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ARTICLE

Ascorbic Acid Is the Only Bioactive That Is Better Preserved by High Hydrostatic Pressure than by Thermal Treatment of a Vegetable Beverage

Francisco J. Barba, Maria J. Esteve, and Ana Frigola*

Nutrition and Food Chemistry, University of Valencia, Avenida Vicent Andrés Estellés s/n, 46100 Burjassot, Spain

Variations in levels of antioxidant compounds (ascorbic acid, total phenolics, and total carotenoids), total antioxidant capacity, and color changes in a vegetable (tomato, green pepper, green celery, onion, carrot, lemon, and olive oil) beverage treated by high hydrostatic pressure (HHP) were evaluated in this work. The effects of HHP treatment, four different pressures (100, 200, 300, and 400 MPa) and four treatment times for each pressure (from 120 to 540 s) were compared with those of thermal treatment (90−98 °C for 15 and 21 s). High pressure treatment retained significantly more ascorbic acid in the vegetable beverage than thermal treatment. However, no significant changes in total phenolics were observed between HHP treated and thermally processed vegetable beverage and unprocessed beverage. Color changes (a^*, b^*, L, chroma, $h°$, and ΔE) were less for pressurized beverage than thermally treated samples compared with unprocessed beverage.

KEYWORDS: HHP; vegetable beverage; bioactive compounds; ascorbic acid; total antioxidant capacity and color

INTRODUCTION

Currently there is a growing tendency for the consumption of minimally processed food products. Dietary recommendations for healthy eating include the consumption of fruit and vegetables, whose health effects are ascribed, in part, to different phytochemicals such as carotenoids, phenolic compounds, and ascorbic acid (AA). These bioactive compounds are beneficial components present in functional foods, and they have been implicated in the reduction of degenerative human diseases, mainly because of their antioxidant potential. Moreover, researchers have used AA as a quality indicator in fruits and vegetables because it is a sensitive bioactive compound providing an indication of the loss of other vitamins and therefore acting as a valid criterion for other organoleptic or nutritional components (*1*, *2*).

Consumption of vegetable beverages, with such a diverse composition, could help fulfill the recommendation to eat more vegetables and achieve the health benefits derived from its bioactive compound combination. "Gazpacho" is a typical meal of the Mediterranean diet, which can be defined as a cold soup which contains vegetables (tomato, cucumber, pepper), olive oil, and other minor components (onion, garlic, wine vinegar, and sea salt) (*3*).

Thermal treatment, commonly used for preserving food, promotes organoleptic and nutritional losses, affecting AA, phenolic compounds, carotenoids, antioxidant capacity, and other parameters such as color and browning index (*4*). Color is an important quality characteristic of fruits and vegetables and one of the major factors affecting sensory perception and consumer acceptance of foods because it is connected with the perception of flavor, sweetness, and other characteristics that appear to be representative of the quality of these products such as browning of fruits and vegetables. Browning of raw fruits and vegetables due to mechanical injury during postharvest handling and processing is an important cause of quality and value loss; it not only reduces the visual quality but also results in undesirable changes in flavor and loss of nutrients due to enzymatic browning and turbidity (*5*).

To achieve a balance between food quality and safety, there is a need to optimize conventional processing techniques currently applied in the food industry and to develop nonthermal processing techniques such as high hydrostatic pressure (HHP) processing. HHP technology is one of the nonthermal physical techniques that are being investigated to be used by the food industry as an alternative to thermal treatment because it inactivates and inhibits microorganisms and degrades enzymes (*6*). HHP processing preserves nutritional value with only a minimal effect on the product quality and delicate sensory properties of fruits and vegetables owing to its limited effect on the covalent bonds of low molecular mass compounds such as color and flavor compounds. Several studies have been conducted in which HHP has been applied to various matrices, especially tomatoes and derived products and other vegetable beverages (*7*−*9*).

In addition, at this stage of development of HHP technology, evaluating the influence of process variables on the bioactive compounds, total antioxidant capacity, color, and browning index is a key factor in defining treatment conditions to avoid the loss of these important properties of foods and to obtain a food beverage with high benefits for the health of the consumer. Thus, the purpose of this study was studied the impact of high pressure treatments on bioactive compounds, antioxidant activity, color, and browning

*To whom correspondence should be addressed. Phone: +34 963544955. Fax: +34 963544954. E-mail: ana.frigola@uv.es.

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index in a vegetable (tomato, green pepper, green celery, onion, carrot, lemon, and olive oil) beverage and established the degradation kinetics of these compounds depending on the treatments applied.

MATERIALS AND METHODS

Samples. The vegetable beverage was prepared by mixing the following ingredients purchased from a local supermarket in Valencia (Spain): tomato (*Lycopersicon esculentum* Mill., 33%), green pepper (*Capsicum annuum* L., Italian pepper, 17%), green celery (*Apium graveolens* L., 8.5%), cucumber (*Cucumis sativus* L., 4%), onion (*Allium cepa* L., 4%), carrot (*Daucus carota* L., 4%), lemon (*Citrus limon* L, 1.7%), salt (1.7%), virgin olive oil (SOS Cuétara, SA, Madrid, Spain, 0.8%), and water to 100%.

