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Hyaluronidase Inhibiting Activity and Radical Scavenging Potential of Flavonols in Processed Onion

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ABSTRACT: The flavonol content and anti-inflammatory and antioxidant activities of onion treated by high-pressure processing (HPP) and HPP combined with freeze-drying and pulverization (HPP-FD-P) were evaluated. *Allium cepa* L. var. *cepa*, 'Recas' was treated at T1 (200 MPa/25 °C/5 min), T2 (400 MPa/25 °C/5 min), and T3 (600 MPa/25 °C/5 min). After treatment, HP-treated and untreated samples were frozen (diced onion, HP-treated). Subsequently, part of the diced samples was freeze-dried and pulverized (pulverized onion, HP-treated and freeze-dried). Flavonol content and anti-inflammatory and antioxidant activities (hyaluronidase inhibiting activity, $NO^{\bullet}$, $ABTS^{\bullet+}$, and $DPPH^{\bullet}$ scavenging capacity, ferric reducing antioxidant power, and antioxidative capacity by photochemiluminescence) were measured in nonhydrolyzed and hydrolyzed extracts. Hydrolysis was carried out in order to evaluate the effect of HPP and HPP-FD-P on both anti-inflammatory and antioxidant activities of extracts mainly containing aglycone forms. HPP-FD-P increased quercetin 3,4′-diglucoside, quercetin 4′-glucoside, quercetin 3-glucoside, and isorhamnetin 3,4′-diglucoside extractability. The present study suggests that HPP (especially treatment at 400 MPa) and HPP-FD-P may be of benefit for obtaining functional ingredients from onion, as suggested by increased $NO^{\bullet}$ scavenging capacity and maintenance of the antioxidant activity mainly in hydrolyzed extracts.

KEYWORDS: *flavonols, HPLC-ESI-MS, antihyaluronidase activity, $NO^{\bullet}$ scavenging capacity, antioxidant properties, onion, high-pressure processing, freeze-drying, pulverization*

INTRODUCTION

The complex biochemical composition of onions has been widely studied as a source of many biological components, whose properties have shown to have important benefits on human health.[1,2] Its bioactivity has been proved through multiple mechanisms, such as the demonstration of its antioxidant,[3−5] anti-inflammatory,[6,7] and antibacterial properties.[8] Most of these mechanisms, which have been proved under *in vitro* and *in vivo* conditions, have resulted in positive human health effects attributed in part to the presence of polyphenols.[9,10] In this sense, it is widely known that onion is one of the richest sources of dietary flavonols and contributes to a large extent to the overall intake of flavonols.[2]

It is important to highlight that a well-balanced dietary intake has been associated with a reduced risk of various pathological conditions such as cardiovascular diseases, cancer, neurodegenerative diseases, and diabetes[11−13] and has also been recognized as a contributor to allergy control in epidemiological studies.[14,15] Moreover, polyphenols, specifically flavonols such as quercetin and its glycoside conjugates, are believed to be a key factor in the prevention of these pathologies.[10,16−19]

Inflammation is a complicated process that usually accompanies disease in most cases. The evidence links oxidative stress to inflammation, which together plays an important role in the pathogenesis of several diseases.[20] In this context, the modulation of biological antioxidant levels and inflammatory responses, by means of natural products, would be a useful tool aimed at improving the general state of health of individuals.[21−23] Hence, the modulation of nutritional and phytochemical value of our diet is an interesting field of study since chronic diseases might also be prevented through the intake of fruit and vegetables[24] with an adequate amount of substances with antioxidant and anti-inflammatory properties. Hyaluronidase enzyme has been recognized in a number of physiological and pathological processes, including inflammation.[25] The modulation of hyaluronidase by suitable inhibitors will be useful for normal homeostasis in the body. Therefore, identification and characterization of hyaluronidase inhibitors would be valuable for recognition of compounds with anti-inflammatory activity. In fact, a large number of scientific publications show the inhibition of hyaluronidase activity as a measure of the anti-inflammatory activity of different compounds present in plant-derived products.[26−36]

The application of advanced food processing technologies provides a unique opportunity for food manufacturers to retain

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bioactive content similar to that found in fresh fruits and vegetables or even to increase its extractability. High-pressure (HP) technology is considered an innovative process that covers the requirements for safer products, satisfying a growing demand for minimally processed foods that maintain their organoleptic quality.[37] Interestingly, food processing by high pressure may modify the localization, concentration, chemical structure, and activity of polyphenols. The effects of high-pressure processing (HPP) in vegetables (mainly on plant cell integrity) could result in the modification of the phytochemical or bioactive compound extractability, bioaccessibility, and functionality.[38−41]

Among the drying processes, freeze-drying is recognized as the best process for maintaining the health-promoting capacity and nutritional characteristics of plant products, including onion products, for long-term storage.[42−45] Onion HPP could offer safe new onion products with similar organoleptic properties to fresh onion that additionally could provide potential human health benefits. In addition, dried onions are an attractive alternative to fresh onions for both domestic and industrial use.[46] Consequently, HPP plus freeze-drying and pulverization in onions could be a process for obtaining a functional food ingredient that is safe and very easy to use for consumers, with great versatility and an extra added value due to its potential biological activity. Therefore, the aim of the present study was to evaluate the changes in flavonol content and anti-inflammatory and antioxidant activities of onion treated by high-pressure processing and HPP combined with freeze-drying and pulverization (HPP-FD-P).

MATERIALS AND METHODS

Chemicals. Methanol and acetonitrile (HPLC-grade) were provided by Lab-Scan (Dublin, Ireland). Hyaluronidase from bovine testes, hexadecytrimethylammonium bromide (CTAB), sulfanilamide, sodium nitropusside dehydrate, iron(III) chloride hexahydrate, phosphate-buffered saline, Folin−Ciocalteu's phenol, gallic acid, 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium persulfate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), quercetin, and isorhamnetin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hyaluronic acid-60 (potassium salt) from human umbilical cord was from USBiological (Swampscott, MA, USA). *N*-(1-Naphthyl)ethylenediamine dihydrochloride and 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) were obtained from Fluka Chemie AG (Buchs, Switzerland). Antioxidant capacity of water-soluble substances (ACW) and antioxidant capacity of lipid-soluble substances (ACL) kits were from Analytik Jena AG (Jena, Germany). Quercetin and isorhamnetin glucosides were purchased from Extrasynthèse (France).

Plant Material. Raw onions (*Allium cepa* L. var. *cepa*, 'Recas') were supplied by Cebacat (Asociación Catalana de Productores-Comercializadores de Cebolla, Lleida, Spain). Onions were harvested in January 2011 in Spain, and their bulbs were free of external damage and stored at 4 °C until processing (5 days later). The weights of whole bulbs ranged from 126 to 224 g and presented an equatorial and apical diameter between 60 and 75 mm and 61−78 mm, respectively. The onions were hand-peeled, cut into 10 mm pieces, packaged in very low gas permeability bags type Doypack (Polyskin XL, Flexibles Hispania, S.L.), and treated by high pressure.

