Research Article

Received: 26 September 2016 Revised: 14 April 2017 Accepted article published: 8 May 2017 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jsfa.8418

Cowpea fresh pods – a new legume for the market: assessment of their quality and dietary characteristics of 37 cowpea accessions grown in southern Europe

Ioannis Karapanos,[a*] Anastasia Papandreou,[a] Marianna Skouloudi,[a] Despoina Makrogianni,[a] Juan A Fernández,[b] Eduardo Rosa,[c] Georgia Ntatsi,[a] Penelope J Bebeli[a] and Dimitrios Savvas[a]

Abstract

BACKGROUND: Cowpea is traditionally cultivated in some regions of southern Europe for its dried seeds; however, there is a scarcity of information on the quality and dietary characteristics of fresh pods, which are occasionally used in folk diets. This paper aims at covering this gap in knowledge, thereby contributing to the dissemination of fresh cowpea pods as a novel product for the market. The quality and dietary characteristics of pods from 37 accessions (*Vigna unguiculata* ssp. *unguiculata* and ssp. *sesquipedalis*) grown in southern Europe were assessed in an attempt to provide information on pod quality and nutritional properties and to identify relationships between quality traits and accession origin.

RESULTS: Pods from the *sesquipedalis* accessions were heavier and larger, and reached commercial maturity 2 days later, than those from the *unguiculata* accessions. There were also large differences in the quality and dietary characteristics of the accessions. The pods of most accessions were rich in proteins, chlorophylls, carotenoids and phenolics, and showed high antioxidant activity and low concentrations of nitrates and raffinose-family oligosaccharides. Cluster analysis based on quality, dietary or antinutritional traits did not reveal any apparent grouping among the accessions. All the quality characteristics were independent of accession origin and subspecies.

CONCLUSION: Most of the accessions produced fresh pods of good quality and high dietary value, suitable for introduction in the market and/or for use as valuable genetic material for the development of new improved varieties.

Supporting information may be found in the online version of this article.

Keywords: leguminous vegetables; quality; local populations; *Vigna unguiculata*; green pods; dietary and antinutritional factors

INTRODUCTION

Cowpea (*Vigna unguiculata* ssp. *unguiculata* (L.) Walp. and *V. unquiculata* ssp. *sesquipedalis* (L.) Verdc.) is the most important food legume cultivated in the sub-Saharan and tropical Savanna regions of Africa, and its dry seeds considerably contribute to the diet of millions of people in this area.[1] It is also traditionally cultivated in the Mediterranean Basin, as it adapts well to arid conditions due to its drought tolerance.[2,3] Cowpea is not widespread in Europe, although it is traditionally cultivated in some regions of southern Europe,[4] for the production of dry seeds and occasionally in folk markets as green pods (vegetable cowpea). Climate changes will probably offer the possibility to expand this crop to other regions. Two different pod types can be identified: the 'common cowpea' (produced by the subspecies *unguiculata*) with fresh pods of 10–30 cm length, and the 'asparagus bean' or 'yard long bean' (produced by the subspecies *sesquipedalis*) with green to pale green pods of 40–80 cm or more in length.[5]

In Europe, cowpea is mainly cultivated using local populations ('landraces'), which exhibit wide variability, so that they are well adapted to a variety of soil and climatic conditions, or are able to produce satisfactory yields under low-input farming systems.[6] It is of interest that about 64% of a total of more than 30 000 accessions of the *Vigna unguiculata* species that are characterized and preserved in genebanks around the

* *Correspondence to: I Karapanos, Laboratory of Vegetable Production, Department of Crop Science, Agricultural University of Athens, Iera Odos 75, 118 55 Athens, Greece. E-mail: karapanos@aua.gr*

a *Department of Crop Science, Agricultural University of Athens, Athens, Greece*

b *Department of Plant Production, Universidad Politécnica de Cartagena, Cartagena, Spain*

c *Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal*

world are local populations/accessions, whereas only 5.7% are improved cultivars or breeder lines (data provided by Genesys PGR, https://www.genesys-pgr.org/acn/search?q = cowpea). Thus the collection, characterization and preservation of these accessions are of major importance for this valuable genetic material, which can be used either as source of new cultivars with improved features or as a gene pool of useful traits in breeding programs. However, to date, the main objectives of most cowpea breeding programs has been to increase yields or improve resistance to diseases and pests, while the quality, dietary or antinutritional properties of the improved varieties have gained hardly any attention.[7]

To our knowledge, there is no published information on the bioactive compounds of cowpea fresh pods, and the respective data on the physicochemical properties of pods are scarce.[8] Therefore, this study was commissioned to analyze several morphological, quality, dietary and antinutritional characteristics of the fresh pods of 37 cowpea accessions grown in southern Europe.

MATERIALS AND METHODS

Plant material

Thirty-six cowpea accessions and one commercial variety (*V. unguiculata* ssp. *unguiculata* and *V. unguiculata* ssp. *sesquipedalis*), of which nineteen originated from Portugal, nine from Spain, and nine from Greece (supporting information, supplementary Table S1), were cultivated in the experimental field of the Laboratory of Vegetable Production at the Agricultural University of Athens, Greece (37° 59′ 10″ N, 23° 42′ 29″ E, altitude 24 m), in spring–summer 2014. Details on the cultivation techniques and conditions are given by Lazaridi *et al.*[9]

Determination of the suitable stage for harvesting and morphological traits of fresh pods

The suitable harvest stage of fresh pods from each accession/variety to be consumed as vegetables was defined as days from anthesis to the stage when pods had reached their maximum possible length, but retained their green colour and tenderness. Flowers were tagged at the anthesis stage, while pods from two plants per replicate (four replicates per tested genotype) were harvested at the suitable growth stage, immediately transferred to the laboratory, where their length (cm) was measured and fresh weight (FW; g) recorded using a laboratory scale with an accuracy of 0.01 g (model PM 600, Mettler-Toledo Inc., Greifensee, Switzerland). For all measurements, 6–10 pods (20–40 g of pods) were used per replicate in each tested accession. In the accession BGE038474 the yield of pods was minimal, so that most of the assessments could not be performed due to the lack of plant material.

Assessment of quality, dietary and antinutritional characteristics of fresh pods

Sample preparation

All pods were transferred to liquid nitrogen within 15 min after harvesting, homogenized in a Waring blender and kept in glass vials at −80 °C until use for chemical analyses. All subsequent analyses were performed with fresh homogenized samples of cowpea pods, using four replicates per accession, each replicate consisting of pods originating from a respective replicate in the field.

