Phenolic compounds, microstructure and viscosity of onion and apple products subjected to *in vitro* gastrointestinal digestion

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ABSTRACT

Microstructure, viscosity and their relationship with bioaccessibility of phenolic compounds in onion and apple products (untreated and HPP) and commercial quercetin supplement throughout a dynamic gastrointestinal digestion (GID) model were investigated. In non-digested (ND) samples, untreated and HPP-onion presented higher total phenolic and flavonol content (TFC-HPLC and TPC-FC) than apple counterparts. TFC-HPLC decreased throughout GID phases in all samples studied. TFC-HPLC bioaccessibility was higher in onion (~17.6%) than in apple (~10%) and in quercetin supplement (0.027%). HPP did not improve TFC-HPLC bioaccessibility. Throughout GID, onion and apple showed a significant decrease in both consistency (K) and apparent viscosity at 10 s^{-1} but higher values were found in apple. These data agree with TFC-HPLC and TPC-FC decrease and with the lower bioaccessibilities of apple compared with onion. Food matrix had a more significant effect than HPP on TFC-HPLC bioaccessibility, which is related to the rheological behavior of the GID-phases.

Keywords: High-pressure processing; *In vitro* gastrointestinal digestion; Bioaccessibility; Phenolic compounds; Viscosity; Microstructure

Industrial relevance: High-pressure processing (HPP) (400 MPa at 25 °C during 5 min) combined with freeze-drying enhanced significantly flavonols extractability (TFC-HPLC) in onion and apple and in some cases their bioaccessibility. Bioaccessibility of bioactive compounds in each food matrix is being required by industrials and consumers concerned to know the actual amount of bioactive compounds that are available for intestinal absorption. The change of the matrices viscosity studied throughout *in vitro* gastrointestinal digestion (GID) could predict the bioaccessibility of these bioactive compounds. HPP could be proposed as a strategy for increasing the extractability of bioactive compounds in vegetable derived products.

1. Introduction

The beneficial effects of a high intake of plant derived products on human health have been attributed to the presence in their composition of bioactive compounds which include vitamin C and E, dietary fiber, carotenoids and phenolic compounds, especially flavonoids (Aguilera, Martin-Cabrejas, & de Mejia, 2016; Lewandowska et al., 2016; Liu, 2013). Onions (*Allium cepa* L.) and apples (*Malus domestica*) are recognized as the major dietary sources of flavonoids, mainly the flavonol quercetin as its aglycone or as *O*-glycosylated derivatives (Roldán-Marín, De Ancos, Cano, & Sánchez-Moreno, 2012). Quercetin and its derivatives are bioactive compounds that nowadays are receiving great attention due to their antioxidant, anti-inflammatory, anti-diabetes, antiestrogenic, cardioprotective, anticarcinogenic, and neuroprotective properties, among others (Erlund, 2004; Guo & Bruno, 2015; Lee & Mitchel, 2012). In fact, numerous commercial quercetin derived products obtained from onions and apples are available as nutraceuticals (Tomé-Carneiro & Visioli, 2016). However, the type and level of the bioactive compounds can vary markedly between species, cultivar, climatic, agronomic, harvest and postharvest conditions and food processing (Williams et al., 2003).

High-pressure processing (HPP) have been proposed as an alternative to traditional thermal processing technologies to obtain safe, nutritive fresh-tasting plant derived products avoiding the degradation of nutrients and bioactive compounds (Rodríguez-Roque et al., 2015). In addition, HPP may alter the food matrix resulting in a major extractability and bioaccessibility of bioactive compounds in different plant foods such as onion, persimmon, fruit juices and beverages (Plaza, Colina, De Ancos, Sánchez-Moreno & Cano, 2012; Vázquez-Gutiérrez et al., 2013).

The beneficial effects of quercetin and its derivative compounds on health depend not only of large amounts of them in the ingested foods but also on their bioaccessibility. The bioaccessibility is defined as the fraction of bioactive compound released from its food matrix in the gastrointestinal tract available for the intestinal absorption (Carbonell-Capella et al., 2014). Phenolic compounds are usually bound to carbohydrates of the cell wall meanwhile others such as flavonoids may stay in the cytosol or in the vacuoles. Thus, the bioaccessibility of phenolic compounds require the disruption of the cell walls and cellular compartments (Bohn, 2014; Kamiloglu, Capanoglu, Bilen, Gonzales, Grootaert, de Wiele & Van Camp, 2016). *In vitro* gastrointestinal digestion (GID) models have been widely used to determine the bioaccessibility of phenolic compounds obtaining results that are well correlated with those obtained *in vivo* (Bermudez-Soto, Tomás-Barberán, & García-Conesa, 2007; Tagliazucchi, Verzelloni, Betolini, & Conte, 2010). These studies have been carried out by determining different total phenolic families using spectrophotometric methods (Bouayed, Hoffmann, & Bohn, 2011) or by the identification and quantification of phenolic compounds by LC-DAD (Bouayed, Deußer, Hoffmann, & Bohn, 2012) and LC-MS (Kamiloglu et al., 2016).

It is well known that rheology is a tool widely used to characterize the internal structure of complex fluids as colloidal suspensions and emulsions. Digest from the small intestine is a complex aqueous suspension of undigested particulate matter and solubilized nutrients, together with other components such as secreted enzymes, bile, and mucin (Shelat et al., 2015). The authors reported that rheology could play an important role in controlling digestive features. Hence, rheological measurements such as viscosity are important factors to be considered in *in vitro* models because they could be related with bioaccessibility of the phenolic compounds.

The aim of this work was to study the changes in the microstructure and viscosity of onion and apple powder samples (untreated and HPP), as well as of commercial quercetin supplement, throughout different phases of an *in vitro* GID model, and their potential relationships with the bioaccessibility of phenolic compounds (total phenolic and total flavonol content). A comparative study between the bioaccessibility of total phenolic and flavonol compounds calculated by spectrophotometric and HPLC-DAD methods were also carried out.

2. Materials and methods

2.1. Reagents

2.1.1. Analysis of phenolic compounds

Methanol (HPLC-grade) was supplied by Lab-Scan (Dublin, Ireland). Folin-Ciocalteu's phenol reagent, ferulic acid, gallic acid, quercetin, quercetin-3-glucoside, quercetin-3,4'-diglucoside and isorhamnetin-3-glucoside were purchased from Sigma-Aldrich (St Louis, MO, USA).

2.1.2. Simulated gastrointestinal digestion

Citrate buffer (pH 6, C-999), α -amylase (from *Aspergillus oryzae*, A-9857), pepsin (from porcine gastric mucosa, P-7012), trypsin (from bovine pancreas, T-8253), pancreatin (from porcine pancreas, P-7012) and bile (porcine bile extract, B-8631) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Lipase (Rhizopus lipase, F-AP15) was obtained from Amano Enzyme, Inc. (Nagoya, Japan).

