Food and Nutrition Sciences, 2013, 4, 109-118
http://dx.doi.org/10.4236/fns.2013.48A014 Published Online August 2013 (http://www.scirp.org/journal/fns)

Extraction of β-Carotene, Vitamin C and Antioxidant Compounds from *Physalis peruviana* (Cape Gooseberry) Assisted by High Hydrostatic Pressure

Vilbett Briones-Labarca[1,2*], Claudia Giovagnoli-Vicuña[1], Paola Figueroa-Alvarez[1,2], Issis Quispe-Fuentes[1], Mario Pérez-Won[1,2]

[1]Department of Food Engineering, Universidad de La Serena, Av. Raúl, Bitrán s/n, La Serena, Chile; [2]Center for Advanced Studies in Arid Zones, CEAZA, Av. Raúl Bitrán s/n, La Serena, Chile.
Email: *vbriones@userena.cl

Received April 2nd, 2013; revised May 2nd, 2013; accepted May 9th, 2013

ABSTRACT

High hydrostatic pressure assisted extraction (HHPE) has several advantages when compared to traditional extraction methods, which frequently cause degradation and loss of target components and might consume large volumes of environmentally unfriendly solvents. The aim of this study was to develop an assisted extraction method using high hydrostatic pressure (HHPE) and to evaluate both HHPE and conventional extraction methods for β-carotene, antioxidant compounds and vitamin C from cape gooseberry. β-carotene and compounds with antioxidant activity (2,2-diphenyl-1-picrylhydrazyl radical assay (DPPH*) or radical scavenging activity; ferric reducing antioxidant power assay (FRAP)) were extracted using HHPE for 5 min, 10 min and 15 min at 500 MPa, while vitamin C was extracted at 500 MPa for 30 s, 60 s and 90 s. Processing significantly affected ($p \leq 0.05$) the β-carotene content of all samples, increasing retention by 8%, 14% and 15% at 500 MPa after 5 min, 10 min and 15 min of HPPE, respectively. The highest antioxidant content determined by DPPH* and FRAP assays was obtained in a sample treated at 500 MPa for 15 min, showing increases of 26% and 51%, respectively, compared with an untreated sample. The ascorbic acid content of fresh cape gooseberry was 26.31 mg·100 g^{-1}. In fact, the ascorbic acid levels were significantly higher for all high-pressure-treated samples compared to this of conventionally extracted sample ($p \leq 0.05$), exhibiting increases of 9%, 41% and 53% at 500 MPa after 30 s, 60 s and 90 s of HPPE, respectively. Thus, the application of HHPE produced higher β-carotene content, antioxidant compounds and vitamin C content and required less extraction time compared to other extraction methods. The pharmaceutical and food industries can benefit by using high pressure extraction technology.

Keywords: Cape Gooseberry; High Hydrostatic Pressure; β-Carotene; Vitamin C

1. Introduction

Fruits have been associated with a protective role in maintaining health. Studies have revealed the favourable effects of fruit intake against risk factors for chronic disease and a significant relationship between high intake and low total mortality, mortality from coronary heart disease and mortality from cancer [1-4]. These beneficial effects have been attributed to the various antioxidants found in fruits, including polyphenols, ascorbic acid, carotenoids and others [2].

The cape gooseberry (*Physalis peruviana*), which belongs to the *Solanaceae* family, is a species native to the Andes region of South America [5]. Cape gooseberries are annuals or short-lived perennials and are more or less hairy. The flowers are bell-shaped, but the most distinctive feature is the fruiting calyx, which enlarges to cover the fruit and hangs downwards like a lantern [6]. One remarkable aspect of the fruit is its colour. Cape gooseberry fruit contains a large amount of secondary metabolites such as provitamin A, minerals, vitamin C, vitamin B-complex and polysaccharides. These secondary metabolites exhibit antibacterial, antiviral, antioxidant, anti-inflammatory and anti-carcinogenic properties [6]. Although there are some reports on the use of traditional methods to extract these valuable components, there are no studies concerning the use of high hydrostatic pres-

*Corresponding author.

sure extraction (HHPE).

Carotenoids are responsible for the orange colour of the cape gooseberry [7,8]. Carotenoids are fat-soluble pigments that have significant antioxidant potential, with the main carotenoids being lycopene and β-carotene. One of the most important characteristics of carotenes, particularly β-carotene, is their provitamin A activity, considering that vitamin A deficiency is one of the main nutritional problems of populations in developing countries [9]. Humans cannot synthesise vitamin A and rely on the intake of provitamin A compounds from foods. Once absorbed by human bodies, provitamin A carotenoids are metabolised to form vitamin A [10].

The main biological form of vitamin C is L-ascorbic acid (AA), but its reversibly oxidised form is dehydroascorbic acid (DHA) [9,11-14]. However, human beings cannot synthesise AA, which needs to be supplied through food. Fruits and vegetables are known to be the best sources of vitamin C, and it is important to determine their AA and DHA contents to estimate their total vitamin C content [11,13].

High hydrostatic pressure extraction (HHPE) is a novel technique used for the extraction of active ingredients from plant materials; it operates under very high pressures ranging from 100 to 1000 MPa, and it has been recognised as an environment-friendly technology by the US. Food and Drug Administration, being extensively applied in the pharmaceutical, metallurgical and food industries. HHPE is considered an alternative extraction method that is proven to be faster and more effective than other extraction methods [15]. Recently, some authors have reported that HHPE can reduce the processing time and obtain higher extraction yields than other extraction methods and has no adverse side effects on the activity or structure of bioactive components [15-19]. Thus, HHPE has some advantages with respect to the extraction of natural products or bioactive compounds. Furthermore, this technology has been used successfully for the extraction of flavonoids from propolis [19], flavonoids from lychee [20], anthocyanins from grape skin [17], ginsenosides from the roots of Panax ginseng [21], ginsenosides from Panax quinquefolium [22], flavones and salidroside from Rhodiola sachalinensis [23], corilagin from longan [20], anthocyanins from grape by-products [17], icariin from Epimedium and polyphenols from green tea [15].

The objective of the present study was to investigate the effects of high hydrostatic pressure on extraction efficiency and compare the effectiveness of HHPE with that of conventional extraction (CE) based on the carotenoid and vitamin C contents and the antioxidant activeties determined by DPPH and FRAP for cape gooseberry. This study could help to better utilise cape gooseberry as a readily accessible source of natural antioxidants in the food or pharmaceutical industry.