High-Pressure Processing. Diced packed onions were exposed to 200, 400, and 600 MPa for a 5-min hold time and processing unit temperature of 25 °C. The high-pressure unit had a 2925 mL vessel, a 900 MPa maximum pressure level, and a 100 °C maximum temperature (High Pressure Iso-Lab System, model FPG7100:9/2C, Stansted Fluid Power Ltd., Essex, UK). The pressure buildup time and temperature were recorded in all experiments. The rates of compression and decompression were both 3 MPa/s. During HPP, the vessel was thermostatically controlled at 25 °C. During compression, the temperature increased to a maximum of 0.5, 1.7, or 5 °C at 200, 400, or 600 MPa, respectively. The pressure-transmitting medium was water. The processing treatments were carried out in triplicate on three different days (3 samples/day for a total of 9 samples for each treatment and group over a 3-day period) with their corresponding samples as control (0 MPa).

After high-pressure treatment, HP-treated (200 MPa/25 °C/5 min, 400 MPa/25 °C/5 min, and 600 MPa/25 °C/5 min) and untreated or non-HP-treated (0 MPa/25 °C/5 min) samples were immediately frozen with liquid nitrogen (diced onion, HP-treated). Subsequently, part of the diced samples was freeze-dried in a lyophilizer (model Lyoalfa, Telstar, S.A., Barcelona, Spain) and pulverized with a pestle and mortar until obtaining a fine powder (final size particle ≤250 μm) (pulverized onion, HP-treated and freeze-dried). Samples were stored at −20 ± 0.5 °C until their analyses. Moisture content was determined from the weight of the samples before and after freeze-drying.

Sample Preparation. Nonhydrolyzed and hydrolyzed extracts were prepared in order to evaluate the effect of HPP on both anti-inflammatory and antioxidant activities of extracts mainly containing flavonol glycosides or aglycone forms, respectively.

Extraction. Each onion sample (1 or 15 g of freeze-dried powder or diced onion pieces, respectively) was homogenized with 25 mL of methanol/formic acid/water (50:5:45, v/v/v) in an ultrahomogenizer (model ES-270, Omni International Inc., Gainesville, VA, USA) at 8000 rpm for 4.5 min. The mixtures were centrifuged (13000*g*, 4 °C, 10 min), and the solid residue was re-extracted with 15 mL of the same extraction solvent. The two resulting supernatants were combined and filtered with six layers of cheesecloth and transferred to an amber glass round-bottomed flask, and then the organic solvent was removed by evaporation in vacuum at 35 °C to reach a final 10 mL volume. Three extracts were prepared from each onion sample.

Hydrolysis. An extraction procedure was carried out similar to that above, placing the mixture obtained after ultrahomogenization in an amber glass round-bottomed flask with 5 mL of 6 M HCl, fluxed with nitrogen, refluxed at 90 °C for 4 h, and then allowed to cool. The mixture was centrifuged (13000*g*, 4 °C, 10 min), and the supernatant separated and evaporated under vacuum at 35 °C to reach a final 10 mL volume. Three extracts were prepared from each onion sample.

Determination of Total Phenolic Content (TPC). Total phenolic content of the nonhydrolyzed and hydrolyzed extracts was determined according to the Folin−Ciocalteu method as previously described,[47] with minor modifications. The assay was carried out by adding 7.5 mL of deionized water to 1 mL of each extract and then 0.5 mL of the Folin−Ciocalteu reagent. After 5 min of incubation, 1 mL of a saturated sodium carbonate solution was added. The mixture was shaken and incubated for 1 h in the dark, and the absorbance was read at 760 nm. The determination was performed in triplicate. Results were expressed as milligrams (mg) of gallic acid equivalents per 100 g of fresh weight (fw).

Identification and Quantification of Flavonols. *HPLC-DAD Procedure.* The analytical HPLC system consisted of an Agilent 1100 series LC coupled with a quaternary pump with integrated degasser, autosampler, thermostated column compartment, and diode array detector (DAD) (Agilent Technologies, Waldbroon, Germany). Separation of flavonoids was performed using a reverse phase C_{18} Hypersil ODS stainless steel column (250 mm × 4.6 mm, 5 μm) (Teknokroma, Barcelona, Spain). The mobile phase consisted of a linear gradient of acetonitrile with 0.1% formic acid (solution B) in 0.1% formic acid/water (solution A) as follows: 10 to 26% B (0−40 min); 26 to 65% B (40−50 min); 65 to 10% B (50−52 min); and isocratic 10% B (52−55 min). The injection loop was 40 μL and the flow rate was fixed at 1 mL/min. Runs were monitored with the UV−visible DAD set at 360 and 280 nm. Data acquisition and analysis were carried out with an Agilent ChemStation. Identification of flavonols was carried out by HPLC by comparing the retention time and UV−visible absorption spectrum with those of the authentic commercial standards and comparing with the data of onion flavonols reported in the literature. Quantification was achieved employing external standard calibration using methanolic solutions of quercetin 4′-glucoside

Table 1. Total Phenols and Flavonol Content of Onion Subjected to High-Pressure Processing (HPP) and HPP Combined with Freeze-Drying and Pulverization (HPP-FD-P)[a]