2.2. Plant material

Onions (*Allium cepa* L. var *cepa*, 'Recas') from Carabaña, Madrid (Spain) and apples (*Malus domestica*, 'Golden Delicious') from Aragón (Spain) were obtained in a Madrid local supermarket in May of 2015 and storage at 4 °C for two days until use. Physicochemical and chemical characteristics of initial plant material are shown in Table S1. Dietary quercetin supplement in capsules of 500 mg (Solaray, Nutraceutical Corp. USA) were purchased in a Madrid local pharmacy. Onions were hand-peeled (only the external layer) and cut into 10 mm cubes. Apples were washed and cut in slices with skin of 10 mm thick. Cubes of onion and slices of apples (200 g) were packaged in very low gas permeability plastic bags (BB4L, Cryovac, Barcelona, Spain) and sealed with vacuum. After packaging, half of the onion and apple samples were immediately frozen with liquid nitrogen and lyophilized (Lyophilizer model Lyoalfa, Telstar S.A., Barcelona, Spain) (0.13 mbar, -90 °C). Lyophilized samples were pulverized using an ultracentrifugal grinder ZM 200 (Retsch GmbH, Haan, Germany) obtaining a fine powder (particle size ≤ 0.5 mm) and maintained at -20 °C until analysis.

2.3. High pressure processing (HPP)

The other half of the packaged onion and apple samples were treated in a high hydrostatic pressure unit with a vessel of 2925 mL capacity, a maximum pressure of 900 MPa, and a working temperature ranged between -10 to 60 °C (High pressure Iso-Lab System, Model FPG7100:9/2C, Stansted Fluid Power LTD., Essex, UK). Two bags of packed onions or apples were introduced in the vessel of the pressure unit filled with pressure medium (water) and treated at 400 MPa with a holding time of 5 min and a maximum temperature of 35 °C. HPP conditions were selected in accordance with previous studies (González-Peña et al., 2013). The compression rate was 500 MPa/min and the decompression was instantaneous. Pressure, time and temperature were controlled and monitored by a computer program during the process. After treatments,

pressurized onion and apple samples were immediately frozen with liquid nitrogen and lyophilized as indicated above.

2.4. *In vitro* dynamic gastrointestinal digestion (GID)

A dynamic gastrointestinal digester (DGD) was used to digest onion and apple powder samples and commercial quercetin supplement. The digester employed and the gastrointestinal digestion procedure have been previously described (Fernández-Jalao, Sánchez-Moreno, & De Ancos, 2017; Villemejane et al., 2016). The DGD consists of two successive serial compartments simulating stomach and small intestine. Peristaltic movements in both compartments stomach and small intestine are simulated. The digest transit was regulated by opening or closing the peristaltic valve pumps that connect the compartments. Temperature at 37 ± 1 °C, pH and enzymes secretions were computer controlled (Fernández-Jalao et al., 2017),

In vitro digestion process was performed with 27 g of freeze-dried powder onion and apple and 500 mg of commercial quercetin supplement from a pool of 5 capsules. The digestion process included several consecutive enzymatic treatments. Thus, simulated saliva secretions (α -amylase, citrate buffer and electrolyte solution) (pH 6), simulated gastric secretions (hydrochloric acid, gastric electrolytes solution, gastric lipase and pepsin) (pH 2), and simulated intestinal secretions (sodium bicarbonate, intestinal electrolytes solution, pancreatin, bile and trypsin) (pH 6.5 -7) were introduced into compartments by computer-controlled pumps. The digestion took 360 min (from 0 to 120 min in the stomach and from 120 to 360 min in the small intestine) at 37 °C in absence of light and under anaerobic conditions by injecting nitrogen gas in the system. Two different *in vitro* gastrointestinal digestion procedures have been done for each product (onion powder, apple powder or quercetin supplement) according to that

described by Fernández-Jalao et al. (2017). To monitor the release of phenolic compounds from onion (or apple or quercetin supplement) at different stages of digestion, in comparison with the non-digested (ND) products, aliquots from the artificial saliva treatment [oral-phase (OP)], gastric digest (GD) and intestinal digest (ID) were separated and acidified to pH 2. Then, these aliquots were frozen and stored at -20 °C until analysis. The rest of the intestinal digest at pH 2 was centrifuged (Sigma Laboratory Centrifuge at 6K15) at 3890 g at 4 °C for 60 min and the supernatant were separated. This supernatant represents the soluble fraction (SF) (or bioaccessible fraction) of the product sample according to previously described procedure (Cilla et al., 2012). The SF was immediately frozen and stored at -20 °C until its analysis.

Bioaccessibility defined as the portion of bioactive compounds (BC) that is released from the food matrix into the gastrointestinal tract and thus become available for intestinal absorption was determined in the SF using the next equation and expressed as percentage:

$$\text{Bioaccessibility (\%)} = (\text{BC}_{\text{digested}} / \text{BC}_{\text{non-digested}}) \times 100$$

With:

$\text{BC}_{\text{digested}}$ = Concentration of BC in the soluble fraction of digested sample

$\text{BC}_{\text{non-digested}}$ = Concentration of BC in the non-digested sample

2.5. Analysis of Phenolic Compounds

2.5.1. Preparation of phenolic extracts from non-digested (ND) products

Untreated and HPP powder samples (0.5 g of onion and 1 g of apple) were extracted with 12.5 mL of methanol/water (80:20, v/v) in an ultrahomogenizer at 7000 rpm for 4.5 min (model ES-270, Omni International Inc., Gainesville, VA, USA). The mixtures were centrifuged at 8000 g at 4 °C for 15 min, using a refrigerated centrifuge

(Thermo Scientific Sorvall, mod. Evolution RC, Thermo Fisher Scientific Inc., USA). The pellets were re-extracted with 12.5 mL of extraction solvent and centrifuged again. The two supernatants were combined and concentrated to approximately 2 mL using a rotatory evaporator at 40 °C. Methanol was added to reach a final volume of 10 mL. Methanolic extracts were stored at -20 °C until analysis. Each sample was extracted in duplicate and analyzed two times.

2.5.2. Preparation of phenolic extracts from GID phases

Aliquots (10 g) from different phases of GID (OP, GD and ID) were lyophilized and extracted with 10 mL of methanol (1.5 mL for soluble fraction). The mixture was vigorously stirred in a vortex for 2 min and 10 min more with a magnetic stir plate. The mixtures were centrifuged at 8000 g at 4 °C for 15 min, using a refrigerated centrifuge (Thermo Scientific Sorvall, mod. Evolution RC, Thermo Fisher Scientific Inc., USA). The supernatant was separated and used in subsequent analysis. Each sample was extracted in duplicate and analyzed two times. For each ND extract and fractions of each GID phase (OP, GD, ID and SF), different analysis were performed as indicated below.

2.5.3. Total Phenolic Content by Folin-Ciocalteu (TPC-FC)

TPC-FC of undigested samples and different digestion phases was performed by according to the procedure described in González-Peña et al. (2013) and Fernández-Jalao et al. (2017). Folin-Ciocalteu method was used to quantify the sample's reducing capacity due to other antioxidants such as ascorbic acid, citric acid, simple sugars, or certain amino acids also are detected by this assay (Huang, Ou, & Prior, 2005). All the samples were analyzed in duplicate and expressed as mg of gallic acid equivalents (GAE) per gram of dry weight (dw).

2.5.4. Total flavonol content by spectrophotometric assay (TFC-S)

The analysis of TFC-S was carried out according to the methodology described by Bonoli et al. (2004) using an Ultrospec 4300 pro UV-vis-Spectrophotometer (GE Amersham Biosciences Pharmacia, Sweden). Quantification was achieved using quercetin as external standard calibration curve in the range from 1 to 50 µg/mL. All the samples were analyzed in duplicate and expressed as mg of quercetin equivalents (QE) per gram of dry weight (dw).