Chemicals. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ABTS (2,2′-azinobis(3-ethylbenzothiazoline 6-sulfonate)), Folin−Ciocalteu reagent, and fluorescein sodium salt were purchased from Sigma-Aldrich (Steinheim, Germany). Gallic acid was purchased from UCB (Brussels, Belgium). Hexane (LC grade), potassium hydroxide, and hydrogen peroxide were purchased from Scharlau (Barcelona, Spain). Sodium and disodium phosphate, L(+)-ascorbic acid, acetonitrile (special grade), magnesium hydroxide carbonate (40−45%), and 2,2′-azobis-(2-amidinopropane) dihydrochloride (AAPH) were purchased from Panreac (Barcelona, Spain), and ethanol, diethyl ether, methanol, and sodium chloride (special grade) from Baker (Deventer, The Netherlands). Chloroform was obtained from Merck (Darmstadt, Germany).

Thermal Treatment. An Armfield FT74P unit with a plate exchanger was used to treat the vegetable beverage. The beverage, placed in a feed tank, was pumped through the heat exchanger to achieve the treatment conditions (90 °C, 98 °C, for 15 and 21 s, respectively). After heating, the samples were cooled in an ice/water bath (Armfield FT61, UK), packed, and then stored under refrigeration (4 ± 1 °C) until needed for analysis.

HHP Treatment System. The samples, inserted in PE-LD bottles, were placed in polyethylene bags filled with water and heat-sealed (MULTIVAC Thermosealer) before being placed in the HHP unit (High Pressure Food Processor; EPSI NV, Belgium). The pressurization liquid was a mixture of water and glycol. The pressure level, pressurization time, and temperature were controlled automatically. The treatment time stated in this study does not include come-up and come-down times. The samples were pressurized at 100, 200, 300, and 400 MPa for specific times in a range from 2 to 9 min at a maximum temperature of 30 °C (initial temperature 25 °C). All the treatments were applied in triplicate, with three bottles per replicate. Immediately after pressurization, the samples were transferred to an ice/water bath (Armfield FT61, UK), packed, and then stored under refrigeration (4 ± 1 °C) until needed for analysis.

Ascorbic Acid. A Metrohm 746 VA trace analyzer (Herisau, Switzerland) equipped with a Metrohm 747 VA stand was used. The working electrode was a Metrohm multimode electrode operated in the dropping mercury mode. A platinum wire counter electrode and a saturated calomel reference electrode were used. The vegetable beverage (5 mL) was diluted to 25 mL with the extraction solution (oxalic acid 1%, w/v, trichloroacetic acid 2%, w/v, sodium sulfate 1%, w/v). After vigorous shaking, the solution was filtered through a folded filter (Whatman 1). Oxalic acid (9.5 mL) 1% (w/v) and 2 mL of acetic acid/sodium acetate 2 M buffer (pH = 4.8) were added to an aliquot of 0.5 mL of filtrate, and the solution was transferred to the polarographic cell. The following instrumental conditions were applied: DP50, mode DME, drop size 2, drop time 1 s, scan rate 10 mV s^{-1}, initial potential −0.10 V. Determinations were carried out by using the peak heights and standard additions method (*10*).

Total Phenolic Compounds. The total phenol contents of the samples were determined using the Folin−Ciocalteu method (*11*). The measuring was done at 750 nm in a Perkin-Elmer UV/vis Lambda 2 spectrophotometer (Perkin-Elmer, Jügesheim, Germany). Results were expressed as gallic acid equivalents (mg L^{-1}).

Total Carotenoids. Total carotenoid extraction was carried in accordance with Lee and Castle (*12*), and the values were calculated in accordance with Ritter and Purcell (*13*) using an extinction coefficient of β-carotene, $E^{1\%} = 2505$.

Antioxidant Capacity. *ABTS Assay*. The method used was as described by Zulueta et al. (*14*) based on the capacity of a sample to inhibit the ABTS radical (ABTS•+) compared with a reference antioxidant standard (Trolox). The radical was generated using potassium persulfate. The solution was diluted with ethanol until it reached an absorbance of 0.70 at 734 nm. Once the radical was formed, 2 mL of ABTS•+ was mixed with 100 μL of appropriately diluted vegetable beverage and the absorbance was measured at 734 nm once per minute for 3 min.

ORAC Assay. The ORAC assay used, with fluorescein (FL) as the "fluorescent probe," was that described by Zulueta et al. (*14*). The automated ORAC assay was carried out on a Wallac 1420 VICTOR^2 multilabel counter (Perkin-Elmer, USA) with fluorescence filters for an excitation wavelength of 485 nm and an emission wavelength of 535 nm. The measurements were made in plates with 96 white flat-bottom wells (Sero-Wel, Bibby Sterilin Ltd., Stone, UK). The reaction was performed at 37 °C as the reaction was started by thermal decomposition of AAPH in 75 mM phosphate buffer (pH 7.0) because of the sensitivity of FL to pH.