Total soluble solids content (TSSC) and titratable acidity (TA)

The TSSC of fresh pods was measured from the juice of the homogenized samples at 20 °C using a portable refractometer (model HR32B, Schmidt & Haensch GmbH & Co., Berlin, Germany). TA was determined by titration with NaOH in water extracts of homogenized samples, up to pH 8.1, and results were expressed as mg malic acid kg^{-1} FW.

Content of chlorophyll and carotenoids + xanthophylls

The green colour of pods was assessed by measuring the chlorophyll content in acetone extracts of homogenized samples, following the methods of Arnon[10] and Lichtenthaler and Buschmann.[11] Absorbance of the extracts at 663 and 647 nm was measured in a spectrophotometer (model Lambda 1A, PerkinElmer, Waltham, MA, USA) and the chlorophyll content was calculated according to the equations referred by Lichtenthaler and Buschmann.[11] Carotenoids + xanthophylls were quantified in the same extracts by measuring the absorbance at 470 nm, according to the method of Lichtenthaler and Buschmann.[11] The chlorophyll and carotenoids + xanthophylls levels were expressed as mg kg^{-1} FW.

Total phenolics content

Total phenolics were quantified using the Folin–Ciocalteu method[12] in methanolic extracts. The homogenized samples were mixed with 80% (v/v) methanol in water, the mixtures were stirred for 2 h at room temperature and, after centrifugation, the supernatants were decanted and the pellets were resuspended in 80% methanol, following the same procedure. The combined supernatants were used for measurements according to the Folin–Ciocalteu method, and absorbance was measured in a spectrophotometer at 765 nm. Gallic acid was used as standard and the results were expressed as mg gallic acid equivalents (GAE) kg^{-1} FW.

Total antioxidant activity

Total antioxidant activity based on the DPPH and ferric reducing antioxidant power (FRAP) methods was measured using the methanolic extracts used for the assessment of total phenolics.

The antioxidant activity was measured using the scavenging capacity of the samples towards DPPH radical (2,2-diphenyl-1-picrylhydrazyl), based on the method of Brand-Williams *et al.*[13] For this, 0.1 mL extract was added to 3.9 mL DPPH solution (0.06 mmol L^{-1}), mixed and kept for 10 min in the dark at room temperature. Absorbance of the solution was measured at 515 nm in a spectrophotometer, and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) solutions (0–35 µmol L^{-1}) were used as reference. Results were expressed as µmol Trolox equivalents (TE) kg^{-1} FW.

For the assessment of total antioxidant activity using the FRAP assay, the method described by Benzie and Strain[14] was followed. A 0.05 mL extract was mixed with 3 mL freshly prepared FRAP reagent and, after maintaining the solution at 37 °C for 30 min, its absorbance was measured at 593 nm in a spectrophotometer at room temperature, using ascorbic acid solutions (0–1 mmol L^{-1}) as standards. The results were expressed as mmol ascorbate kg^{-1} FW.

Protein content

Total proteins were quantified by the Bradford method,[15] using 1 mL homogenized samples diluted in 5 mL extraction solution

(100 mmol L^{-1} Tris–HCl, pH 7.5; 4 mmol L^{-1} reduced glutathione; 4% soluble PVP (Sigma-Aldrich PVP-40)). The mixture was kept at 5 °C in the dark for 2 h. After centrifugation (5300 × *g*, 15 min, 10 °C), 0.1 mL of the supernatant was used for protein analysis. Absorption was measured at 595 nm using a spectrophotometer, and bovine serum albumin (BSA) solutions of known concentrations were used as reference. Results were expressed as g protein kg^{-1} FW.

Nitrate content

Homogenized samples were mixed with deionized/distilled water and, after incubation at 45 °C for 1 h and centrifugation (5300 × *g*, 15 min, 22 °C), the supernatants were used for the quantification of nitrates following the method of Cataldo *et al.*[16] Absorbance was measured in a spectrophotometer at 410 nm, using KNO_3 solutions of known concentrations as reference. The results were expressed as mg NO_3^- kg^{-1} FW.

Identification and quantification of soluble sugars

Soluble sugars were resolved, identified and quantified by high-performance liquid chromatography (HPLC), using a revised version of the method described by Piccaglia and Galleti.[17] In brief, soluble sugars were extracted by washing the homogenized samples three times with 80% ethanol, combining the supernatants and removing ethanol by evaporation using an N_2 stream while keeping the tubes in a water bath at 65 °C. Sugars were dissolved in H_2O (HPLC grade), and the solutions were filtered using syringe filters of 0.2 µm pore size (Chromafil PET 20/15 MS, Macherey-Nagel GmbH & Co. KG, Düren, Germany) and kept in Eppendorf tubes at −80 °C until analysis by HPLC.

Sugars were resolved and quantified using a Shimadzu Prominence HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with a refractive index detector (model ERC-7511, Erma Inc., Tokyo, Japan) and either a Supelco Supelcosil LC-NH_2 column (25 cm × 4.6 mm i.d.; Sigma-Aldrich, St Louis, MO, USA) to resolve glucose, fructose, sucrose and maltose, or a Phenomenex Rezex RPM Monosaccharide Pb^{+2} column (30 cm × 7.8 mm i.d.; Phenomenex Inc., Torrance, CA, USA) to analyze the raffinose-family oligosaccharides (RFOs: raffinose, stachyose, verbascose). In the Supelco column, which was kept at a temperature of 30 °C, a mixture of 80% acetonitrile and 20% HPLC grade H_2O (Fisher Scientific, Hampton, NH, USA) was used as mobile phase at a flow rate of 1 mL min^{-1}. In the Phenomenex column, which was kept at 75 °C, 100% HPLC-grade H_2O was used as mobile phase at a flow rate of 0.6 mL min^{-1}. In both cases the injection volume was 20 µL. Sugars were identified by their retention times in relation to known standards and the results were expressed as g kg^{-1} FW. Individual RFOs were not distinctly resolved, and so total RFOs were measured and quantified using stachyose standards of known concentrations, since stachyose was found to be the predominant RFO sugar in all the samples. The detection level was 50 mg L^{-1} for fructose, glucose, sucrose and maltose, and 30 mg L^{-1} for total RFOs.