2.5.5. Total flavonol content by HPLC-DAD (TFC-HPLC)

Total flavonol content was determined as the sum of individual flavonols that was separated, identified and quantified by HPLC-DAD according to the procedure described by González-Peña et al. (2013). The quantification was achieved using standards calibration curves of quercetin, quercetin-3-glucoside, quercetin-3,4'-diglucoside and isorhamnetin-3'-glucoside in the range from 0.4 to 550 µg/mL. All the samples were analyzed in duplicate and expressed as mg per gram of dry weight (dw).

2.6. Microstructure

A light microscope (Nikon Eclipse 80i, Nikon Co., Ltd., Tokyo, Japan) was used to study the structure of different GID phases (ND, OP, GD, and ID) according to Hernández-Carrión et al. (2015). The autofluorescence of the samples containing phenolic compounds was observed while using a mercury arc lamp with a FITC filter ($\lambda_{\text{ex max}}=482 \text{ nm}$, $\lambda_{\text{em max}}=536 \text{ nm}$) as excitation source. A drop of sample was placed on a microscope slide, covered with a cover slip and visualised at 4x. The images were captured and stored at 1280 x 1024 pixels using the microscope software (NIS-Elements F, Version 4.0, Nikon, Tokyo, Japan). The software interfaced directly with the microscope, enabling image recording.

2.7. Viscosity measurements

Steady shear tests were carried out with a dynamic Kinexus Pro Rotational Rheometer (Malvern Instruments Ltd., Worcestershire, UK) equipped with a cone and plate geometry (4° cone angle, 40 mm diameter) and a gap of 0.150 mm. Samples of different GID phases (ND, OP, GD, and ID) were placed into the plates at 37 °C and a cover was used to maintain the samples at the specified temperature. Temperature was controlled to within 0.1 °C by Peltier elements in the lower plates kept at 37 °C. The SF was discarded for the viscosity measurements as the presence of precipitates in the liquid phase prevented the adequate measure of the viscosity. Before measurement, a pre-shear was done at shear rate of 100 s⁻¹ for 5 min for temperature setting, standardizing the shear rate of each sample, as well as avoiding particle trapping near the tip of the cone (Shelat et al., 2015). Then, flow curves were obtained as a function of shear rate ranging from 100 to 0.1 s⁻¹. The power law model (Eq. (1)) was used to describe the shear rate effect on apparent viscosity values of the samples:

$$\eta_a = K\dot{\gamma}^{n-1} \quad (1)$$

Where η_a is the apparent viscosity (mPa s), K is the consistency coefficient (mPa s ^{n}), $\dot{\gamma}$ is the shear rate (s⁻¹), and n is flow behavior index.

A shear rate at 10 s⁻¹ has been used to mimic oral conditions (Espinal-Ruiz, Restrepo-Sánchez, Narvaez-Cuenca, & McClements, 2016; Pal, 2011). The maximum shear stress generated by the small intestine has been reported to be about 1.2 Pa (Hardacre, Lentle, Yap, & Monro, 2016). The authors reported that a maximum shear stress of 1.2 Pa would generate shear rates of about 10 s⁻¹ at an apparent viscosity of 0.1 Pa s ($\eta_{a,0.1}$) and 0.1 s⁻¹ at an apparent viscosity of 10 Pa s ($\eta_{a,10}$). Linear correlations between experimental apparent viscosity and predicted values by power law models at shear rates of 0.1, 7.5 and 100 s⁻¹ were also established in order to test the accuracy of

the predictive models. Measurements were performed in triplicate on two different *in vitro* digested samples ($n = 6$).

2.8. Statistical analysis

One-way analysis of variance (ANOVA) of the results followed by the least significant difference test (LSD) were carried out to determine significant differences ($P < 0.05$) in the concentration and bioaccessibility of bioactive compounds, as well as in the viscosity of the samples in relation to the three factors studied (GID phase, HPP and food matrix). Two-way analysis of variance (ANOVA) was also performed to study separately the main effects (GID phase and HPP, as well as GID phase and food matrix) and the interaction effects (GID phase $\times$ HPP and GID phase $\times$ food matrix, respectively). All statistical analyses were performed with StatgraphicsPlus 5.1 (Statistical Graphics Corporation, Inc., Rockville, MD, USA). The results are reported as mean $\pm$ standard deviation.

3. Results and discussion

3.1. Effects of *in vitro* GID phase, HPP and food matrix (onion, apple and quercetin supplement) on total flavonol and total phenolic content and their bioaccessibility

Table 1 shows the effect of the *in vitro* GID phases (OP, GD, ID and SF), in comparison with non-digested (ND) samples, on the content and bioaccessibility of total phenolic content (TPC-FC) and total flavonol content determined by spectrophotometric methods (TFC-S) and by HPLC-DAD (TFC-HPLC) in onion and apple products modulated by two different factors, food matrix and HPP. Also a quercetin supplement was subjected to a GID.

Initially, onion product (untreated and non-digested) presented significantly ($P<0.05$) higher total phenolic compounds and total flavonol content than apple product (Table 1). Regarding the methodology used, total flavonol content in untreated and non-digested apples determined by spectrophotometric assays (TFC-S) (0.27 mg QE/g dw) was similar to those calculated by HPLC-DAD (0.26 mg/g dw) (TFC-HPLC). However, TFC-HPLC in onion (8.65 mg/g dw) was 2.26-fold higher than TFC-S (3.82 mg/g dw) (Table 1).

When considering HPP effects on non-digested samples (ND), HPP-onion showed a significantly ($P<0.05$) higher TPC-FC, TFC-S and TFC-HPLC (6%, 9% and 13%, respectively) than its corresponding untreated sample (Table 1). Also, HPP increased 30% the TFC-HPLC value in apple product although this trend was not detected by spectrophotometric assays (TFC-S). Different effect on the extraction of total phenolic compounds due to HPP and depending on the type of assay (spectrophotometry or HPLC-DAD) was also observed in fruit-juices beverages (Rodriguez-Roque et al., 2015). These results are in accordance with those found in the literature that shown how HPP could produce changes in the membrane permeability and disruption of cell walls favoring the release of phenolic compounds from tissues improving their extractability (Fernández-Jalao et al. 2017; González-Peña et al., 2013; Rodriguez-Roque et al., 2015; Vázquez-Gutiérrez et al., 2013). However, HPP produced a significant decrease ($P<0.05$) of 14% in the total phenolic content (TPC-FC) in apple. These different results found for TPC-FC in onion and apple products are in accordance with the fact that the increase of extraction of bioactive compounds by HPP depends on both the treatment intensity and the food matrix (Barba, Esteve & Frigola, 2012). For example, Plaza et al. (2012) found significant increases by 86% in the extractability of total carotenoids in the astringent and less maturity (stage III) persimmon fruit cv. Rojo

Brillante meanwhile with non-astringent persimmon with similar maturity stage, the same HPP produced a significant decrease (~60%) of total carotenoids extracted. Table S1 shows the different physicochemical and chemical composition of onion and apple products that can modulate the effect of HPP favoring or not the extraction of bioactive compounds depending on the intensity and duration of the treatment. Therefore, taking into account numerous published results, the effect of HPP on bioactive compounds must be separately studied in each food matrix (Barba et al., 2012 Vázquez-Gutiérrez et al., 2013; Rodríguez-Roque et al., 2015).