2. Materials and Methods

2.1. Reagents and Other Materials

The following HPLC-grade reagents were used [the purity grade of the reagents is reported as a percentage]: methanol (Tedia, USA) [99.9], acetonitrile (Vetec, Brazil) [99.8], ethyl acetate (Merck, Darmstadt, Germany) [99.9], metaphosphoric acid (Merck, Germany) [90.5 - 99.5], ascorbic acid (Aldrich Company Ltd., St. Louis, MO, USA) [99], potassium dihydrogen phosphate (Merck, Darmstadt, Germany), hexadecytrimethyl-ammonium bromide (Sigma-Aldrich Company Ltd., St. Louis, MO, USA) and acetic acid (Vetec, Brazil) [99.7]. Milli-Q water was obtained through a Millipore filter system (Millipore Co., USA).

2.2. Sample Preparation

Cape gooseberries *(Physalis peruviana)* were purchased from a local market (La Serena, Chile). The calyx was removed, and thefruit was washed, dried and stored at −20˚C until the moment the experiments were performed.

2.3. Physicochemical Analysis

The crude protein content was determined using the Kjeldahl method with a conversion factor of 6.25. The lipid content was analysed gravimetrically following Soxhlet extraction. The crude fibre was estimated by the acid/alkaline hydrolysis of insoluble residues. The crude ash content was estimated by incineration in a muffle furnace at 550˚C. All methodologies followed the recommendations of the Official Method of analysis [24]. The available carbohydrate content was estimated by difference. The moisture level was determined by means of AOAC method No. 93406 [24]. The pH was measured using an EXTECH Instrument microcomputer pH-vision 246072 (Waltman, MA, USA); the level of titrimetric acidity was expressed as citric acid. The soluble solids were measured using a refractometer (ABBE, 1T, Tokyo, Japan), which measures the refraction indices of both solid and liquid samples in a fast and accurate way and whose scale ranges from 0˚ to 95˚Brix. The water activity (a_w) was measured at 25˚C using a water activity instrument (Novasina, model TH-500, Pfaffikon, Lachen, Switzerland). All measurements were performed in triplicate. All solvents and reagents were purchased from Sigma-Aldrich Company Ltd. (St. Louis, MO, USA) and Merck KGaA (Darmstadt, Germany) and were of analytical grade.

2.4. Determination of Ascorbic Acid (Vitamin C)

2.4.1. Conventional Extraction

The ascorbic acid (AA) extraction method was tested using 5% metaphosphoric acid (MPA) as the extracting solution, according to the method by Campos *et al.* [13] with some modifications. Thirty millilitres of the extracting solution was added to 5 g Cape gooseberry samples; then, the sample was ground in a food-grade blender (Ultra-Turrax, T25 Basic, Ika Labortechnik, Staufen, Germany) for 5 min and filtered through a double-layer cheese cloth. The filtrate was centrifuged for 15 min at 5000 rpm at 10˚C. The supernatant was stored in a refrigerator at approximately 5˚C until chromatographic analysis, which was performed on the same day.

2.4.2. Extraction Assisted by High Hydrostatic Pressure (HHPE)

Thirty millilitres of the extracting solution was added to a 5 g sample of cape gooseberries; then, the sample was ground in a food-grade blender (Ultra-Turrax, T25 Basic, Ika Labortechnik, Staufen, Germany) for 5 min. The pureed cape gooseberry samples were packed individually with extraction solution (meta-phosphoric acid to 5%) and hermetically sealed in high-density-polyethylene bags. The packaged samples were placed in a cylindrical loading container at room temperature and pressurised at 500 MPa for 30, 60 and 90 seconds. Water was employed as a pressure-transmitting medium at a ramp rate of 17 MPa/s; the decompression time was less than 5 s. A 2 L processing unit (Avure Technologies Incorporated, Kent WA, USA) was used to pressurise the samples. The samples were filtered through a double-layer cheese cloth. The filtrates were centrifuged for 15 min at 5000 rpm at 10˚C. The supernatant was stored in a refrigerator at approximately 5˚C until chromatographic analysis, which was performed on the same day.

2.4.3. Chromatographic Conditions

Ascorbic acid (AA) analysis was performed using an Agilent 1200 series HPLC system (Santa Clara, CA), which featured a model G1311A quaternary pump, a model G1367B autosampler, a model G1316A column oven and a model G13150 photodiode array detector. The column used was an Agilent ZORBAX Eclipse XDB-C18 measuring 4.6 mm × 150 mm with a 5 μm bead size, which was connected to an Eclipse XDB-C18 guard column. Immediately prior to HPLC analysis, each sample was filtered through a Millipore filter (0.22 μm) before use. A 20 ml aliquot of the supernatant was injected directly into a reverse phase C18 HPLC column. AA was detected and quantified based on UV absorbance at 245 nm.

The mobile phase was a 5 mM solution of hexadecytrimethyl ammonium bromide as the ion-pairing agent and 50 mM potassium dihydrogen phosphate as the buffer at pH 4.5. The flow rate was fixed at 0.8 ml/min at room temperature under isocratic elution conditions. Detection was performed with a 486 Absorbance Detector (Waters, Milford, MA) set at 245 nm. Under these conditions, the AA peak was eluted after approximately 6.1 - 6.2 min. AA standards in the range of 15 - 120 μg of ascorbic acid/ml in 5% meta-phosphoric acid (MPA) were run on the HPLC with every assay for use as external standards, and the calibration curve was used to quantify AA in the samples. Total AA was estimated after the reduction of dehydroascorbic acid (DHA) with dithiotreitol ($C_4H_{10}O_2S_2$). The results were expressed as mg vit.C/100 g sample.

2.5. Determination of Carotenoids

2.5.1. Conventional Extraction

Conventional extraction was performed in triplicate using the method described by KoKa *et al.* [25] with some modifications. Cape gooseberry puree (5 g) was extracted in a 50 ml hexane:acetone:ethanol (50:25:25, v/v) mixture using a shaker. The extract was vacuum-filtered through a Buchner funnel. The residue was re-extracted until it became colourless. The filtrates were combined in a separatory funnel and washed with 50 ml distilled water. The water phase was discarded, and Na_2SO_4 (2 g) was added as a desiccant. The hexane phase was transferred into a 250 ml round-bottom flask. The solvent was evaporated using a rotary evaporator under reduced pressure at 40˚C. The cape gooseberry puree concentrate was dissolved in hexane and diluted to a final volume of 50 ml.

