Effects of high hydrostatic pressure (HHP) on bioaccessibility, as well as antioxidant activity, mineral and starch contents in Granny Smith apple

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Abstract

The aim of this work was to study the effect of high hydrostatic pressure on the bioaccessibility of specific nutrients (antioxidant, minerals and starch) in apple and to establish processing conditions that maximise the health benefits. The apple was pressurised at 500 MPa during 2, 4, 8 and 10 min. The antioxidant activity, mineral and starch content and bioaccessibility of apple samples were significantly affected by the processing and digestion conditions. Therefore, these results indicated that in vitro digestion has a noticeable effect on the antioxidant concentration, IC50, with much lower values (a smaller IC50 value corresponds to a higher antioxidant activity) of apple samples compared with those untreated and non-digestion. Apple has the highest calcium content (30.33 ± 1.94 mg/100 g), iron (14.46 ± 3.49 mg/100 g) and zinc (6.22 ± 0.91 mg/100 g). High hydrostatic pressure increased the mineral contents availability by 2.11–303.00% for calcium, 4.63–10.93% for iron and 8.68–28.93% for zinc. The dialysability and solubility of calcium, iron and zinc with respect to the values for the untreated sample were reduced by this high pressure technique. Consumption of apple under high hydrostatic pressure may supply substantial antioxidants, mineral and starch, which may provide health promoting and disease preventing effects.

1. Introduction

The diet is important for human health because it is associated with the morbidity and mortality in the chronic diseases, such as cardiovascular disease, cancer, hypertension and obesity. Several investigations have estimated that one-third of all cancer cases and one-half of cardiovascular diseases and hypertension can be attributed to diet (Reddy & Martijn, 2004; Wolfe, Wu, & Liu, 2003).

Fruits contain many different dietary phytonutrients which contribute to the prevention of degenerative diseases caused by oxidative stress (Kaur & Kapoor, 2001). The intake of food rich in phenolic acids, polyphenols and flavonoids scavenges free radicals such as peroxyde, hydroperoxide or lipid peroxyl, thus inhibiting the oxidative mechanism that leads to degenerative diseases (Halvorsen et al., 2006; Lam, Woo, Leung, & Cheng, 2007; Pellegrini et al., 2003; Yi-Zhong, Luo, Mei, & Corke, 2004).

Apples are one of the most frequently consumed fruits in the world, preferably in Europe, and constitute a main source of phenolic compounds such as (Vinson, Su, Zubik, & Bose, 2001; Yang & Lui, 2009) and their consumption is associated to reduced risk of several diseases (Boyer & Liu, 2004). The polyphenol profiles of all varieties of apples are practically identical, but concentrations may range from 0.1 to 5 g total polyphenols/kg fresh wt and may be as high as 10 g/kg in certain varieties of cider apples (Guyot, Marnet, Laraba, Sanoner, & Drilleau, 1998; Sanoner, Guyot, Marnet, Molle, & Drilleau, 1999).

Minerals are needed by the body in different amounts, depending on the element, to maintain good health. The terms trace minerals or trace elements can refer to essential, non-essential, or toxic elements which are found in very small amounts in human body (Promchan & Showatana, 2005). Iron (Fe), zinc (Zn) and calcium (Ca) are essential nutrients that are often lacking in human diets, either due to insufficient intake or to poor absorption of food. In developing countries, deficiencies of Fe and Zn lead to much suffering and death. In industrialised countries, chronic Ca deficiency is one of the important causes of reduced bone mass and osteoporosis in the elderly (Frossard, Bucher, Mächler, Možafar, & Hurrell, 2000). Insufficient mineral intake for children in the first year of life, when growth is accelerated, especially a lack of iron, calcium, and zinc, is responsible for diseases such as iron deficiency anaemia, rickets, osteoporosis, and immune diseases. Early mineral deficiency also can lead to an increase in infectious diseases, which cannot only influence immediate health but also may have an
important impact on adult health (Cámara, Amaro, Barbera & Clemente, 2005; Febles, Arias, Hardisson, Rodriguez-Alvarez, & Sierra, 2001).

The methods of culinary preparation also have a marked effect on the polyphenol and antioxidant content of foods. For example, simple peeling of fruit and vegetables can eliminate a significant portion of polyphenols because these substances are often present in higher concentrations in the outer parts than in the inner parts. Cooking may also have a major effect. Onions and tomatoes lose between 75% and 80% of their initial quercetin content after boiling for 15 min, 65% after cooking in a microwave oven, and 30% after frying (Crozier, Lean, McDonald, & Black, 1997). It has to be taken into account the way in which apple is processed and consumed when considering its utility in preventing cardiovascular disease and obtaining the maximum health effects. The apple has to be eaten raw or moderately cooked for obtaining those beneficial effects.

Since the nineties, increase in the consumption of fruit and fruit products has been considered as a major issue by the European Union. In the same way consumer demand for more “fresh appearing”, more convenient and healthier fruit and fruit products has led to increase the research on minimal preservation techniques like high pressure processing (Bull et al., 2004; Houška et al., 2006), modified atmosphere packaging (Soliva-Fortuny, Elz-Martínez, & Martín-Beloso, 2004; Soliva-Fortuny & Martín-Belloso, 2003), or biopreservation (Janisiewicz, Conway, & Leverentz, 1999; Leverentz et al., 2006; Trias, Badosa, Montesinos, & Bañeras, 2008). In order to extend the shelf life of these products, they are usually processed thermally using methods such as hot water immersion; however, these treatments can cause a reduction in antioxidant capacity (Dewanto, Wu, Adom, & Liu, 2002). High hydrostatic pressure processing uses water as a medium to transmit pressures from 300 to 700 MPa to foods resulting in a reduction in microbial loads and thus extending shelf life. This can be achieved without heating and therefore the method could be useful for preserving the antioxidant capacity of the foods (Cheftel, 1992; Farr, 2003; Mertens & Knorr, 1992).

