour, width and length of borage and centaurea flowers were not measure due to the small size of the petals, which difficult the correct measurement.

Water activity (a_w) was determined with a portable water activity meter (Novasina, LabSwift-aw, Lachen, Switzerland).

Weight was measured in a digital balance (Kern ACJ/ACS, Balingen, Germany).

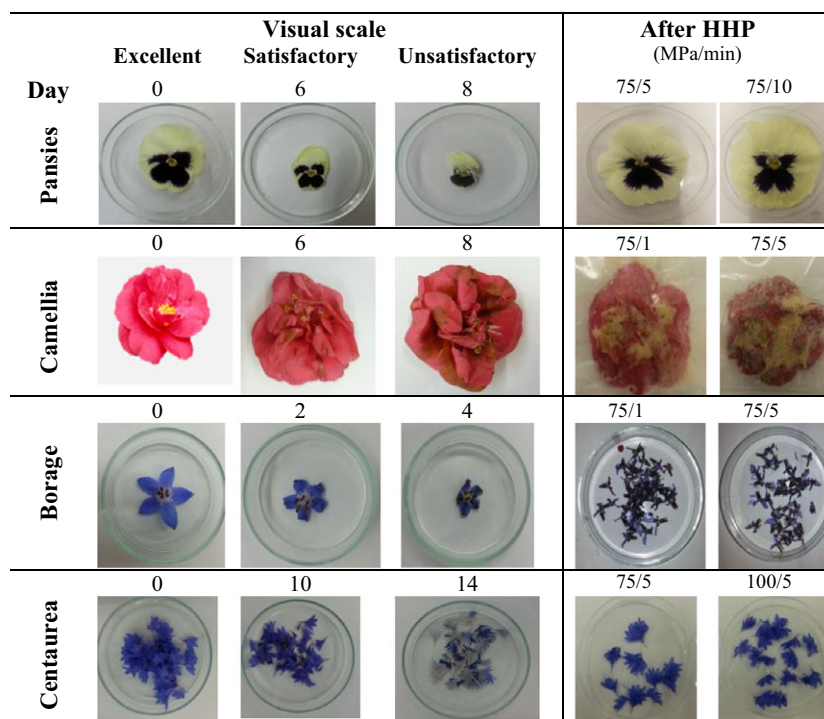


Figure 1 Visual scales and visual appearance after applying HHP treatments to pansies, camellia, borage and centaurea. [Colour figure can be viewed at wileyonlinelibrary.com]

Weight loss was determined according to the following equation: $WL = (M_0 - M)/(M_0) \times 100$, where M_0 is the initial mass of fresh pansies before HHP treatment, M is the mass of pansies after HHP treatment.

Storage

After selection of the best HHP treatment to apply to each flower, namely $75/5 \text{ MPa min}^{-1}$ and $100/5 \text{ MPa min}^{-1}$ for pansies and centaurea, respectively, the flowers' quality was also evaluated during refrigerated storage. Fresh (control) and HHP-treated pansies and centaurea were stored at 4°C until presenting unsatisfactory visual appearance. Every day, at the same time, photographs of the flowers were taken and the a_w , WL, dimensions and colour were measured, as described in the previous section. As unsatisfactory results were observed for borage and camellia HHP treated, even when low pressures were applied, no studies along storage were done for both flowers. As pansies looked suitable after HHP treatment and along storage, the microbial quality and bioactivity of these flowers were evaluated. The methods used are described in the following section.

Bioactivity of pansies

Extraction conditions

The extraction conditions used were those described by Li *et al.* (2014), with slight modifications. Dried

flower powder (0.5 g) was extracted with 50 mL of water:acetone (6:4, v/v) at 37°C for 30 min under agitation (900 rpm, IKA, RCT Model B, Staufen, Germany). The solution was filtered, and the final volume was adjusted to 40 mL.

Flavonoids

Total flavonoid content was determined by the method described by Viuda-Martos *et al.* (2011). Flavonoids were quantified using a standard curve of quercetin ($10\text{--}160 \mu\text{g mL}^{-1}$), being the results expressed in mg of quercetin equivalents/g freeze-dried flower (mg QE g^{-1} freeze-dried flower).

Hydrolysable tannins

The content of hydrolysable tannins was determined by the method described by Elfalleh *et al.* (2012). Different concentrations of tannic acid ($0.025\text{--}1.6 \text{ g L}^{-1}$) were used for calibration. Results were expressed in mg of tannic acid equivalents/g freeze-dried flower (mg TAE g^{-1} freeze-dried flower).