2.5.2. Extraction Assisted by High Hydrostatic Pressure

Extraction assisted by high hydrostatic pressure was performed in triplicate. Cape gooseberry (5 g) puree was extracted in a 50 ml hexane:acetone:ethanol (50:25:25, v/v) mixture using a shaker; samples were hermetically sealed in high-density-polyethylene bags. The packaged samples were placed in a cylindrical loading container at room temperature and pressurised at 500 MPa for 5, 10 and 15 minutes with pulses of 1 minute each. The treated mixture was vacuum-filtered through a Buchner funnel. The filtrates were combined in a separatory funnel and washed with 50 ml distilled water. The water phase was discarded, and Na_2SO_4 (2 g) was added as a desiccant. The hexane phase was transferred into a 250 ml round-bottom flask. The solvent was evaporated using a rotary evaporator under reduced pressure at 40˚C. The cape

gooseberry puree concentrate was dissolved in hexane and diluted to a final volume of 50 ml. The cape gooseberry puree concentrate was used to determine the total carotenoid content by spectrophotometry.

2.5.3. Spectrophotometric Determination of Total Carotenoids

The concentration of carotenoids in the Cape gooseberry puree concentrate solution was then determined at 450 nm using a UV–Vis spectrophotometer (Spectronic instruments, 20 Genesys™, USA). External calibration with authenticated β-carotene standard solutions (103 mg/ml - 824 mg/ml) in hexane was used to quantify the carotenoids in the solutions. The carotenoid content was expressed as β-carotene equivalents (βCE) in mg 100 g^{-1} of sample.

2.6. Determination of Antioxidant Capacity

2.6.1. Conventional Extraction

Conventional extraction was carried out at 0.1 MPa by weighing 6 g of Cape gooseberry into Erlenmeyer flasks containing 80% aqueous methanol (250 mL) using a solid/liquid ratio of 1:2. Extraction was carried out in a sonicator bath for 30 min. The extraction was filtered through Whatman #1 filter paper (Whatman International Limited, Kent, England). The solid filter cake was re-extracted by repeating the above-mentioned steps under the same conditions. The two filtrates were combined and transferred into a 250 ml round-bottom flask. The solvent was evaporated using a rotary evaporator under reduced pressure at 40˚C. The cape gooseberry puree concentrate was dissolved in 80% methanol and diluted to a final volume of 50 ml.

2.6.2. Extraction Assisted by High Hydrostatic Pressure

Six-gram samples of Cape gooseberry puree were packed individually with extraction solution (80% methanol) and hermetically sealed in high-density-polyethylene bags. The packaged samples were placed in a cylindrical loading container at room temperature and pressurised at 500 MPa for 5, 10 and 15 minutes with pulses of 1 minute each. The treated mixture was filtered through Whatman #1 filter paper (Whatman International Limited, Kent, England) and transferred into a 250 ml round-bottom flask. The solvent was evaporated using a rotary evaporator under reduced pressure at 40˚C. The cape gooseberry puree concentrate was dissolved in 80% methanol and diluted to a final volume of 50 ml.

2.6.3. Determination of DPPH Radical Scavenging Activity

The DPPH assay was performed according to the method developed by Brand-Williams *et al.* [26], as slightly modified by Kim *et al.* [24]. A solution of 50 μM DPPH in 80% (v/v) methanol was stirred for 40 min. The absorbance of the solution was adjusted of 0.650 to 0.020 mM at 517 nm using fresh 80% (v/v) methanol. Then, 0.1 ml of standard or sample was mixed with 2.95 ml of DPPH solution and incubated for 30 min in the dark, covered with aluminium foil. The concentration of DPPH in the reaction medium was calculated from a calibration curve (the reference synthetic antioxidant is Trolox at a concentration of 0.08 to 1.28 mM in an 80% methanol solution, tested under the same conditions) obtained by linear regression. The total antioxidant capacity determined by the DPPH assay was expressed as μmol Trolox equivalents per g sample (μM TE g^{-1} sample). This assay was performed to facilitate the comparison with the ferric reducing antioxidant power (FRAP) assay. All measurements were carried out in triplicate.

2.6.4. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay procedure described by Benzie and Strain [28] was employed, with some modifications. This method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous, coloured form in the presence of antioxidants. Briefly, the FRAP reagent contained 2.5 ml of a 10 mm/L TPTZ (2,4,6-tripyridy-s-triazine, Sigma-Aldrich Company Ltd., St. Louis, MO, USA) solution in 40 mmol/L HCl with 2.5 ml of 20 mm/L $FeCl_3$ and 25 ml of 0.3 mol/L acetate buffer at a pH of 3.6; the solution was prepared freshly and warmed to 37˚C. Sample aliquots of 30 μL were mixed with 90 μL distilled water and 900 μL FRAP reagent, and the absorbance of the reaction mixture was measured spectrophotometrically (Spectrophotometer, Spectronic instruments, 20 Genesys™, USA) at 593 nm after incubation at 37˚C for 2 hours. The concentration of FRAP was calculated from a calibration curve obtained by linear regression. The results are expressed in activity equivalent to Trolox (μM/g sample fresh). The reference was the synthetic antioxidant Trolox at a concentration of 100 to 1500 μM in methanol solution 80%, which was tested under the same conditions.

2.7. Statistical Analysis

Two-way analysis of variance (ANOVA) (Statgraphics Plus® 5.1 software, Statistical Graphics Corp., Herndon, USA) was used to indicate significant differences among samples. Significance testing was performed using Fisher's least significant difference (LSD) test; differences were determined to be statistically significant when $p \leq 0.05$. The Multiple Range Test (MRT) included in the statistical program was used to test the existence of ho-

mogeneous groups within each of the parameters analysed. For all types of samples, three different batches (n = 3) were considered and analysed separately in the statistical analysis. Each replication considered at least two samples for the parameters

3. Results and Discussion

3.1. Effect on Physico-Chemical Properties

Table 1 shows the mean values and standard deviations of the moisture content, protein, fat, crude fibre, ash, available carbohydrates, pH, % acidity, soluble solids, and water activity of cape gooseberry samples, both untreated and treated with high hydrostatic pressure (500 MPa at 5, 10 and 15 min). A significant ($p \leq 0.05$) increase in the moisture content relative to that of the untreated sample was apparent for all samples treated with high hydrostatic pressure (HHP). The highly significant increase in moisture content may have been due to increased water absorption by proteins because it is known that high hydrostatic pressure can increase the hydration of proteins [29]. The protein content was significantly ($p \leq 0.05$) lower in cape gooseberry samples treated at 500 MPa than that in the control sample (untreated sample) at all times, probably because the increase in moisture exerts a dilution effect on the other constituents of the high-pressure-treated cape gooseberry samples, as was reflected in the levels of crude protein observed after high-pressure treatment; meanwhile, the fat, ash, available carbohydrates, acidity and soluble solids value of the untreated sample were significantly lower compared to the treated cape gooseberry sample ($p > 0.05$). In the same table, the water activity, which is an indicator of water availability, is shown to be the same for all of the samples, and the pH and crude fibre exhibit an increasing tendency in the treated Cape gooseberry sample with respect to the untreated apple sample and are significantly different ($p \leq 0.05$). The HHP-induced changes in the proximal composition observed in this study are in agreement with data regarding oysters treated with HHP [30].

