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 composi-tion 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. Theleaves 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 colormaintained throughout the shelf-life. The nutritional composition was in general stable during storage, showing some significant (p b 0.05) variation in certain water-soluble vitamins.

Keywords:
Pea shoots, Nutritional composition, Carotenoid profile, Flavonoid profile, Storage

1. Introduction

The consumption of green leafy vegetables is recommended due to their high content of vitamins, minerals and antioxidant phytochemi- cals, 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 ingre- dients ("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 mix- tures and their quality and safety is strictly dependent on the mainte- nance 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

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 weredivided 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 determi- nations. Vitamins, minerals, carotenoids and flavonoids were deter- mined 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 b L* b 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 es- timated 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 differ- ence. All proximate composition analyses were done, at least, in tripli- cate. 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, calci- um, magnesium, iron, manganese and zinc were analyzed with flame atomization (FAAS) (ContrAA 700, Analytik Jena, Germany), while cop- per was determined by electrothermal (EAAS) atomization. Phospho- rous 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 centrifu- gation (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 centri- fuged (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 moni- tored 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 proper- ties and MS information with the values obtained from available stan- dards 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 N 0.998) and β-carotene (6.25–250 μg mL^{-1}, R^2 N 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 extracted with 70% MeOH in a pressurized liquid extraction system (ASE 200, Dionex, Sunnyvale, CA, USA) with 11 mL extraction cells and following a procedure previously described (Miron, Plaza, Bahrim, Ibáñez, & Herrero, 2011). The extraction conditions were the following: extraction time, 20 min; temperature, 70 °C; pressure 10 MPa; flush volume, 60%. The extracts were first dried in a Rotavapor R-210 (Büchi, Labortechnik AG, Flawil, Switzerland) and later freeze-dried (Labconco Corporation, Missouri, USA). The dried extracts were re-

dissolved in 70% methanol (5 mg mL^{-1}) and filtered through a 0.45 μm disposable syringe filter. For the study of acyl flavonoid deriva- tives, an alkaline hydrolysis was carried out to eliminate acid moieties (p-coumaroyl, caffeoyl, feruloyl and sinapoyl), following the procedure described by Francisco et al. (2009).

The analyses of both extracts (native and hydrolyzed) were carried out on 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 electrospray interface. The column was a Zorbax Eclipse XBD C18 (5 μm, 150 × 4.6 mm) (Agilent, Santa Clara, CA) and the mobile phases (A: 0.1% formic acid; B: methanol with 0.1% formic acid), elut- ed with the following gradient: 0 min, 95% A; 4 min, 95% A; 20 min, 73% A; 50 min, 5% A; 57 min, 99% A; 58 min, 99% A; 60 min, 95% A.

A flow rate used was of 0.7 mL min^{-1} and the injection volume of 10 μL. The UV–vis spectra were recorded from 200 to 550 nm and the chromatograms were monitored at 330 nm. The MS detector operated under ESI negative ionization mode, with dry temperature of 350 °C; dry gas flow of 12 L min^{-1}; nebulizer gas pressure of 40 psi and 3500 V of capillary voltage. A mass scan range was set from m/z 100 to 1000 and MS/MS automatic mode was also used. The flavo- noids were characterized according to their retention time, UV–vis and mass spectra compared to information available in the literature and available commercial standards. To quantify the flavonoid con- tents, a calibration curve was obtained from seven different concen- trations of quercetin-3-O-glucoside (linear range: 1.0–66.7 μg mL^{-1}, R^2 N 0.999) and kaempferol-3-O-glucoside (linear range: 0.2–14.8 μg mL^{-1}, R^2 N 0.999). All quercetin derivatives were quantified though the quercetin-3-O-glucoside calibration curve, while kaempferol- 3-O-glucoside calibration curve was used in the case of kaempferol derivatives. The results were grouped as quercetin and kampferol derivatives, and the values presented represent the sum of the individ- ual quercetin and kaempferol derivatives compounds, respectively, found in the extracts. The results were expressed in mg/100 g of fresh weight (f.w.), as mean ± standard deviation of two extracts.

