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Assessment of nutritional and metabolic profiles of pea shoots: The new ready-to-eat baby-leaf vegetable

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ABSTRACT

Pea-shoots are a new option as ready-to-eat baby-leaf vegetable. However, data about the nutritional composition and the shelf-life stability of these leaves, especially their phytonutrient composition is scarce. In this work, the macronutrient, micronutrient and phytonutrients profile of minimally processed pea shoots were evaluated at the beginning and at the end of a 10-day storage period. Several physicochemical characteristics (color, pH, total soluble solids, and total titratable acidity) were also monitored. Standard AOAC methods were applied in the nutritional value evaluation, while chromatographic methods with UV–vis and mass detection were used to analyze free forms of vitamins (HPLC-DAD-ESI-MS/MS), carotenoids (HPLC-DAD-APCI-MS^n) and flavonoid compounds (HPLC-DAD-ESI-MS^n). Atomic absorption spectrometry (HR-CS-AAS) was employed to characterize the mineral content of the leaves. As expected, pea leaves had a high water (91.5%) and low fat (0.3%) and carbohydrate (1.9%) contents, being a good source of dietary fiber (2.1%). Pea shoots showed a high content of vitamins C, E and A, potassium and phosphorous compared to other ready-to-eat green leafy vegetables. The carotenoid profile revealed a high content of β-carotene and lutein, typical from green leafy vegetables. The leaves had a mean flavonoid content of 329 mg/100 g of fresh product, mainly composed by glycosylated quercetin and kaempferol derivatives. Pea shoots kept their fresh appearance during the storage being color maintained throughout the shelf-life. The nutritional composition was in general stable during storage, showing some significant ($p < 0.05$) variation in certain water-soluble vitamins.

1. Introduction

The consumption of green leafy vegetables is recommended due to their high content of vitamins, minerals and antioxidant phytochemicals, as well as low content of fat and carbohydrates (Rico, Martín-Diana, Barat, & Barry-Ryan, 2007). Minimally processed vegetables sold as ready-to-eat salads are a convenient way to include vegetables in the diet. To increase variety and attract even more consumers, the fresh-cut producers seek for new varieties of leafy vegetables to add to ready-to-eat salad mixtures (Martínez-Sánchez et al., 2012). Pea shoots were recently presented as a ready-to-eat vegetable, and are recognized as a popular specialty vegetable in some parts of Asia and Africa that is gaining popularity in the United States and Europe (Miles & Sonde, 2003).

Peas (*Pisum sativum*) are among the most consumed vegetables worldwide, with a registered global production of 15 million tons in 2010 (FAO, 2013). It is normally consumed as a seed food, and is a good source of proteins, vitamins and minerals (Martins, 2010). The consumption of leaves of the pea plants, also known as pea shoots, is not as common as eating the peas. They are harvested in a very early maturation stage, when the leaves and tendrils are tender, crispy and have an intense pea flavor (Miles & Sonde, 2003). This baby-leaf green leafy vegetable can be eaten raw in salads, or cooked with others ingredients ("Pea shoots, 2013"). Accordingly to Miles and Sonde (2003), pea shoots are a very perishable product with a high market value, when compared to other common leafy vegetables. As a minimally processed vegetable, pea leaves can be packed solely or in ready-to-eat salad mixtures and their quality and safety is strictly dependent on the maintenance of refrigerating conditions during storage (Rico et al., 2007).

The pea plant is one of the most-studied vegetables, being a well-established classic model for genetics and agronomic studies (Edelenbos, Christensen, & Grevsen, 2001; Hamada & El-Enany, 1994; Wong, Bhalla, Ottenhof, & Singh, 2008). Its origins are in Middle East and Mediterranean regions, integrating the diet of early civilizations (Smýkal, Coyne, Redden, & Maxted, 2013). The nutritional composition of peas is published in official nutritional tables (Martins, 2010). On the other hand, the nutritional quality of pea shoots is not mentioned. There are however some nutritional allegations of being rich in vitamin C and A in the producers' *website* ("Pea shoots, 2013"). Specific scientific data regarding the nutritional composition of pea shoots is scarce, being most of the available information based in the generalization of the

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green leafy vegetables composition (Miles & Sonde, 2003). In this context, the objective of this work was to characterize and compare physicochemical characteristics as well as nutritional quality and phytonutrients composition of minimally processed pea shoots stored under refrigerated conditions. Color, total soluble solids (TSS), total titratable acidity (TTA), pH, macronutrient composition and also minerals, vitamins, carotenoids and flavonoids contents of pea shoots were assessed.