Starch content

Starch quantification in cowpea pods was based on the methods of Dekker and Richards and Barham and Trinder.[18,19] In order to remove any trace of soluble sugars from the pellets left after the extraction of sugars, the sugar extraction procedure was repeated three times and all supernatants were discarded. Washed pellets were treated with NaOH for the gelatinization of starch, and neutralized by acetic acid. Supernatants were mixed with amyloglucosidase solution (A7420, from *Aspergillus niger*, 30–60 units mg^{-1} protein; Sigma-Aldrich), and after 1 h incubation NaOH was added to terminate the amylolytic activity of amyloglucosidase. In this solution, glucose was quantified, using the GOD-POD colorimetric method. Starch solutions of known concentrations (0–1 g L^{-1}) were used as reference and the results were expressed as g starch kg^{-1} FW.

Statistical analysis

All data obtained from the morphological and chemical assessments were analyzed by applying one-way analysis of variance following a completely randomized design with thirtyseven treatments (varieties/accessions) and four replicates per treatment. Means were separated by Tukey's HSD test, ($P < 0.05$). All possible correlations among measured variables (quality or dietary characteristics) were assessed to ascertain whether there were similar trends in the performance of the various accessions in each variable. Pearson's correlation coefficients (*r*) were statistically evaluated using the *t*-test.

Data from the chemical analyses were subjected to cluster analysis to test for the presence of groups among the accessions showing similar quality or dietary characteristics. The parameters selected as variables for sorting accessions into groups/clusters were sensory characteristics (using TSSC and TA), colour (chlorophyll and carotenoids + xanthophylls content), antioxidant activity (total phenolics content and total antioxidant activity, assessed by FRAP and DPPH methods), soluble sugars content (glucose, fructose, sucrose and maltose) and antinutritional factors (content in RFOs and nitrates). All statistical tests were performed using the StatGraphics Centurion XVI statistical package (StatPoint Technologies Inc., Warrenton, VA, USA).

RESULTS AND DISCUSSION

Suitable stage for harvesting and pod morphological characteristics

The pods reached commercial maturity 8–10 days after anthesis (DAA) in all accessions of ssp. *unguiculata*, and 10–12 DAA in all accessions of ssp. *sesquipedalis* (data not shown). Harvesting pods at 14 DAA provided the greatest pod weight and length, but resulted in loss of tenderness and juiciness, and accumulation of fibers (data not shown). These data agree with Omueti *et al.*[20] in Nigeria, who reported that green cowpea pods harvested 7–10 DAA were of optimal quality and dietary characteristics, whereas those harvested at 10–14 DAA were larger and had a higher dry matter content.

Pod FW ranged between 3 and 6 g in the accessions of ssp. *unguiculata*, whereas it was significantly heavier (8–17 g) in ssp. *sesquipedalis*, with the exception of the accession BGE040818, which produced pods of comparable weight to the ssp. *unguiculata* (Fig. 1). Accordingly, pod length in the ssp. *unguiculata* accessions ranged between 11 and 20 cm and in the ssp. *sesquipedalis* between 30 and 60 cm (data not shown).

Sensory characteristics (TSSC and TA)

Large variations in TSSC (Fig. 2) and TA (data not shown) were observed among the accessions. TSSC (Fig. 2) exceeded 5 °Brix in all accessions and in some cases reached 6.5 °Brix (Cp 4877, Vg59, AUA2) and even 7.6 °Brix (Cp 5647) – values comparable to

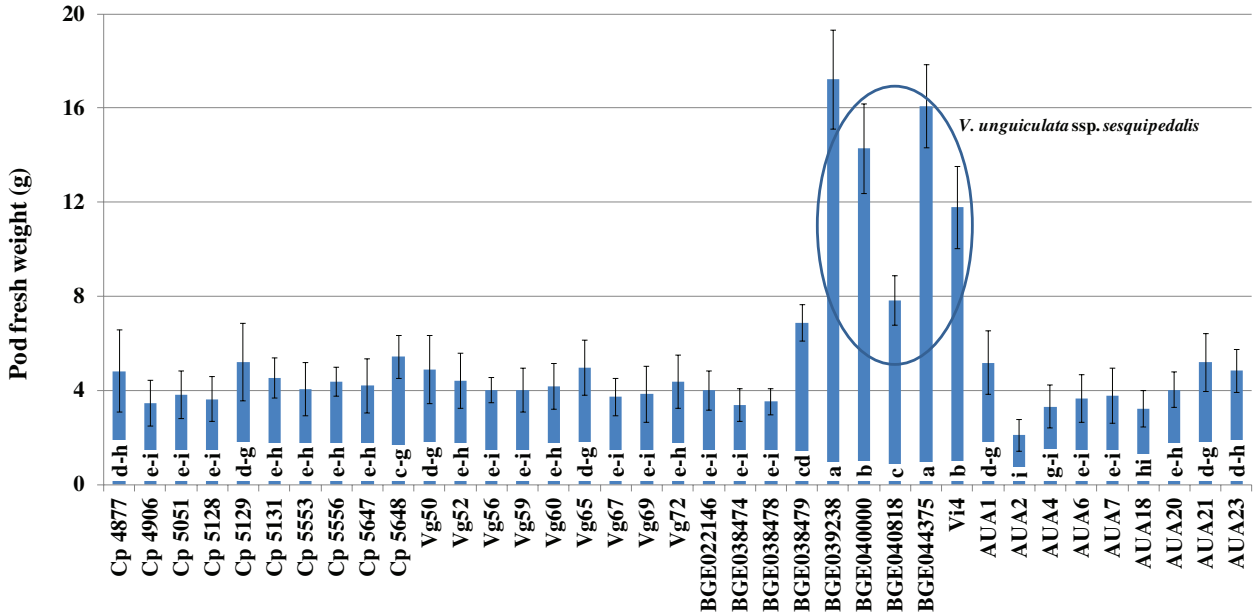

Figure 1. Mean FW (g) of cowpea fresh pods from 37 accessions/varieties originated from southern Europe and harvested at the suitable stage for consumption as vegetables. Bars are mean ± standard deviation ($n = 4$). Means followed by different letters do not differ significantly ($P < 0.05$, Tukey's HSD test).