3.2. Ascorbic Acid Content

Ascorbic acid is an important nutrient in fruits and vegetables and is also widely used as an antioxidant to prevent enzymatic browning in processing fruits [31]. Furthermore, ascorbic acid acts as an oxygen scavenger for the removal of molecular oxygen in enzymatic reactions, but at high temperatures it may react to form carbonylic compounds that are responsible for non-enzymatic browning [32]. Ascorbic acid is classified as a hydro-soluble vitamin, which is the reason for its abundance in fruits with water content that exceeds 50% [5].

The ascorbic acid contents of cape gooseberry purées after extraction conventional and high hydrostatic pressure are presented in **Figure 1**. The ascorbic acid content of fresh cape gooseberry was 26.31 mg 100 g^{-1}, which is in the range of values previously reported by Puente *et al.* (2010) [5] for cape gooseberry pulp. The ascorbic acid content in treated cape gooseberry purée ranged from 28.49 - 40.33 mg 100 g^{-1} pulp. In fact, the ascorbic acid levels were significantly higher for all high-pressure-

Table 1. Effect of high hydrostatic pressure on the proximal composition, pH, acidity (% citric acid), soluble solids (˚Brix) and water activity (Aw) in untreated and treated cape gooseberry. Values are mean ± standard deviation of triplicates; values followed by the same letter in the same row are not significantly different ($p \leq 0.05$).

Parameters	Untreated cape gooseberry sample 0.1 MPa	Treated cape gooseberry samples		
		500 MPa/5 min	500 MPa/10 min	500 MPa/15 min
Moisture (g/100 g)	77.30 ± 0.32^{a}	80.90 ± 1.07^{b}	80.75 ± 0.75^{b}	80.75 ± 0.75^{b}
Protein (g/100 g)	2.01 ± 0.03^{a}	1.16 ± 0.05^{b}	1.11 ± 0.11^{b}	1.62 ± 0.10^{c}
Fat (g/100 g)	0.90 ± 0.10^{a}	0.76 ± 0.05^{b}	0.83 ± 0.05^{ab}	0.65 ± 0.02^{c}
Crude fiber (g/100 g)	5.67 ± 0.01^{a}	6.36 ± 0.02^{b}	7.33 ± 0.01^{c}	7.25 ± 0.02^{d}
Ash (g/100 g)	1.88 ± 0.11^{a}	1.54 ± 0.07^{bc}	1.57 ± 0.07^{b}	1.42 ± 0.01^{c}
Available carbohydrates (g/100 g)	12.24 ± 0.23^{a}	9.28 ± 1.08^{b}	8.42 ± 0.69^{b}	8.02 ± 0.62^{b}
pH*	3.80 ± 0.01^{a}	4.20 ± 0.00^{b}	4.20 ± 0.01^{b}	4.20 ± 0.00^{b}
Acidity** (% citric acid)	1.24 ± 0.18^{a}	0.85 ± 0.02^{b}	0.84 ± 0.01^{b}	0.84 ± 0.02^{b}
Soluble solids (˚Brix)	15.0 ± 0.06^{a}	11.0 ± 0.06^{b}	10.9 ± 0.06^{c}	11.0 ± 0.00^{b}
Water activity*	0.931 ± 0.002^{a}	0.931 ± 0.001^{a}	0.931 ± 0.001^{a}	0.930 ± 0.001^{a}

*Adimensional and **by difference.

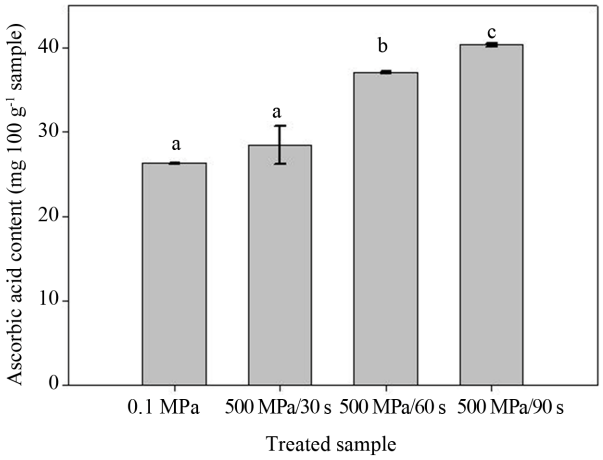

Figure 1. The vitamin C content of cape gooseberry obtained using CE and HHPE. Values are mean ± standard deviation (n = 3). Identical letters above the bars indicate no significant difference (p < 0.05).

treated samples than for samples obtained by conventional extraction ($p \leq 0.05$), showing increases of 9%, 41% and 53% after HPPE at 500 MPa for 30 s, 60 s and 90 s, respectively. The ascorbic acid content of the treated sample (46 mg·100 g^{-1}) is higher than that of most fruits, such as pear (4 mg·100 g^{-1}), apple (6 mg·100 g^{-1}), and peach (7 mg·100 g^{-1}), and it is somewhat comparable to that of orange (50 mg·100 g^{-1}) and strawberry (60 mg·100 g^{-1}) [6]. This vitamin plays an important role in human nutrition, including the growth and maintenance of tissues and the production of neurotransmitters, hormones and immune system responses.

The concentration of vitamin C is the most important indicator of the nutritional quality of fruit. The Recommended Daily Allowance (RDA) of vitamin C in the United States (Institute of Medicine of the National Academy of Sciences. Food and Nutrition Board) for adults between 31 and 50 years of age is 90 mg·day^{-1} for men and 75 mg·day^{-1} for women. Assuming that a standard cape gooseberry contains at least 4 mg of vitamin C per 10 g of cape gooseberry pulp, only 20 units of cape gooseberry are required to obtain all of the vitamin C required, according to the RDA for a standard serving in the USA.