Total monomeric anthocyanin

The total monomeric anthocyanin contents in the flower extracts were estimated by the pH differential method, as described by Bchir *et al.* (2012). The monomeric anthocyanin pigment contents (mg Cy 3-glu g^{-1} freeze-dried flower) were calculated by the following equation:

$$A \times MW \times DF \times 1000 \times 25/(\epsilon \times 1 \times M) \quad (3)$$

where $A = (A_{510\text{ nm}} - A_{700\text{ nm}})_{\text{pH } 1.0} - (A_{510\text{ nm}} - A_{700\text{ nm}})_{\text{pH } 4.5}$, MW = molecular weight (449.2), DF = dilution factor, ϵ = molar absorptivity ($26\,900\text{ L mol}^{-1}\text{ cm}^{-1}$) and M = initial sample mass. All measurements were performed in triplicate.

Total phenolics

The total phenolics of each sample were determined by the Folin–Ciocalteu method, described by Falcão *et al.* (2007). A calibration curve was obtained with gallic acid ($0.25\text{--}5\text{ mg L}^{-1}$), and the results expressed in mg gallic acid equivalents per gram freeze-dried flower (mg GAE g^{-1} freeze-dried flower).

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

DPPH radical scavenging activity was determined by the procedure described by Delgado *et al.* (2010), using $300\text{ }\mu\text{L}$ of flower extract (diluted 50 fold). Antioxidant activity was expressed by the percentage of scavenging effect according to eqn 4:

$$\text{DPPH radical scavenging effect (\%)} = \frac{A_{\text{DPPH}} - A_{\text{Sample}}}{A_{\text{DPPH}}} \times 100 \quad (4)$$

A_{DPPH} was the absorbance of the DPPH solution and A_{Sample} the absorbance in the presence of the sample. The blank was made with the solvent used in the extraction of the samples.

Reducing power

The reducing power of the extracts was determined by the procedure described by Delgado *et al.* (2010), being the results the absorbance values read at 700 nm .

Microbial quality of pansies

Samples (Fresh and HHP treated) at the beginning of storage (0 days) and after 20 days of storage ($4\text{ }^{\circ}\text{C}$) were collected to determine the microbial quality of pansies. Three grams of sample was mixed with 27 mL of sterile peptone water solution and homogenised in a Stomacher. Decimal dilutions were prepared in the same diluent and plated on appropriate media in duplicate. The growth media and incubation conditions were the following for the studied micro-organisms: (i) total mesophilic: Plate Count Agar (PCA; Merck, Algés, Portugal) for 2 days at $30\text{ }^{\circ}\text{C}$; (ii) yeasts and moulds: Rose Bengal Chloramphenicol Agar (RBC agar; Merck) incubated at $27\text{ }^{\circ}\text{C}$ for 5 days; (iii) lactic acid bacteria (LAB): Man, Rogosa and Sharpe Agar (MRS agar; Merck) incubated at $37\text{ }^{\circ}\text{C}$ for 3 days; and (iv) total coliforms and *Escherichia coli* by the SimPlate[®] method. All counts were expressed as $\log_{10}\text{ cfu g}^{-1}$ fresh sample.

Statistical analysis

The results were analysed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test with $\alpha = 0.05$. This treatment was carried out using SPSS version 18.0 program (SPSS Inc., Chicago, IL, USA).

Results and discussion

Flowers' characteristics along cold storage

Regarding pansies, borage, centaurea and camellias cold storage (Fig. 1), borage was the most sensitive, preserving satisfactory quality during the shortest period (only 1 day). On contrary, centaurea maintained a satisfactory quality for 12 days. Pansies and camellias showed an intermediate behaviour, maintaining a satisfactory quality for 6 days. These results show that the flowers exhibit different behaviours after harvest and storage, probably due to the existence of morphological differences between these flowers.