2.3. Statistical analysis

Data were expressed as mean ± standard deviation and the differ- ences between the two days of sampling (day 1 and day 10) were tested by the one-way ANOVA. Normal distribution of data in the different samples was assessed by Kolmogorov–Smnirnov test. Statistical signifi- cance was defined for a p b 0.05 (95% confidence level). The statistical analyses were carried out using the Statistica 8.0 software (Statsoft Inc., Tulsa, USA).

3. Results and discussion

Convenience is the key factor that leads consumers to choose mini- mally processed vegetables, increasing the intake of fresh products in their diets (Barrett, Beaulieu, & Shewfelt, 2010). Although it could not be directly appreciated by the consumer, the nutritional quality of these products is also becoming a choosing factor, due to an increased perception of the possibility of preserving health by choosing a balanced diet (Poiroux-Gonord et al., 2010). Due to the lack of specific informa- tion about the nutritional composition of pea shoots, this work focused on achieving a comprehensive characterization of nutritional quality of these leaves. Micronutrient composition (vitamins and minerals) of pea leaves was evaluated, knowing that it is determined by genetic factors, but it also reflects the agronomic practices and environmental condi- tions during their growth and storage (Hanson, Yang, Chang, Ledesma, & Ledesma, 2011; Santos et al., 2012). Carotenoids and flavonoids are phytonutrients with recognized beneficial antioxidant properties (Poiroux-Gonord et al., 2010). Their content is also affected by intrinsic and external factors. The levels of each nutrient were compared during the storage period to evaluate the stability of the bioactive compounds.

3.1. Evolution of physicochemical characteristics

The evolution of physicochemical characteristics analyzed in pea shoot is presented in Table 1. These parameters monitored the evolution of quality characteristics with major influence on the consumer's choice: color/appearance and flavor (Barrett et al., 2010). The color of minimally processed vegetables can suffer changes during storage, being the loss of greenness and the appearance of a yellowish tonality signs of the onset of senescence reactions (Barrett et al., 2010; Kidmose, Edelenbos, & R.N., 2002). Pea shoots leaves showed a dark green color (Hue angle of 122º) that was constant during the storage period. No significant differences (p b 0.05) were found between a* values from the beginning and end of storage, which corroborates the preservation of the green tonality. Relatively to the other color parameters, the changes were less than 6% of the initial value recorded, being the fresh appearance maintained throughout the refrigerated storage. Normally, an evolution of L* values lower than 3 units is not detectable by consumers (Tomás-Callejas, Boluda, Robles, Artés, & Artés-Hernández, 2011). A slight increase of lightness (L*) values (between 7 and 9%) was also described during storage of red chard baby leaves (Tomás-Callejas et al., 2011), whereas a decrease of L* values was registered in watercress baby leaves (Hinojosa et al., 2013). The levels of TSS revealed a slight decrease (− 18%) during storage, being followed by a 20% increase of the TTA (Table 1). A similar evolution was found in the TSS of swiss chard leaves during storage (Roura, Davidovich, & del Valle, 2000). The high respiration rate, normally associated to initial stages of maturation, can lead to the consumption of the sugars included in the TSS fraction, leading to a de- crease of these values during storage. These changes are directly related to metabolic processes, corresponding to the evolution of the leaf me- tabolism during storage (Roura et al., 2000), and could also represent a change in the sensorial properties of the product (especially, sweet- ness and sourness sensation) (Barrett et al., 2010). However, in pea shoots the small TSS and TTA variation, together with the even less pro- nounced variation of the pH (−4%), points to an overall preservation of flavor characteristics, when leaves are stored under low temperatures (3 ± 1 °C). These results highlight the possibility of preserving the pea shoots quality for at least 10 days after harvested, when properly processed and stored.

Table 1
Variation of physicochemical parameters through the storage period (mean value ± standard deviation; § means no significant variation between sampling days (p b 0.05); TSS: total soluble solids; TTA: total titratable acidity).

Pea shoots		
	Day 1	Day 10
Color parameters		
L	45.9 ± 2.3	47.3 ± 2.0
a	−18.6 ± 0.9§	−18.8 ± 0.6§
b	29.4 ± 2.2	31.4 ± 2.2
C	34.8 ± 2.1	36.6 ± 2.1
Hue angle	122.3 ± 1.7	120.9 ± 1.4
TSS (%)	6.28 ± 0.15	5.34 ± 0.26
pH	6.42 ± 0.07	6.18 ± 0.05
TTA (%)[1]	0.18 ± 0.03	0.22 ± 0.01

[1] % of citric acid.

