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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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A B S T R A C T

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, IC_{50}, with much lower values (a smaller IC_{50} 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 scavenge free radicals such as peroxide, 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 flavonoid intake in the European diet, after onions and tea (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993; Scalbert & Williamson, 2000). Apples are an important source of phenols and antioxidants (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 & Shiowatana, 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, Mozafar, & 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

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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, Elez-Martíınez, & Martín-Belloso, 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 & Shiowatana, 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 & Aguilera, 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 (a_w) 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, Tokio, Japan) which measures refraction 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:

$$(\%)\ \mathrm{TAA} = [1 - (\mathrm{Abssample}/\mathrm{Abscontrol})] \times 100 \quad (1)$$

where TAA is the total antioxidant activity and Abs is the absorbance. IC_{50}, 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. IC_{50} was determined from a graph of antioxidant capacity