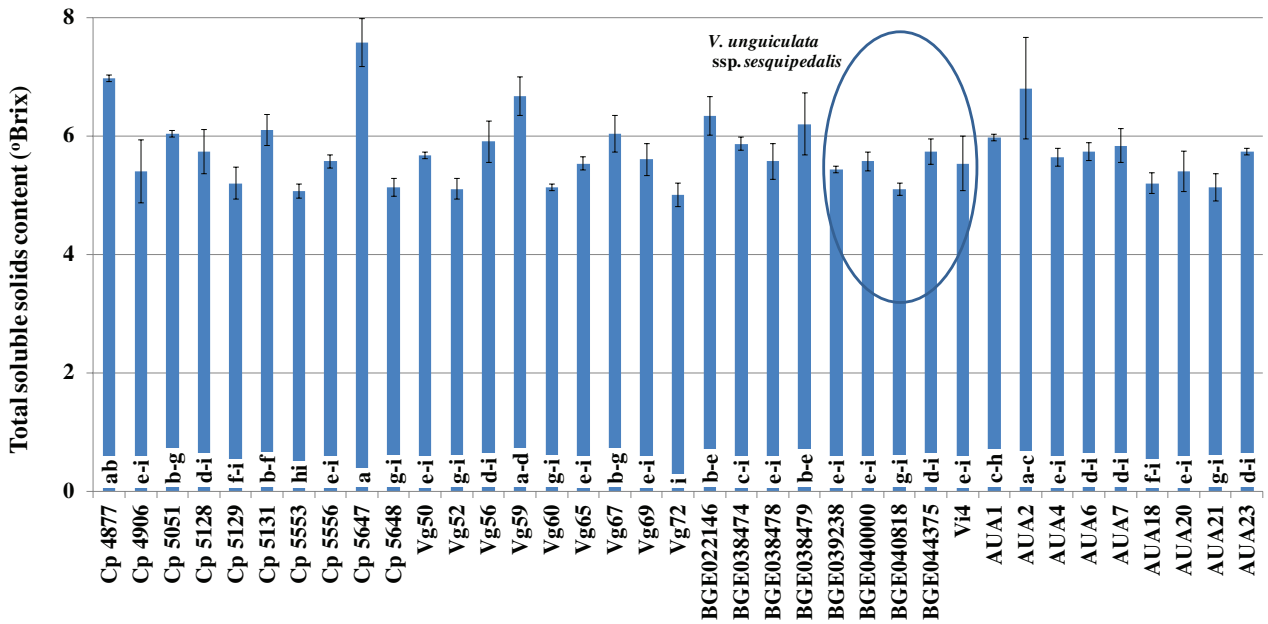

Figure 2. TSSC (°Brix) of cowpea fresh pods from 37 accessions/varieties originating from southern Europe and harvested at the suitable stage for consumption as vegetables. Bars are mean ± standard deviation ($n = 4$). Means followed by different letters do not differ significantly ($P < 0.05$, Tukey's HSD test).

those of snap bean (*Phaseolus vulgaris* L.).[21] TA ranged between 1.7 and 2.8 g malic acid kg^{-1} FW, which is also comparable to the values reported for snap bean.[22] Unlike the size characteristics of pods (weight and length), the determinations of TSSC and TA did not show any consistent differences between the accessions of ssp. *unguiculata* and ssp. *sesquipedalis*. As sugars and acidity are both key components in fruit taste,[23] and high levels of both TSSC and TA are determinants of a satisfactory taste, especially in fleshy fruits, pods from accessions Cp 5647 and Cp 4877 would probably be preferred by consumers (having the highest values in both TSSC and TA), whereas Vg 52, which exhibited the lowest values, might be the least popular. However, no correlation was found between the TSSC and TA content in pods of any of the tested accessions.

Soluble sugars content

The soluble sugars profile of cowpea pods was complex, consisting of monosaccharides (glucose, fructose), disaccharides (sucrose, maltose) and RFOs (mainly stachyose, secondly raffinose and, in some accessions, traces of verbascose), as well as a number of non-identified sugars (supporting information, supplementary Fig. S1). A comparison of the total area of all chromatogram peaks

Table 1. Content of fructose, glucose, sucrose, maltose and starch (g kg^{-1} FW) of cowpea fresh pods of 37 accessions/varieties harvested at the suitable stage for consumption as vegetables