Measurement of Instrumental Color. The color of the different beverages was measured with a Hunter Labscan II spectrophotometric colorimeter (Hunter Associates Laboratory Inc., Reston, VA) controlled by a computer that calculated color coordinates from the reflectance spectrum (*15*). The results were expressed in accordance with the CIELAB system with reference to illuminant D65 and with a visual angle of 10°. The samples were placed in an optical glass tray, using the white plate of the colorimeter as the background (standard white plate no. LS 13681 11/86, $X = 78.50$, $Y = 83.32$, $Z = 87.94$). This background was used to standardize the measurements. The results were expressed as tristimulus values corresponding to the CIELAB uniform color space (CIE 1978) (L^*: lightness [0 = black, 100 = white], a^* [$-a^*$ = greenness, $+a^*$ = redness], and b^* [$-b^*$ = blueness, $+b^*$ = yellowness]). Three consecutive measurements of each sample were taken. These values were then used to calculate hue degree (h^0 = arctangent [b^*/a^*]), chroma [$C = (a^{*2} + b^{*2})^{1/2}$], which is the intensity or color saturation, and total differences of color [$\Delta E = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$].

Physicochemical Parameters. °Brix was determined by measurement of the refraction index with an Atago model RX-1000 digital refractometer. The determination of pH was based on the potentiometric measurement at 20 °C. It was determined in a Crison GLP 21 pH meter equipped with a temperature compensation sensor at 20 °C. The results were expressed to two decimal places. To measure the turbidity index, a sample was centrifuged, the supernatant was taken, the absorbance at 660 nm was measured (*16*), and the browning index was obtained directly by measuring the absorbance at a wavelength of 420 nm, measured against ethanol (*17*).

Statistical Analysis. Significant differences between the results were calculated by analyses of variance (ANOVA) and the possible interactions between the parameters. An LSD test was applied to indicate the samples between which there were differences. A multiple regression analysis was performed for each parameters to study the influence of pressure and time of treatment Also the correlations between a pair of variables were studied. To gain insight into the data structure a multivariate analysis called principal component analysis (PCA) to describe the relations between variables and observations.

All statistical analyses were performed using Statgraphics Plus 5.0 (Statistical Graphics Corporation, Inc., Rockville, MD).

RESULTS AND DISCUSSION

To establish the effect of the HHP treatment on the vegetable beverage, different pressures (100, 200, 300, and 400 MPa) were applied for different times (from 120 to 540 s), and in all cases the results were compared with the untreated vegetable beverage. The beverage was also subjected to a thermal treatment (90 and 98 °C for 15 and 21 s) to evaluate the effects in relation with the untreated fresh vegetable beverage. To compare this effect with the HHP beverages, it is important first to establish the HHP pasteurization conditions. Saucedo (*18*) studied the same vegetable beverage used in this work and demonstrated 5-log reduction of *Escherichia coli* CECT 433 after HHP (200 MPa/9 min or 225 MPa/5 min), and the minimum reduction level demanded by USFDA (*6*) was obtained. Plaza et al. (*8*) achieved a maximum logarithmic reduction of 3.2 and 3.7 for total counts at 150 MPa/60 °C/15 min and 350 MPa/60 °C/15 min, respectively.

Table 1. Values of Various Antioxidant Compounds (Mean ± Standard Deviation of Three Replicates of Each Sample) in the Vegetable Beverage Treated by HHP (100, 200, 300, and 400 MPa) from 120 to 540 s and Thermal Processing (90−98 °C during 15 and 21 s)[a]

treatment	time (s)	ascorbic acid (mg/100 mL)	total phenolics (mg gallic/L)	total carotenoids (mg/100 mL)
untreated	0	18.0 ± 0.6 a	746.9 ± 90.5 a	0.262 ± 0.035 abc
TP 90 °C	15	17.0 ± 0.1 bcde	848.7 ± 56.0 a	0.271 ± 0.012 ab
	21	16.4 ± 0.2 def	807.8 ± 60.7 a	0.272 ± 0.018 ab
TP 98 °C	15	16.5 ± 0.3 cdef	732.6 ± 21.9 a	0.256 ± 0.012 abcd
	21	15.9 ± 0.1 f	820.7 ± 31.0 a	0.281 ± 0.023 a
HHP 100 MPa	120	17.7 ± 0.1 ab	684.9 ± 28.3 a	0.195 ± 0.018 cdef
	300	17.0 ± 0.4 bcde	737.0 ± 31.1 a	0.178 ± 0.005 ef
	420	16.5 ± 0.4 def	702.9 ± 26.1 a	0.135 ± 0.004 f
	540	16.4 ± 0.3 def	735.1 ± 56.1 a	0.187 ± 0.011 def
HHP 200 MPa	120	17.6 ± 0.4 ab	768.1 ± 8.3 a	0.171 ± 0.016 ef
	300	17.5 ± 0.3 ab	782.6 ± 20.0 a	0.181 ± 0.009 ef
	420	16.5 ± 0.0 def	678.6 ± 41.6 a	0.141 ± 0.016 ef
	540	16.4 ± 0.1 ef	724.9 ± 10.5 a	0.200 ± 0.021 bcdef
HHP 300 MPa	120	17.5 ± 0.2 abc	750.0 ± 53.2 a	0.145 ± 0.007 f
	300	17.0 ± 0.2 abcde	761.5 ± 88.2 a	0.187 ± 0.001 def
	420	16.4 ± 0.2 ef	780.1 ± 55.7 a	0.167 ± 0.032 ef
	540	16.4 ± 0.1 def	758.4 ± 67.6 a	0.221 ± 0.009 abcde
HHP 400 MPa	120	17.5 ± 0.2 abc	805.1 ± 37.0 a	0.188 ± 0.030 def
	300	17.3 ± 0.3 abcd	718.9 ± 53.3 a	0.157 ± 0.014 ef
	420	16.4 ± 0.0 def	754.9 ± 14.5 a	0.153 ± 0.002 ef
	540	16.4 ± 0.1 ef	726.2 ± 58.8 a	0.165 ± 0.025 ef