Effect of HHP on flowers' characteristics

Visual appreciation

The four edible flowers subjected to HHP showed different behaviours. Pansies subjected to the three-first pressure treatments combinations ($75/5$, $75/10$ and $150/5\text{ MPa min}^{-1}$) showed the best appearance; however, only the first two binomials maintained a similar appearance to fresh flowers (Fig 1). On contrary, the flowers submitted to the treatment of $150/5\text{ MPa min}^{-1}$ seemed more fragile (Figure S2). After application of the other three combinations, the colour of the flowers changed and mixed (Figure S2). At a pressure of 450 MPa , the flowers swelled for both holding times. When comparing visual appearance after applying HHP with the proposed visual scale, pansies subjected to the first two binomials of pressure/time can be classified as excellent (similar to fresh flowers (0 days)), while flowers submitted at $150/5\text{ MPa min}^{-1}$ were only rated as very good. The remaining binomials did not resemble any level of the proposed scale, being visually considered unsatisfactory. According to the proposed visual scale, the best binomials to be applied in the future to pansies will be $75/5$ and $75/10\text{ MPa min}^{-1}$. Camellia and borage flowers treated at low pressure and time ($75/1\text{ MPa min}^{-1}$) showed unsatisfactory appearance (Fig 1). So, this technology cannot be used to increase their shelf life. Centaurea subjected to the two-first pressure treatment combinations ($75/5$ and $100/5\text{ MPa min}^{-1}$) showed the best appearance, while the flowers submitted to the others binomials seemed damage (Fig. 1 and Figure S2). Until now, some studies have been conducted on other edible flowers (broccolis and cauliflower). For

example, cauliflower treated at 350 MPa maintained its firmness, but it underwent slight browning of the outer portions (Arroyo *et al.*, 1999), while broccoli treated at 600 MPa and 75 °C did not show any detectable effect on chlorophyll *a* and *b*, responsible for the green colour (Butz *et al.*, 2002). These results show that broccolis and cauliflower are more HHP resistant than the edible flowers that were studied in the present work. Thus, each type of flower has its own HHP behaviour, being necessary to perform experiments in order to find out the best pressure/time binomial.

Colour, dimensions, water activity (a_w) and weight loss

Colour and dimensions were measured only in pansies (Tables S2 and S3), due to the difficulty of measuring these parameters in centaurea. A_w and WL of both flowers are presented in Table S3. As borage and camellia flowers were damaged after HHP treatments, they were not evaluated on these parameters.

When analysing the colour of the white part on pansies, significant differences ($P < 0.05$) were observed in almost colour parameters, indicating some effect of HHP treatments on pansies colour (Table S2). In general, colour parameters did not show a constant trend, explaining the inexistence of significant variations on the ΔE^* parameter between treatments ($P = 0.060$). Nevertheless, all HHP treatments caused visible variations on flower's global colour, with ΔE^* of up to 3 units indicating colour changes and appreciable to the human eye (Trivellini *et al.*, 2014). When observing the results of the violet part, it could be stated that this region was more HHP sensitive than the white part, because all Δ values, independently of the parameter, were much higher than those obtained for the white part. Even though no significant differences on Δa^* , Δb^* and Δc^* were observed between treatments ($P = 0.082$, 0.139, 0.159, respectively), the last three HHP treatments (150/10, 450/5 and 450/10 MPa min⁻¹) presented higher ΔE^* values than the other three treatments indicative of higher changes in pansies' overall colour. These results suggest that pansies of different colours will behave differently under HHP and thus each variety must be tested individually, with one-colour pansies probably being less affected.

Regarding dimensions variations (length and width) of pansies, no significant differences were observed between treatments ($P = 0.321$, 0.537, respectively) probably due to the high standard deviations determined, linked to the difficulty of measuring the pansies' dimensions, as previously explained (Table S3). Nevertheless, all HHP caused a reduction in the flowers' dimensions, expressed by the negative values of changes in dimensions, being the lowest variation obtained at the lowest binomial: 75/5 MPa min⁻¹.

Concerning WL of pansies (Table S3), significant differences were found among the six binomials of pressure/time ($P < 0.01$), varying between 4.3% (75/5 MPa min⁻¹) and 13.8% (450/5 MPa min⁻¹). The application of higher pressures induced higher changes, more than 10%. Although all the values determined were lower than the WL observed along storage, WL is undesirable because it will mean a loss of economic revenue and must be minimised. For centaurea, no significant differences were detected between the different pressure/time treatments applied, being the WL < 5% ($P = 0.649$).

Water activity (a_w) is one of the most critical factors in determining quality and safety of food because it affects its shelf life, safety, texture, flavour and smell (Jangam & Mujumdar, 2010). However, after application of HHP to pansies, the a_w values continued to be high, similar to those of fresh flowers (0.980 ± 0.005), and no significant differences between treatments were observed ($P = 0.458$) (Table S3). Centaurea flowers had also high values of a_w after all treatments applied; however, the binomial 75/5 MPa min⁻¹ showed a significant ($P < 0.01$) lower value (0.955 ± 0.001) than other treatments.