Bioavailability is a term used to describe the proportion of a nutrient in food that can be utilised for normal body functions. Many techniques have been proposed for quantification of bioavailability. The most reliable methods for bioavailability studies are in vivo measurement of absorption in humans with or without using a labelling technique (Promchan & Shiwatana, 2005). Human in vivo studies are, however, time-consuming, very expensive, and complicated, and produce variable results. In-vitro methods are being extensively used at present since they are rapid, safe, and do not have the ethical restrictions of in vivo methods. In-vitro methods either simulate the digestion and absorption processes (for bioavailability) or only the digestion process (for bioaccessibility) and the response measured is the concentration of a nutrient in some kind of final extract (Parada & Aguillera, 2007). The in vitro method developed in 1981 by Miller, Schricker, Rasmussen, and Van Campen (1981) in particular, has been found to provide availability measurements that correlate well with human in vivo studies.

The effects of the food matrix on the bioavailability or bioaccessibility of antioxidant minerals and starch have not been examined in much detail. Direct interactions between this component and some components of food, such as binding to proteins and polysaccharides, can occur, and these interactions may affect digestion and absorption.

The objective of this study was to study the effect of high pressure on the bioaccessibility of specific nutrients (antioxidant, minerals and starch) in apple and to establish processing conditions that maximise the health benefits.

2. Materials and methods

2.1. Materials

Apples (Granny Smith) were purchased from a local market (La Serena, Chile) and stored at 4 °C until the moment of the experiment. Before processing, the apples were peeled with a stainless steel knife and immediately cellular debris on the cut surfaces were removed by rinsing with distilled water.

2.2. High hydrostatic pressure treatments

The apple samples were packed individually and hermetically sealed in high density polyethylene bags. Packaged apple samples were loaded in a cylindrical loading container and high hydrostatic pressure treated at 500 MPa for 2, 4, 8 and 10 min at 20 °C in a processing unit (Avure Technologies Incorporated. Kent, WA, USA) using water as the pressure-transmitting medium. The time to reach the designated pressure was less than 10 s, and depressurisation was less than 5 s. Pressurisation was carried out at ambient temperature.

2.3. Physico-chemical analysis

The apples samples were peeled, cut, and the pulp portion was homogenised in a blender (Ultra-Turrax, T25 Basic, Ika Labortechnik, Staufen, Germany) and then used for chemical analysis. The crude protein content was determined using the Kjeldahl method with a conversion factor of 6.25 (AOAC No. 960.52). The lipid content was analysed gravimetrically following Soxhlet extraction (AOAC No. 960.39). The crude fibre was estimated by acid/alkaline hydrolysis of insoluble residues (AOAC No. 962.09). The crude ash content was estimated by incineration in a muffle furnace at 550 °C (AOAC No. 923.03). The available carbohydrate was estimated by difference. The moisture content of the samples was determined by means of the AOAC methodology No. 934.06 (AOAC, 1990). All methodologies followed the recommendations of the Association of Official Analytical Chemists (AOAC, 1990).

The pH was measured using a potentiometer (Extech Instruments, Microcomputer pH-Vision 246072, Waltham, Massachusetts, USA); the level of titratable acidity was expressed as malic acid and it was determined according to the AOAC methodology No.924.15. The water activity (aw) was measured at 25 °C by means of a water activity instrument (Novasina, model TH-500, Pfäffikon, Lachen, Switzerland). Soluble solids were measured using a refractometer (ABBE, 1T, Tokyo, Japan) which measures refractive indices both of solid and liquid samples in a fast and accurate way and its scale ranges from 0.0 to 95 °Brix. All determinations were done in triplicate. All solvents and reagents were purchased from Sigma–Aldrich Company Ltd. (St. Louis, MO, USA) with analytical grade.

2.4. Determination of DPPH radical scavenging activity

Free radical scavenging activity of the samples was measured according to the procedure described by Brand-Williams, Cuvelier, and Berset (1995). The total antioxidant activity (TAA) was expressed as the percentage inhibition of the DPPH radical and was determined by the following equation:

\[
\text{TAA} = \left[ 1 - \left( \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \right] \times 100
\]

where TAA is the total antioxidant activity and Abs is the absorbance. IC50, which is the concentration required to obtain a 50% antioxidant capacity, is typically employed to express the antioxidant activity and to compare the antioxidant capacity of various samples. IC50 was determined from a graph of antioxidant capacity.
2.6. In-vitro digestion