	diced onion, HP-treated				pulverized onion, HP-treated and freeze-dried			
	0 MPa	200 MPa	400 MPa	600 MPa	0 MPa	200 MPa	400 MPa	600 MPa
Nonhydrolyzed Extract								
total phenols (mg GAE/100 g fw)	95.66 ± 2.54 bB	87.92 ± 3.31 aA	104.64 ± 5.14 cB	83.76 ± 1.33 aA	85.71 ± 3.03 *a*A	91.63 ± 5.45 *a*A	85.62 ± 2.36 *a*A	82.37 ± 2.48 *a*A
quercetin 3-glucoside (μmol/100 g fw)	2.64 ± 0.23 *b*B	2.03 ± 0.30 *ab*A	2.52 ± 0.52 *ab*A	1.62 ± 0.27 *a*A	1.49 ± 0.13 aA	2.53 ± 0.27 bA	2.14 ± 0.12 b*A*	2.18 ± 0.23 bA
quercetin 4′-glucoside (μmol/100 g fw)	56.52 ± 1.66 bB	38.81 ± 6.33 abA	46.45 ± 12.39 ab*A*	32.72 ± 6.25 aA	26.34 ± 1.93 aA	38.50 ± 0.89 bA	44.66 ± 2.53 c*A*	42.99 ± 4.01 bcA
quercetin 3,4′-diglucoside (μmol/100 g fw)	140.64 ± 10.14 bB	108.28 ± 13.92 aA	115.55 ± 17.88 abA	88.15 ± 7.71 aA	92.68 ± 9.66 aA	120.16 ± 7.26 bA	101.31 ± 6.73 aA	124.04 ± 6.49 bB
quercetin 7,4′-diglucoside (μmol/100 g fw)	0.176 ± 0.014 *a*B	0.417 ± 0.217 *ab*A	0.895 ± 0.086 *b*B	0.140 ± 0.010 *a*A	0.109 ± 0.018 aA	0.268 ± 0.075 bA	0.155 ± 0.014 aA	0.140 ± 0.043 aA
quercetin 3,7,4′-triglucoside (μmol/100 g fw)	0.027 ± 0.018 aA	0.020 ± 0.003 aA	0.121 ± 0.008 cB	0.088 ± 0.011 bB	0.014 ± 0.001 aA	0.018 ± 0.005 aA	0.010 ± 0.001 aA	0.014 ± 0.004aA
isorhamnetin 4′-glucoside (μmol/100 g fw)	15.39 ± 0.69 bB	10.65 ± 1.03 aA	12.38 ± 1.87 ab*A*	9.00 ± 1.99 aA	8.42 ± 0.25 *a*A	12.33 ± 3.66 *ab*A	11.46 ± 0.48 *b*A	13.38 ± 1.09 *ab*B
isorhamnetin 3,4′-diglucoside (μmol/100 g fw)	2.35 ± 0.37 bcB	1.86 ± 0.29 abA	2.65 ± 0.41 cB	1.61 ± 0.25 aA	1.13 ± 0.18 aA	2.61 ± 0.41 bA	2.04 ± 0.32 bA	2.81 ± 0.44 bB
Hydrolyzed Extract								
total phenols (mg GAE/100 g fw)	283.13 ± 4.77 bB	275.48 ± 12.84 bB	257.38 ± 10.25 aB	285.26 ± 7.19 bB	178.48 ± 6.91 aA	214.66 ± 14.90 bA	192.07 ± 17.64 abA	166.99 ± 23.38 aA
quercetin (μmol/100 g fw)	37.05 ± 11.51 aA	52.66 ± 5.03 aB	57.83 ± 12.55 aB	43.91 ± 4.70 aB	30.37 ± 4.24 *a*A	23.87 ± 9.99 *a*A	28.64 ± 4.51 *a*A	33.90 ± 3.63 *a*A
isorhamnetin (μmol/100 g fw)	7.01 ± 0.49 aB	7.61 ± 1.00 aB	7.95 ± 0.80 aB	8.84 ± 0.95 aA	1.64 ± 0.14 *a*A	3.55 ± 0.51 *ab*A	5.50 ± 0.21 *bc*A	7.34 ± 0.86 *c*A

[a]All high-pressure treatments were carried out at 25 °C for a 5-min hold time. Small letters in the same row indicate significant differences ($p < 0.05$) for the same compound within the same group (diced and pulverized) for different high-pressure treatments (ANOVA and posterior Tamhane's T2 and Tukey's *b post hoc* tests were used as appropriate). Italic small letters indicate Tamhane's T2 *post hoc* test. Capital letters in the same row indicate significant differences ($p < 0.05$) for the same compound within the same high-pressure treatment for different groups (diced and pulverized) (Student's *t* test). Italic capital letters indicate that equal variances have not been assumed.

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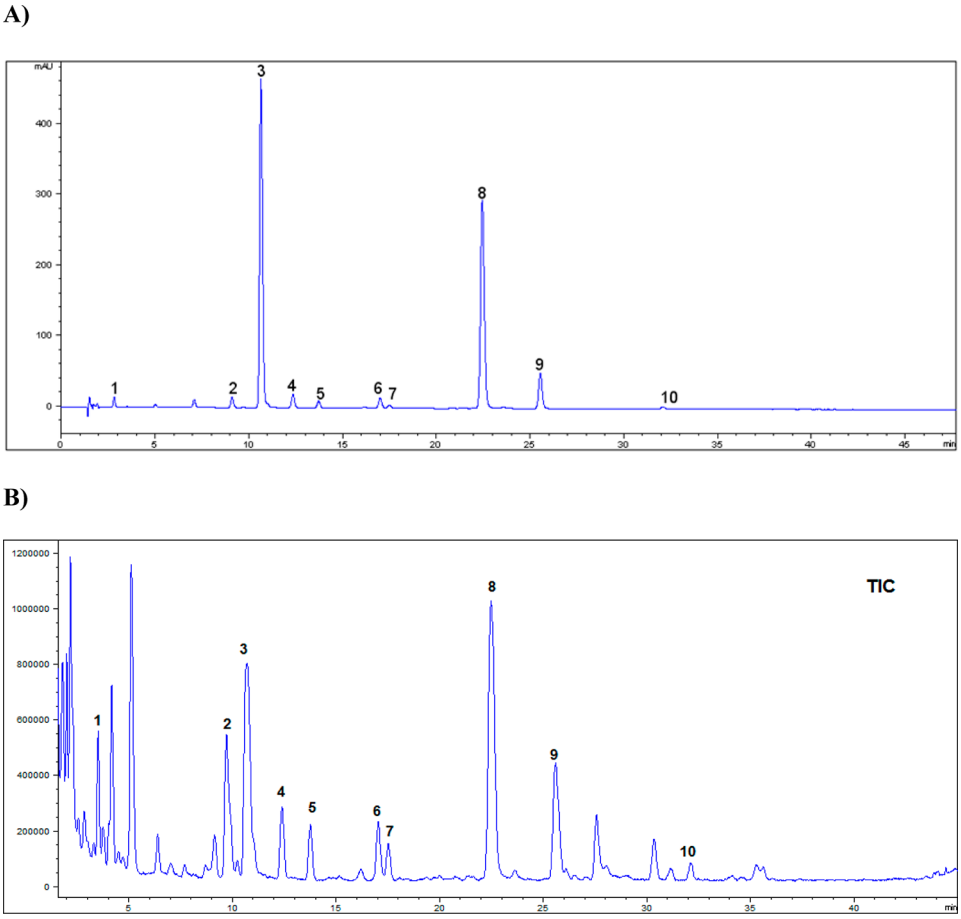

Figure 1. (A) HPLC-DAD chromatogram at 360 nm and (B) total ion chromatogram (TIC) of nonhydrolyzed onion extract (untreated onion). For compound identification see Table 2.

(Q4G), quercetin 3,4′-diglucoside (Q3,4G), quercetin 3-glucoside (Q3G), quercetin, and isorhamnetin, in the range from 10 to 150 μg/mL. All determinations were performed in triplicate and expressed as μmol per 100 g of fw.