2. Material and Methods

2.1. Samples

Minimally processed pea shoots (*Pisum sativum*) were obtained from a producer (Odemira, Portugal). Upon arrival to the laboratory, one day after processed (washed, cut and packed), pea shoots were divided in two groups. One was prepared for analysis and the second was stored under refrigerated conditions (3 ± 1 °C) for 10 days. About 200 g of fresh leaves from each group were used for color, TSS, TTA, pH and macronutrient analyses. The fresh leaves were grinded in a knife mill and used for protein, fat, ash and dietary fiber determinations. Vitamins, minerals, carotenoids and flavonoids were determined in freeze-dried pea shoots samples (Telstar Cryodos-80, Terrassa, Barcelona), that were powdered in a knife mill (GM 200, RETSCH, Haan, Germany) and stored protected from light, oxygen and heat until analysis.

2.2. Quality analysis

2.2.1. Physicochemical characteristics

Leaves color parameters L*, a* and b* were determined with a tri-stimulus colorimeter (CR-400Chroma Meter, Konica Minolta, Japan), where L* defines the lightness ($0 < L^* < 100$) variation. Parameters a* define the red (+) to green (−) and b* the blue (−) to yellow (+) chromaticity. These were used to calculate the hue angle ($h^o = \arctan(b^*/a^*)$) and chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) values. The equipment was set up for illuminant D65 with 10º observer angle and calibrated using a standard white plate. Forty measurements were made in different leaves at each sampling day. Total soluble solids (TSS) were determined on pea shoots juice, obtained by grinding 10 g of fresh leaves in a knife mill, in a Digital Refractometer (ºBrix, HI 9680, Hanna Instruments, EUA). The pH was measured with a pH-meter (Crison Instruments, Barcelona, Spain) in 10 g of leaves homogenized in 20 mL of deionised water (AOAC, 2000). Total titratable acidity (TTA) was determined accordingly to the Official method 942.15 (AOAC, 2000). Briefly, 10 grams of fresh leaves were homogenized in 100 ml of deionized water and then titrated with 0.1 M NaOH to pH 8.1 and expressed as the units of citric acid (mg/100 g) on a fresh weight (f.w.) basis.

2.2.2. Nutritional Composition

The water, protein (factor of 6.25), fat, ashes and total dietary fiber contents were determined accordingly to the AOAC (2000) methods, in the samples after one and ten days of storage. Protein content was estimated by the Kjeldahl method, fat by Soxhlet extracting method, whereas ash content was determined by incineration at 600 ± 15 °C and dietary fiber by an enzymatic gravimetric method. All values were presented as a percentage, being carbohydrates calculated by difference. All proximate composition analyses were done, at least, in triplicate. Energy was calculated according Atwater Factors (Otten, Hellwig, & Meyers, 2006).

Mineral composition was evaluated by a High Resolution-Continuum Source Atomic Absorption Spectrophotometric (HR-CS-AAS) method optimized by Santos, Oliva-Teles, Delerue-Matos, and Oliveira (2014). Briefly, 150 mg of freeze dried pea shoots were digested with 9 ml of nitric acid diluted with ultrapure water (43.3%) by microwave assisted digestion (MARS-X, CEM, Mathews, NC, USA). Potassium, sodium, calcium, magnesium, iron, manganese and zinc were analyzed with flame atomization (FAAS) (ContrAA 700, Analytik Jena, Germany), while copper was determined by electrothermal (EAAS) atomization. Phosphorous content was measured according to the 4500-P standard method (Greenberg, Clesceri, & Eaton, 1992) the vanadomolybdophosphoric acid colorimetric method in a UV–vis spectrophotometer (Evolution™ 300, Thermo Scientific, Waltham, MA, USA). Four replicates of pea shoots from each sampling day were used in minerals determination.