[a] a−h: Different letters indicate significant statistical differences in function of the applied treatment.

This corroborates the finding that the food matrix is one of the factors that modifies microorganism resistance when HHP is applied (*19*−*21*). The values of pH and °Brix (data not shown) in the untreated vegetable beverage were (4.21 ± 0.02) and (4.20 ± 0.01), respectively, with no statistically significant changes occurring when the various treatments were applied. The turbidity index (TI) of the untreated vegetable beverage was 0.26 ± 0.01, but it increased significantly ($p < 0.05$) when the sample was treated thermally, for all the treatments applied. The TI obtained for the pasteurized sample was 0.42 ± 0.01 and 0.59 ± 0.04 when it was heated at 90 °C for 15 and 21 s, and when the treatment conditions were 98 °C for 15 and 21s, the values obtained were 0.56 ± 0.03 and 0.37 ± 0.01. However, no statistically significant changes were seen when the various treatments with HHP were applied. The browning index (BI) of the untreated beverage (0.076 ± 0.001) increased significantly ($p < 0.05$) when the various HHP treatments were applied (0.080 ± 0.001 − 0.087 ± 0.001). Similar results were found by Saldo et al. (*22*) in apple juice and by Bull et al. (*23*) after high pressure treatment of orange juice (600 MPa/60 s/20 °C). However, we did not observe any changes when the samples were treated with heat.

The results obtained for ascorbic acid, phenolic compounds, total carotenoids, antioxidant capacity, and color are shown in **Tables 1**−**4**. Indexes indicate the existence of statistically significant differences ($p < 0.05$) between the different results obtained by applying an ANOVA test. The ascorbic acid concentration in the untreated vegetable beverage was 18.0 ± 0.6 mg/100 mL. These values are in the range of those previously reported by other authors for various vegetable soups (*24*). The ascorbic acid concentration decreased significantly ($p < 0.05$) in all cases after pasteurization, with losses of 11% when the temperature applied was 98 °C for 21 s. In the samples treated with HHP, there was also a significant decrease ($p < 0.05$) in the concentration of ascorbic acid when the treatment time was equal to or greater than 420 s, but the losses did not exceed 9% in any of the cases. These results were in accordance with those reported by other authors: for example Sánchez-Moreno et al. (*25*) reported 91% retention of ascorbic acid in orange juice after HPP at 400 MPa/40 °C/1 min. Patras et al. (*26*, *27*) did not obtain significant changes after applying different HHP treatments (400, 500, 600 MPa/10−30 °C/15 min) in strawberry and tomato purée, obtaining ascorbic acid retentions higher than 90% after HPP 600 MPa and, along the same lines, Yen and Lin (*28*) reported that 89% of the initial content of ascorbic acid in strawberry coulis and strawberry nectar was retained after treatment at 400 MPa/20 °C/30 min.

Table 2. Values of Total Antioxidant Capacity Determined by ABTS and ORAC Assay (Mean ± Standard Deviation of Three Replicates of Each Sample) in the Vegetable Beverage Treated by HHP (100, 200, 300, and 400 MPa) from 120 to 540 s and Thermal Processing (90−98 °C during 15 and 21 s)[a]