Storage behaviour—comparison between HHP-treated and untreated pansies and centaurea

Only pansies and centaurea showed good appearance after HHP treatment, showing borage and camellia to be more HP sensitive, being the structure destroyed more quickly even at low pressures. So only, the behaviour of pansies and centaurea has been studied along storage. Even though untreated centaurea maintained good appearance until 8 days of storage at 4 °C, centaurea treated at 100/5 MPa min⁻¹ only presented good aspect for 1 day (Figure S3). So, HHP did not increase centaurea's shelf life. Untreated (fresh) pansies maintained good condition until 4 days of storage (4 °C), while pansies treated at 75/5 MPa min⁻¹ presented good aspect for 20 days (Figure S3). The different behaviour of pansies and centaurea may be due to their different epidermis structures, as pansies present superhydrophobic structures called papillae (Schulte *et al.*, 2011; Weryszko-Chmielewska & Sulborska, 2012), while centaurea does not, consisting the centaurea florets of elonged cells, with straight walls (Chiru *et al.*, 2013).

Comparing the a_w and WL (Figure S4a,b), both HHP-treated and untreated pansies had similar behaviours until 8 days of storage. After that period, the a_w of HHP-treated samples continued to decrease until 0.564. Regarding dimensions (Figure S4c), untreated samples shrank faster than HHP treated for the same storage period. Concerning colour, the overall colour difference (ΔE^*) showed some variability, with a slight

increase along storage time, being again more perceivable that the violet part on the untreated and treated samples suffered higher colour changes than the white part (Figure S4d). Due to their intense colour, anthocyanin's drainage from damaged cells is probable the cause for these observations. Furthermore, when comparing untreated and HHP-treated samples of the white or violet parts, it was stated that the HHP-treated samples presented slight higher ΔE^* values than the untreated along time, with some exceptions.

Bioactivity of pansies

The highest values of total phenolics, hydrolysable tannins and flavonoids were detected in HHP-treated pansies in day 0 (Table 1). These higher values determined in pansies pressurised at 75 MPa when compared to untreated might be associated with structural alteration of the cells provoked by the HHPs, yielding a higher amount of extracted metabolites (Ferrari *et al.*, 2011). Other possible explanation is that the higher concentrations of those compounds are a physiological response of the flower to stress conditions at higher pressurisation levels (Ortega *et al.*, 2013). HHP-treated pansies after 20 days of storage showed a decrease in all bioactive compounds contents, as well

as in the antioxidant activity measured by the DPPH radical scavenging assay, which can be explained with changes in the activity of enzymes involved in their synthesis, as well as, the presence of oxygen that may cause compounds' oxidation and the occurrence of pressure-induced degradation of polyphenols (Ferrari *et al.*, 2011; Ortega *et al.*, 2013). On contrary, during storage of untreated pansies (0–20 days) the values of these bioactive compounds and antioxidant activity measured by the DPPH assay increased, showing that cold storage might have induced the production of these compounds. When comparing untreated and HHP-treated pansies after 20 days of storage, no significant differences were observed for total phenolics, flavonoids, DPPH radical scavenging effect and reducing power. Furthermore, HHP-treated pansies after 20 days of storage had higher contents of bioactive compounds (except total monomeric anthocyanins) and antioxidant activity measured by the DPPH assay than fresh flowers (day 0). So, the bioactivity of pansies was not influenced negatively by HHP.

Microbial quality of pansies

There were no significant differences between untreated and treated pansies in day 0, except for yeasts counts,

Table 1 Total phenolics, hydrolysable tannins, flavonoids, total monomeric anthocyanins, DPPH radical scavenging effect and reducing power in untreated and HHP-treated pansies at 0 and 20 days of storage