Considering the effects of *in vitro* gastrointestinal digestion (GID), two-way ANOVAs showed that during the GID of onion and apple products, either the phase of the *in vitro* GID or HPP, as well as the interaction between them exerted a significant influence ($P<0.05$) on TPC-FC, TFC-HPLC (Fig. 1) and on TFC-S (data not shown). Therefore, in both onion and apple products, the effect of HPP on TPC-FC and TFC-HPLC was dependent on the individual *in vitro* GID phase analyzed. Thus, TFC-HPLC in onion and apple products (untreated and HPP) (Fig. 1A and 1B) and TFC-S in the quercetin supplement (Table 1) showed a continuous decrease from ND sample to ID phase. On the contrary, the highest total phenolic compounds (TPC-FC) value in onion and apple products (untreated and HPP) was obtained in the intestinal phase (ID) (Fig. 1G and 1H). A detailed explanation is given below.

3.1.1. Total flavonol content (TFC). Total flavonol content analyzed by HPLC (TFC-HPLC) progressively decreased ($P<0.05$) throughout the different GID phases in both untreated and HPP onion and apple products. Thus, OP and GI phases retained by 89-87% and 79-81%, respectively, the total flavonol content (TFC-HPLC) of native onion in untreated and HPP products (Table 1). These results previously published (Fernández-Jalao et al. (2017) were compared with those obtained with apple product.

Thus, in untreated apple, a recovery of 85% of native TFC-HPLC after OP and GD phases has been achieved as in onion (Table 1). Similar results were found in the literature for apples (cv. Mutzu and Golden) with a recovery of 80-85% of the initial total flavonols after GD phase (Bouayed et al., 2012). However, HPP-apple showed lower recovery of the native TFC-HPLC (47%) than HPP-onion (79%) after gastric digestion (GD) (Table 1). In general, flavonol compounds showed relatively good stability under gastric conditions depending on the food matrix and their physicochemical characteristics. For example, around 75-80% of initial amount of quercetin derivatives in apples were released in the GD phase (Bouayed et al., 2012) and by 75% in a blended fruit juices (Rodriguez-Roque, Rojas-Graü, Elez-Martínez & Martín-Belloso, 2013).

The transition from acid gastric to mild basic intestinal environment produced a significant loss of total flavonols (26%) in all the products studied in the present study except for HPP-apple where a significant increase of 25% was achieved. This increase of quercetin derivatives concentration in the ID was previously detected in blended fruit juices, where rutin increased 25% its initial concentration (Rodriguez Roque et al., 2013), and in apples (~10%) (Bouayed et al., 2011) suggesting an efficient extraction of these compounds under intestinal conditions.

Approximately by 60% of the native TFC-HPLC was bioaccessible in the ID phase of onion and apple products (untreated and HPP) (Table 1). However, significant differences in the bioaccessibility of total flavonols were found depending on HPP and food matrix. Although TFC-HPLC in the ID of HPP-onion (1.70 mg/g dw) was a 10% higher than in the untreated onion (1.54 mg/g dw), no significant differences ($P<0.05$) were found between their bioaccessibilities (17.47 - 17.80%), so in this case HPP did not improve the bioaccessibility of total flavonols in onion (Fernández-Jalao et al.,

2017). Also, HPP did not improve the TFC-HPLC bioaccessibility in apple product moreover a significant decrease by 23% was observed (Table 1). Higher bioaccessibilities to those found for onion and apple products in the present study were reported for the flavonols rutin (22.2%) and quercetin (28.9%) in a blended fruit juice (Rodriguez-Roque et al., 2013) and in total flavonols in apples (~50%) (Bouayed et al., 2012). The differences between the flavonol bioaccessibilities calculated in the present study and those found in the literature may be due to the different reasons such as we used a dynamic GID equipment and centrifugation to obtain the soluble fraction (SF).

In the present study, the bioaccessibility of TFC-HPLC in apple (11.57%) was significant lower than in onion (17.80%). These results agree with those obtained by Hollman et al., (1997) that suggested that the bioaccessibility of the flavonol quercetin in onion was higher than in apple as a consequence of the chemical structure of the main flavonols present in their matrices. Thus, the main flavonols in onion are glucoside derivatives of quercetin and they seemed to be more bioaccessible than quercetin-3-galactoside mainly present in apples. The results obtained in this research agree with those obtained in other studies that showed how the bioaccessibility of phenolic compounds depend on the combined effect of food matrix and the type of processing applied (Rodriguez-Roque et al., 2015).

The spectrophotometric assay (TFC-S) detected approximately 1.77 - 2.26 times lower total flavonol content than HPLC-DAD in the different GID phases of onion and was not able to show the decrease of total flavonols after oral and gastric phase (OP and GD). However, the bioaccessibilities calculated by TFC-S (16.55-17.99%) in untreated and HPP-onion was similar to those observed by TFC-HPLC (17.80-17.47%) (Table 1). Contrary behaviour was found with apple product. The spectrometric assay detected between 1.2 and 5 times higher total flavonol content in the different apple GID phases

than HPLC-DAD. In fact, the total flavonol bioaccessibility in apple determined by TFC-S (~ 60 %) was significant higher than by HPLC-DAD (8.9-11.57%). Higher total flavonol bioaccessibility in apple (~ 60 %) than in onion (16.55-17.99 %) determined by spectrophotometric assay was not in accordance with the published data that indicated that quercetin glucosides in onion are more bioaccessibles than the quercetin-3-galactoside present in apple (Hollman et al., 1997). The different pH and the enzymes used to simulate the conditions of the different GID phases in combination with the different composition of apple and onion (Table S1) may be interfering with the reagent of the spectrophotometric assay and these results could be not comparable with those obtained by HPLC-DAD. This is the first time that the effect of GID on total flavonols was carried out at the same time with two different food matrices and two different assays (HPLC-DAD and spectrophotometry). The results obtained by spectrophotometric assays were more dependent on the composition of food matrix (apple or onion) and on the complexity of the reaction medium (GID phases) than those obtained by HPLC.

In conclusion, total flavonol bioaccessibility of processed foods must be separately studied in each food matrix and for specific processing parameters applied such as intensity and duration of HPP, taking into account also the type of analytical assay employed.

In addition, a quercetin supplement was submitted to the same GID process than onion and apple products. A progressively decreased of TFC-HPLC ($P<0.05$) throughout the different GID phases was observed as happened with onion and apple products. In fact, approximately by 3% of the initial TFC-HPLC in the supplement was observed in the ID (Table 1). It is evident that although the TFC-HPLC content in the quercetin supplement (736.10 mg/g dw) was significantly higher than in onion and

apple samples (8.65 and 0.26 mg/g dw, respectively), the concentration of TFC-HPLC was nearly 8-fold higher in the soluble fraction (SF) of onion product than in the SF of quercetin supplement being the bioaccessibility of TFC-HPLC in the supplement (0.027 %) much lower than in onion and apple products (~17.5% and 10%, respectively). Similar results were previously reported by Hollman et al. (1995). These results showed an important effect of the food matrix in the TFC-HPLC bioaccessibility being more bioaccessible when they are embedded in the plant tissue than in form of a supplement without food matrix.