Table 2
Nutritional composition of pea shoots during storage (mean value ± standard deviation relative to fresh weight (f.w.); * means a significant variation (p b 0.05) between sampling days).

Pea shoots		
	Day 1	Day 10
Macronutrient composition	%	
Water	91.5 ± 0.2	91.6 ± 0.1
Protein	4.0 ± 1.0	3.1 ± 0.2
Fat	0.3 ± 0.0	0.4 ± 0.0
Ash	0.9 ± 0.0	0.7 ± 0.1
Dietary Fiber	2.0 ± 0.1	2.2 ± 0.1
Sugar (by difference)	1.2 ± 0.9	2.0 ± 0.2
Energy[1] (/100 g f.w)	24.1 Kcal (102.2 kJ)	
Micronutrient composition ± sd	mg/100 g f.w.	
Water-soluble vitamins		
Ascorbic Acid (C)	153.94 ± 2.81	174.05 ± 19.30
Thiamine (B1)	0.18 ± 0.02	0.19 ± 0.01
Riboflavin (B2)	0.13 ± 0.04	0.13 ± 0.01
Nicotinamide (B3)	0.14 ± 0.02*	0.10 ± 0.00*
Pantothenic Acid (B5)	0.64 ± 0.03*	1.19 ± 0.03*
Pyridoxine (B6)	0.02 ± 0.00*	0.05 ± 0.00*
Fat-soluble vitamins		
Vitamin E (α-tocopherol)	2.65 ± 0.09*	3.66 ± 0.07*
Vitamin A (RAE eq.)[2]	1.42 ± 0.01	1.36 ± 0.06
Minerals		
Potassium	315.97 ± 8.30*	332.4 ±
Sodium	5.08 ± 0.24*	6.46 ±
Calcium	57.59 ± 2.69	58.17 ±
Magnesium	27.20 ± 1.60	27.84 ±
Phosphorus	96.14 ± 1.81	90.12 ±
Iron	1.47 ± 0.02	1.48 ± 0.02
Manganese	0.35 ± 0.02	0.33 ± 0.01
Zinc	0.46 ± 0.03	0.49 ± 0.01
Copper	0.14 ± 0.01*	0.12 ±

[1] Calculated accordingly to Atwater Factors (Otten et al., 2006).

[2] RAE = Retinol activity equivalent.

3.2. *Nutritional composition*

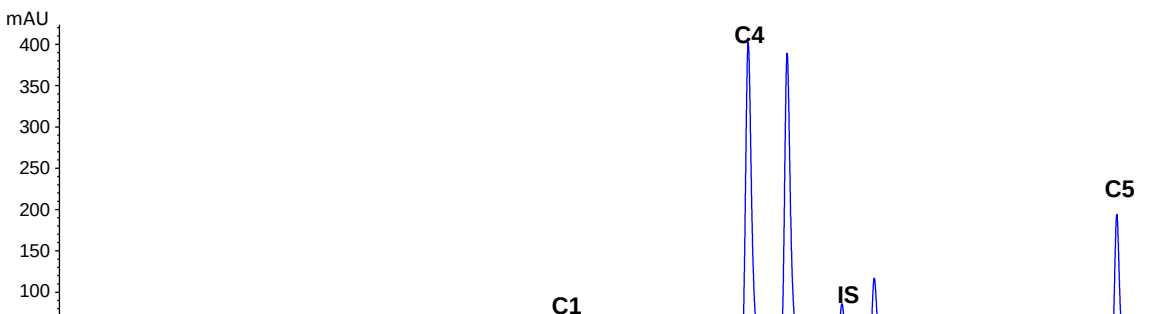
The macro and the micronutrient contents of pea shoots are present- ed in Table 2. The macronutrient composition was similar to other baby leaf vegetables, like spinach, lamb's lettuce or watercress (Martins, 2010). However, these leaves showed a higher percentage of protein (approximately 3%) and dietary fiber (approximately 2%) than the ones found in lettuces (1.8% and 1.3% of protein and dietary fiber, respectively), commonly present in ready-to-eat salads (Martins, 2010). All macronutrients showed a stable content during storage.