HPLC-ESI-MS Analysis. Identification of flavonols was achieved by HPLC-MS applying identical chromatographic conditions used for HPLC-DAD analysis. Analyses were performed using an Agilent 1100 series LC, comprised of a quaternary pump with integrated degasser, autosampler, thermostated column compartment, and DAD, coupled with an Agilent G1946D quadrupole mass spectrometer (Agilent Technologies, Waldbroon, Germany). Data acquisition and analysis were carried out with an Agilent ChemStation. Ionization was achieved by an atmospheric pressure electrospray ionization (ESI) source, operated in negative and positive ion mode, with the electrospray capillary voltage set to +3500 and −3500 V, respectively, fragmentor 150 V, a nebulizing gas flow rate 12 L/h, nebulizer pressure 45 psig, and drying temperature 350 °C. Mass spectrometry data were acquired in the scan mode (mass range 100−1000 m/z).

Determination of Anti-inflammatory Activity. *Hyaluronidase Inhibiting Activity Assay.* Onion extract preparation and the enzymatic assay were carried out according to the method of Bralley et al.[48] with slight modifications. Ethanol/water extracts (50:50, v/v) were diluted appropriately with acetate buffer (0.2 M sodium acetate-acetic acid, pH 6.0, containing 0.15 M NaCl). Both hyaluronic acid (4 mg/mL) and hyaluronidase (1 mg/mL) stock solutions were prepared daily in acetate buffer. The reaction was started by mixing in a culture tube 300 μg of hyaluronic acid, 0−250 μL of onion extract, acetate buffer, and 30 μg of hyaluronidase enzyme and then immediately incubated at 37 °C for 15 min. The reaction was ended by adding 2 mL of 2.3% CTAB (w/v) in 2% NaOH. Absorbance values were measured at 400 nm (Ultrospec 4300 Pro, Biochrom Ltd., Cambridge, England) against a blank, after 10 min at room temperature. Results were expressed as follows: % inhibition = $[(OD_s - OD_c)/\Delta OD_c \times 100]$, where OD_s is the optical density of the sample, OD_c is the optical density of the control, and ΔOD_c is the increase in optical density of the control.

Nitric Oxide Radical ($NO^{\bullet}$) Scavenging Capacity Assay. The activity was determined according to the method by Hazra et al.[49] The reaction mixture contained 10 mM sodium nitroprusside (SNP) and 100 μL of onion extract to reach a final 3 mL volume, containing phosphate-buffered saline (pH 7.4). After incubation at 25 °C for 150 min, 0.5 mL was removed, transferred, and vortexed with 1 mL of sulfanilamide (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min. Then, 1 mL of naphthylethylenediamine dihydrochloride (NED) (0.1%, w/v) was added, and the mixture was incubated at 25 °C for 30 min. The diazotization of nitrite ions with sulfanilamide and subsequent coupling with NED generated a pink chromophore, whose intensity was measured spectrophotometrically at 540 nm against a blank sample. Results were expressed as μmol of trolox equivalents per 100 g of fw.

Determination of Antioxidant Activity. *2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic Acid Radical Cation ($ABTS^{\bullet+}$) Scavenging Capacity Assay.* According to the method of Re et al.,[50] including an adaptation of the method to 96-well microplate format, ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS with potassium persulfate ($K_2S_2O_8$). Absorbance was measured at 734 nm in a microplate reader. All samples were run in triplicate. Results were expressed as μmol of trolox equivalents per 100 g of fw.

2,2-Diphenyl-1-picrylhydrazyl Radical ($DPPH^{\bullet}$) Scavenging Capacity Assay. The method described by Sánchez-Moreno et al.,[51] including an adaptation of the method to 96-well microplate format, was followed. Absorbance was measured at 515 nm in a microplate

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Table 2. Flavonol Compounds in Onion Using HPLC-DAD and HPLC-ESI-MS[a]

peak no.	t_R (min)	λ_{max} (nm)	major ESI$^-$ peak m/z $[M-H]^-$ and fragments	major ESI$^+$ peak m/z $[M+H]^+$ and fragments	MW (M)	compound name	peak identification
1	2.79	255, 265, 344	787	789, 627	788	quercetin 3,7,4′-triglucoside (Q3,7,4G)	MS
2	9.08	255, 366	625, 463	627, 465, 303	626	quercetin 7,4′-diglucoside (Q7,4G)	MS
3	10.62	255, 264, 344	625, 463	627, 465, 303	626	quercetin 3,4′-diglucoside (Q3,4G)	ST
4	12.33	256, 344	639	641, 479, 317	640	isorhamnetin 3,4′-diglucoside (I3,4G)	MS
5	13.70	254, 266, 344	653	655, 465, 303	654	quercetin derivative	MS
6	16.98	256, 354	463	465, 303, 105	464	quercetin 3-glucoside (Q3G)	ST
7	17.66	254, 266, 354	653, 463	655, 493, 303	654	quercetin derivative	MS
8	22.43	250, 366	463, 301	465, 303	464	quercetin 4′-glucoside (Q4G)	ST
9	25.53	252, 366	477	479, 317	478	isorhamnetin 4′-glucoside (I4G)	MS
10	32.06	255, 370	301	303	302	quercetin	ST

[a]Peak identification: ST, comparison of spectra and retention time with commercial standards; MS, tentatively identified by using databases.

reader. All samples were run in triplicate. Results were expressed as μmol of trolox equivalents per 100 g of fw.

Ferric Reducing Antioxidant Power (FRAP) Assay. The total antioxidant potential of a sample was also determined using the ferric reducing antioxidant power assay by Benzie and Strain,[52] including an adaptation of the method to 96-well microplate format. All samples were run in triplicate. Results were expressed as μmol of trolox equivalents per 100 g of fw.

Antioxidative Capacity by Photochemiluminescence (PCL) Assay. The measurement was determined by using the PHOTOCHEM system (Analytik-Jena). The method combines the photochemical generation of radicals with a very sensitive technique of chemiluminometric detection. In this system, the superoxide radical $O_2^{\bullet-}$ is created by interaction of oxygen with intermediate products of the photoinduced luminol; then part of these $O_2^{\bullet-}$ radicals are quenched by all antioxidants present in the samples, and remaining radicals are quantified by luminescence-detection reaction. This method allows for the quantification of the antioxidant capacity of both hydrophilic and lipophilic substances against the superoxide radical, by means of two commercial kits: antioxidative capacity in water-soluble substances (ACW) and antioxidative capacity in lipid-soluble substances (ACL). Both determinations were carried out according to manufacturer specifications of the commercial kits. All samples were run in triplicate. Results were expressed as μmol of ascorbic acid equivalents for ACW-soluble substances and μmol of trolox equivalents for ACL-soluble substances per 100 g of fw.