Landrace/variety	Fructose	Glucose	Sucrose	Maltose	Starch
Cp 4877	6.82 ± 1.35ab	10.82 ± 1.55a	2.26 ± 0.25c–i	0.929 ± 0.109a–d	2.45 ± 1.08c–k
Cp 4906	4.37 ± 0.54c–j	7.20 ± 0.78b–g	0.96 ± 0.14 h–j	0.565 ± 0.042c–g	3.21 ± 0.47c–i
Cp 5051	2.93 ± 0.69 h–m	5.78 ± 1.24c–j	1.14 ± 0.20f–j	0.722 ± 0.099a–g	2.79 ± 0.68c–k
Cp 5128	1.63 ± 0.24 m	4.02 ± 0.59 fg–j	2.96 ± 0.26c–e	0.937 ± 0.277a–d	3.33 ± 1.16c–h
Cp 5129	1.79 ± 0.08 lm	2.47 ± 0.23j	1.29 ± 0.17f–j	0.575 ± 0.042c–g	0.31 ± 0.12 k
Cp 5131	2.49 ± 0.43i–m	5.14 ± 0.66d–j	2.45 ± 0.47c–h	0.944 ± 0.043a–c	4.99 ± 0.76b–d
Cp 5553	2.40 ± 0.37j–m	3.90 ± 0.89 g–j	1.94 ± 0.52c–j	0.874 ± 0.237a–e	2.91 ± 0.14c–k
Cp 5556	7.09 ± 0.36a	8.32 ± 1.60a–d	1.73 ± 0.48c–j	0.835 ± 0.117a–e	1.83 ± 0.51f–k
Cp 5647	5.77 ± 0.60a–c	9.75 ± 1.16ab	0.52 ± 0.09j	1.053 ± 0.213ab	0.48 ± 0.15i–k
Cp 5648	3.03 ± 0.17 g–m	5.41 ± 0.40c–j	1.39 ± 0.16e–j	0.656 ± 0.150b–g	4.01 ± 1.68b–f
Vg50	3.69 ± 1.11d–l	6.32 ± 1.57c–i	2.34 ± 0.57c–i	0.676 ± 0.053b–g	3.17 ± 0.91c–j
Vg52	4.41 ± 0.58c–i	6.26 ± 1.12c–i	2.02 ± 0.28c–j	0.723 ± 0.042a–g	1.73 ± 0.55f–k
Vg56	3.08 ± 0.13 g–m	5.04 ± 0.29d–j	1.56 ± 0.05d–j	0.664 ± 0.018b–g	0.92 ± 0.21 g–k
Vg59	1.78 ± 0.47 lm	3.55 ± 0.71 h–j	2.38 ± 0.26c–i	0.689 ± 0.062a–g	5.22 ± 0.34bc
Vg60	2.03 ± 0.34 k–m	3.57 ± 0.74 h–j	1.55 ± 0.17d–j	0.652 ± 0.080b–g	4.72 ± 0.17b–e
Vg65	3.53 ± 0.45e–m	5.70 ± 0.95c–j	2.61 ± 0.58c–h	0.827 ± 0.172a–e	2.37 ± 0.79d–k
Vg67	3.01 ± 0.40 h–m	5.12 ± 1.24d–j	1.11 ± 0.15f–j	0.809 ± 0.163a–e	2.58 ± 0.37c–k
Vg69	3.13 ± 0.49f–m	5.60 ± 0.91c–j	1.01 ± 0.48 g–j	0.735 ± 0.157a–g	3.12 ± 0.87c–j
Vg72	3.95 ± 0.84c–k	6.51 ± 1.43b–i	1.44 ± 0.20d–j	0.812 ± 0.140a–e	0.91 ± 0.70 g–k
BGE022146	4.50 ± 0.58b–g	7.14 ± 0.41b–g	2.70 ± 0.56c–f	0.838 ± 0.089a–e	1.79 ± 0.35f–k
BGE038474	NA	NA	NA	NA	NA
BGE038478	2.77 ± 0.66 h–m	4.12 ± 0.91e–j	1.74 ± 0.40c–j	0.697 ± 0.001a–g	3.37 ± 0.33c–h
BGE038479	2.95 ± 0.74 h–m	6.92 ± 1.00b–h	5.98 ± 2.02b	1.124 ± 0.161a	0.85 ± 0.26 g–k
BGE039238	3.20 ± 0.80e–m	5.80 ± 1.46c–j	3.36 ± 0.39c	0.315 ± 0.047 g	2.03 ± 0.13e–k
BGE040000	4.90 ± 0.33b–h	6.42 ± 1.76b–i	3.10 ± 0.55 cd	0.541 ± 0.105c–g	3.54 ± 0.96c–g
BGE040818	4.45 ± 0.50c–i	7.38 ± 0.63b–f	1.35 ± 0.46e–j	0.530 ± 0.132c–g	2.43 ± 0.79c–k
BGE044375	5.58 ± 0.65a–d	8.78 ± 0.97a–c	2.66 ± 0.41c–g	0.505 ± 0.081d–g	2.82 ± 0.56c–k
Vi4	4.50 ± 0.58c–h	8.81 ± 1.26a–c	1.95 ± 0.25c–j	0.474 ± 0.035e–g	1.72 ± 0.15f–k
AUA1	3.11 ± 0.23f–m	4.97 ± 0.41d–j	1.82 ± 0.74c–j	0.540 ± 0.089c–g	6.62 ± 1.52b
AUA2	2.43 ± 0.68j–m	7.66 ± 0.72a–e	5.59 ± 0.70b	0.780 ± 0.117a–f	9.96 ± 1.03a
AUA4	5.07 ± 0.69b–f	7.22 ± 1.01b–g	1.68 ± 0.07d–j	0.808 ± 0.024a–e	0.40 ± 0.14jk
AUA6	3.27 ± 0.49e–m	4.72 ± 0.29e–j	1.65 ± 0.09d–j	0.623 ± 0.025b–g	2.60 ± 0.25c–k
AUA7	2.26 ± 0.58 k–m	3.22 ± 0.99ij	1.79 ± 0.38c–j	0.657 ± 0.156b–g	5.13 ± 0.87b–d
AUA18	2.96 ± 0.52 h–m	4.53 ± 0.20e–j	1.98 ± 0.20c–j	0.729 ± 0.178a–g	3.59 ± 0.91c–g
AUA20	5.15 ± 0.83a–e	10.87 ± 1.46a	0.72 ± 0.09ij	0.600 ± 0.029c–g	0.59 ± 0.31 h–k
AUA21	3.89 ± 0.45c–k	4.65 ± 0.96e–j	1.13 ± 0.36f–j	0.752 ± 0.093a–g	9.96 ± 0.92a
AUA23	3.67 ± 0.69e–l	7.40 ± 1.43b–e	9.48 ± 0.26a	0.393 ± 0.046 fg	10.18 ± 0.12a

Data are mean ± standard deviation ($n = 4$). Means in columns followed by different letters do not differ significantly ($P < 0.05$, Tukey's HSD test). ND, not detected; NA, not available.

with the respective area of the peaks identified as known sugars revealed that about 60–80% (depending on the accession) of the total amount of sugars in each chromatogram were identified and quantified. As a result, the sum of individual sugars (Table 1 and Fig. 5) did not correlate with pod TSSC, since the sum of quantified sugars represents only a part of the total soluble sugars content in each variety/accession, and its contribution to total sugars depends on the tested accession. Although all quantified sugars were present in pods from all accessions, the respective chromatograms varied considerably, suggesting large differences not only in the sugar content but also in the number and quantity of non-identified ones.

Pods from all accessions contained more glucose (2.47–10.87 g kg^{-1} FW) than fructose (1.63–6.82 g kg^{-1} FW) and a close correlation between glucose and fructose content was observed ($r = 0.84$, significant at $P < 0.001$). The sucrose content (0.52–9.48 g kg^{-1} FW) was less than that of fructose (except in Vg59, BGE038479, BGE039238, AUA2 and AUA3), whereas the maltose content (0.31–1.12 g kg^{-1} FW) was invariably lower compared to that of sucrose (except in Cp 5647) (Table 1). Cowpea pods are therefore different in this respect from those of snap bean, which are reported to contain more fructose than glucose, followed by sucrose.[24] As fructose is almost twice as sweet as glucose, cowpea pods are expected to have a less sweet taste than those of snap bean with similar total sugar levels. Apart from glucose–fructose, all other sugar combinations showed no correlation. The accessions AUA20, Cp 4877, Cp 5556, BGE044375, AUA23 and Cp 5647 exhibited the highest sum of the sugars quantified.

Starch content

Significant differences were observed in the starch content among the tested genotypes (0.31–10.18 g kg^{-1} FW, Table 1).