treatment	time (s)	ABTS (mM trolox)	ORAC (mM trolox)
untreated	0	1.61 ± 0.10 abc	3.70 ± 0.22 a
TP 90 °C	15	1.40 ± 0.16 abcdefg	2.99 ± 0.04 bcd
	21	1.35 ± 0.08 adefg	2.75 ± 0.29 de
TP 98 °C	15	1.80 ± 0.16 ch	3.20 ± 0.07 bfg
	21	1.75 ± 0.19 ch	3.28 ± 0.11 bf
HHP 100 MPa	120	1.10 ± 0.03 gi	2.73 ± 0.11 e
	300	1.13 ± 0.07 gi	2.73 ± 0.04 e
	420	1.28 ± 0.02 efg	3.07 ± 0.07 bcf
	540	1.21 ± 0.03 g	2.46 ± 0.06 h
HHP 200 MPa	120	1.32 ± 0.03 defg	3.19 ± 0.05 bfg
	300	1.51 ± 0.13 abcdef	3.36 ± 0.07 gi
	420	1.66 ± 0.15 bch	3.63 ± 0.25 a
	540	1.62 ± 0.14 abcdh	3.60 ± 0.11 ai
HHP 300 MPa	120	1.23 ± 0.10 g	3.09 ± 0.11 bcf
	300	1.33 ± 0.02 defg	3.28 ± 0.03 bf
	420	1.26 ± 0.03 efg	3.19 ± 0.05 bfg
	540	1.24 ± 0.02 fg	3.22 ± 0.01 bfg
HHP 400 MPa	120	1.11 ± 0.03 gi	2.91 ± 0.08 cde
	300	1.58 ± 0.09 abcd	3.58 ± 0.08 ai
	420	1.56 ± 0.22 abcde	3.61 ± 0.03 ai
	540	1.50 ± 0.09 abcdef	4.08 ± 0.11 j

[a] a−h: Different letters indicate significant statistical differences in function of the applied treatment.

Table 3. Values of a^*, b^*, and L^* (Mean ± Standard Deviation of Three Replicates of Each Sample) of Vegetable Beverage Treated by HHP (100, 200, 300, and 400 MPa) from 120 to 540 s and Thermal Processing (90−98 °C during 15 and 21 s)[a]

treatment	time (s)	a^*	b^*	L^*
untreated	0	2.84 ± 0.09 a	18.21 ± 0.38 a	27.89 ± 0.16 ef
TP 90 °C	15	3.52 ± 0.02 gh	24.11 ± 0.18 hi	25.86 ± 0.05 b
	21	3.32 ± 0.04 def	23.51 ± 0.48 h	25.30 ± 0.24 a
TP 98 °C	15	3.94 ± 0.04 i	25.56 ± 0.12 j	26.32 ± 0.06 c
	21	3.57 ± 0.03 h	24.31 ± 0.29 i	27.68 ± 0.04 de
HHP 100 MPa	120	3.28 ± 0.03 def	18.58 ± 0.16 ab	28.42 ± 0.13 ij
	300	3.41 ± 0.01 fg	18.73 ± 0.10 abc	28.89 ± 0.03 kl
	420	3.88 ± 0.01 i	19.74 ± 0.23 ef	29.51 ± 0.09 m
	540	3.79 ± 0.07 i	19.49 ± 0.02 de	29.28 ± 0.03 m
HHP 200 MPa	120	4.18 ± 0.05 j	20.80 ± 0.05 g	29.21 ± 0.10 lm
	300	4.36 ± 0.06 k	21.01 ± 0.03 g	29.39 ± 0.10 m
	420	4.22 ± 0.03 jk	19.37 ± 0.09 cde	27.77 ± 0.09 def
	540	3.86 ± 0.02 i	18.91 ± 0.10 abcd	27.49 ± 0.05 d
HHP 300 MPa	120	3.36 ± 0.05 efg	20.32 ± 0.06 fg	28.08 ± 0.13 ghi
	300	3.02 ± 0.04 b	19.22 ± 0.15 bcde	27.56 ± 0.22 de
	420	3.21 ± 0.04 cde	19.19 ± 0.10 bcde	27.57 ± 0.19 de
	540	2.79 ± 0.06 a	19.04 ± 0.09 bcde	27.59 ± 0.11 de
HHP 400 MPa	120	3.20 ± 0.04 cd	18.28 ± 0.22 a	28.25 ± 0.08 hij
	300	3.17 ± 0.07 bcd	18.58 ± 0.43 ab	27.92 ± 0.12 efg
	420	3.42 ± 0.08 fgh	19.06 ± 0.37 bcde	28.38 ± 0.14 ij
	540	3.12 ± 0.03 bc	18.83 ± 0.07 abcd	28.56 ± 0.05 jk

[a] a−h: Different letters indicate significant statistical differences in function of the applied treatment.