Statistical Analysis. Descriptive statistics (including means and SDs) were used to summarize the data. Full factorial design with two factors, group (diced, pulverized) and treatment (0 MPa/25 °C/5 min, 200 MPa/25 °C/5 min, 400 MPa/25 °C/5 min, and 600 MPa/25 °C/5 min) was carried out. Two-way analysis of variance (ANOVA) was performed to study separately the main effects (group and treatment) and the interaction effect (group × treatment). As a significant interaction effect was observed in most of the variables, ANOVA, comparing the means within the same group for different treatments and within the same treatment for different groups, was performed. Levene's test was applied to verify the homogeneity of the variances. Tamhane's T2 (equal variances not assumed) and Tukey's *b* (equal variances assumed) *post hoc* tests and Student's *t* test were used to compare pairs of means and determine statistical significance at the $p \leq 0.05$ level. The correlations within variables were examined by Pearson correlation. All analyses were performed by using the IBM SPSS Statistics 19 Core System (SPSS Inc., an IBM Company).

RESULTS AND DISCUSSION

Quantification of Total Phenolic Content. The total phenolic content of the nonhydrolyzed and hydrolyzed onion samples is shown in Table 1. The highest level of TPC in nonhydrolyzed samples was noted in diced onion subjected to 400 MPa (9.4%) compared with untreated onion, while onion treated at 200 and 600 MPa showed a decrease of 8.1% and 12.4%, respectively. In contrast, an opposite effect was observed in hydrolyzed extracts, which showed a significant decrease (9.1%) in diced onion treated at 400 MPa, whereas treatments at 200 and 600 MPa showed no significant effect on the TPC. In addition, there was no significant effect of HPP on the levels of total phenols in pulverized onion (nonhydrolyzed extracts). Enhanced TPC (20.3%) was only related with pulverized samples subjected to 200 MPa in hydrolyzed extracts, suggesting that the combination of this HP treatment and freeze-drying favored the extraction of TPC. Previous studies showed a rise of approximately 12% in TPC for pressures at 100 and 400 MPa combined with high (50 °C) and low (5 °C) temperatures, respectively.[47] Butz et al.[53] found that any pressure treatment above 100 MPa induced browning of diced onions due to an enzymatic browning reaction involving polyphenoloxidases, which could explain the reduction in TPC found in some cases in the present study.

Identification and Quantification of Flavonols. Figure 1A shows the HPLC-DAD chromatogram recorded at 360 nm of the nonhydrolyzed onion extract from untreated onion (non-HP-treated). Also, the total ion chromatogram (TIC) of the HPLC-MS analysis is shown in Figure 1B. Eight compounds were identified in the HPLC-DAD chromatogram at 360 nm based on chromatographic retention time, UV−visible absorption maxima, and published data.[54] The identity of these compounds was confirmed by high-performance liquid chromatography (HPLC) and electrospray ionization-mass spectrometry (ESI-MS), both in positive and negative ion mode (Table 2). The two major flavonols present in the onion samples were quercetin 3,4′-diglucoside (Q3,4G) and quercetin 4′-glucoside (Q4G). The rest of the detected flavonol compounds, quercetin 3,7,4′-triglucoside (Q3,7,4G), quercetin 7,4′-diglucoside (Q7,4G), quercetin 3-glucoside (Q3G), isorhamnetin 3,4′-diglucoside (I3,4G), and isorhamnetin 4′-glucoside (I4G), represented about 10% of the total concentration of the identified flavonols. The hydrolyzed onion extract chromatogram revealed two peaks that were identified according to commercial standards as quercetin and isorhamnetin aglycones.

The effects of HPP and HPP-FD-P on onion flavonol profile of the nonhydrolyzed and hydrolyzed extract are shown in

Table 1. As shown in Table 1, in general, the freeze-drying process significantly diminished the content of quercetin and isorhamnetin glucosides comparing untreated diced and pulverized onion.

The major flavonol identified in nonhydrolyzed onion extract was quercetin 3,4′-diglucoside (Q3,4G), which represented approximately 65% of total flavonols in untreated diced onion. Q3,4G content in diced onion diminished by pressurization without significant differences among the different high-pressure levels. In pulverized samples, an increase in Q3,4G content was observed at 200 MPa (29.7%) and 600 MPa (33.8%) compared with the untreated samples (0 MPa). Quercetin 4′-glucoside (Q4G) corresponded to approximately 26% of total flavonols in untreated diced onion. In diced onion, there were no significant differences in Q4G concentration between treatments at 200 and 400 MPa comparing with untreated onion, whereas there was a significant reduction for samples treated at 600 MPa. In pulverized onion, Q4G content increased 69.6% and 63.2% when pressures of 400 and 600 MPa were applied, respectively. Therefore, onion HPP-FD-P increased Q4G and Q3,4G extractability without affecting quercetin concentration. These results are in agreement with the results reported by Roldán-Marín et al.[47]

Quercetin and isorhamnetin aglycone values corresponded to hydrolyzed extracts because their content in nonhydrolyzed extracts was not quantifiable. In diced and pulverized onion, quercetin concentration was not affected by HPP, as no significant differences were found among different treatments. In agreement with TPC, in pulverized samples, quercetin content was significantly lower than in diced samples for all HP-treated samples, indicating that freeze-drying produced a loss of quercetin. In diced onion, isorhamnetin concentration was not affected by HPP, as no significant differences were found among different treatments comparing with untreated onion. Interestingly, in pulverized onion, isorhamnetin concentration increased when pressures of 400 and 600 MPa were applied comparing with untreated onion. In addition, when comparing the same HP treatment for diced and pulverized onion, a significant decrease was found in pulverized onion non-HP-treated and treated at 200 and 400 MPa, less pronounced with higher pressures, suggesting that HPP prevented the losses resulting from freeze-drying and pulverization. The increase in flavonol content found in onion treated by high-pressure and freeze-drying could be due to the disruption of the onion vegetative vacuoles where these phenolic compounds are confined. Another hypothesis could be that HPP could help in the extraction of polyphenols bound to the cell wall membrane.[47,55,56] However, it has to be taken into account that HPP could cause structural changes in food matrixes, which may affect bioactive compound bioaccessibility.[38]

Anti-inflammatory Activity. *Hyaluronidase Inhibiting Activity.* Taking into account that both inflammation and oxidation play an important role in the disease pathogenesis, research efforts are concentrated on the evaluation of anti-inflammatory and antioxidant activity of plant foods. Therefore, the evaluation of the potential *in vitro* anti-inflammatory activity by the measurement of hyaluronidase activity was carried out. Hyaluronidases are enzymes capable of hydrolyzing hyaluronic acid, one of the most abundant constituents of the extracellular matrix, which is ubiquitously distributed in body tissues.