3.3. Carotenoid Content

The high-pressure treatment of foods is not expected to have an adverse effect on low-molecular-weight components such as flavouring agents, pigments and vitamins because covalent bonds are not disrupted by the level of pressure that is normally used [33]. Research on the effects of high-pressure treatment on carotenoids in fruits and vegetables has focussed on tomatoes and tomato products, orange juice, persimmons and vegetables [34-38].

The provitamin A (β-carotene) contents of cape gooseberry purées after extraction using conventional methods and high hydrostatic pressure are presented in **Figure 2**. The β-carotene content of fresh cape gooseberry was 1074.67 ± 6.41 mg·100 g^{-1} sample, which is higher than that previously reported by Ramadan and Morsel [39] (432 mg·100 g^{-1} pulp) and Puente *et al.* [5] (1460 mg·100 g^{-1} pulp). Processing affected ($p \leq 0.05$) the β-carotene content at all times, showing 8%, 14% and 15% increases in retention at 500 MPa after 5 min, 10 min and 15 min of HPPE, respectively). Therefore, cape gooseberry could be a novel source of nutraceuticals or bioactive components of natural origin that can be utilised in food processing as natural additives and obviate the need for artificial additives. The availability of compounds can also be affected by HHP treatment due to changes in the molecular organisation of the lipid-peptide complex and disruption of the structure of the phosphatidic acid bilayer membrane.

These effects would lead to changes in the function of membrane-bound proteins that control ion permeability [40]. For instance, HHP treatment is known to affect vitamin stability and extraction yield, such as that of vitamin A in orange juice, [41] lycopene in tomato puree [36, 42], carotenoids in gazpacho and tomato puree [35,43] and tomato juices [44]. Carotenoids and vitamin A content have also been studied in persimmon puree [35].

Data published in the study "Global prevalence of vitamin A deficiency in populations at risk 1995-2005" by the World Health Organisation in 2009 indicate that 190 million preschool-age children and 19.1 million pregnant

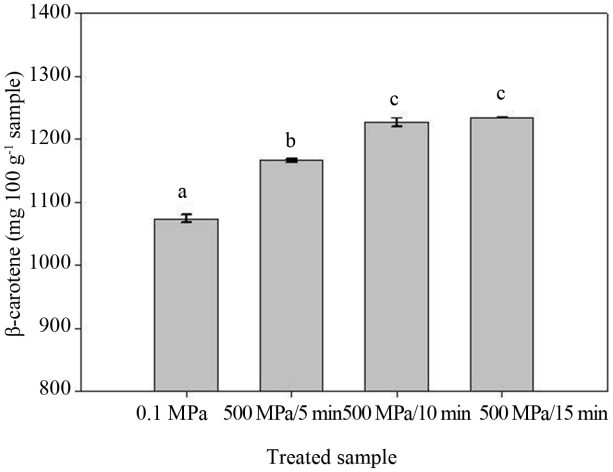

Figure 2. The β-carotene content of cape gooseberry obtained using CE and HHPE. Values are mean ± standard deviation (n = 3). Identical letters above the bars indicate no significant difference (p < 0.05).

women had serum retinol levels of less than 0.7 μmol/L, which is the lower limit of normal and below which is considered a state of vitamin A deficiency. The Institute of Medicine of the National Academy of Sciences (USA) has established recommended daily allowance levels for the population according to age, with an additional distinction for pregnant and lactating women. For adults between 31 and 50 years of age, for example, the Recommended Daily Allowance (RDA) value is 108 mg·day^{-1} for men and 84 mg·day^{-1} for women.

3.4. Antioxidant Capacity of Cape Gooseberry by DPPH and FRAP Assay

Table 2 shows the antioxidant capacity of the fruit extracts determined as Trolox equivalents (μM TE g^{-1} sample) using the DPPH and FRAP assays. The highest antioxidant content (86.80 ± 0.38 μM TE g^{-1} sample) was observed in the samples treated at 500 MPa for 15 min, followed by those treated at 500 MPa/10 min and 500 MPa/5 min (85.77 ± 2.27 μM TE g^{-1} sample and 66.92 ± 0.07 μM TE g^{-1} sample) and the control (68.89 ± 1.16 μM TE g^{-1} sample) using the DPPH assay.

Meanwhile, the values for total antioxidant activity determined by FRAP were 2.10%, 22.72% and 50.53% higher in samples treated at 500 MPa for 5 min, 10 min and 15 min, respectively, compared to the untreated sample (see **Table 1**). HHP significantly affected the extraction, allowing more antioxidants to be obtained from the extracts.

Interest in the antioxidant properties of fruits has recently increased [45,46], some of the medicinal properties of the fruit of *P. peruviana* L. are associated with the fruit's antioxidant capacity. Puente *et al.* [5] have reported the following values in terms of DPPH free radical scavenger (DPPH method) activity for fresh cape gooseberry: 210.82 ± 9.45 and 192.51 ± 30.13 μmol 100 g^{-1} sample; they have also reported antioxidant activity as the ability of the samples to reduce metals (FRAP), reporting values of 192.51 ± 30.13 and 54.98 ± 7.14 mg gallic acid 100 g^{-1} sample.

Table 2. Comparison between DPPH radical scavenging activity and FRAP, which measures the ability of a sample to reduce metals from cape gooseberry, after the application of CE and HHPE; values are expressed as μM TE g^{-1} sample. Values are mean ± standard deviation of triplicates; values followed by the same letter in the same column are not significantly different ($p \leq 0.05$).

Samples	DPPH (mM TE g^{-1} cape gooseberry)	FRAP (mM TE g^{-1} cape gooseberry)
0.1 MPa untreated	68.89 ± 1.16^{a}	82.83 ± 0.19^{a}
500 MPa/5 min	66.92 ± 0.07^{a}	84.57 ± 0.20^{a}
500 MPa/10 min	85.77 ± 2.27^{b}	97.60 ± 0.14^{b}
500 MPa/15 min	86.80 ± 0.38^{b}	124.68 ± 2.43^{c}

HHP increased the extraction yields due to its aptitude for deprotonating charged groups and disrupting salt bridges and hydrophobic bonds in cell membranes, which may lead to higher permeability [17,47].