Regarding the micronutrient composition, pea shoots showed to be a good source of vitamin C, Vitamin E and vitamin A. Vitamin C repre- sented more than 96% of the total vitamin content, followed by vitamin E and vitamin A. These vitamins levels were higher than the ones found in other common green leafy vegetables, especially in the case of vitamin C (Santos et al., 2012). Regarding the water-soluble vitamins from the B group, the highest values were found for pantothenic acid (B_5). The mineral composition revealed that pea shoots are also a good source of potassium and phosphorous. On the other hand, sodium, calcium and magnesium contents were lower than the levels found in other baby-leaf vegetables (Santos et al., 2014). Concerning the microminerals (iron, manganese, zinc and copper), they represent 0.5% of the total mineral content determined. Iron was the most abun- dant, but zinc levels were higher than those reported in other common baby-leaf vegetables (Santos et al., 2014), revealing that these leaves could enhance the zinc content of a ready-to-eat salad. In this sense, the micronutrient composition, especially vitamin C, vitamin E, vitamin A, potassium and zinc contents, are the most distinguishable character- istics of pea shoots in relation to other baby-leaf vegetables.

The nutritional quality of pea shoots revealed some variations throughout storage, mostly in their vitamin content. Pyridoxine re- vealed the highest variation (+57%) between sampling days, followed by pantothenic acid (+46%), nicotinamide (−35%) and vitamin E (+28%). This behavior was also described in other leafy vegetables, being referred in some studies the occurrence of vitamin synthesis in the post-harvest period (Sánchez-Mata, Cámara, & Díez-Marqués, 2003; Santos et al., 2012). Moreover the enzymatic action against the conjugated form of the vitamins could originate higher levels of free form of the vitamin throughout the storage (Hounsome, Hounsome, Tomos, & Edwards-Jones, 2009; Santos et al., 2012). The mineral content showed less variation, and could be considered stable during the studied period.

3.3. *Carotenoid profile*

The fat-soluble pigments extracted from pea shoots (carotenoids and chlorophylls) were successfully separated by the chromatographic conditions employed (see Fig. 1). From the obtained UV–vis spectra information it was possible to identify 26 compounds, corresponding 12 to carotenoids and the other to chlorophyll a and b and their deriva- tives. A tentative identification of the six main separated carotenoid compounds was accomplished by comparing the UV–vis and MS spectra information provided by the two detectors (i.e., DAD and MS) with commercial standards and data from the literature (Britton, Liaaen- Jensen, & Pfander, 2004; Castro-Puyana et al., 2013; Crupi, Milella, & Antonacci, 2010). Information about characteristic UV–Vis spectra, $[M + H]^+$, and the main fragments obtained by MS^2 experiments for the different carotenoids is presented in Table 3, together with their quantification in the two sampling days. In MS detection the caroten- oids were detected as protonated molecules $[M + H]^+$, with exception



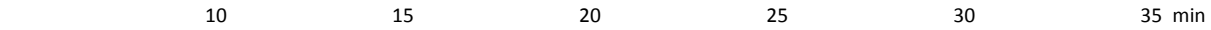


Fig. 1. HPLD-DAD chromatogram (450 nm) of the carotenoid profile of peas shoots. For peak identification and information see Table 3 (*c*, correspond to carotenoid compounds not completely identified).

Table 3
Carotenoids from pea shoots: retention time (Rt), UV–vis maxima, mass spectral data, tentative identification and concentration (mean value ± standard deviation relative to fresh weight (f.w.); * means a significant variation (p b 0.05) between sampling days).

Peak	Rt	λ max (nn)	$[M + H]^+$ (m/z)	MS^2 main fragment ions	Identification	Quantification	
						Day 1	Day 10
						mg/100 g	mg/100 g
C1	18.4	416, 440, 469	601.3	583.4, 565.3 509.4, 491.3,	Violaxanthin	0.3 ± 0.1	0.6 ±
C2	19.3	414, 436, 464	601.5	583.4, 565.4 491.2	Neoxanthin	1.5 ± 0.1	1.6 ±
C3	20.0	398, 422, 449	601.3	583.4, 491.4, 221.1	Luteoxanthin	0.4 ± 0.2*	1.2 ±
C4	23.5	423sh, 445, 473	551.4[a]	551.4, 533.4, 495.3, 429.3	Lutein[b]	12.1 ± 0.2	12.7 ±
C5	33.3	428sh, 452, 479	537.4	537.4, 481.2, 441.4, 399.2	all-*trans*-β-Carotene[b]	13.9 ± 0.1	13.2 ±
C6	34.1	342, 424sh, 448, 472	537.4	537.4, 481.3, 444.3, 413.3	*cis*-β-Carotene	3.1 ± 0.0	3.1 ±
					Total carotenoides	31.3 ± 0.6	32.4 ±

sh, spectral shoulder.
[a] $[M + H\text{-}H_2O]^+$.
[b] Identification corroborated using commercial standards.