Table 4. Values of Chroma, Hue Angle ($h°$), and ΔE (Mean ± Standard Deviation of Three Replicates of Each Sample) of Vegetable Beverage Treated by HHP (100, 200, 300, and 400 MPa) from 120 to 540 s and Thermal Processing (90−98 °C during 15 and 21 s)[a]

treatment	time	chroma	$h°$	ΔE
untreated	0	18.43 ± 0.38 a	81.15 ± 0.11 j	0 a
TP 90 °C	15	24.36 ± 0.13 ij	81.70 ± 0.09 k	6.27 ± 0.11 g
	21	23.75 ± 0.47 i	81.95 ± 0.25 k	5.93 ± 0.50 g
TP 98 °C	15	25.87 ± 0.13 k	81.23 ± 0.05 j	7.60 ± 0.14 h
	21	24.57 ± 0.29 j	81.64 ± 0.07 k	6.14 ± 0.29 g
HHP 100 MPa	120	18.86 ± 0.16 abc	80.00 ± 0.13 fgh	0.80 ± 0.04 bc
	300	19.04 ± 0.10 abc	79.68 ± 0.04 f	1.26 ± 0.06 c
	420	20.12 ± 0.23 ef	78.87 ± 0.10 de	2.47 ± 0.10 e
	540	19.85 ± 0.04 de	79.00 ± 0.18 e	2.11 ± 0.06 de
HHP 200 MPa	120	21.22 ± 0.06 gh	78.65 ± 0.10 cd	3.21 ± 0.09 f
	300	21.45 ± 0.02 hi	78.28 ± 0.15 b	3.52 ± 0.06 f
	420	19.83 ± 0.09 de	77.72 ± 0.08 a	1.81 ± 0.06 d
	540	19.20 ± 0.10 bcd	78.39 ± 0.02 bc	1.26 ± 0.03 c
HHP 300 MPa	120	20.60 ± 0.07 fg	80.61 ± 0.12 i	2.19 ± 0.08 de
	300	19.45 ± 0.15 cde	81.07 ± 0.05 j	1.10 ± 0.08 c
	420	19.46 ± 0.10 cde	80.52 ± 0.08 i	1.11 ± 0.05 c
	540	19.24 ± 0.10 bcd	81.65 ± 0.14 k	0.89 ± 0.04 bc
HHP 400 MPa	120	18.55 ± 0.22 ab	80.08 ± 0.02 gh	0.55 ± 0.04 b
	300	18.85 ± 0.43 abc	80.32 ± 0.03 hi	0.55 ± 0.33 b
	420	19.36 ± 0.37 cd	79.84 ± 0.15 fg	1.17 ± 0.24 c
	540	19.09 ± 0.07 bc	80.60 ± 0.12 i	0.96 ± 0.06 bc

[a] a−h: Different letters indicate significant statistical differences in function of the applied treatment.

Total phenolics appeared to be relatively resistant to the effect of processing. High pressure and thermal treatment did not have a significant effect on the levels of phenol compounds. Patras et al. (*26*) observed similar results in tomato purées. On the other hand, in our study, total phenolics did not vary significantly ($p > 0.05$) in the thermally treated vegetable beverage. These results are in accordance with those found by Gil-Izquierdo et al. (*29*) in orange juice after pasteurization (75 and 95 °C, 30 s).

Total carotenoids were particularly affected, HHP processed samples having a lower total carotenoid content ($p < 0.05$) than that of unprocessed samples. These results were similar to those found by Patras et al. (*26*) for tomato purées when they applied HHP of 400 and 500 MPa/15 min. Plaza et al. (*8*) also found a decrease, but not a significant one ($p > 0.05$), in total carotenoid content after HHP treatment (150 MPa/60 °C/15 min). On the other hand, in our study, the pasteurization treatment did not significantly affect the total carotenoid contents, and in some cases there was even an increase in total carotenoids. Patras et al. (*26*) obtained similar results in carrot purées treated at 70 °C ≥ 2 min.

ABTS and ORAC assays are the ones most popularly used in electron transfer and hydrogen atom transfer methods, respectively. The measured antioxidant capacity of a sample depends on which technology and which free radical generator or oxidant is used in the measurement. For research, therefore, it is important to measure total antioxidant capacity by using different methods and comparing and discussing the results, as there is no official method. The antioxidant capacity of fresh sample (untreated) was 1.61 ± 0.10 and 3.70 ± 0.22 mM Trolox for the ABTS and ORAC assays, respectively. Upon comparing the antioxidant capacity results obtained with the ABTS method and the ORAC method (**Figure 1**), we found good correlations ($r = 0.823$, $p < 0.05$).

To evaluate pressure and time influence in the HPP treatment, we performed a multiple regression analysis, and the results obtained demonstrated that only pressure significantly affected the antioxidant capacity values obtained by the ABTS and ORAC assays (correlation coefficient = 0.5124, SE = 0.2215,

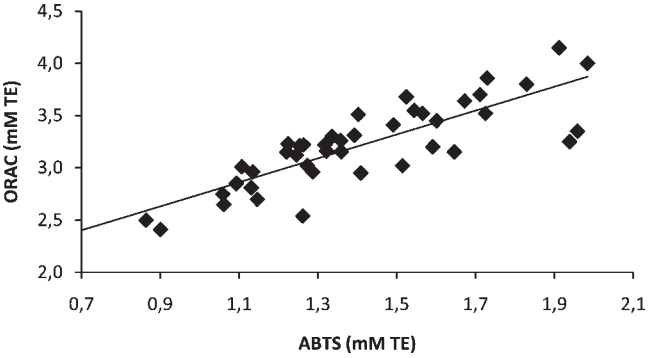

Figure 1. Correlation of ORAC and TEAC values in the samples in processed vegetable beverage.