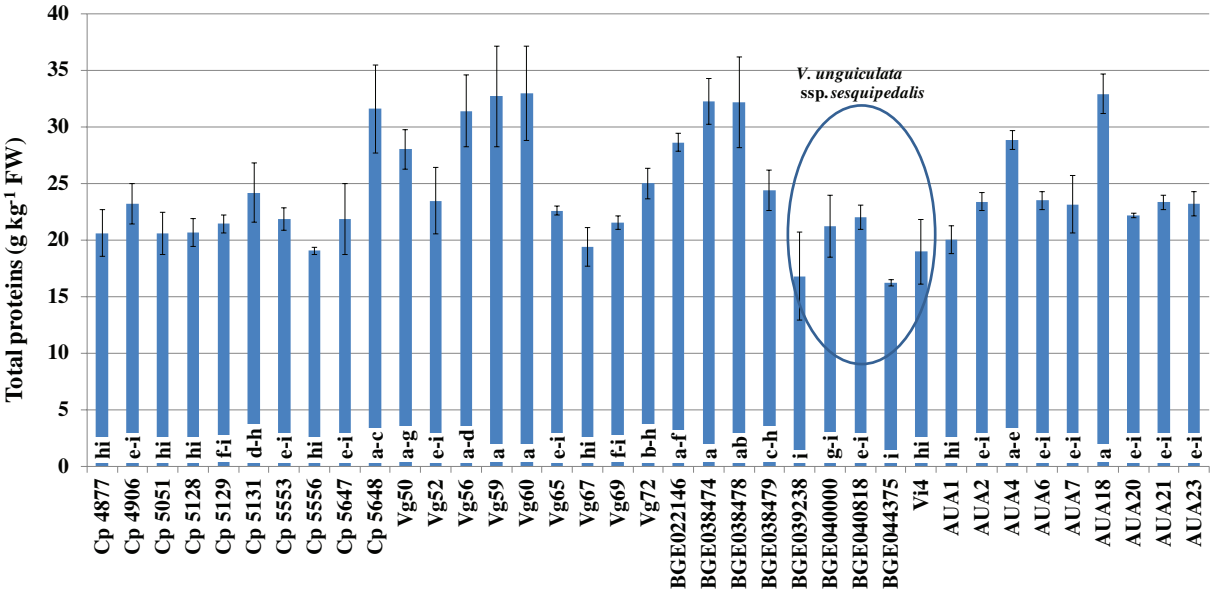

Figure 3. Total soluble proteins content (g kg^{-1} FW) of cowpea fresh pods from 37 accessions/varieties originated from southern Europe and harvested at the suitable stage for consumption as vegetables. Bars are mean ± standard deviation ($n = 4$). Means followed by different letters do not differ significantly ($P < 0.05$, Tukey's HSD test).

Greek accessions (AUA2, AUA21 and AUA23) accumulated starch at significantly higher levels than those from Spain and Portugal, although their content in soluble sugars was also high. Again, no relationship between individual or total sugars and starch content was evident. Since starch in the pods of legumes mainly accumulates in the developing seeds, its content in cowpea pods starts to increase considerably at 9 DAA, which corresponds to a quick onset of seed development.[20] The high starch content possibly indicates excessive development of seeds in relation to pod walls, a negative quality characteristic in the fresh pods of cowpea and snap beans. As in snap beans, the percentage of seeds to pod walls can be used as a good index of pod maturity.[25] Consequently, a low starch content might be used as a criterion to select cowpea accessions with small seeds in relation to pod coat at the stage suitable for pod harvest.

Soluble proteins content

It is known that pods and immature seeds of legumes contain lower amounts of proteins than dry seeds of the same species.[26] Nevertheless, with a protein content of 16.2–32.9 g protein kg^{-1} FW (Fig. 3), cowpea pods could be considered moderately high to rich in proteins compared with other legume vegetables. Compared with cowpea pods, snap bean pods contain a significantly lower amount of proteins (on average 18 g kg^{-1} FW, which is 25% less than that found in cowpea pods in this study), while pea green pods (*Pisum sativum* L.) have a similar range. Only the immature green seeds of pea and broad bean (*Vicia faba* L.) have a higher protein content (120% and 190% higher, respectively)[27] than that of cowpea pods, but the former need an extra growth period to reach harvest stage. In particular, pea and broad bean plants are ready for harvest after 2.5–3.5 months from sowing and seeds need more than 3 weeks from anthesis to reach commercial maturity.[28] Thus cowpea pods need a much shorter time to be produced and therefore much lower inputs. Protein content is one of the most important dietary features in legumes and a primary criterion for genotype selection in breeding programs that aim at dietary improvement of pulses. However, in the case of fresh pods, since proteins are accumulated in developing seeds, selection for its high protein content may result in the selection of pods with a high seed-to-pod coat ratio, similar to that mentioned for starch, although no correlation between starch and protein content was found in this study.

Although large differences were observed among accessions, the protein content in the accessions of ssp. *sesquipedalis* was consistently lower than in most ssp. *unguiculata* accessions (Fig. 3).

Content of chlorophyll and carotenoids + xanthophylls

The chlorophyll content was used in this study as an indication of pod green colour intensity. There were large differences among the tested accessions (37.5–213.5 mg kg^{-1} FW) irrespective of their country of origin (Table 2). However, most of the ssp. *sesquipedalis* accessions (BGE039238-Vi4) had a lower chlorophyll content than most of the ssp. *unguiculata* accessions, reflecting their pale-green colour at harvest. Overall, larger pods did not necessarily have a less intense green colour, as no negative correlation between pod weight and chlorophyll concentration was found. The carotenoids + xanthophylls content of pods was low and varied considerably among the accessions (Table 2). Again, chlorophyll and carotenoids + xanthophylls did not show any correlation.