of lutein (peak C4) identified by the dehydrated fragment ion $[M + H\text{-} H_2O]^+$ (Castro-Puyana et al., 2013). This ion was also detected as a fragment ion in the other hydroxylated carotenoids (peaks C1, C2 and C3) at a m/z of 583 $[M + H\text{–}H_2O]^+$. These three compounds had the same protonated molecules at a m/z of 601 $[M + H]^+$ and share the same fragmentation ions. Their identification was based on the different relative intensities of the main MS^2 fragment ions and on their maxima UV–vis spectra. The fragmentation profile of violaxanthin (C1) showed five different main ions (Table 3), while in the other xanthophylls (neoxanthin (C2) and luteoxanthin (C3)) only 3 of those ions were present. The compounds C5 and C6 also presented a similar MS spec- trum, however, in this case the presence in compound C6 of shift of absorption maxima (approximately 4 nm with respect to the C5) and a low peak at 342 nm permitted the identification of *cis*-β-carotene.

Pea shoots revealed a mean carotenoid content of 31.9 ± 0.8 mg/ 100 g (f.w.), being β-carotene and lutein the main carotenoids of the pea leaves composition, representing each about 40% of the total carot- enoid content (Table 3). The other xanthophylls were present in lower amounts, being this a typical carotenoid profile of a dark green leafy vegetable (Reif, Arrigoni, Schärer, Nyström, & Hurrell, 2013). Although there are no specific value proposed for the daily intake of carotenoids, there are epidemiological evidence suggesting that higher blood con- centrations of β-carotene and other carotenoids obtained from foods are associated with a lower risk of several chronic diseases and reduc- tion of eye diseases (Otten et al., 2006). Recent studies have linked the consumption of fruit and vegetables and a higher carotenoid intake with a lower risk of invasive bladder cancer among women (Park et al., 2013) and esophageal cancer (Ge, Xing, Yu, & Shen, 2013). In this sense, pea shoots can be considered a good source of these com- pounds, especially β-carotene, that has provitamin-A activity, and lutein that is a component of the human retina. Besides, all carotenoids would contribute to the antioxidant properties of this product.

The carotenoid degradation is common during the senescence of the leaves, being also affected by the presence of light during post-harvest storage (Kidmose et al., 2002). In pea shoots, the global carotenoid content was stable during the storage period, revealing, once more, the stability of the nutritional quality of this product.

3.4. Flavonoid characterization

The presence of several flavonoid glycosides was observed in the native extract of pea shoots. However, before the characterization of