Several free forms of water-soluble vitamins (C, B_1, B_2, B_3, B_5, B_6 and B_9) and fat-soluble vitamins (Pro-vitamin A and E (α-tocopherol)) were assessed by HPLC-MS/MS and HPLC-DAD methods described by Santos, Mendiola, Oliveira, Ibáñez, and Herrero (2012). Briefly, 250 mg of freeze dried sample was extracted with 16 mL of 10 mM ammonium acetate/methanol 1:1 (v/v) in an ultrasound bath for 15 minutes. After centrifugation (14000 g; 15 min) the supernatant was concentrated under nitrogen stream and injected into a HPLC–ESI-MS/MS system (Thermo Scientific, San Jose, CA, USA) to determine the water-soluble vitamin content. The solid residue was re-extracted twice with ethyl acetate (0.1% BHT) (6 + 6 mL) in an ultrasound bath (15 min). After centrifuged (14000 *g*, 15 min), the two supernatants were combined and dried under nitrogen stream. The residue was re-dissolved in 3 mL of ethyl acetate and injected in a HPLC-DAD system (Agilent 1100 Santa Clara, CA, USA) to determine fat-soluble vitamin content of the samples. Pea shoot vitamin contents were determined along the storage period (day 1 and day 10). The results were expressed as mg/100 g, with exception for vitamin A, expressed as mg Retinol Activity Equivalent (RAE) calculated accordingly to the following equation: 1 mg RAE = 12 mg β-carotene (Otten et al., 2006).

2.2.3. Carotenoid profile

The extraction procedure used to study the carotenoid profile was described previously for the analysis of fat-soluble vitamins (Santos et al., 2012). Once re-dissolved, the extract was filtered through a 0.45 μm nylon filter and injected in a HPLC-DAD-APCI-MS^n system. The equipment used was an Agilent 1200 liquid chromatograph (Agilent, Santa Clara, CA, USA) equipped with an autosampler, a DAD, and directly coupled to an ion trap mass spectrometer (Agilent ion trap 6320) via an atmospheric pressure chemical ionization (APCI) interface, using an YMC C30 analytical column (5 μm particle size, 250 × 4.6 mm i.d.) (YMC, Schermbeck, Germany). The mobile phases (A: methanol/water, 90:10 v/v; B: Methyl *tert*-butyl ether/methanol/water, 90:6:4, v/v/v) eluted in the following gradient: 0 min, 6.5%B; 8 min, 6.5%B; 43 min, 100%B; 46 min, 6.5%B; 55 min, 6.5%B. The flow rate was 1 mL min^{-1} and the injection volume 10 μL. The DAD recorded the spectra from 220 to 700 nm, and the chromatograms were monitored at 450 nm. MS analysis was conducted with APCI in positive ionization mode using the following parameters: capillary voltage, −3.5 kV; drying temperature, 350 °C; vaporizer temperature, 400 °C; drying gas flow rate, 5 L/min; corona current (which sets the discharge amperage for the APCI source), 4000 nA; nebulizer gas pressure, 60 psi. A range from m/z 150 to m/z 1300 was acquired and MS/MS automatic mode was used on the more abundant ions in the MS spectra to identify the principal fragmentation ions. The major carotenoids were identified by combining absorption spectroscopic data, chromatographic properties and MS information with the values obtained from available standards and data reported in the literature. To quantify the carotenoids, six different concentrations were used to construct a calibration curve of lutein (linear range 10–200 μg mL^{-1}, $R^2 > 0.998$) and β-carotene (6.25–250 μg mL^{-1}, $R^2 > 0.999$). All xanthophylls were quantified as lutein equivalents, while the carotene isomers were quantified as β-carotene equivalents. The results were expressed in mg/100 g of fresh weight (f.w.), as mean ± standard deviation of two extracts from each sampling day.

2.2.4. Flavonoid compounds characterization

The flavonoids present in pea shoots were analyzed by an HPLC-DAD-ESI-MS^n method. Briefly, 500 mg of freeze dried pea shoots were