$p = 0.0001$, correlation coefficient = 0.5894, SE = 0.3384, $p = 0.0004$, respectively). With HHP treatments there was a decrease in the antioxidant capacity of the vegetable beverage. Results obtained at 100, 200, 300, and 400 MPa were 1.10 ± 0.15, 1.50 ± 0.18, 1.26 ± 0.06, and 1.58 ± 0.03 mM Trolox, when they were determined with the ABTS assay, and 2.75 ± 0.24, 3.44 ± 0.22, 3.19 ± 0.09, and 3.54 ± 0.45 mM Trolox, respectively, when they were determined with the ORAC assay. Other authors have also reported that high pressure processing either increases or does not affect the antioxidant activity of blackberry purées, tomato purées, and carrot purées (*26*, *27*). Sánchez-Moreno et al. (*30*) stated that the total scavenging activity (DPPH) in aqueous and organic fractions of tomato purée was unaffected by a HP treatment of 400 MPa/25 °C/15 min. Similar results were obtained by Plaza et al. (*8*) after applying 150 MPa/60 °C/15 min and 350 MPa/60 °C/15 min in a vegetable soup.

On the other hand, a significant increase ($p < 0.05$) in the ABTS and ORAC values of the vegetable beverage was detected after conventional thermal processing at 98 °C for 15 and 21 s.

The instrumental color parameters of the vegetable beverage as affected by thermal and high pressure processing are shown in **Tables 3** and **4**. With the various HHP treatments applied, the CIE L^* values of the unprocessed vegetable beverage (27.89 ± 0.16) were modified significantly ($p < 0.05$) with high pressure and thermal processing. The HHP processed samples were in the range (27.49 ± 0.05 − 29.51 ± 0.09), very close to the unprocessed beverage, while thermal treatment caused a decrease in L^* values, as also observed by Daoudi et al. (*31*) in gazpacho, Soliva-Fortuny et al. (*32*) in avocado purées, and Quevedo et al. (*33*) in pears.

The color parameters were influenced by browning, especially the L^* value, which has been used as a browning indicator in fruits and vegetables (*32*−*34*). In the present study, a positive correlation ($p = 0.0006$) between BI and L^* was also observed in the processed samples. The CIE a^* values (2.84 ± 0.09) gradually changed toward a more positive direction, (2.79 ± 0.06 − 4.36 ± 0.06) for the high pressure processed vegetable beverage, and (3.32 ± 0.04 − 3.94 ± 0.04) for the pasteurized vegetable beverage. The increase in CIE a^* values in the vegetable beverage is similar to the results found by Patras et al. (*26*) for high pressure processed tomato and carrot purées. CIE b^* values (18.21 ± 0.38) also increased with processing. The values for the high pressurized vegetable beverage were (18.28 ± 0.22 − 21.01 ± 0.03), and for the pasteurized samples a statistically significant ($p < 0.05$) increase in b^* values (23.51 ± 0.48 − 25.56 ± 0.12) was also observed.

The a^* and b^* color parameters had a positive correlation ($p = 0.0181$). A significant negative correlation ($p = 0.0002$) was observed between BI and the b^* color parameter. Saldo et al. (*22*) established a similar correlation in apple juice processed by high

Table 5. Coefficients of Discriminant Function for the Different Bioactive Compounds and Physicochemical Parameters Studied[a]

	1	2	3	4
ascorbic acid	0.3019	−0.2306	−0.5221	0.7160
total phenolics	−0.1008	0.8436	−0.7325	0.3309
total carotenoids	−0.2441	−0.9488	−0.8795	0.2517
ABTS	−0.3933	−1.0347	0.4434	0.0980
ORAC	0.7155	1.0202	−1.5872	0.9035
pH	−0.1435	0.5012	−0.2336	−0.2180
°Brix	−0.1281	−0.3935	0.5074	−0.2564
TI	1.3156	−1.3133	0.5118	0.5880
BI	0.1997	0.5557	−0.7562	−0.1412
a^*	10.7378	14.1229	−1.2622	−0.7914
b^*	25.3895	73.0132	9.2705	40.8983
L^*	1.7748	−2.4850	1.2910	0.4420
chroma	−31.5617	−84.9458	−7.1865	−43.1849
$h°$	5.43277	8.4365	−0.5913	−2.0325
ΔE	−2.5836	3.9140	−1.9460	1.8822

[a] TI, turbidity index; BI, browning index.

pressure. The color intensity (chroma), hue angle (h°), and ΔE^* calculated from the color parameters L^*, a^*, and b^* of the various nonthermal and thermal treatments are shown in **Table 4**.