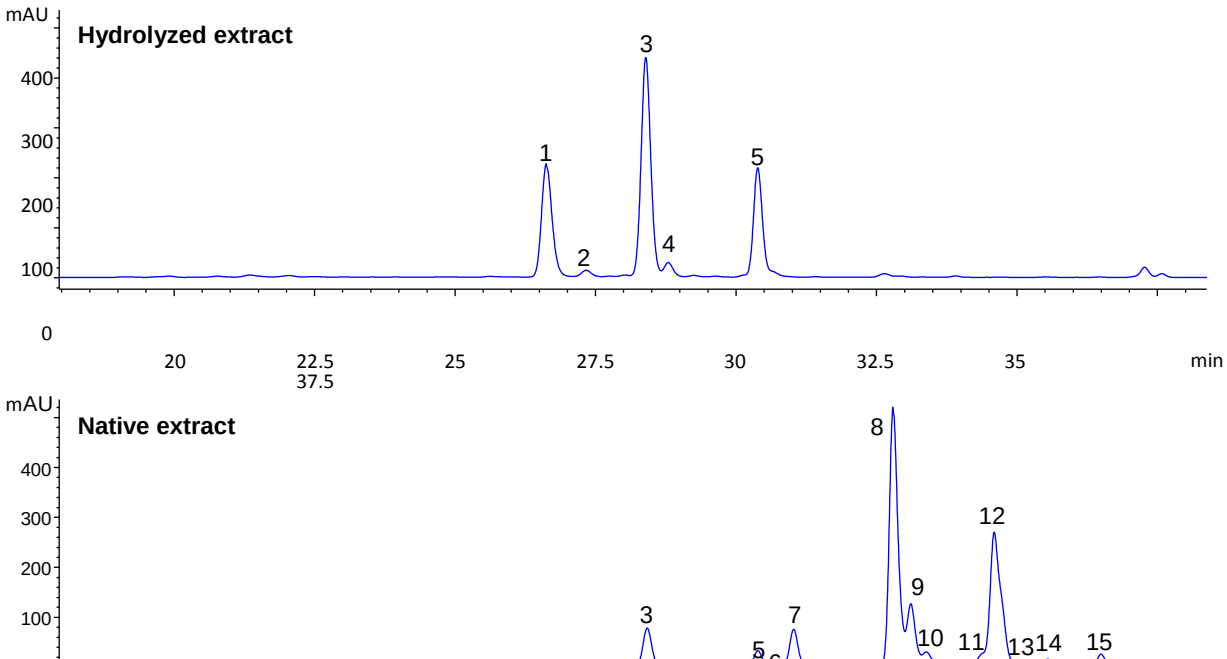


Fig. 2. HPLD-DAD chromatograms (330 nm) of the hydrolyzed and native extracts of pea shoots. Compound identification: 1 — p-coumaric acid; 2 — ferulic acid; 3 — quercetin-3-O- sophorotrioside; 4 — sinapic acid; 5 — kaempferol-3-O-sophorotrioside; 6 — isomer from 5; 7 — quercetin-3-(caffeoyl-diglucoside)-7-glucoside; 8 — quercetin-3-(p-coumaroyl- diglucoside)-7-glucoside; 9 — quercetin-3-feruloylsophoroside-7-glucoside; 10 — quercetin-3-O-(glucuronide-diglucoside)-7-glucoside; 11 — kaempferol-3-sinapoylsophotrioside; 12 — kaempferol-3-(p-coumaroyl-diglucoside)-7-glucoside; 13 — kaempferol-3-feruloylsophoroside-7-glucoside; 14 — kaempferol-3-O-sophorotrioside-7-glucoside; 15 — isomer from 12.

Table 4
Flavonoids from pea shoots: retention time (Rt), UV–vis maxima, mass spectral data, MS^2 fragmentation pattern and tentative identification. Peak numbers as in Fig. 2 (sh, spectral shoulder).

Peak	Compounds	RT (min.)	UV–Vis maxima (nm)	$[M-H]^-$ (m/z)	MS^2 main fragment ions (m/z) (%)					
	Glycosylated flavonoid from pea shoots hydrolyzed extract									
					(−120)	(−162)	(−176)	(−342)	(−486)	
3	Quercetin-3-O-sophorotrioside	28.8	256; sh 268; 352	787	667 (100)	625 (100)		445 (30)	301 (19)	
5	Kaempferol-3-O-sophorotrioside	30.7	266; 347	771	651 (23)	609 (100)		429 (22)	285 (22)	
	Others glycosylated flavonoid from pea shoots native extract									
10	Quercetin-3-O-(glucuronide-diglucoside)-7-glucoside	33.6	256; sh268; 354	963			787(100)			
14	Kaempferol-3-O-sophorotrioside-7-glucoside	35.8	266; 349	933		771 (100)				
	Acylated flavonoid derivatives from pea shoots native extract									
					(−146)	(−162)	(−176)	(−206)	(−338)	
					(−p-coumaroyl)	(−caffeoyl)	(−feruloyl)	(−sinapoyl)	(−feruloyl-glucoside)	
7	Quercetin-3-(caffeoyl-diglucoside)-7-glucoside	31.3	252; sh 268:332	949		787 (100)				
8	Quercetin-3-(p-coumaroyl-diglucoside)-7-glucoside	33.1	226; 316	933	787 (100)					
9	Quercetin - 3-feruloylsophoroside-7-glucoside	33.3	251; sh 268; 331	963			787 (100)			
11	Kaempferol-3-sinapoylsophotrioside	34.1	266; 336	979				771 (100)		
12	Kaempferol-3-(p-coumaroyl-diglucoside)-7-glucoside	34.9	268; 316	917	771 (100)					
13	Kaempferol-3-feruloylsophoroside-7-glucoside	35.3	268; 320	947			771 (100)			609(5)