In the present study, the ability of onion treated by HPP and HPP-FD-P to inhibit *in vitro* hyaluronidase activity was evaluated (Table 3). In diced onion, HPP resulted in significant decreases in anti-inflammatory capacity (∼48%) in the treated onion in comparison with the untreated onion, regardless of the pressure level. In pulverized onion, also decreases were found at the three levels of pressure, although the reduction was significant in onion treated at 600 MPa (44%), suggesting that the combination of HPP and freeze-drying did not favor the antihyaluronidase capacity. In order to explain the effects found in the inhibition of hyaluronidase activity by the processed onion, the antihyaluronidase capacity of quercetin and quercetin derivatives, which are suggested to be partially responsible for such activity in onion, was tested. Quercetin and Q3,4G at 750 μM exhibited about 27% and 38% inhibition of hyaluronidase activity, respectively. The potential of flavonoids working as hyaluronidase enzyme inhibitors was described by Kuppusamy et al.[57] Currently, it is also known that certain types of flavonols demonstrate good anti-inflammatory activity in models of induced inflammation, even working (as quercetin for example) as antiatherosclerotic agents.[58,59] In the present study, a positive correlation was observed among quercetin and isorhamnetin contents and inhibition of hyaluronidase activity among both the diced and the pulverized onion ($R = 0.635$, $p < 0.001$ and $R = 0.602$, $p = 0.002$). However, it is important to note that anti-inflammatory potential of the onion extracts is not exclusively influenced by flavonoids but also depends on the levels of other compounds that could be modified by high-pressure treatments, as well as by the subsequent freeze-drying and pulverization processes.

Nitric Oxide Radical ($NO^{\bullet}$) Scavenging Capacity. The controversial role of nitric oxide (NO) has been investigated in various *in vitro* and *in vivo* models. The autoxidation of NO may generate the nitrosating agent nitrous anhydride (N_2O_3), and the overproduction of NO could induce the generation of $ONOO^-$, which is a powerful oxidant originating in the reaction of NO and $O_2^{\bullet -}$ (both abundant species in inflammatory areas), exhibiting a wide array of tissue-damaging effects.[60] It is thought that the phenolic constituents present in onion could contribute to regulate inflammatory responses by reducing the formation of reactive nitrogen species. Furthermore, this property has recently been found in onion sprouts.[61] In the present study, the extracts obtained from onion subjected to HPP displayed a significantly higher scavenging of $NO^{\bullet}$ than the extracts obtained from non-HP-treated onion. For nonhydrolyzed and hydrolyzed onion extracts, HPP had a significant effect, increasing the $NO^{\bullet}$ scavenging for treatments at 200 and 400 MPa in both diced and pulverized onion (Tables 3 and 4). This confirms that the extracts obtained from onion subjected to HPP are able to establish a competitive relation with oxygen, reacting with the NO generated from the sodium nitroprusside present in the assay. In addition, these results showed that the combination of HPP and freeze-drying and pulverization did not modify the effect of HPP on $NO^{\bullet}$ scavenging potential. In a recent study, the hypothesis that antioxidant and anti-inflammatory responses are involved in the marked antiatherogenic effect produced by onion extracts in atherosclerotic rats was reinforced.[62]

The full factorial two-way ANOVA to study separately the main effects (group and treatment) and the interaction effect (group × treatment) revealed that both factors (group and treatment) showed a significant effect ($p < 0.001$). Lyophilization and pulverization had substantial significant effects on hyaluronidase activity ($F = 1227.524$, $p < 0.001$) compared with treatment ($F = 15.362$, $p < 0.001$). Whereas $NO^{\bullet}$ scavenging

Table 3. Anti-inflammatory and Antioxidant Activity Parameters in Onion Subjected to High-Pressure Processing (HPP) and HPP Combined with Freeze-Drying and Pulverization (HPP-FD-P) in Nonhydrolyzed Extract[a]

	diced onion, HP-treated				pulverized onion, HP-treated and freeze-dried			
	0 MPa	200 MPa	400 MPa	600 MPa	0 MPa	200 MPa	400 MPa	600 MPa
inhibition of hyaluronidase activity (%)	18.66 ± 0.57 bB	10.63 ± 0.84 aB	11.70 ± 0.80 aB	6.52 ± 0.53 aB	7.98 ± 0.54 *b*A	4.96 ± 0.16 *ab*A	5.34 ± 0.61 *ab*A	4.46 ± 0.13 *a*A
scavenging of $NO^{\bullet}$ (μmol TE/100 g fw)	32.12 ± 4.48 aA	42.67 ± 5.51 bA	42.60 ± 3.48 bA	29.04 ± 4.31 aB	30.69 ± 4.80 *a*A	56.96 ± 7.55 *b*B	46.10 ± 6.44 *b*A	21.87 ± 1.31 *a*A
$ABTS^{\bullet+}$ (μmol TE/100 g fw)	126.63 ± 2.35 *c*B	100.75 ± 11.47 *abc*A	121.83 ± 1.67 *bB*	83.01 ± 6.85 *a*A	86.05 ± 6.02 aA	98.90 ± 4.04 bA	107.95 ± 5.69 c*A*	87.42 ± 4.86 aA
$DPPH^{\bullet}$ (μmol TE/100 g fw)	58.77 ± 4.91 cA	49.62 ± 4.86 bA	49.93 ± 6.14 bA	35.93 ± 3.20 aA	54.50 ± 2.09 bA	63.79 ± 4.45 cB	58.00 ± 3.76 bcB	41.95 ± 4.68 aB
FRAP (μmol TE/100 g fw)	137.51 ± 3.00 *cB*	110.51 ± 8.21 *b*A	121.21 ± 5.33 *b*A	87.84 ± 1.96 *a*A	105.16 ± 1.55 a*A*	116.16 ± 5.18 bA	125.70 ± 1.40 cA	105.19 ± 2.80 aB
PCL-ACL (μmol TE/100 g fw)	56.03 ± 0.83 *c*B	41.55 ± 0.53 *bB*	55.39 ± 5.23 *c*B	33.55 ± 0.62 *aA*	27.29 ± 2.98 aA	34.84 ± 2.53 ab*A*	40.21 ± 3.86 bA	29.04 ± 3.64 a*A*
PCL-ACW (μmol AA/100 g fw)	207.48 ± 10.02 *abA*	200.45 ± 5.16 *ab*A	262.43 ± 23.30 *bA*	192.74 ± 10.99 *aA*	246.44 ± 27.34 a*A*	290.44 ± 36.60 aB	279.38 ± 17.83 aA	278.62 ± 17.57 aB

[a]All high-pressure treatments were carried out at 25 °C for a 5-min hold time. Small letters in the same row indicate significant differences ($p < 0.05$) for the same activity within the same group (diced and pulverized) for different high-pressure treatments (ANOVA and posterior Tamhane's T2 and Tukey's *b post hoc* tests were used as appropriate). Italic small letters indicate Tamhane's T2 *post hoc* test. Capital letters in the same row indicate significant differences ($p < 0.05$) for the same activity within the same high-pressure treatment for different groups (diced and pulverized) (Student's *t* test). Italic capital letters indicate that equal variances have not been assumed.