The mineral elements (Ca, Fe and Zn) were measured using an atomic absorption spectrophotometer (AAS; Shimadzu Instruments, Inc., SpectrAA-220, Kyoto, Japan) as described previously (Sebastiá, Barberá, Farré, & Lagarda, 2001). For the analysis of calcium and zinc, the apple sample (1 g) placed in platinum crucibles, were dry ashing in a furnace at 525 °C for 12 h. The ash was then weighed and put in solution in 5 ml HNO3/HCl (3/2). The solution was filtered to eliminate the silica, recovered in a 250 ml flask, added with pure HNO3, heated and diluted. In the case of calcium, lanthanum chloride was added to the mineral solution (final concentration 1%) to avoid interference from phosphate. For the analysis of iron, the sample (0.5 g) was placed in a 100 ml-flask with 5 ml HCl/HNO3 (3/1), boiled for 2 h with a flowing back system and the solution was then filtered and recovered in a 50 ml flask. All measurements were carried out using standard flame operating conditions, as recommended by the manufacturer. The calibration of measurements was performed using commercial standards. The reproducibility values were within 2.0% for all zinc, calcium and iron. All determinations were done in triplicate and solvents and reagents were purchased from Sigma–Aldrich Company Ltd. (St. Louis, MO, USA) with analytical grade.

2.5. Determination of the mineral content (zinc, iron and calcium)

The mineral elements (Ca, Fe and Zn) were measured using an atomic absorption spectrophotometer (AAS; Shimadzu Instruments, Inc., SpectrAA-220, Kyoto, Japan) as described previously (Sebastiá, Barberá, Farré, & Lagarda, 2001). For the analysis of calcium and zinc, the apple sample (1 g); placed in platinum crucibles, were dry ashing in a furnace at 525 °C for 12 h. The ash was then weighed and put in solution in 5 ml HNO3/HCl (3/2). The solution was filtered to eliminate the silica, recovered in a 250 ml flask, added with pure HNO3, heated and diluted. In the case of calcium, lanthanum chloride was added to the mineral solution (final concentration 1%) to avoid interference from phosphate. For the analysis of iron, the sample (0.5 g) was placed in a 100 ml-flask with 5 ml HCl/HNO3 (3/1), boiled for 2 h with a flowing back system and the solution was then filtered and recovered in a 50 ml flask. All measurements were carried out using standard flame operating conditions, as recommended by the manufacturer. The calibration of measurements was performed using commercial standards. The reproducibility values were within 2.0% for all zinc, calcium and iron. All determinations were done in triplicate and solvents and reagents were purchased from Sigma–Aldrich Company Ltd. (St. Louis, MO, USA) with analytical grade.

2.6. Bioaccessibility of the mineral soluble fraction

Aliquots of 20 ml of mixture were transferred to 50 ml centrifuge tubes and centrifuged at 5000 rpm for 1 h at 4 °C, and the supernatants were used to determine the mineral content (solubilised fraction). The minerals were then analysed by atomic absorption spectrophotometry (AAS; Shimadzu Instruments, Inc., SpectrAA-220, Kyoto, Japan). Calibration of measurements was performed using commercial standards. Solubility percentage was determined by the following equation:

\[
\text{Solubility (\%)} = \frac{A}{B} \\
\]

where A is the soluble mineral content (mg/100 g), and B is the total mineral content (calcium, zinc or iron) of the sample (mg/100 g). All determinations were done in triplicate.

2.6.1. Bioaccessibility of DPPH radical scavenging activity

Mixture at 4 °C were centrifuged (35 min, 5000 rpm) and supernatants were removed. The residues were washed twice with 5 ml of distilled water, and all supernatants were combined. The mixture was collected in a boiling flask, which had been weighed previously, and concentrated to dryness at 60 °C in a vacuum rotavapor (Buchi, model RE-121, Flawil, Switzerland). The dried extract sample was used as test sample to determine the antioxidant capacity content (free radical scavenging activity) using the 2,2-di-phenyl-1-picryl-hydrazyl (DPPH) method (Brand-Williams et al., 1995) with some modifications. All determinations were done six-fold and the solvents and reagents were purchased from Sigma–Aldrich Company Ltd. (St. Louis, MO, USA) and were of analytical grade.

2.6.2. Bioaccessibility of the mineral soluble fraction

2.6.3. Bioaccessibility of the mineral dialysed fraction

Dialysis comprises gastric steps, common to that of the solubility method, followed by intestinal step in which dialysis is included. The dialysis bag (molecular mass cut-off value 12000 Da) containing 12.5 ml of water and an amount of NaHCO3 equivalent to the titratable acidity was placed in the flasks. The titratable acidity was measured in an aliquot of the gastric digest, and is defined as the amount of 0.5 N sodium hydroxide required to attain a pH of 7.5. After 30 min of incubation (dialysis), the pancreatic-bile mixture (0.001 g of pancreatin and 0.006 g bile salts/g of aliquot) was added, and the dialysis was continued for another 2 h at 37 °C under stirring. Dialysates of iron, calcium and zinc content were determined by atomic absorption spectrophotometry (AAS; Shimadzu Instruments, Inc., SpectrAA-220, Kyoto, Japan). Calibration of measurements was performed using commercial standards.

The mineral (calcium, iron and zinc) in vitro bioaccessibility was calculated as the percentage of the element dialysed of the total amount present in the aliquot (% dialysability). The dialysis percentage was determined by the following equation:

\[
\text{Dialysis (\%)} = \frac{A}{B} \times 100 \\
\]

where Y is the element content of the dialysates mineral fraction (mg mineral element/100 g), and Z is the total mineral (calcium, iron or zinc) content of the sample (mg mineral element/100 g grain). All determinations were done in triplicate.