The thermally treated and HHP processed vegetable beverage had a higher color purity (chroma) than the unprocessed vegetable beverage (18.43 ± 0.38). The changes for the thermally processed samples (23.75 ± 0.47 − 25.87 ± 0.13) were especially significant. The results found by Patras et al. (*26*) for tomato purées were similar. The increase in color intensity (chroma) in the nonthermally treated vegetable beverage was probably due to the activity of browning enzymes not yet fully inactivated. A negative correlation ($p = 0.0003$) was observed. Similar characteristics were found by Saldo et al. (*22*) when they studied the effects of ultrahigh-pressure in apple juices. The hue angle values of the untreated sample (81.15 ± 0.11) were not modified or increased in the thermally processed samples. Similar results were found by Saldo et al. (*22*), but a decrease in hue angle values was established in HHP processed samples. Patras et al. (*26*) found similar results for carrot purées.

In the vegetable beverage, the total color change (ΔE) in all the processed samples was significantly different ($p < 0.05$) than the unprocessed samples. The total color difference (ΔE^*) indicates the magnitude of the color difference. It has been considered (*35*) that a ΔE^* of 2 would be a noticeable visual difference for a number of situations. The ΔE^* values were lower for the vegetable beverage treated by HHP than those obtained after thermal processing in all cases (see **Table 4**). It is quite clear that the application of HHP had a smaller effect on color changes than thermal processing. For the pressurized vegetable beverage, ΔE was about or less than 3.52 ± 0.06, but for the heat treated beverage, the color change was more intense, reaching a maximum of 7.60 ± 0.14. Dede et al. (*36*) and Patras et al. (*26*) reported similar results for carrot and tomato juice and for strawberry and blackberry purées. They observed that high pressure treatment produced a lower color difference in comparison with fresh sample than thermally processed juices or purées.

A significant negative correlation was observed between ΔE^* and ascorbic acid ($p = 0.0483$). Patras et al. (*27*) observed similar results in nonthermally processed samples of strawberry purées. In addition, an increase in the concentration of total carotenoids correlated positively with an increase in ΔE^* ($p = 0.0001$).

A principal components analysis (PCA) was performed on all samples (untreated, thermal treatments, and HHP) and variables to obtain relationships between the parameters studied. **Table 5** shows the four principal components. They accounted for 80.7% of the variability in the original data. **Figure 2** shows the influence of the first two components obtained (PC1 and PC2). A discriminant analysis was performed, establishing as a classification variable the treatment applied: untreated, thermal treatment (90 and 98 °C), and HHP (100, 200, 300, and 400 MPa). Six statistically significant ($p < 0.05$) discriminate functions were obtained (**Table 5**). The first function had a relative weight of 82.9% and the second was responsible for 3%. **Figure 3** shows the differences between the treatments applied. It can be seen that it is possible to differentiate the vegetable beverage studied depending on the treatment applied, although beverages treated at 100 and 200 MPa were only slightly differentiated (function 1). The model was able to discriminate 100% of the samples. In conclusion, in general, HHP treatment did not significantly degrade the concentrations of ascorbic acid in the vegetable beverage and did not significantly improve extractability of carotenoids and total phenolics in the vegetable beverage. Total differences in color (ΔE) and color intensity (chroma) were higher in the thermally processed samples than in the pressure treated vegetable beverage. Thus, further research is required to elucidate the effects of HHP process parameters on bioactive compounds and color in foods, and further studies dealing with the effects of HHP treatments in vegetable beverages during storage are needed. In any case, we can conclude that HHP treatment has good prospects for use in the food industry as an alternative to thermal pasteurization.

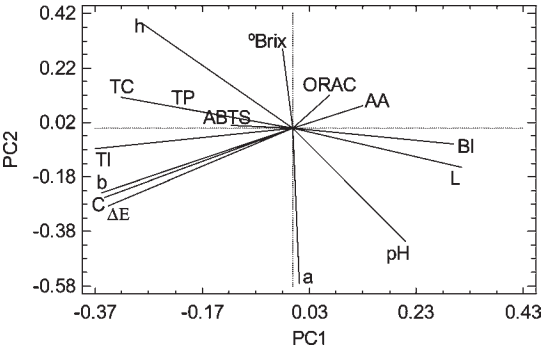

Figure 2. Principal components plot of fresh, HHP, and thermally treated vegetable beverage (TC, total carotenoids; TP, total phenols; TI, turbidity index; C, chroma; BI, Browning index; AA: ascorbic acid).

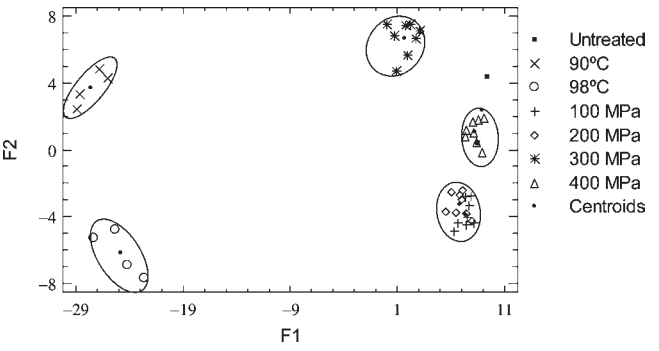

Figure 3. Discriminant function for the different treatments applied in the vegetable beverage.

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