According to Le Chatelier's theory [48], the volume of a system tends to decrease under pressure. During this process, the extracting solvent is absorbed into cells to integrate with bio-active components. Moreover, pressurised cells exhibit increased permeability. As the hydrostatic pressure increases, the amount of solvent that enters cells and the amounts of compounds that permeates out to the solvent increase. The equilibrium in the solvent concentration between the inside and outside of cells is established during the pressure-holding period. When the high pressure is suddenly released, the cell wall is disrupted to release the cytoplasm, which contains a high concentration of target material; thus, a short extraction period is enough to harvest a high concentration of extract. Under high pressure, larger molecules (proteins, starches, etc.) are denatured and do not enter the solvent; thus, the concentration of impurities in HHP extracts is lower than that in extracts obtained using other methods. Therefore, compounds are more accessible to extraction up to the equilibrium point [47].

FRAP, ABTS, DPPH and ORAC are the most common methods used to determine antioxidant capacity in vitro. It is recommended that at least two of these methods be used. FRAP measures the ability of a sample to reduce metals, while ABTS, DPPH and ORAC measure a sample's free radical scavenging capacity. From a chemistry standpoint, in FRAP and ABTS, there is a SET (Single Electron Transfer) reaction, while in ORAC there is a HAT (Hydrogen Atom Transfer) reaction; meanwhile, DPPH combines both reaction types [49-51]. Therefore, the FRAP assay is based on electron transfer reactions, whereas the DPPH assay evaluates both electron transfer and hydrogen atom transfer reactions [50]. Hence, similar antioxidant compounds might react differently in different assays.

4. Conclusion

In the present study, raw cape gooseberry extracts obtained by HHPE were compared with those obtained by a conventional extraction method. HHPE provided a higher extraction yield and required less extraction time. The greater vitamin C and β-carotene contents and the stronger antioxidant activity (FRAP and DPPH) of cape gooseberry extracts obtained by HHPE were also observed. Thus, HHPE could be used as an alternative to conventional extraction to extract bioactive compounds from

cape gooseberry. A further comparison of the operational costs between HHPE and CE are needed to assess commercial use.

5. Acknowledgements

The authors wish to acknowledge the financial support of Fondecyt Regular n° 1120069, CEAZA and the Research Department of Universidad de La Serena (DIULS). We also thank the members of the Main Laboratory of Universidad de La Serena for their service and assistance.

REFERENCES

[1] D. Hervert-Hernández, O. García, J. L. Rosado and I. Goñi, "The Contribution of Fruit and Vegetables to Dietary Intake of Polyphenols and Antioxidant Capacity in a Mexican Rural Diet: Importance of Fruit and Vegetable Variety," *Food Research International*, Vol. 44, No. 5, 2011, pp. 1182-1189. doi:10.1016/j.foodres.2010.09.021

[2] G. Du, M. Li, F. Ma and D. Liang, "Antioxidant Capacity and the Relationship with Polyphenol and Vitamin C in Actinidia Fruits," *Food Biotechnology*, Vol. 113, No. 2, 2009, pp. 557-562.

[3] M. Ozgen, R. N. Reese, A. Tulio, J. Scheerens and R. Miller, "Modified 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid (ABTS) Method to Measure Antioxidant Capacity of Selected Small Fruits and Comparison to Ferric Reducing Antioxidant Power (FRAP) and 2,2'-Diphenyl-1-oicrylhydrazyl (DPPH) Methods," *Journal Agricultural and Food Chemistry*, Vol. 54, No. 4, 2006, pp. 1151-1157. doi:10.1021/jf051960d

[4] E. M. Kuskoski, A. G. Asuero, A. M. Troncoso, J. Mancini-Filho and R. Fett, "Aplicación de Diversos Métodos Químicos Para Determinar Actividad Antioxidante en Pulpa de Frutos," *Ciência e Tecnologia de Alimentos*, Vol. 25, No. 4, 2005, pp. 726-732. doi:10.1590/S0101-20612005000400016

[5] L. A. Puente, C. A. Pinto-Muñoz, E. S. Castro and M. Cortés, "Physalis Peruviana Linnaeus, the Multiple Properties of a Highly Functional Fruit: A Review," *Food Research International*, Vol. 44, No. 7, 2010, pp. 1733-1740. doi:10.1016/j.foodres.2010.09.034

[6] M. F. Ramadan, "Bioactive Phytochemicals, Nutritional and Funtional Properties of Cape Gooseberry (Physalis Peruviana): An Overview," *Food Research International*, Vol. 44, No. 7, 2011, pp. 1830-1836. doi:10.1016/j.foodres.2010.12.042

[7] A. M. Castro, L. Rodriguez and E. M. Vargas, "Dry Gooseberry (*Physalis peruviana L*) with Pretreatment of Osmotic Dehydration," *Revista de la Facultad de Química Farmacéutica*, Vol. 15, No. 2, 2008, pp. 226-231.

[8] M. F. Ramadan and J. Morsel, "Oil Goldenberry (Physalis Peruviana)," *Journal of Agricultural and Food Chemistry*, Vol. 51, No. 4, 2003, pp. 969-974. doi:10.1021/jf020778z

[9] P. C. Cardoso, A. P. B. Tomazini, P. C. Stringheta, S. M. R. Ribeiro and H. M. Pinheiro-Sant'Ana, "Vitamin C and Carotenoids in Organic and Conventional Fruits Grown in Brazil," *Food Chemistry*, Vol. 126, No. 2, 2011, pp. 411-416. doi:10.1016/j.foodchem.2010.10.109

[10] L. M. Laur and L. Tian, "Provitamin A and Vitamin C Contents in Selected California-Grown Cantaloupe and Honeydew Melons and Imported Melons," *Journal of Food Composition and Analysis*, Vol. 24, No. 2, 2011, pp. 194-201. doi:10.1016/j.jfca.2010.07.009

[11] K. M. Phillips, M. T. Tarragó-Trani, S. E. Gebhardt, J. Exler, K. Y. Patterson, D. B. Haytowitz, P. R. Pehrsson and J. M. Holden, "Stability of Vitamin C in Frozen Raw Fruit and Vegetable Homogenates," *Journal of Food Composition and Analysis*, Vol. 23, No. 3, 2010, pp. 253-259. doi:10.1016/j.jfca.2009.08.018

[12] C. Mertz, P. Brat, C. Caris-Veyrat and Z. Gunata, "Characterization and Thermal Lability of Carotenoids and Vitamin C of Tamarillo Fruit (*Solanum betaceum Cav*.)," *Food Chemistry*, Vol. 119, No. 2, 2010, pp. 653-659. doi:10.1016/j.foodchem.2009.07.009