3.1.2. Total phenolic compounds (TPC-FC). During GID of both untreated and HPP-onion, the TPC-FC value, or the antioxidant capacity value measure by the reducing capacity of the samples, significant increased from the non-digested (ND) samples up to the ID phase (Table 1). The OP and GD phases maintained unchanged TPC-FC of the native onion product. However, the transit from GD to ID produces a significant increase of TPC-FC in the untreated and HPP onion by 78% and 95%, respectively. The TPC-FC concentration in the ID of HPP-onion was 17% higher than in untreated-onion. In consequence, a significant increase ($P<0.05$) by 6.5% of the bioaccessibility of TPC-FC was observed in HPP-onion (95.33%) in comparison with untreated-onion (89.45%) (Table 1).

However, TPC-FC of apple (untreated and HPP) followed different behavior as that observed for onion samples in the first phases of GID. Thus, TPC-FC significant ($P<0.05$) decreased from ND to OP (19-26%) and in the transit from OP to GD (19%). Similar results were found after gastric digestion of different apples where total phenolic in GD was 35% lower than in non-digested product (Bouayed et al., 2011). As in onion, the transit of apple digest from acid gastric to mild basic intestinal environment produced a significant increase ($P<0.05$) of TPC-FC of 66% and 126% for

untreated and HPP-apple, respectively (Table 1). These behavior was also observed with total flavonol content (TFC-HPLC) in both onion and apple (untreated and HPP). Intestinal digestion environment could facilitate the release of phenolic compounds bounded to the vegetable matrix, transforming part of them into other structural forms or release other compounds that could be more sensitive to Folin-Ciocalteu reagent (Bouayed et al., 2011; Bohn, 2014).

3.2. Microstructure of onion and apple powder samples and quercetin supplement.

Fluorescence microscopy allows observing the presence of phenolic compounds, due to their autofluorescence (Pawlikowska–Pawlega et al., 2007). Fig. 2 shows the changes occurred in untreated and HPP onion and apple products, both in non-digested and digested samples. Non important structural differences are observed between untreated and HPP samples, when comparing the ND onion and apple samples.

Regarding the influence of *in vitro* GID in onion, the digestive phases that most seem to influence the autofluorescence of the phenolic compounds are the gastric and intestinal phases. In both untreated and HPP-onion, the increase in fluorescence intensity together with the high extent of the product structural disintegration observed in these GID phases are related to a significant release of phenolic compounds from the food matrix. These effects are largely appreciated in the ID and in the HPP-onion product if compared to ND product. These results are in agreement with the significantly higher values of TPC-FC observed in GD and ID for both untreated and HPP-onion (Table 1).

Regarding the apple tissue, there were no substantial changes in the structure and fluorescence of the phenolic compounds in the ND apple product during *in vitro* GID. However, there is a remarkable fluorescence increase during intestinal phase (ID)

in the apple subjected to HPP. This increase can be related with a higher extractability of TPC-FC and TFC-HPLC in this phase if compared to untreated sample as observed in Table 1. On the other hand, disintegration during *in vitro* GID is smaller in apple than in onion tissue, which could explain the higher extractability values of TPC-FC and TFC-HPLC found in onion. The different food matrices seem to disintegrate differently during *in vitro* GID, and thus, food matrix has a decisive influence on the extractability of the phenolic compounds.

The images corresponding to quercetin supplement show that the intense fluorescence signal observed in non-digested is lost during *in vitro* digestion.

All the observations are consistent with the TPC-FC and TFC-HPLC values found in onion, apple and quercetin supplement, as it was discussed in the previous section.

3.3. Viscosity measurements

Changes in the apparent viscosity versus shear rate of untreated onion powder and quercetin supplement at the different *in vitro* GID phases are shown in Fig. 3. Similar curves to untreated onion ones (Fig. 3A) were also obtained for HPP-onion and both untreated and HPP-apple powders (data not shown). As the apparent viscosity decreased with an increase in shear rate, all the samples showed non-Newtonian shear-thinning behavior due to rearrangement in the conformation of the molecules in the dispersion as a result of shearing. As can be seen in Fig. 3B, in quercetin supplement the different GID phases showed very close flow curves. However, in untreated onion (Fig. 3A) there were differences among the GID phases, showing a stepwise decrease in viscosity throughout *in vitro* GID.

Plots of interactions from the different two-way ANOVAs carried out are shown in Fig. 1S. For onion powder, two-way ANOVA showed significant ($P<0.05$) *in vitro* GID phase and HPP main effects for both the consistency coefficient (K) and the apparent viscosity at 10 s^{-1} ($\eta_{a,10}$), as well as for the flow behavior index (n) (data not shown). Binary GID phase $\times$ HPP interaction was also significant for both K and $\eta_{a,10}$ (Figs. S1A, S1B) and therefore, the effect of pressurization on these properties was dependent on the *in vitro* GID phase considered. In turn, for apple powder, the GID phase also had a significant effect ($P<0.05$) on both K and $\eta_{a,10}$ values, but it was not observed neither HPP nor interaction significant effects (Figs. S1C, S1D). For *in vitro* GID phase and food matrix (onion and apple powders) main effects (Figs. S1E-S1H), in untreated powders the binary GID phase $\times$ food matrix interaction had no significant effect on both K and $\eta_{a,10}$ values (Figs. S1E, S1F) evidencing that both main effects were no dependent. In addition, food matrix had no significant effect on the K values of HPP powders (Fig. S1G). Finally, regarding *in vitro* GID phase and food matrix (onion and quercetin supplement) main effects (Figs. S1I, S1J), either main effects or interaction had a significant effect ($P<0.05$) on the values of both K and $\eta_{a,10}$, clearly reflecting that in this case the effect of GID phase was very different at each matrix.

3.3.1. *In vitro* GID phase effect

Table 2 shows the mean values of both rheological properties (K and $\eta_{a,10}$) in untreated and HPP-treated onion and apple products, as well as in commercial quercetin supplement, before digestion (ND) and after the different *in vitro* GID phases (OP, GD, and ID). The K and $\eta_{a,10}$ values of onion and apple products were much higher than those of commercial quercetin throughout *in vitro* GID. In addition, for the four untreated and HPP-treated onion and apple samples, both K and $\eta_{a,10}$ values decreased

significantly ($P<0.05$) during the simulated *in vitro* GID. Therefore, the different GID phases would appear to exert a diluting effect on the digests. In general, there were a significant decrease in the values of K and $\eta_{a,10}$ after the OP in comparison to the ND samples. This diluting effect has been previously reported by other authors (Espinal-Ruiz et al., 2016; Morell et al., 2015) as a consequence of the incorporation of enzymes and liquids during *in vitro* GID process.

On the other hand, the *in vitro* GID phase main effect also exerted a significant influence ($P<0.05$) on the K and the $\eta_{a,10}$ values of commercial quercetin supplement. Nevertheless, either K or $\eta_{a,10}$ values were quite similar throughout *in vitro* GID phases. This could be due to lack of a “tissue (solid) matrix” in commercial quercetin supplement unlike onion and apple samples. Unexpectedly, the gastric digest (GD) had the highest consistency and viscosities values.