Parameters	HHP untreated		HHP treated	
	0 days	20 days	0 days	20 days
Total phenolics (mg GAE g ⁻¹ freeze-dried flower)	12.2 ± 0.5 ^a	19.2 ± 0.9 ^b	27.3 ± 1.0 ^c	19.8 ± 0.4 ^b
Hydrolysable tannins (mg TAE g ⁻¹ freeze-dried flower)	26.6 ± 1.3 ^a	48.3 ± 0.6 ^c	51.8 ± 3.1 ^c	37.4 ± 0.7 ^b
Flavonoids (mg QE g ⁻¹ freeze-dried flower)	60 ± 6 ^a	134 ± 10 ^b	182 ± 6 ^c	137 ± 10 ^b
Total monomeric anthocyanins (mg Cy 3-glu g ⁻¹ freeze-dried flower)	0.09 ± 0.02 ^a	0.18 ± 0.03 ^b	0.18 ± 0.03 ^b	0.08 ± 0.02 ^a
DPPH radical scavenging effect (%) [*]	12.0 ± 0.5 ^a	24.0 ± 3.9 ^b	32.7 ± 1.1 ^c	22.1 ± 0.3 ^b
Reducing power (Abs _{700 nm})	0.35 ± 0.01 ^a	0.34 ± 0.01 ^a	0.35 ± 0.01 ^a	0.36 ± 0.01 ^a

Values are expressed as: Mean ± SD. Values with the same letter in the same row are not statistically different ($P > 0.05$).

^{*}Percentage relative to a flower extract diluted 50-fold.

Table 2 Mean counts (log cfu g⁻¹ ± SD) of total aerobic mesophilic, yeasts, moulds, total coliforms, *Escherichia coli*, psychrotrophic bacteria and lactic acid bacteria examined in untreated and HHP-treated pansies at 0 and 20 days of storage

Conditions								
Samples	Days	Total aerobic mesophilic	Yeasts	Moulds	Total coliforms	<i>E. coli</i>	Psychrotrophic bacteria	Lactic acid bacteria
HHP untreated	0	7.14 ± 0.01 ^b	5.95 ± 0.03 ^a	<2 ^a	1.30 ± 0.43 ^a	<1	7.07 ± 0.82 ^a	<2
	20	8.97 ± 0.24 ^c	6.11 ± 0.14 ^{a,b}	4.72 ± 1.02 ^b	3.34 ± 0.11 ^b	<1	9.08 ± 0.29 ^b	<2
HHP treated	0	7.20 ± 0.01 ^b	6.35 ± 0.05 ^b	<2 ^a	1.15 ± 0.21 ^a	<1	7.11 ± 0.01 ^a	<2
	20	6.32 ± 0.05 ^a	6.19 ± 0.09 ^{a,b}	<2 ^a	2.80 ± 0.28 ^b	<1	9.11 ± 0.01 ^{a,b}	<2

Values with the same letter in the same column are not statistically different ($P > 0.05$).

indicating that 75 MPa/5 min were not sufficient to cause a significant decrease in micro-organisms load (Table 2). However, after 20 days of storage untreated pansies had higher micro-organism counts than treated pansies, namely for total aerobic mesophilic count and moulds, suggesting some protection of the HHP treatment. *E. coli* and lactic acid bacteria were not detected in any sample.

Conclusion

In summary, borage and camellia flowers subjected to HHP showed unsatisfactory appearance even when low pressures and small times were applied. On contrary, pansies and centaurea flowers submitted at 75 and 100 MPa, during 5 min, showed similar appearance to the fresh flowers. Nevertheless, flowers treated with high pressures showed perceivable changes to the consumer.

During storage (4 °C), HHP-treated centaurea rapidly showed an unsatisfactory appearance compared to the fresh samples. On contrary, HHP induced the production of bioactive compounds in pansies and these maintained good appearance until 20 days of storage, even though they were more dried. No significant differences on microbial load were observed between untreated and HHP-treated pansies in day 0; however, after 20 days of storage, untreated pansies had higher micro-organism counts than HHP treated. So, lower pressures and short holding times may be a promising technology to increase the shelf life of pansies.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Visual scales developed to evaluate the appearance of four edible flowers.

Figure S2. Visual appearance of pansies, camellia, centaurea and borage after applying HHP treatments.

Figure S3. Visual appearance of untreated and HHP treated pansies (75/5 MPa min⁻¹) and centaurea (100/5 MPa min⁻¹) along storage.

Figure S4. HHP treated (75/5 MPa min⁻¹) and untreated pansies during storage for 20 days, for: a_w (A), WL (%) (B), dimension changes (%) (C) and ΔE^* (D).

Table S1. Description of the visual scales established for pansies, camellia, centaurea and borage.

Table S2. Color changes for L^* (ΔL^*), a^* (Δa^*), b^* (Δb^*), c^* (Δc^*), h^* (Δh^*) and total color difference (ΔE^*) of pansies.

Table S3. Weight loss, a_w and changes in dimensions of white/violet pansies and centaurea subjected to different combinations of pressure and time.