[13] F. M. Campos, S. R. M. Ribeiro, C. M. D. Lucia, H. M. Pinheiro-Sant'Ana and P. C. Stringheta, "Optimization of Methodology to Analyze Ascorbic and Dehydroascorbic Acid in Vegetables," *Quim Nova*, Vol. 32, No. 1, 2009, pp. 87-91. doi:10.1590/S0100-40422009000100017

[14] Y. Hernández, M. G. Lobo and M. González, "Determination of Vitamin C in Tropical Fruits: A Comparative Evaluation of Methods," *Food Chemistry*, Vol. 96, No. 4, 2006, pp. 654-664. doi:10.1016/j.foodchem.2005.04.012

[15] S. Zhang, Z. Junjie and W. Changzhen, "Novel High Pressure Extraction Technology," *International Journal Pharmaceutics*, Vol. 278, No. 2, 2004, pp. 471-474. doi:10.1016/j.ijpharm.2004.02.029

[16] X. Jun, S. Deji, L. Ye and Z. Rui, "Comparison of *in Vitro* Antioxidant Activities and Bioactive Components of Green Tea Extracts by Different Extraction Methods," *International Journal Pharmaceutics*, Vol. 408, No. 1-2, 2011, pp. 97-101. doi:10.1016/j.ijpharm.2011.02.002

[17] M. Corrales, S. Toepfl, P. Butz, D. Knorr and B. Tauscher, "Extraction of Anthocyanins from Grape By-Products Assisted by Ultrasonics, High Hydrostatic Pressure or Pulsed Electric Fields: A Comparison," *Innovative Food Science and Emerging Technologies*, Vol. 9, No. 1, 2008, pp. 85-91. doi:10.1016/j.ifset.2007.06.002

[18] J. Ahmed and H. S. Ramaswamy, "High Pressure Processing of Fruit and Vegetables," *Stewart Postharvest Review*, Vol. 2, No. 5, 2006, pp. 1-8. doi:10.2212/spr.2006.5.9

[19] S. Zhang, J. Xi and C. Z. Wang, "Effect of High Hydrostatic Pressure on Extraction of Flavonoids in Propolis," *Food Science and Technology International*, Vol. 11, No. 3, 2005, pp. 213-216. doi:10.1177/1082013205054420

[20] K. N. Prasad, B. Yang, M. Zhao, N. Ruenroengklin and Y. Jiang, "Application of Ultrasonication or High-Pressure Extraction of Flavonoids from Litchi Fruit Pericarp," *Journal of Food Process Engineering*, Vol. 32, No. 6, 2009, pp. 828-843. doi:10.1111/j.1745-4530.2008.00247.x

[21] S. Zhang, C. Ruizhan and W. Changzheng, "Experiment Study on Ultrahigh Pressure Extraction of Ginsenosides," *Journal of Food Engineering*, Vol. 79, No. 1, 2007, pp. 1-5. doi:10.1016/j.jfoodeng.2005.12.048

[22] S. Zhang, R. Chen, H. Wu and C. Wang, "Ginsenoside Extraction from *Panax quinquefolium* L. (American Ginseng) Root by Using Ultrahigh Pressure," *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 41, No. 1, 2006, pp. 57-63. doi:10.1016/j.jpba.2005.10.043

[23] S. Zhang, H. Bi and C. Liu, "Extraction of Bio-Active Components from Rhodiola Sachalinensis under Ultrahigh Hydrostatic Pressure," *Separation and Purification Technology*, Vol. 57, No. 2, 2007, pp. 277-282. doi:10.1016/j.seppur.2007.04.022

[24] Association of Official Analytical Chemists (AOAC), "Official Methods of Analysis," 13th Edition, Washington DC, 1990.

[25] N. Koka, H. S. Burdurlu and F. Karadeniz, "Kinetics of Colour Changes in Dehydrated Carrots," *Journal of Food Engineering*, Vol. 78, No. 2, 2007, pp. 449-455. doi:10.1016/j.jfoodeng.2005.10.014

[26] W. Brand-Williams, M. E. Cuvelier and C. Berset, "Use of Free Radical Method to Evaluate Antioxidant Activity," *Lebensmittel Wissenschaft und Technologie*, Vol. 28, No. 1, 1995, pp. 25-30.

[27] D. O. Kim, K. W. Lee, H. J. Lee and C. Y. Lee, "Vitamin C Equivalent Antioxidant Capacity (VCEAC) of Phenolic Phytochemicals," *Journal Agricultural and Food Chemistry*, Vol. 50, No. 13, 2002, pp. 3713-3717. doi:10.1021/jf020071c

[28] I. F. F. Benzie and J. J. Strain, "The Ferric Reducing Ability of Plasma (FRAP) as a Measure of 'Antioxidant Power': The FRAP Assay," *Analitycal Biochemistry*, Vol. 239, No. 1, 1996, pp. 70-76. doi:10.1006/abio.1996.0292

[29] J. L. Silva, D. Foguel and C. A. Royer, "Review: Pressure Provides New Insights into Protein Folding, Dynamics and Structure," *Trends in Biochemical Sciences*, Vol. 26, No. 10, 2001, pp. 612-618. doi:10.1016/S0968-0004(01)01949-1

[30] M. Cruz-Romero, M. Smiddy, C. Hill, J. P. Kerry and A. L. Kelly, "Effects of High Pressure Treatment on Physicochemical Characteristics of Fresh Oysters (*Crassostrea gigas*)," *Innovative Food Science and Emerging Technologies*, Vol. 5, No. 2, 2004, pp. 161-169. doi:10.1016/j.ifset.2004.01.002

[31] K. S. Lee and A. A. Kader, "Preharvest and Postharvest Factors Influencing Vitamin C Content of Horticultural Crops," *Postharvest Biology and Technology*, Vol. 20, No. 3, 2000, pp. 207-220. doi:10.1016/S0925-5214(00)00133-2

[32] P. Elez-Martínez, R. C. Soliva-Fortuny and O. Martín-Belloso, "Comparative Study on Shelf Life of Orange Juice Processed by High Intensity Pulsed Electric Fields or Heat Treatment," *European Food Research and Technology*, Vol. 222, No. 3-4, 2006, pp. 321-329. doi:10.1007/s00217-005-0073-3