3.3.2. HPP effect

The ND, OP and GD fractions in HPP-treated onion powders presented significantly ($P<0.05$) higher K and $\eta_{a,10}$ values than their untreated counterparts (Table 2). Only, HPP had no significant effect on the rheological properties of the ID fraction in both digested onion products. Therefore, the HPP in onion powder exerted an important effect on the flow behavior of the digested samples throughout *in vitro* GID. Likely, the HPP affected the cell wall and membrane permeability of HPP-onion, favoring the diffusion of soluble material to the apoplast (Vázquez-Gutiérrez et al., 2014). The authors just cited reported that when 400 MPa at 25°C during 5 min were applied to onion, solubilization of the cell wall material was observed and cells were distorted. This would explain the loss of turgor in these samples, and therefore the increase of the K and $\eta_{a,10}$ values observed in the HPP-onion powders as compared with

their untreated counterparts (Table 2). Hence, this phenomenon could explain the higher values of TPC-FC observed in all the GID phases of HPP-onion in comparison with those of untreated ones (Table 1). It seems that 400 MPa disrupted the cell wall of the onions releasing the phenolic compounds bound to carbohydrates of the cell wall (Bohn, 2014) to be available to the organism. Also Gonzalez et al. (2010) and Vázquez-Gutiérrez et al. (2013) found loss of cell integrity and damage on the cell membranes in HPP-onions. On the other hand, it has been reported by Vázquez-Gutiérrez et al. (2014) that HPP can cause deprotonation of charged groups and disruption of salt bridges and hydrophobic bonds in onion, resulting in conformational changes and protein denaturation (US FDA 2000), which could affect their solubility. In addition, these authors also shown that onions treated with 400 MPa at 25 °C had significantly higher ($P<0.05$) shear force values than untreated ones due to cell wall degradation favoring a better contact between the pectic compounds and the enzyme pectin methyl esterase.

Conversely, in apple powders, only significant differences as a consequence of the HPP effect were observed between ID fractions, which were significantly higher in the untreated cases (Table 2). Hernández-Carrión et al. (2014) found that the damage caused to the texture in sweet pepper cell tissue was less noticeable with 500 MPa for 15 min at 25 °C, probably because this treatment provided suitable conditions for inactivating enzymes such as polygalacturonase. Hence, it seems that at 400 MPa for 5 min at 25 °C the composition or structure of the cell wall of the apple is more resistant to the HPP than those of the onion as discussed below.

3.3.3. Food matrix effect

Regarding food matrix (onion vs. apple) main effect, in all the GID phases, both K and $\eta_{a,10}$ were significantly higher in the untreated apple powder than in the onion one

(Table 2). These differences may be only associated with the different chemical composition and structure of both raw tissues included their cell wall. For example, raw apple sample contains pectin, more total fiber and less moisture than raw onion one, and moreover, it has a more acid pH than raw onion (Table S1). The presence of dietary fibers in the emulsions is likely to alter the rheological properties of the gastrointestinal fluids, which may impact the rate and extent of digestion by altering mixing and mass transport processes (Espinal-Ruiz et al., 2016). These authors observed that emulsions containing pectin had a higher viscosity due to the ability of pectin molecules to increase the effective volume fraction of the dispersed phase.

However, in HPP products, ND and ID onion fractions had significantly ($P<0.05$) higher K values than their HPP-apple counterparts, whereas the contrary occurred when comparing the GD fraction of both pressurized food matrices, reflecting different responses of both pressurized matrices to the digestion conditions. It is worth mentioning that apple matrix contains starch, and it is well known that if sufficiently high content is present, HPP induces either gelatinization of starch in excess water or “rapid retrogradation” occurring inside intact granules (Vallons et al., 2014). Therefore, HPP-induced starch gelatinization and retrogradation might be partially responsible for the higher rheological properties of HPP-apple products in GD as compared to HPP-onion counterpart. Briones-Labarca et al. (2011) reported that the bioaccessibility of the antioxidant activity, mineral and starch content were significantly affected by HPP and digestion conditions in apple.

In turn, when comparing the quercetin supplement with the untreated onion powder (Table 2), in all the GID phases the K and $\eta_{a,10}$ values of the onion were significantly higher than those of the quercetin powder.

It is interesting to note that onion powder showed a progressive decrease of K and $\eta_{a,10}$ values and TFC-HPLC from ND to ID. However, in quercetin supplement, this behavior was not observed for K and $\eta_{a,10}$ values that remaining almost constant and significantly lower than in onion but also TFC-HPLC significant decreased approximately 87% from ND to ID. This decrease of TFC-HPLC in quercetin supplement (97%) was higher than in onion (60%) and led to a lower TFC-HPLC bioaccessibility (0.027%) in the supplement than in onion (17-18%). That means that different matrices seem to affect the digest conditions and the rheological behavior, at the same time, and, therefore, the bioaccessibility final of the TFC-HPLC. Hence, TFC-HPLC in quercetin supplement resulted to be less bioaccessible than in tissue matrices. These results may be due to different factors such as the bioaccessibility of flavonols depends on their chemical structure. In onion and apple, the main flavonols are β -glycosides of quercetin which are more bioaccessible than quercetin aglycone in the commercial supplement (Hollman et al. 1995). Other reason could be the poor solubility of quercetin aglycone in the digestive tract meanwhile TFC-HPLC are disperse in the onion and apple tissues making it more bioaccessible (Wiczowski et al. 2008). Hence, it seems that the different structures of flavonols could influence the rheological steady properties (K and $\eta_{a,10}$) of these matrices and could be used to predict the phenolic compounds bioaccessibility. Viscosity through the GID could be related to bioaccessibility of bioactive compounds such as phenolic compounds present in the different matrices studied (onion, apple and quercetin supplement), being the chemical structure of these bioactive compounds an important factor.

3.3.4. Predictive models for the apparent viscosity at each GID phase

According to Hardacre et al. (2016), physiological shear rate levels during *in vitro* intestinal digestion range from 0.1 to 10 s⁻¹. For all products tested, a prediction of the change of the viscosity throughout *in vitro* GID tract can be done based on power law models describing the evolution of viscosity as a function of shear rate. In this way, viscosity power law model was applied to the average experimental data of the apparent viscosity vs. shear rate (from 0.1 to 100 s⁻¹) before (ND) and throughout *in vitro* GID of each product (Table S2). The lower the value of the power law index (n), the greater the viscosity decreases with shear rate. The values of n ranged between 0.2 and 0.5, corroborating previous findings in small ID (Shelat et al., 2015). For untreated samples, the better fits after each GID phase corresponded to reconstituted apple powder, likely due to its higher initial viscosity, whereas the worse fits corresponded to the more fluid extracts of commercial onion quercetin powder. In addition, in both untreated and HPP onion and apple products worse fits corresponded to GD and ID fractions, probably due to that lower shear rates are needed for imitating peristalsis speed in these digestion stages. Kozu et al. (2014) reported that the maximum shear rate in the liquid gastric contents was below 20 s⁻¹ at standard value of peristalsis speed in healthy adults (2.5 mm s⁻¹). Nevertheless, a lower R^2 was also obtained for fitting the viscosity values of HPP apple sample after simulated OP (Table S2).