Table 4. Anti-inflammatory and Antioxidant Activity Parameters in Onion Subjected to High-Pressure Processing (HPP) and HPP Combined with Freeze-Drying and Pulverization (HPP-FD-P) in Hydrolyzed Extract[a]

	diced onion, HP-treated				pulverized onion, HP-treated and freeze-dried			
	0 MPa	200 MPa	400 MPa	600 MPa	0 MPa	200 MPa	400 MPa	600 MPa
scavenging of $NO^{\bullet}$ (μmol TE/100 g fw)	215.89 ± 10.27 *a*A	289.22 ± 24.56 *b*A	303.04 ± 40.61 *b*B	299.12 ± 23.22 *b*B	205.89 ± 7.60 aA	252.61 ± 33.09 bA	239.54 ± 20.56 bA	174.59 ± 14.92 aA
$ABTS^{\bullet+}$ (μmol TE/100 g fw)	258.43 ± 10.49 aB	259.76 ± 6.09 aB	338.82 ± 16.66 bB	263.42 ± 7.36 aB	189.99 ± 6.18 bA	156.18 ± 14.09 aA	202.20 ± 14.12 bA	150.45 ± 6.82 aA
$DPPH^{\bullet}$ (μmol TE/100 g fw)	1226.58 ± 18.30 bB	1194.90 ± 44.33 abB	1220.28 ± 34.45 abB	1158.04 ± 42.94 aB	781.83 ± 12.36 *a*A	741.42 ± 14.33 *a*A	736.10 ± 17.52 *a*A	731.80 ± 54.89 *a*A
FRAP (μmol TE/100 g fw)	434.48 ± 7.68 *a*B	533.28 ± 22.96 *b*B	538.44 ± 6.99 *bB*	512.29 ± 14.99 *b*B	278.34 ± 4.30 *a*A	301.27 ± 22.06 *ab*A	355.01 ± 45.74 *abA*	315.00 ± 7.93 *b*A
PCL-ACL (μmol TE/100 g fw)	102.65 ± 11.14 *a*B	121.42 ± 7.77 *bc*B	125.15 ± 4.69 *c*B	106.90 ± 4.16 *abB*	62.84 ± 6.81 aA	61.13 ± 5.83 aA	67.26 ± 6.26 aA	54.92 ± 13.54 a*A*
PCL-ACW (μmol AA/100 g fw)	416.66 ± 51.40 *abB*	501.94 ± 52.15 *ab*B	593.13 ± 0.99 *bB*	466.33 ± 28.94 *a*B	230.51 ± 9.76 a*A*	238.15 ± 29.09 aA	266.92 ± 16.92 a*A*	230.34 ± 44.34 aA

[a]All high-pressure treatments were carried out at 25 °C for a 5-min hold time. Small letters in the same row indicate significant differences ($p < 0.05$) for the same activity within the same group (diced and pulverized) for different high-pressure treatments (ANOVA and posterior Tamhane's T2 and Tukey's *b post hoc* tests were used as appropriate). Italic small letters indicate Tamhane's T2 *post hoc* test. Capital letters in the same row indicate significant differences ($p < 0.05$) for the same activity within the same high-pressure treatment for different groups (diced and pulverized) (Student's *t* test). Italic capital letters indicate that equal variances have not been assumed.

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activity was mainly influenced by treatment (F = 48.017, p < 0.001), the main effect of the group was not significant in nonhydrolyzed extracts. The knowledge of these interactions may redirect future investigations toward the most advisable variables.

Antioxidant Activity. The effects of HPP and HPP-FD-P on the antioxidant activity were determined by four different antioxidant assays (Tables 3 and 4). The bioanalytical assays applied in this work differ in terms of reaction mechanisms and oxidant and target species. The knowledge of the behavior of the extracts under different types of stress is highly valuable information in order to generate a complete antioxidant profile. It has to be mentioned that free-radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. Therefore, these assays have been considered as easy and useful spectrophotometric methods to screen/measure the radical scavenging capacity of food constituents and plant extracts.[63]

2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid Radical Cation ($ABTS^{\bullet+}$) Scavenging Capacity. In nonhydrolyzed extracts, $ABTS^{\bullet+}$ values showed a significant decrease in diced onion subjected to 400 and 600 MPa, while in pulverized samples this activity was significantly increased in samples subjected to 200 and 400 MPa, by 14.9% and 25.4%, respectively, suggesting a positive effect of the combination of HPP and freeze-drying. The two-way ANOVA revealed similar significant (p < 0.001) effects of group (F = 48.241) and treatment (F = 46.312) and a significant interaction between group and treatment (F = 28.906, p < 0.001).

In hydrolyzed extracts, HPP caused an increase in antioxidant activity determined by the $ABTS^{\bullet+}$ assay in diced onion by HP treatment at 400 MPa (31.1%). However, in pulverized onion, reduction in this antioxidant activity was observed at 200 and 600 MPa. The two-way ANOVA showed that the effect of group was higher (F = 924.016, p < 0.001) than the effect of treatment (F = 73.883, p < 0.001), concordantly with the higher values found in diced onion compared with pulverized onion within each treatment.

These findings in processed onion support the theory that activity modifications may be dependent on food matrix and processing parameters. HPP and/or HPP-FD-P may modify the extractability of bioactive compounds, and as a result small differences in antioxidant activity (mainly on soluble fractions) may be reported.[41]

2,2-Diphenyl-1-picrylhydrazyl Radical ($DPPH^{\bullet}$) Scavenging Capacity. In nonhydrolyzed extracts, $DPPH^{\bullet}$ values showed a decrease in antioxidant activity at the three levels of pressure in diced samples, whereas in pulverized samples, onion treated at 200 MPa showed an increase in antioxidant capacity (17.0%), indicating a positive effect of the combination of HPP and freeze-drying.

In hydrolyzed extracts, the effect of HPP on antioxidant activity determined by $DPPH^{\bullet}$ assay showed that all high-pressure treatments maintained the antioxidant activity of the untreated samples, in diced and pulverized samples, except in diced onion treated at 600 MPa, which showed a slight reduction (5.6%), showing that the combination of HPP and freeze-drying did not modify the effect of HPP on antioxidant activity determined by the $DPPH^{\bullet}$ assay, in agreement with the $NO^{\bullet}$ scavenging potential of these samples. The two-way ANOVA appointed a much higher effect of group (F = 1815.606, p < 0.001) compared with the effect of treatment (F = 5.357, p = 0.004), in agreement with the $ABTS^{\bullet+}$ assay.

The effect of HPP on solid foods has been poorly studied. However, Butz et al.[38] showed important quality changes in carrots and tomatoes treated at 500 and 800 MPa, in which antioxidant activity suffered a slight decrease. Likewise, other recent work screening the effect of HPP at 500 MPa on the activity of antioxidants from "Granny Smith" apples reflected a noticeable effect on the $DPPH^{\bullet}$ radical scavenging activity of both digested and undigested samples, depending on the length of exposure.[64]

Ferric Reducing Antioxidant Power. Regarding the measurement of the antioxidant activity by the FRAP assay in nonhydrolyzed extracts, HPP had a clear differential effect on diced and pulverized onion. Significant decreases were observed in diced onion at the three levels of pressure, while an increase in antioxidant activity by the FRAP assay was observed for treatments at 200 and 400 MPa (∼15.0%) in pulverized onion, suggesting a positive effect of the combination of HPP and freeze-drying. Concordantly, the analysis of two-way ANOVA supported that high-pressure treatment was the main factor that modified this reducing power (F = 81.000, p < 0.001), while group effect was not significant.