[33] B. Tauscher, "Pasteurization of Food by Hydrostatic High Pressure: Chemical Aspects," *Zeitung Lebensmittel Unters Forschung*, Vol. 200, No. 1, 1995, pp. 3-13. doi:10.1007/BF01192901

[34] A. Fernandez-Garcia, P. Butz and B. Tauscher, "Effects of High-Pressure Processing on Carotenoid Extractability, Antioxidant Activity, Glucose Diffusion, and Water Binding of Tomato Puree (*Lycopersicon esculentum Mill.*)," *Journal of Food Science*, Vol. 66, No. 7, 2001, pp. 1033-1038. doi:10.1111/j.1365-2621.2001.tb08231.x

[35] B. De Ancos, E. Gonzalez and M. P. Cano, "Effect of High-Pressure Treatment on the Carotenoid Composition and the Radical Scavenging Activity of Persimmon Fruit Purees," *Journal of Agricultural and Food Chemistry*, Vol. 48, No. 8, 2000, pp. 3542-3548. doi:10.1021/jf990911w

[36] B. Krebbers, A. Matser, S. W. Hoogerwerf, R. Moezelaar, M. M. M. Tomassen and R. W. Van der Berg, "Combined High-Pressure and Thermal Treatments for Processing of Tomato Puree: Evaluation of Microbial Inactivation and Quality Parameters," *Innovative Food Science and Emerging Technologies*, Vol. 4, No. 4, 2003, pp. 377-385. doi:10.1016/S1466-8564(03)00045-6

[37] P. Butz, R. Edenharder, A. Fernandez Garcia, H. Fister, C. Merkel and B. Tauscher, "Changes in Functional Properties of Vegetables Induced by High Pressure Treatment," *Food Research Institute Studies*, Vol. 35, No. 2-3, 2002, pp. 295-300. doi:10.1016/S0963-9969(01)00199-5

[38] J. McInerney, C. Seccafien, C. Stewart and A. Bird, "Effects of High Pressure Processing on Antioxidant Activity, and Total Carotenoid Content and Availability, in Vegetables," *Innovative Food Science and Emerging Technologies*, Vol. 8, No. 4, 2007, pp. 543-548. doi:10.1016/j.ifset.2007.04.005

[39] M. F. Ramadan and J. T. Mörsel, "Impact of Enzymatic Treatment on Chemical Composition, Physicochemical Properties and Radical Scavenging Activity of Goldenberry (*Physalis peruviana* L.) Juice," *Journal of the Science of Food and Agriculture*, Vol. 87, No. 3, 2007, pp. 452-460. doi:10.1002/jsfa.2728

[40] H. Dornenburg and D. Knorr, "Cellular Permeabilization of Cultured Plant Tissues by High Electric Field Pulses or Ultra-High Pressure for the Recovery of Secondary Metabolites," *Food Biotechnology*, Vol. 7, No. 1, 1993, pp. 35-48. doi:10.1080/08905439309549844

[41] B. De Ancos, S. Sgroppo, L. Plaza and M. P. Cano, "Possible Nutritional and Health Related Value Promotion in Orange Juice Preserved by High-Pressure Treatment," *Journal of the Science of Food and Agriculture*, Vol. 82, No. 8, 2002, pp. 790-796. doi:10.1002/jsfa.1093

[42] W. Qiu, H. Jiang, H. Wang and Y. Gao, "Effect of High Hydrostatic Pressure on Lycopene Stability," *Food Chemistry*, Vol. 97, No. 3, 2006, pp. 516-523. doi:10.1016/j.foodchem.2005.05.032

[43] L. Plaza, C. Sánchez-Moreno, B. De Ancos and M. P. Cano, "Carotenoid Content and Antioxidant Capacity of Mediterranean Vegetable Soup (*gazpacho*) Treated by Highpressure/ Temperature During Refrigerated Storage," *European Food Research and Technology*, Vol. 223, No. 2, 2006, pp. 210-215. doi:10.1007/s00217-005-0174-z

[44] S. Dede, H. Alpas and A. Bayindirli, "High Hydrostatic

Pressure Treatment and Storage of Carrot and Tomato Juices: Antioxidant Activity and Microbial Safety," *Journal of the Science of Food and Agriculture*, Vol. 87, No. 5, 2007, pp. 773-782. doi:10.1002/jsfa.2758

[45] H. Liu, N. Qiu, H. Ding and R. Yao, "Polyphenols Contents and Antioxidant Capacity of 68 Chinese Herbals Suitable for Medical or Food Uses," *Food Research International*, Vol. 41, No. 4, 2008, pp. 363-370. doi:10.1016/j.foodres.2007.12.012

[46] C. K. R. Vijaya, D. Sreeramulu and M. Raghunath, "Antioxidant Activity of Fresh and Dry Fruits Commonly Consumed in India," *Food Research International*, Vol. 43, No. 1, 2010, pp. 285-288. doi:10.1016/j.foodres.2009.10.006

[47] G. V. Barbosa-Canovas, U. R. Pothakamury, E. Palou and B. G. Swanson, "In Non Thermal Preservation of Foods," Marcel Dekker, New York, 1998.

[48] Z. Shou-qin, B. Hui-min and L. Chang-Jiao, "Extraction of Bio-Active Components from *Rhodiola sachalinensis* under Ultrahigh Hydrostatic Pressure," *Separation and Purification Technology*, Vol. 57, No. 2, 2007, pp. 277-282. doi:10.1016/j.seppur.2007.04.022

[49] M. C. Foti, C. Dasquino and C. Geraci, "Electron-Transfer Reaction of Cinnamic Acids and Their Methyl Esters with the DPPH Radical in Alcohol Solutions," *Journal of Organic Chemistry*, Vol. 69, No. 7, 2004, pp. 2309-2314. doi:10.1021/jo035758q

[50] R. L. Prior, X. Wu and K. Schaich, "Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements," *Journal of Agricultural and Food Chemistry*, Vol. 53, No. 10, 2005, pp. 4290-4302. doi:10.1021/jf0502698

[51] J. Pérez-Jiménez, S. Arranz, M. Tabernero, M. E. Díaz-Rubio, J. Serrano, I. Goní and F. Saura-Calixto, "Updated Methodology to Determine Antioxidant Capacity in Plant Foods, Oils and Beverages: Extraction, Measurement and Expression of Results," *Food Research International*, Vol. 41, No. 3, 2008, pp. 274-285. doi:10.1016/j.foodres.2007.12.004