Models fitted for the apparent viscosity at each phase were used for doing predictions at 0.1, 7.5 and 100 s⁻¹ shear rates. Very good linear correlations were found between experimental values of untreated and HPP onion and apple samples at highest shear rate (100 s⁻¹) and the values predicted by the power law models at each GID phase (Fig. 4A). At 7.5 s⁻¹, the determination coefficients of the linear fits were higher for both untreated and HPP-onion powders ($R^2 > 0.99$) than for apple ones (Fig. 4B). In turn, at the lowest shear rate (0.1 s⁻¹), only high R^2 were established between

experimental and predicted values for both untreated onion and apple powders (Fig. 4C), especially for the former. With regard to quercetin supplement, the Fig. 4D shows at the same time the linear correlations found between experimental and predicted viscosity values at 7.5 and 100 s⁻¹ rates. No significant linear correlation was observed at the lowest rate (0.1 s⁻¹), and again, the R^2 decreased with decreasing the shear rate tested. Models fitted proved to be adequate for making predictions in the above mentioned shear rate range, although predictions should be considered with caution in apple for lower shear rates (both 0.1 and 7.5 s⁻¹).

4. Conclusions

HPP seemed to increase the extraction of total flavonol content (TFC-HPLC) and total phenolic content (TPC-FC) in the non-digested apple and onion powder and also during the *in vitro* GID of these products, but this effect did not result in a significant increase of their bioaccessibilities. The bioaccessibility of TPC-FC in onions (89-95%) was higher than in apples (81-85%). Also, TFC-HPLC bioaccessibility was higher in onions (17-18%) than in the apples (9-12%) or in the quercetin supplement (0.028%). These results evidence the importance of food matrix and the processing parameters applied on total phenolic compounds and total flavonols bioaccessibility.

Fluorescence microscopy confirms that food matrices studied disintegrate differently during *in vitro* GID and this parameter has a decisive influence on the extractability of the phenolic compounds.

Regarding rheology, onion and apple (untreated and HPP) and quercetin supplement showed non-Newtonian shear-thinning behavior with differences (in K and $\eta_{a,10}$ values) among the flow curves of the different *in vitro* GID phases except for quercetin supplement which were very close. The food matrix effect (onion vs. apple)

seems to be more relevant than HPP effect. In this way, apple showed the higher values of K and $\eta_{a,10}$ in matrices non-treated because of the different chemical structure of their flavonols and its content of pectin and starch. High correlations were found between apparent viscosity experimental values of untreated and HPP onion and apple matrices at the highest shear rate (100 s^{-1}) and the values predicted by the power law fits at each GID phase.

Therefore, the change of viscosity throughout GID could predict the bioaccessibility of TFC-HPLC in the different matrices studied (onion, apple and quercetin supplement), which depends on their different chemical structure.

Conflict of interest statement

Authors declare no conflict of interest.

Acknowledgements

This study has been funded by the Spanish projects AGL2013-46326-R and AGL2016-76817-R (Ministry of Economy, Industry and Competitiveness).

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compounds (TPC) in onion and apple powders (21). Main effects were in
815 *vitro* GID phases and food matrix performed on TFC-HPLC and TPC-FC in both
816 untreated and HPP powders.

817 **Fig. 2.** Light microscopy micrographs of untreated and high-pressure processing (HPP)
818 onion, apple and quercetin supplement products corresponding to the different *in vitro*
819 gastrointestinal digestion (GID) phases. Magnification: 4x.

820 **Fig. 3.** Apparent viscosity changes versus shear rate at the different *in vitro*
821 gastrointestinal digestion (GID) phases for (A) Untreated onion powder, (B)
822 Commercial quercetin supplement.

823 **Fig. 4.** Experimental viscosity at different shear rates vs. predicted values by power law
824 models at the different *in vitro* gastrointestinal digestion (GID) phases for (A) Untreated
825 and HPP onion and apple powders at 100 s⁻¹, (B) Untreated and HPP onion and apple
826 powders at 7.5 s⁻¹, (C) Untreated onion and apple powders at 0.1 s⁻¹, (D) Commercial
827 quercetin supplement at 7.5 and 100 s⁻¹.

Table 1. Effects of *in vitro* dynamic gastrointestinal digestion (GID), high-pressure processing (HPP) and food matrix on total flavonol and total phenolic content of onion and apple products and commercial quercetin supplement

Digestion Phase	Treatment	Onion powder			Apple powder			Quercetin Supplement
		Total Flavonol Content (TFC-S) (mg QE/g dw)	Total Flavonol Content (TFC-HPLC) ¹ (mg/g dw)	Total Phenolic Content (TPC-FC) (mg GAE/g dw)	Total Flavonol Content (TFC-S) (mg QE/g dw)	Total Flavonol Content (TFC-HPLC) (mg/g dw)	Total Phenolic Content (TPC-FC) (mg GAE/g dw)	Total Flavonol Content (TFC-HPLC) (mg/g dw)
Non-digested (ND)	Untreated	3.82±0.19 ^A _b *	8.65±0.02 ^A _b *	4.17±0.05 ^D _b *	0.27±0.007 ^B _a	0.26±0.01 ^A _b	3.42±0.23 ^A _a	736.10±36.81 ^A
Oral-phase (OP)	Untreated	3.35±0.06 ^B _b *	7.50±0.08 ^B _b *	4.34±0.13 ^C _a *	0.26±0.03 ^B _a	0.22±0.01 ^B _a	2.54±0.16 ^B _a	492.05±7.08 ^B
Gastric digest (GD)	Untreated	3.38±0.12 ^B _b *	7.03±0.15 ^C _b *	4.91±0.14 ^B _b *	0.32±0.02 ^A _a	0.22±0.02 ^B _a	2.06±0.17 ^C _a	71.19±0.61 ^C
Intestinal digest (ID)	Untreated	2.92±0.03 ^C _b *	5.17±0.25 ^D _b *	7.41±0.11 ^A _b *	0.37±0.04 ^A _b	0.16±0.004 ^C _b	3.41±0.23 ^A _b	20.54±0.39 ^D
Soluble fraction (SF)	Untreated	0.64±0.06 ^D _b *	1.54±0.17 ^E _a *	3.73±0.10 ^E _b *	0.16±0.03 ^C _a	0.03±0.003 ^D _a	2.78±0.23 ^B _a	0.20±0.01 ^E
Bioaccessibility (%)	Untreated	16.55±2.26 ^a *	17.80±1.93 _a	89.45±2.57 _a	59.72±7.38 _a	11.57±1,11 _a	81.47±5.39 _a	0.027±0.001
Non-digested (ND)	HPP	4.17±0.16 ^A _a *	9.75±0.02 ^A _a *	4.43±0.06 ^C _a *	0.28±0.01 ^{B,C} _a	0.34±0.003 ^A _a	2.93±0.14 ^B _b	
Oral-phase (OP)	HPP	4.02±0.15 ^A _a *	8.68±0.08 ^B _a *	4.44±0.22 ^C _a *	0.23±0.03 ^C _a	0.19±0.02 ^B _a	2.37±0.18 ^{C,D} _a	
Gastric digest (GD)	HPP	4.37±0.15 ^A _a *	7.69±0.75 ^C _a *	5.06±0.11 ^B _a *	0.31±0.04 ^B _a	0.16±0.03 ^C _b	2.20±0.22 ^D _a	
Intestinal digest (ID)	HPP	3.28±0.20 ^B _a *	5.81±0.25 ^D _a *	8.66±0.32 ^A _a *	0.53±0.06 ^A _a	0.20±0.01 ^B _a	4.99±0.13 ^A _a	
Soluble fraction (SF)	HPP	0.75±0.05 ^C _a *	1.70±0.06 ^E _a *	4.22±0.08 ^D _a *	0.17±0.008 ^D _a	0.03±0.002 ^D _a	2.51±0.20 ^C _b	
Bioaccessibility (%)	HPP	17.99±0.62 ^a *	17.47±0.64 ^a *	95.33±2.16 ^a *	59.93±3.73 _a	8,91±0,67 _b	85.53±3.99 _a	