2.7. Determination of starch

2.7.1. Determination of resistant starch (RS)

The method used was that proposed by Góñi, García-Diz, Matas, and Saura-Calixto (1996) and modified by Morales, Escarpa, and González (1997). The main features were: (a) removal of protein; (b) hydrolysis with α-amylase; (c) isolated of RS; (d) dispersion in KOH 2 M; (e) hydrolysis with amyloglucosidase; (f) measurement of glucose released.

Triplicate portions (100 mg) of apple sample were preincubated into centrifuge tube with a pepsin solution containing 1 g of pepsin (No. 7000, Sigma–Aldrich, St. Louis, MO, USA) for 60 min at 40 °C under stirring for resistant starch removal. Then, samples were taken out of water bath and left to cool to room temperature and were added to KCl–HCl buffer (pH 6.9, 0.1 M) and 0.10/100 ml solution of sodium azide. Furthermore, starch was hydrolysed at 37 °C for 16 h by adding 1 ml of the α-amylase solution (40 mg de α-amylase (No. 3176, Sigma–Aldrich, St. Louis, MO, USA) per ml of tri-maleate buffer). After α-amylase hydrolysis, samples were centrifuged at 5000 rpm for 5 min, and the supernatants were discarded. The residue (isolated RS) was dispersed in water before adding KOH (2 M) at room temperature with constant shaking for 30 min. The suspension was incubated at 60 °C for 30 min with 80 μl of amyloglucosidase from Aspergillus niger (No 7420, Sigma–Aldrich, St. Louis, MO, USA) with constant shaking. Then, the
sample was centrifuged at 5000 rpm for 5 min, and the supernatant (RS) was collected and residue was discarded.

The glucose concentration in the supernatant (resistant starch) was determined using a kit (GAGO20, Sigma–Aldrich, St. Louis, MO, USA). The mixture was mixed consistently and left for 30 min in a water bath at 37 °C. The colour absorption was measured at a wavelength of 500 nm using a spectrophotometer (Spectronic Instruments, Spectronic® 20 Genesys™, Chicago, IL, USA); the digestible starch concentration of the apple sample was calculated as mg of glucose x 0.9.

2.7.2. Determination of digestible starch (DS)

The principal steps were: (a) removal of RS; (b) hydrolysis with amyloglucosidase; (c) measurement of glucose released.

Triplicate portions (100 mg) of apple sample were preincubated into centrifuge tube with a pepsin solution containing 1 g of pepsin for 60 min at 40 °C for resistant starch removal. Then, samples were taken out of water bath and left to cool to room temperature and were added to KCl–HCl buffer (pH 6.9, 0.1 M) and sodium azide at 0.10%. Furthermore, starch was hydrolysed at 37 °C for 16 h after adding 1 ml of the α-amylase solution (40 mg de α-amylase per ml de-tri-maleate buffer). After α-amylase hydrolysis, samples were centrifuged at 5000 rpm for 5 min, and the supernatant (DS) were collected and stored in a volumetric flask. The residue was discarded (RS). Then, an aliquot of 20 ml was mixed with 2 ml of 0.4 M sodium acetate buffer, pH 4.75 (pH was adjusted with HCl 2 M). The suspension was incubated at 60 °C for 30 min with 60 μl of amyloglucosidase from A. niger (No. 7420, Sigma–Aldrich, St. Louis, MO, USA) with constant shaking. The glucose liberated in the supernatant (digestible starch) was quantified, as described above for resistant starch (Morales et al., 1997).

2.7.3. Determination of total starch

The method used is based on Holm's method (1986) with some modification. The main modification has been the dispersion in 2 M KOH after pepsin incubation. In samples, such as wheat flour, spaghetti, lentils, beans, raw potatoes and peas, it was necessary after the treatment with pepsin to carry out a centrifugation. All determinations were done in triplicate and the solvents and reagents were purchased from Sigma–Aldrich Company Ltd. (St. Louis, MO, USA) with analytical grade.

2.8. Statistical analysis

The effect of high hydrostatic pressure on the bioaccessibility of specific nutrients (antioxidant, minerals and starch) was estimated using Statgraphics® Plus 5 (Statistical Graphics Corp., Herndon, VA, USA). The results were analysed by analysis of variance (ANOVA). The differences amongst the media were analysed using the least significant difference (LSD) test with a significance level of α = 0.05 and a confidence interval of 95% (p < 0.05). In addition, the multiple range test (MRT) included in the statistical program was used to demonstrate the existence of homogeneous groups within each of the parameters.

3. Results and discussion

3.1. Proximal analyses of raw material

Table 1 shows the mean values and standard deviations of the moisture content, protein, fat, crude fibre, ash, available carbohydrates, water activity, soluble solids, % acidity and pH of samples, both untreated and treated with high hydrostatic pressure (500 MPa for 10 min). A significant (p < 0.05) increase in the moisture content relative to the untreated sample was apparent for the treated sample at 500 MPa/10 min. The highly significant increase in moisture content may be due to increased water absorption by the protein, since it is known that high hydrostatic pressure can increase the hydration of proteins (Silva, Foguel & Royer, 2001). In parallel, the protein content was significantly (p < 0.05) lower in treated apple sample at 500 MPa/10 min than in the control sample (untreated sample) since the increase in moisture probably has a dilution effect on the other constituents of the high pressure-treated apple sample, as was reflected in the levels of crude protein on high pressure treatment; while the ash, fat, crude fibre and pH content of the untreated sample were lower compared to the treated apple sample but were not significantly different (p > 0.05). In the same table, the value of water activity, which is an indicator of water availability, was high for all the samples and the pH exhibited an increase tendency in the treated apple sample compared to the untreated apple sample (p < 0.05).