Total phenolics and antioxidant activity

In most of the accessions tested, the concentration of total phenolics did not exceed 800 mg GAE kg^{-1} FW, which is comparable to the respective values reported for snap beans (780 mg GAE kg^{-1} FW).[27] Nevertheless, in the accessions Vg52, BGE038478, BGE03479, AUA18 and AUA21 the total phenolics exceeded 1400 mg GAE kg^{-1} FW (Table 2). Compared to other common vegetables such as tomato, zucchini, cabbage and Brussels sprouts, cowpea pods have a medium to high content of phenolics, whereas the respective values in broccoli are reported to be two to three times higher.[29,30]

Antioxidant activity, assessed by the FRAP or the DPPH method, showed a close correlation with the phenolics content ($r = 0.96$

Table 2. Content of total chlorophylls, carotenoids and xanthophylls, total phenolics and antioxidant activity as assessed by FRAP and DPPH methods, of cowpea fresh pods of 37 accessions/varieties harvested at the suitable stage for consumption as vegetables

Landrace/ variety	Total chlorophylls (mg kg^{-1} FW)	Xanthophylls +carotenoids(mg kg^{-1} FW)	Total phenolics (mg GAE kg^{-1} FW)	Total antioxidant activity	
				FRAP method (mmol ascorbate kg^{-1} FW)	DPPH method (μmol TE kg^{-1} FW)
Cp 4877	137.1 ± 3.3b–g	12.6 ± 0.2b–i	618.1 ± 42.8d–h	1.98 ± 0.37c–e	23.9 ± 3.1de
Cp 4906	40.2 ± 11.0jk	6.4 ± 1.8e–i	727.0 ± 27.2c–h	2.22 ± 0.16c–e	33.0 ± 1.4de
Cp 5051	62.6 ± 4.5 h–k	1.8 ± 0.4i	645.3 ± 56.1c–h	1.64 ± 0.03de	12.7 ± 4.0e
Cp 5128	60.0 ± 8.3 h–k	ND	577.2 ± 47.2e–h	1.14 ± 0.08e	21.5 ± 0.9de
Cp 5129	118.6 ± 15.3c–h	3.8 ± 0.7 g–i	794.6 ± 162.4c–f	2.68 ± 0.33c–e	51.2 ± 7.8b–e
Cp 5131	109.3 ± 14.5c–i	13.6 ± 3.8b–i	699.2 ± 50.6c–h	1.55 ± 0.10de	32.4 ± 3.0de
Cp 5553	100.0 ± 8.1c–k	12.3 ± 0.2b–i	609.6 ± 65.8d–h	2.12 ± 0.24c–e	33.3 ± 3.0de
Cp 5556	125.5 ± 24.0b–g	20.8 ± 1.6a–c	839.4 ± 39.5 cd	2.81 ± 0.22c–e	39.5 ± 5.3de
Cp 5647	147.5 ± 6.5a–e	23.1 ± 7.4ab	631.7 ± 34.6c–h	2.15 ± 0.24c–e	34.7 ± 2.4de
Cp 5648	105.5 ± 23.7c–i	15.5 ± 3.2b–g	693.0 ± 98.5c–h	2.01 ± 0.25c–e	28.7 ± 5.4de
Vg50	108.2 ± 18.1c–i	29.4 ± 10.5a	744.6 ± 39.2c–g	2.38 ± 0.03c–e	53.1 ± 3.1b–e
Vg52	99.2 ± 13.8c–k	22.1 ± 9.7ab	1425.5 ± 310.6b	6.07 ± 0.68a	172.1 ± 4.8a
Vg56	152.7 ± 24.7a–d	16.7 ± 3.1b–e	738.4 ± 44.2c–gh	2.85 ± 0.77c–e	42.7 ± 3.7c–e
Vg59	85.9 ± 24.3e–k	18.5 ± 5.6a–d	750.3 ± 31.7c–g	2.04 ± 0.17c–e	26.1 ± 1.3de
Vg60	93.7 ± 11.4d–k	2.6 ± 0.5i	521.0 ± 73.2gh	1.66 ± 0.23de	41.3 ± 4.3c–e
Vg65	144.3 ± 34.6b–e	17.2 ± 4.8b–e	679.4 ± 15.8c–h	2.19 ± 0.13c–e	42.1 ± 8.0c–e
Vg67	156.3 ± 39.4a–c	ND	679.4 ± 99.6c–h	2.16 ± 0.25c–e	51.2 ± 8.4b–e
Vg69	183.2 ± 38.5ab	20.9 ± 3.3a–c	689.6 ± 49.8c–h	2.37 ± 0.31c–e	57.2 ± 5.2b–d
Vg72	154.1 ± 26.4a–d	18.5 ± 3.5a–d	801.4 ± 40.9c–f	2.50 ± 0.21c–e	43.7 ± 4.9b–e
BGE022146	136.6 ± 23.1b–g	4.6 ± 1.1f–i	645.9 ± 155.9c–h	2.36 ± 0.51c–e	41.5 ± 17.2c–e
BGE038474	112.0 ± 11.1c–i	10.9 ± 5.0b–i	NA†	NA	NA
BGE038478	118.0 ± 6.1c–i	19.2 ± 1.1a–d	1522.5 ± 271.9b	6.38 ± 1.30a	169.5 ± 29.0a
BGE038479	92.4 ± 9.2d–k	3.3 ± 0.3hi	1809.6 ± 352.5a	7.07 ± 1.89a	183.2 ± 40.2a
BGE039238	57.2 ± 2.4i–k	10.2 ± 0.9b–i	597.7 ± 4.3d–h	2.57 ± 0.05c–e	56.7 ± 7.8b–d
BGE040000	98.2 ± 14.6c–k	16.9 ± 1.1b–e	584.1 ± 94.6e–h	2.43 ± 0.43c–e	54.1 ± 13.6b–e
BGE040818	70.5 ± 2.0 g–k	8.9 ± 1.3d–i	871.7 ± 156.0c	3.82 ± 0.77bc	85.2 ± 24.6b
BGE044375	77.0 ± 14.1f–k	12.3 ± 2.7b–i	489.3 ± 60.9 h	2.05 ± 0.43c–e	47.0 ± 11.6b–e
Vi4	58.8 ± 4.4 h–k	9.3 ± 0.1c–i	627.2 ± 64.5c–h	2.70 ± 0.40c–e	55.1 ± 7.1b–d
AUA1	213.5 ± 14.9a	ND	673.7 ± 18.8c–h	1.64 ± 0.18c–e	47.6 ± 6.5b–e
AUA2	37.5 ± 2.3 l	5.5 ± 0.4e–i	570.4 ± 27.3f–h	1.19 ± 0.09e	19.0 ± 2.1de
AUA4	137.2 ± 26.0b–f	19.5 ± 4.3a–d	693.4 ± 93.7c–h	2.59 ± 0.62c–e	56.8 ± 12.9b–d
AUA6	103.0 ± 10.8c–i	15.0 ± 1.9b–h	767.2 ± 75.4c–g	2.32 ± 0.22c–e	41.1 ± 8.5c–e
AUA7	116.0 ± 17.2c–i	18.7 ± 2.4a–d	726.2 ± 18.6c–h	1.87 ± 0.16de	47.5 ± 5.7b–e
AUA18	116.5 ± 15.8c–i	20.3 ± 0.9a–d	1475.6 ± 196.6b	6.51 ± 1.27a	185.0 ± 19.8a
AUA20	111.2 ± 21.1c–i	8.6 ± 2.2d–i	825.1 ± 166.4c–e	3.39 ± 1.11 cd	82.5 ± 12.8bc
AUA21	102.8 ± 12.0c–i	16.5 ± 2.1b–f	1446.5 ± 153.9b	5.55 ± 0.57ab	154.2 ± 13.0a
AUA23	66.6 ± 10.0 g–k	13.2 ± 1.0b–i	522.2 ± 12.0gh	1.87 ± 0.18c–e	35.4 ± 3.3de