Values are given as mean ($n = 4$) ± standard deviation. ¹ Data published in Fernandez-Jalao et al., (2017). HPP = High-pressure processing; TFC-S = Total flavonol content by spectrophotometric assay; TFC-HPLC = Total flavonol content by HPLC; TPC-FC = Total phenolic content by Folin-Ciocalteu; ^{A-D} Different uppercase letters for the same determination and treatment level (untreated or HPP samples) means significant differences ($P < 0.05$) between GID phases; ^{a,b} Different lowercase letters for the same determination and *in vitro* digestion phase means significant differences ($P < 0.05$) between untreated and HPP samples; * Asterisk means significant differences ($P < 0.05$) between products (onion and apple) for the same determination and GID phase. GAE = Gallic acid equivalents; QE = Quercetin equivalents.

Table 2. Effects of *in vitro* gastrointestinal digestion (GID), high-pressure processing (HPP) and food matrix on the steady shear rheological properties of onion and apple powder products and commercial quercetin supplement.

GID phase	Treatment	Onion powder		Apple powder		Quercetin supplement	
		K (mPa s ⁿ)	$\eta_{a,10}$ (mPa s)	K (mPa s ⁿ)	$\eta_{a,10}$ (mPa s)	K (mPa s ⁿ)	$\eta_{a,10}$ (mPa s)
Non-digested (ND)	Untreated	237±18.3 ^{A_b*}	48.0±10.5 ^{A_b*}	376±13.4 ^{A_a†}	72.2±17.6 ^{A_a†}	12.2±0.178 ^{A,B}	1.60±0.038 ^B
Oral-phase (OP)	Untreated	111±18.1 ^{B_b*}	32.8±7.34 ^{B_b*}	207±15.3 ^{B_a†}	45.6±14.5 ^{B_a†}	10.5±0.472 ^C	1.27±0.087 ^C
Gastric digest (GD)	Untreated	22.3±2.47 ^{C_b*}	4.77±1.11 ^{C_b*}	178±20.7 ^{B_a†}	23.7±8.60 ^{B,C_a†}	12.8±0.373 ^A	1.78±0.076 ^A
Intestinal digest (ID)	Untreated	14.2±0.501 ^{C_a*}	2.00±0.047 ^{C_a*}	23.8±1.82 ^{C_a†}	3.41±0.222 ^{C_a†}	11.7±0.304 ^B	1.52±0.065 ^B
Non-digested (ND)	HPP	546±10.6 ^{A_a†}	88.1±11.8 ^{A_a}	341±65.1 ^{A_a}	74.2±5.98 ^{A_a}	-	-
Oral-phase (OP)	HPP	313±7.13 ^{B_a}	68.6±12.6 ^{B_a†}	340±51.9 ^{A_a}	42.6±6.11 ^{B_a}	-	-
Gastric digest (GD)	HPP	76.4±1.95 ^{C_a}	16.9±1.05 ^{C_a}	189±45.9 ^{B_a†}	18.9±5.10 ^{C_a}	-	-
Intestinal digest (ID)	HPP	15.1±0.325 ^{C_a†}	1.97±0.091 ^{D_a}	13.5±0.874 ^{C_b}	1.98±0.121 ^{D_b}	-	-

Values are given as mean ($n = 6$) ± standard deviation.

^{A–D} Different uppercase letters for each steady rheological property and treatment level (untreated or HPP-treated) means significant differences ($P < 0.05$) between GID phases.

^{a,b} Different lowercase letters for each steady rheological property and for the same GID phase means significant differences ($P < 0.05$) between untreated and HPP-treated samples.

[†] Latin letter alveolar click means significant differences ($P < 0.05$) between products (onion and apple) for the same steady rheological property and GID phase.

*Asterisk means significant differences ($P < 0.05$) between products (onion and quercetin supplement) for the same steady rheological property and GID phase.

K : consistency coefficient from power law model; $\eta_{a,10}$: apparent viscosity at 10 s⁻¹.

Table S1. Physicochemical and chemical characteristics of raw plant material.

Parameters	Onion	Apple
Weight (g)	210.8 ± 16.7	195.5 ± 9.3
Water content (%)	89.0 ± 0.4	81.9 ± 0.3
Soluble solid content (°Brix)	7.4 ± 0.3	13.3 ± 1.1
pH	5.3 ± 0.04	3.60 ± 0.04
Acidity (g citric acid/ 100 g fw)	0.07 ± 0.004	0.13 ± 0.01
Pectin (g/100 g fw)	-	0.40 ± 0.1
Soluble Fiber (g/100 g fw)	0.41± 0.02	0.57± 0.01
Insoluble Fiber (g/100 g fw)	1.45± 0.15	2.03± 0.23
Total Fiber (g/100 g fw)	1.86± 0.11	2.60± 0.07
Vitamin C (mg/100 g fw)	5.70 ± 0.2	12.4 ± 0.9
Ascorbic acid (mg/100 g fw)	4.70 ± 0.3	7.70 ± 0.8

Data are the mean ($n = 3$) ± standard deviation

Table S2

Effects of *in vitro* gastrointestinal digestion (GID) and high-pressure processing (HPP) on power law model ($\eta_a = K \dot{\gamma}^{n-1}$) parameters for the different food matrices tested.

GID phase	Treatment	Onion powder			Apple powder			Quercetin supplement		
		<i>K</i>	<i>n</i> -1	<i>R</i> ²	<i>K</i>	<i>n</i> -1	<i>R</i> ²	<i>K</i>	<i>n</i> -1	<i>R</i> ²
Non-digested (ND)	Untreated	246	-0.601	0.9812	376	-0.641	0.9923	12.2	-0.658	0.8732
Oral-phase (OP)	Untreated	112	-0.479	0.9951	207	-0.587	0.9905	10.5	-0.746	0.9315
Gastric digest (GD)	Untreated	22.5	-0.522	0.9331	181	-0.728	0.9603	12.8	-0.650	0.8745
Intestinal digest (ID)	Untreated	14.2	-0.703	0.9496	23.9	-0.738	0.9786	11.7	-0.688	0.8973
Non-digested (ND)	HPP	547	-0.697	0.9903	342	-0.638	0.9861	-	-	-
Oral-phase (OP)	HPP	313	-0.580	0.9906	340	-0.748	0.9688	-	-	-
Gastric digest (GD)	HPP	76.6	-0.547	0.9753	189	-0.858	0.9797	-	-	-
Intestinal digest (ID)	HPP	15.1	-0.717	0.9479	13.6	-0.629	0.8963	-	-	-

Power law model parameters are given from fits for average curves (*n* = 6).

η_a : apparent viscosity (mPa s); *K*: consistency coefficient (mPa s^{*n*}); $\dot{\gamma}$: shear rate (s⁻¹); *n*: flow behavior index; *R*² = determination coefficient.