The differences in the chemical composition in the same varieties of apples are due to the region of growth, variety, ripening stage during harvesting, agronomic and environmental conditions. About 80% of the apple carbohydrates are soluble sugars: sucrose (~2.1%), glucose (2.4%) and fructose (5.9%). Apples contain about 2.4% of total dietary fibres, and it is proved that they contain sorbitol. Malic acid is the predominant organic acid in apples (80–90% of total acids); its content varies depending on the variety, ripeness, and environmental conditions during growing and storage (Escarpa & Gonzalez, 1998; Van der Luis, Dekker, Skrede, & Jongen, 2002).

3.2. Effect of high hydrostatic pressure on bioaccessibility of DPPH radical scavenging activity

The free radical-scavenging is one of the known mechanisms by which antioxidants inhibit lipid peroxidation (Blokchina, Virolainen, & Fagerstedt, 2003; Rice-Evans, Miller, & Paganga, 1997). The principle of the antioxidant activity is the availability of electrons to neutralise any so-called free radicals. The DPPH radical scavenging activity has been extensively used for screening antioxidants from fruit and vegetable juices or extracts (Robards, Prenzler, Tucker, Swatsigtang, & Glover, 1999; Sanchez-Moreno, 2002). DPPH forms a stable molecule on accepting an electron or a hydrogen atom and thus has applications in the determination of radical scavenging activity of natural products (Agbor, Vinson, Oben, & Ngogang, 2006). The free radical scavenging activity of the extract apples was evaluated by using the DPPH radical scavenging method (Brand-Williams et al., 1995). The antioxidant activities measured in ethanolic extract obtained using DPPH assays from a single extract were measured three times to test the reproducibility of the assays. The dry extracts were dissolved in ethanol in concentrations ranging between 2 and 10 mg/ml. The DPPH radical scavenging activities
of the apple samples were expressed as % radical scavenging, by measuring the absorbance decrease at 517 nm due to disappearance of the DPPH radical because antioxidants in the sample react with DPPH and convert it to 2,2-diphenyl-1-picrylhydrazine. The degree of discoloration indicates the scavenging potential of the antioxidant extract, which is produced by the hydrogen donating ability (Van Gadow, Joubert, & Hannsman, 1997). It is visually noticeable as a discoloration from purple to yellow. The model of DPPH stable radical scavenging is a widely used method to evaluate the antioxidant activities in a relatively short time compared with other methods. Results are presented as % DPPH radical scavenging activity of the apple extracts in mg/ml concentrations and are shown in Fig. 1.

The antioxidant activity of apple samples was significantly affected by the processing and digestion conditions. Fig. 1 illustrates a significant ($p \leq 0.05$) decrease in the concentration of DPPH radical due to the scavenging ability of both sample types with non-digestion and digestion. ANOVA showed significant differences ($p \leq 0.05$) between the antioxidant activities obtained for both apple samples as determined with all the treatments in this study.

The samples without digestion, untreated sample, 500 MPa/8 min and 500 MPa/10 min processed apple showed stronger DPPH scavenging activity activity rather than the sample with 500 MPa/4 min and 500 MPa/2 min, and this difference was found statistically significant ($p \leq 0.05$). However, 10 mg/ml concentration of untreated sample and the same concentration of 500 MPa/4 min showed similar DPPH radical scavenging activity, statistically. The scavenging effect of high pressure at different time and untreated sample on the DPPH radical decreased was in the order of 500 MPa/10 min > untreated sample > 500 MPa/8 min > 500 MPa/4 min > 500 MPa/2 min and 74.9%, 74.9%, 73.3%, 72.3%, and 69.6%, respectively, at the same concentration (10 mg/ml). These results indicated that all treatments of the apple sample have a noticeable effect on the free radical scavenging. The free radical scavenging activity also increased with increasing concentration.

For higher time of treatment (10 min) at high hydrostatic pressure, the sample with in vitro digestion had significantly higher ($p \leq 0.05$) DPPH radical scavenging activity than at lower time of treatment (2, 4, and 8 min). No significant difference was observed between the sample with in vitro digestion treated with high hydrostatic pressure at 2, 4 and 8 min and untreated sample. However, 10 mg/ml concentration of 500 MPa/10 min sample and the same concentration of 500 MPa/8 min and 500 MPa/4 min samples showed similar DPPH radical scavenging activity, statistically, therefore the extracts of the apple samples with in vitro digestion showed maximum hydrogen-donating ability in the presence of DPPH stable radicals at high concentrations. The scavenging effect of high pressure at different time and fresh sample on the DPPH radical decreased in the order of 500 MPa/10 min > 500 MPa/8 min > 500 MPa/4 min > 500 MPa/2 min > untreated sample and 99.9%, 97.9%, 97.7%, 95.9%, and 95.5%, respectively, at the same concentration (10 mg/ml).