the compounds in the native extract, the deacylated compounds were studied in the hydrolysed extract. In this extract 5 major compounds were identified (see Fig. 2), according to their UV–vis spectra as flavonol-3-O-glycosides (peaks 3 and 5) and hydroxycinnamic acids (peaks 1, 2 and 4) (Table 4). The MS analysis of the flavonols revealed the presence of a molecular ion at m/z 787 ($[M-H]^-$) and m/z 771($[M-H]^-$) for peak 3 and 5, respectively. The ions obtained in the MS^2 experiment allowed the identification of these compounds as quercetin (peak 3) and kaempferol (peak 5) glycosylated with a sophorotrioside unit (Table 4). The fragmentation pattern obtained was in agreement with the results described in the literature for the identified compounds (Ferreres, Llorach, & Gil-Izquierdo, 2004). The other compounds found in the hydrolyzed extract correspond to *p*-coumaric acid (m/z 163, $[M-H]^-$), ferulic acid (m/z 193, $[M-H]^-$) and sinapic acid (m/z 223, $[M-H]^-$) (Fig. 2).

The analysis of flavonoid compounds present in the native extract permitted to detect, by their typical UV spectra, 5 flavonol 3-O-glycosides compounds (peaks 3, 5, 6, 10 and 14) and 7 peaks of acylated flavonols (compounds 7, 8, 9, 11, 12, 13 and 15). The first group present- ed an UV–vis spectra with a maximum between 350 and 385 nm, while the second group had a UV–vis spectral shape that resembles the over- lapping of a flavonol spectrum with a hydroxycinnamic acid, with a maximum around 310–330 nm (Carazzone, Mascherpa, Gazzani, & Papetti, 2013). The MS analysis allowed to identify these compounds as quercetin and kaempferol derivatives. Compounds 3 and 5, previous- ly identified in the hydrolyzed extract, were also present in the native pea shoots extract but in minor amounts. The acylated flavonols were the main compounds in the flavonoid profile of pea shoots (see Fig. 2), especially the compound 8 (m/z 933 $[M-H]^-$) identified as quercetin- 3-(p-coumaroyl-diglucoside)-7-glucoside and compound 12 (m/z 917 $[M-H]^-$) identified as kaempferol-3-(p-coumaroyl-diglucoside)-7-glucoside. In both compounds the MS^2 experiment revealed a loss of 146 mu, corresponding to the loss of a p-coumaroyl unit (Table 4). The presence of these compounds was also described in the flavonoid composition of other green leafy vegetables (Lin, Sun, Chen, & Harnly, 2011).

Pea shoots had a mean flavonoid content of 329 ± 1 mg/100 g (f.w.) (see Fig. 3), which indicates pea shoots as a good source of these antiox- idant compounds. Quercetin derivatives represent 67% of the total flavonoids. During the storage, no significant changes ($p > 0.05$) were observed, in agreement with other reports that mention a greater stabil- ity of the glycosylated flavonoids in relation to other phenolic com- pounds like the hydroxybenzoic or hydroxycinnamic acids (Martínez- Sánchez, Marín, Llorach, Ferreres, & Gil, 2006).

Fig. 3. Flavonoid content from pea shoots at the beginning and at end of storage period.

4. Conclusions

The results achieved in the present study demonstrated that fresh pea shoots are a good source of different micronutrients, with sig- nificant amounts of biologically active compounds. The inclusion of these leaves in the diet can contribute to a higher intake of antioxidant compounds like flavonoids, carotenoids and vitamin C, and to the daily requirements of minerals, especially potassium. As a minimally proc- essed vegetable, the pea shoots showed a very good stability of their main quality characteristics, when stored under refrigerated conditions for 10 days. The results presented in this work can be also useful to complete food composition databases with the inclusion of a new option of a nutritious green leafy vegetal.

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