The FRAP values in hydrolyzed extracts indicated that a significant increase was observed in diced onion treated at 200, 400, and 600 MPa (∼21.5%) and also in pulverized onion treated at 600 MPa (13.2%).

These results present detailed information about the impact of HPP on the onion hydrophilic antioxidant activity as determined by the FRAP assay. In the study by McInerney et al.[65] HPP had differential effects on antioxidant activity measured by the FRAP method depending on vegetable type. For broccoli, antioxidant activity was not affected by HPP, for carrots a modest reduction in antioxidant activity at the lower pressure level (400 MPa) was observed, and for green beans a significant increase in antioxidant activity by pressure treatment at 400 and 600 MPa was shown. The authors acknowledge food matrix characteristics and nutrient composition as critical factors influencing antioxidant activity values.

Antioxidative Capacity by Photochemiluminescence. In nonhydrolyzed extracts, the antioxidant activity by the PCL assay for ACL-soluble substances showed some differences. In diced onion, there was a significant reduction in antioxidant activity at 200 and 600 MPa (25.8% and 40.1%, respectively), whereas in pulverized onion the antioxidant activity was increased by HPP at 400 MPa (47.3%). Likewise, the two-way ANOVA for the PCL-ACL assay indicated that the effects of group (F = 108.504), treatment (F = 28.500), and its interaction effect (F = 16.361) were significant (p < 0.001), emphasizing the influence of the group. In diced and pulverized onion, the antioxidant activity by the PCL assay for ACW-soluble substances was not affected by HPP, as no significant differences were found among different treatments.

The measure of the antioxidant activity by the PCL assay for ACL-soluble substances in hydrolyzed extracts revealed that only in diced samples was a significant increase (∼20.1%) found after treatments at 200 and 400 MPa, whereas HPP had no effect in pulverized onion, also in concordance with the two-way ANOVA, which showed a higher main effect of group. In diced and pulverized onion, the antioxidant activity by the PCL assay for ACW-soluble substances was not affected by HPP, as no significant differences were found among different treatments, in accordance with the findings in quercetin and total phenols.

Table 5. Pearson's Correlation Coefficients (R) among Total Phenol Content, Quercetin, Isorhamnetin, and Antioxidant and Anti-inflammatory Activities in Hydrolyzed Onion Extracts Subjected to High-Pressure Processing (HPP) and HPP Combined with Freeze-Drying and Pulverization (HPP-FD-P)

	ABTS$^{\bullet+}$	DPPH$^{\bullet}$	FRAP	PCL-ACL	PCL-ACW	NO$^{\bullet}$
TPC	0.743[a]	0.910[a]	0.835[a]	0.856[a]	0.800[a]	0.598[a]
quercetin	0.775[a]	0.727[a]	0.788[a]	0.761[a]	0.772[a]	0.511[a]
isorhamnetin	0.573[a]	0.680[a]	0.772[a]	0.654[a]	0.703[a]	0.307
ABTS$^{\bullet+}$	1	0.878[a]	0.872[a]	0.911[a]	0.932[a]	0.642[a]
DPPH$^{\bullet}$		1	0.893[a]	0.933[a]	0.917[a]	0.565[a]
FRAP			1	0.923[a]	0.921[a]	0.700[a]
PCL-ACL				1	0.951[a]	0.630[a]
PCL-ACW					1	0.594[a]
NO$^{\bullet}$						1

[a] p-value for Pearson's correlation coefficient <0.01.

Correlation between Flavonol Content and Antioxidant and Anti-inflammatory Activities. The result obtained in this study showed statistical correlations among the flavonol glycoside contents and antioxidant activity values. Q4G content was positively correlated with ABTS$^{\bullet+}$, FRAP, and PLC-ACL values (R = 0.750, R = 0.699, R = 717, p < 0.001, respectively). Q3,4G and I4G contents were correlated with FRAP values (R = 0.617 and R = 0.635, p < 0.001, respectively), while the correlations among Q3G, Q3,4G, Q7,4G, and I4G contents and ABTS$^{\bullet+}$ values was weaker but still significant (p < 0.01). These results are consistent with the findings of other studies,[65−68] which placed phenolic content and flavonoids (quercetin and its glycoside derivatives) in the leading role in oxidative prevention, indicating the presence of functional groups, such as hydroxyl, in the phenolic structure, as a possible explanation for such potential activity. The strong ability to scavenge free radicals and reactive species that showed hydrolyzed extracts was correlated with the high levels of quercetin quantified. In fact, this study showed that the content of quercetin as well as TPC determined in samples from hydrolyzed extracts revealed stronger correlations with antioxidant and anti-inflammatory activities (Table 5) than those reported by the samples from nonhydrolyzed extracts, pointing, in agreement with the literature, to quercetin as one of the most potent scavengers of ROS within the flavonoid family due to its molecular configuration.[69] Furthermore, the effect of HPP on food quercetin content becomes important since hydrolytic mechanisms are involved to release the aglycone form, which enters the enterocyte prior to passage of the metabolites into the circulatory system.[70] Therefore, on the basis of the results obtained in the present study it has been confirmed that the aglycone content and antioxidant activity of hydrolyzed extracts was not negatively affected by HPP. Interestingly, a positive correlation was observed between NO$^{\bullet}$ scavenging values and antioxidant and anti-inflammatory activity values in hydrolyzed extracts (Table 5), supporting the close relationship between inflammation and oxidation.

In conclusion, this is the first time that onion HPP effects on flavonol content and its relationship with the anti-inflammatory and antioxidant activities comparing two different food matrixes, diced and pulverized onion, have been evaluated. Flavonol content was influenced by HPP. HPP-FD-P increased quercetin 3,4′-diglucoside, quercetin 4′-glucoside, quercetin 3-glucoside, and isorhamnetin 3,4′-diglucoside extractability. Freeze-drying and pulverization of fresh onion diminished total phenolic and quercetin and isorhamnetin glycoside content, pointing to the importance of the influence of the processes used for vegetable stabilization on the bioactive compound content. In addition, in general the effect of group was more marked than the effect of treatment for the flavonol content and the antioxidant and anti-inflammatory activities. The present study suggests that HPP (especially treatment at 400 MPa) and HPP-FD-P may be of benefit for obtaining functional ingredients from onion, as suggested by increased NO$^{\bullet}$ scavenging capacity and maintenance of the antioxidant activity mainly in hydrolyzed extracts.

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Notes

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