Other studies have demonstrated that high hydrostatic pressure of vegetables has little influence on their in vitro antioxidant activity and content of carotenoids. However, exposure of plant foods to high hydrostatic pressures has been shown to alter plant matrix structures (McInerney, Seccafien, Stewart & Bird, 2007), which may have impact on the nutritional properties and possible protective effects of the food once processed. Green beans at a pressure of 600 MPa resulted in significant increase in lutein availability compared to untreated samples; this can be due to a disruption of cellular structures in the beans by exposure to high pressures, which might have facilitated the release of lutein within the plant tissue matrix during the in vitro digestion process (McInerney et al., 2007). It is possible that changes to the tissue matrix induced by high hydrostatic pressures, for example disruption of plant cell walls, resulted in the release of compounds with antioxidant actions into the extracellular environment.

Based on the data obtained from this study, all the samples at different pressures are free radical inhibitors or scavengers, as well as primary antioxidants that react with free radicals, which may limit free radical damage occurring in human body. Free radical-induced oxidative damage is strongly implicated in the development of a number of common chronic disease states (Ames, Gold, & Willett, 1995; Diplock et al., 1998) and many of the health benefits associated with consumption of vegetables and fruits have been linked to their potent antioxidant properties (Halliwell, Rafter, & Jenner, 2005). It was reported that oxidative stress, which occurs when free radical formation exceeds the body’s ability to protect itself, forms the biological basis of chronic conditions, such as arteriosclerosis.

The IC50 value, defined as the concentration of extract required for 50% scavenging of DPPH or hydroxyl radicals under the experimental conditions employed is a parameter widely used to measure the free radical scavenging activity (Cuvelier, Richard, & Berzet, 1992); a smaller IC50 value corresponds to a higher antioxidant activity.

The IC50 values were calculated from the regression equations prepared from the concentrations of the extracts and the percentile inhibition of the free radical formation (DPPH assay). The IC50 values were compared with the IC50 Value of THE untreated sample in each system to assess the antioxidant property of treated apple sample with high pressure (Table 2). A lower IC50 value indicates a greater antioxidant activity. ANOVA showed significant differences between the antioxidant activities (IC50) obtained for both sample types (non and with in vitro digestion) as determined with all the treatments in the study. These results show that the in vivo digestion and high hydrostatic pressure have a noticeable effect on the antioxidant activities, IC50, with much lower values of apple samples compared with those without digestion.

The DPPH scavenging activities of the apple extracts (non-digestion) expressed as an IC50 value, ranged from 4.1 to 4.8 mg/ml. The untreated apple sample exhibited the strongest antioxi-
significant differences (p < 0.05), the mean changes between the samples were analysed by one-way ANOVA followed Multiple Ranking Test.

Table 2
Effect of high pressure hydrostatic on DPPH free radical scavenging activity (IC_{50}) in the apple sample with no digestion, and after in vitro digestion for different times.

<table>
<thead>
<tr>
<th>Apple samples</th>
<th>IC_{50}</th>
<th>IC_{500}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>4.07 ± 0.06A</td>
<td>0.28 ± 0.04A</td>
</tr>
<tr>
<td>500 MPa/2 min</td>
<td>4.81 ± 0.42m</td>
<td>0.20 ± 0.06m</td>
</tr>
<tr>
<td>500 MPa/4 min</td>
<td>4.66 ± 0.18m</td>
<td>0.04 ± 0.03m</td>
</tr>
<tr>
<td>500 MPa/8 min</td>
<td>4.58 ± 0.02m</td>
<td>0.08 ± 0.03m</td>
</tr>
<tr>
<td>500 MPa/10 min</td>
<td>4.47 ± 0.01nc</td>
<td>0.09 ± 0.02gn</td>
</tr>
</tbody>
</table>

Same letter in the exponential in the same row and column show there are no significant differences (p > 0.05). The antioxidant activities obtained for the untreated apple sample and all the other samples treated with in vitro digestion at high hydrostatic pressure. The experiments showed that the antioxidant capacity of all apple samples with in vitro digestion at long time at high pressure was higher than of the non-digestion samples. This suggests that the amount of antioxidants released by the apple matrix into the human intestine, and hence also the antioxidant capacity of these samples, may be higher than expected from the data based on chemical extracts. This is a fact that could be taken into account when evaluating the antioxidant capacity of a fruit from a nutritional standpoint. These antioxidants are potentially available in the small gut; the degree to which they produce an antioxidant effect depends on the rate of absorption. Also, the antioxidants that are not released in these digestive enzymatic extracts may enter the colon, where they can be fermented by the microflora, yielding different compounds that may be metabolised and may provide an antioxidant environment.

Oszmianska, Wolniak, Wojdylo, and Wawer (2008) reported that the differences in the antioxidant activities of fruits could be due to their different profiles of phenolic acids, flavonoid compounds and their derivatives. For instance, the antioxidant activities of flavonoids depend on the number of hydroxyl groups in their molecules. Individual apple cultivars vary in their antioxidant activity and the main compounds responsible for it are polyphenols present in peel and flesh (Boyer & Liu 2004; Garcia-Alonso, de Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2004; Guyot et al., 1998). Many studies (Chinnici, Bendini, Galiani, & Ripon, 2004; Patras, Brunton, Da Pieve, Butler, & Downey, 2009) have demonstrated the relationship between the free radical scavenging ability of apples and the polyphenol and antioxidant vitamin content. The main compounds responsible for high antioxidant activity of apples are procyanidins and epicatechins, and – to a lesser extent – chlorogenic acid (Lee, Kim, Kim, Lee, & Lee, 2003).

In the other works, a high hydrostatic pressure treatment at pressures from 400–600 MPa significantly retained more phenols, anthocyanins and ascorbic acid in strawberry purees than thermal treatment. In blackberry purees a greater retention of anthocyanins was noted as compared to the thermally treated purees, and the total anti-radical powers of high pressure treated samples were significantly higher than in fresh and thermally processed samples (Patras et al., 2009).

3.3. Effect of high hydrostatic pressure on minerals bioaccessibility

Mineral bioaccessibility may be affected by the matrix composition of the samples, and the in vitro solubility and dialysis methods used is considered useful for predicting many promoting/hindering dietary factors, as well as the influence of processing on micronutrient availability in food (Hazell & Johnson, 1987). In addition, mineral absorption is correlated to its intestinal solubility. Thus, mineral bioaccessibility can be used to establish trends in the relative bioavailability of minerals, because the chance of mineral uptake increases with higher solubility (Zhu, Glahn, Yeung, & Miller, 2006). The in vitro method for estimating essential minerals has gained popularity due to its promptness and low cost. Some methods, particularly the in vitro development by Miller et al. (1981), have been shown to provide availability measurements that correlate well with various in vivo studies.

The calcium, zinc and iron contents of the apple samples studied are presented in Table 3. As this table shows, the inherent calcium concentration in untreated and 500 MPa/10 min treated samples was lower (not significant differences shown, p > 0.005), almost a third less than the treated samples at 500 MPa for 2 min and 4 min and a fourth lower than the 500 MPa/8 min treated samples. These results were slightly different from those of reported by Gorinstein, et al. (2001), but they were predictable because we studied different cultivars of apples, which were grown in completely different conditions in different geographic regions. Smaller molecules, such as volatile compounds, pigments, vitamins, and other compounds related with the sensory, nutritional, and health promoting are unaffected (Cheftel, 1992; Oey, Van der Plancken, Van Loey, & Hendrickx, 2008) by high hydrostatic pressure processing. The recommended dietary allowances for calcium are 1300–1000 mg day⁻¹ according to age and sex group, for iron are 8–18 mg day⁻¹, while zinc recommendations range between 8 and 11 mg day⁻¹ (Food & Nutrition Board, 2004). Thus, a usual 200 g serving of fruit treated with high pressure (500 MPa/8 min) would contribute 244.5 mg of calcium, 32.1 of iron and 13.5 mg of zinc, respectively.

The bioaccessibility values of calcium, iron and zinc present in untreated and treated apple samples with hydrostatic high pressure, as determined by the in vitro digestibility procedure, are also presented in Table 4. Untreated samples had higher amounts of bioaccessibility calcium than had treated apple sample with high pressure. The bioaccessibility of calcium of the untreated samples ranged from 13.9% to 14.9%, and that of treated samples ranged between 0.9–1.2%, 0.8–1.6%, 0.9–1.2% and 5.7–8.3% for 500 MPa/2 min, 500/4 min, 500 MPa/8 min and 500 MPa/10 min, respectively, of the element present in them. Thus, the bioaccessibility of calcium in the untreated samples was several-fold higher than of those in the treated samples with high pressure.

Among the apple samples, the lowest bioaccessibility of calcium was from the untreated sample at 500 MPa/2 min expressed as % sol-

Table 3
Total mineral content in the apple samples (mg/100 g).

<table>
<thead>
<tr>
<th>Apple samples</th>
<th>Calcium content (mg/100 g)</th>
<th>Iron content (mg/100 g)</th>
<th>Zinc content (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>30.33 ± 1.94*</td>
<td>14.46 ± 3.49*</td>
<td>6.22 ± 0.91*</td>
</tr>
<tr>
<td>500 MPa/2 min</td>
<td>93.81 ± 1.17*</td>
<td>15.13 ± 1.44*</td>
<td>4.76 ± 1.63*</td>
</tr>
<tr>
<td>500 MPa/4 min</td>
<td>92.25 ± 10.44</td>
<td>11.81 ± 2.75*</td>
<td>4.08 ± 0.75*</td>
</tr>
<tr>
<td>500 MPa/8 min</td>
<td>122.13 ± 19.40</td>
<td>16.04 ± 1.41*</td>
<td>6.76 ± 1.37*</td>
</tr>
<tr>
<td>500 MPa/10 min</td>
<td>30.97 ± 3.46*</td>
<td>15.23 ± 1.61*</td>
<td>8.02 ± 1.09*</td>
</tr>
</tbody>
</table>

Different letters in the same column indicate that the values are significantly different (p < 0.05).
3.4.1. Digestible starch (DS)

The enzymatically assessed digestible starch contents of the untreated and treated apple samples with high hydrostatic pressure are presented in Fig. 2. There are few studies on the digestibility of starch in apple. The starch fraction considered digestible (DS) was high for all the samples. The untreated sample contained 75.5 ± 0.7% of digestible starch; this was lower compared with the samples treated with high hydrostatic pressure 76.6 ± 1.7%, 83.8 ± 7.7%, 96.4 ± 7.0% and 100.4 ± 6.5%, at 500 MPa/2 min, 500 MPa/4 min, 500 MPa/8 min and 500 MPa/10 min, respectively. No statistical differences (p > 0.05) were observed between the untreated sample and the sample at 500 MPa/2 min, but there were statistical differences (p < 0.05) with the other samples at 500 MPa/4 min, 500 MPa/8 min and 500 MPa/10 min. The digestible starch content in the apple sample was higher than banana starch (80.0 ± 1.2%). However the value for apple sample was lower than those recently found for Peruvian carrots (95.8 ± 0.6%), lentils (95.1 ± 1.0%) and maize (96.9 ± 2.0%) (Bello-Perez, Sayago-Ayerdí, Méndez-Montecalvo, & Tovar, 2004). The content of digestible starch was affected by pressure to a great extent. When applying a pressure of 500 MPa at room temperature (20 °C) for longer time, the resistant starch content decreased (p < 0.05) and there was a tendency towards a concurrent increase in digestible starch (Fig. 2). These changes may be due to the redistribution of soluble pectin molecules (mainly uronic acids, but to some extent also arabinose and galactose) to insoluble molecules.

3.4.2. Resistant starch (RS)

RS has been defined as the sum of starch and the product of starch degradation not absorbed in the small intestine but is fermented in the large intestine of healthy individuals (Singh & Lim, 2008). The RS content in food, as well as the digestive rate and level of starch, has a positive effect on health. It participates in the reduction of glycomic and insulinemic responses to food, it has hypocholesterolemic effects and protects against colorectal cancer (Asp, Van Amelsvoort & Hautvast,1996; Cassidy, Bingham, & Cummings, 1994).

RS was measured by the procedure of Goñi et al. (1996). The starch fraction considered resistant (RS) was low for all samples (see, Fig. 2). The untreated sample contained 19.6 ± 1.9% of resistant starch; this content of starch was higher compared with the samples treated with high pressure 14.9 ± 1.2%, 4.4 ± 0.5% and 3.1 ± 0.5%, at 500 MPa/4 min, 500 MPa/8 min and 500 MPa/10 min, respectively. No statistical differences (p > 0.05) were observed between the untreated sample and the sample at 500 MPa/2 min, but there were statistical differences (p < 0.05) with the other samples at 500 MPa/4 min, 500 MPa/8 min and 500 MPa/10 min.

The starch in the raw samples is contained within the granules, which are poorly affected by hydrolytic enzymes, and it is therefore mostly indigestible (Bravo, Siddhuraju, & Saura-Calixto, 1998). The variation in the starch digestibility is being influenced by factors, such as degree of crystallinity or the amylose/amylopectin ratio of the starch granules (Englyst et al., 1992).

3.4.3. Total starch (TS)

Starch is a transitional form of carbohydrates in apple and many other fleshy fruits, and is stored in the living chloroplasts or plastids of growing fruits. The biosynthesis and degradation of starch...
are closely associated with fruit development and quality formation (Beruter, 1985; Fan, Matthes, & Patterson, 1995; Irving, Singleton, & Hurst, 1999). Starch is a major substance accumulated during maturation of the apple fruit (Magein & Leurguin, 2000). Starch degradation in apples starts when the fruit reaches the mature stage. Sugars are accumulated in the fruit cells during ripening and the fruit becomes sweet, which is an important attribute for determining fruit quality (Kader, 2000).

The TS of the samples ranged between 81.2% (untreated sample) and 99.8% (500 MPa for 10 min). ANOVA showed significant differences (p < 0.005) between the total starch obtained for the treated apple samples at 500 MPa/10 min and the rest of samples. However, the total starch measured as TS = DS + RS of samples ranged between 95.1% (untreated sample) and 103.5% (500 MPa for 10 min). ANOVA did not show significant differences (p > 0.005) between the total starch obtained for the untreated samples and the samples treated at 500 MPa for 2, 4 and 8 min. For other foods, the highest TS value ranged between 73.71% (polenta) and 87.36% (polished rice).

Granny Smith apples had a significantly greater total starch content than others apples, for example Royal Gala, which suggests that Granny Smith apple is well suited for cooking, whereas Royal Gala is a good eating apple. There are few studies on the starch content of immature apple fruits found in the literature because most studies report the starch content at harvest or measure the starch index (colour scale based on iodine staining of sliced fruit) during fruit development. A total starch content of 53% dry weight was previously reported for Granny Smith apple during development (Stevenson, Domoto, & Jay-lin, 2006), which was less than the results obtained in this study (81.2% dry weight). Brookfield, Murphy, Harker, and MacRae (1997) reported a 28% starch content (dry weight basis) in mature Royal Gala apples, which is substantially less than the values found in this study. The differences could be attributed to the degradation of starch during maturation, which resulted in less starch in mature apples.

4. Conclusions

High pressure processed apple had significantly higher antioxidant capacities, mineral and starch content when compared to untreated samples (p < 0.05). In summary, the antioxidant capacity, mineral and starch content of fruit may be underestimated in the literature because the extraction solvents usually used do not allow the complete release of antioxidant compounds. Similarly, this applies to the starch and mineral content. On the other hand, the analysis of in vitro digestive enzymatic extracts suggests that the antioxidant activity of the fruit in the human gut may be higher than what might be expected from literature data based on measurements of aqueous-organic extracts. It is possible that changes to the tissue matrix induced by high hydrostatic pressures, for example disruption of the plant cell walls, resulted in the release of compounds with antioxidant actions and increased mineral and starch content into the extracellular environment. Consumption of apple under high hydrostatic pressure may supply substantial antioxidants, minerals and starch which may provide health promoting and disease preventing effects.

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References