Data are mean ± standard deviation ($n = 4$). Means in columns followed by different letters do not differ significantly ($P < 0.05$, Tukey's HSD test). ND, not detected; NA, not available.

and $r = 0.94$ respectively, significant at $P < 0.001$), and the two methods also showed a significant correlation with each other ($r = 0.98$, significant at $P < 0.001$). Although several bioactive compounds present in cowpea pods (chlorophyll, carotenoids, ascorbic acid etc.) exhibit considerable antioxidant properties, the results of this study suggest that the high concentration of phenolics may determine the antioxidant activity of cowpea pods, similar to snap beans. Indeed, the concentration of total phenolics in snap beans has been reported to be five to six times higher than that of ascorbic acid,[31] which indicates that phenolics provide a major contribution to their total antioxidant activity.

The total antioxidant activity of cowpea pods found in this study was comparable to the values reported for snap bean.[32] As the antioxidant activity of snap beans ranks within the top ten of those measured in common vegetables,[33] the results of the present study suggest that cowpea pods may also exhibit considerable antioxidant properties. Nevertheless, the antioxidant activity of snap beans is reported to be 1.5–3 times lower than that of vegetables with the highest values, particularly pepper, broccoli, spinach, beet and cauliflower.[33]

Antinutritional factors

Nitrates

Nitrates in vegetables are widely considered as antinutritional factors when they accumulate to excessive levels in green and leafy vegetables, because they pose a potential threat to human

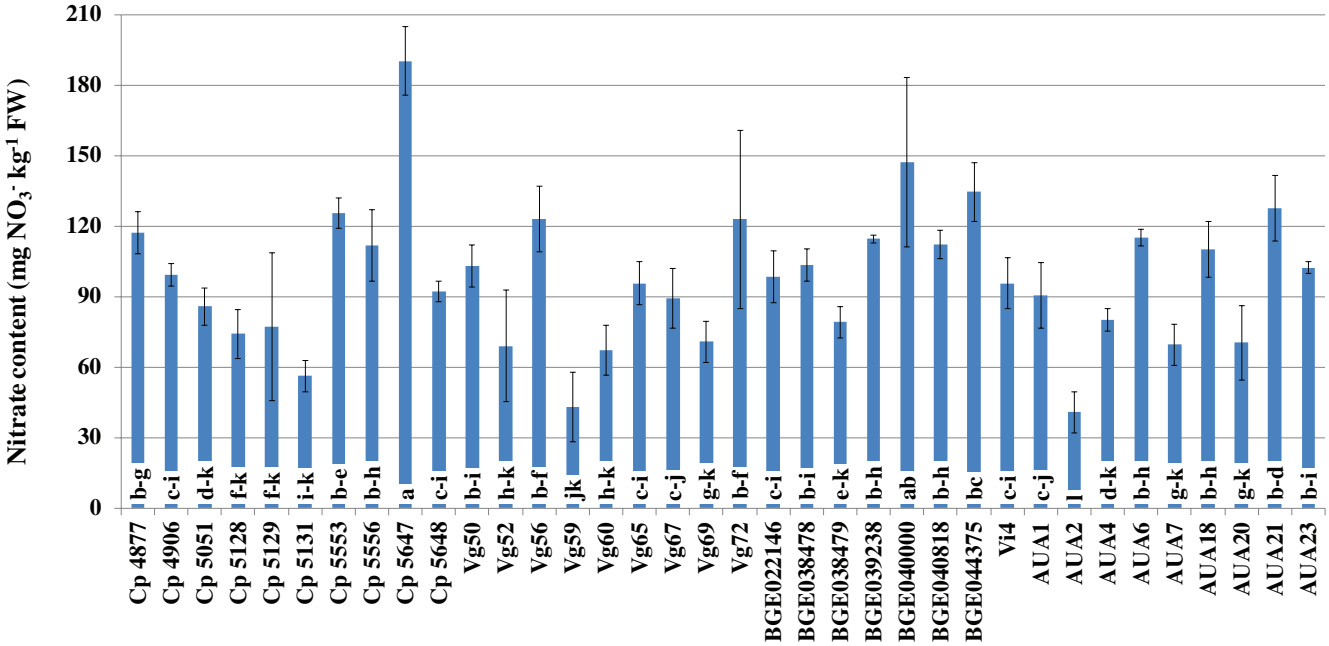

Figure 4. Content in nitrates (mg NO_3^- kg^{-1} FW) of cowpea fresh pods from 37 accessions/varieties originating from southern Europe and harvested at the suitable stage for consumption as vegetables. Bars are mean ± standard deviation ($n = 4$). Means followed by different letters do not differ significantly ($P < 0.05$, Tukey's HSD test).

health.[34] In the present study, the nitrate content in cowpea pods of all accessions ranged from 40.8 to 190.1 mg NO_3^- kg^{-1} FW, the highest level being determined in the accession Cp 5647 (Fig. 4). These values are considerably lower than those commonly found in leafy vegetables. For instance, in wild rocket (*Diplotaxis tenuifolia* (L.) DC.), the nitrate content may reach values as high as 9300 mg kg^{-1}.[34] Snap bean pods cultivated in Greece showed a nitrate content (31–159 mg NO_3^- kg^{-1} FW) comparable with that found in cowpea pods in the present study, which is 6–10 times lower than that commonly found in leafy vegetables (e.g. 443–981 mg NO_3^- kg^{-1} FW in beet leaves).[35] Therefore, cowpea should be characterized as a vegetable with a low nitrate content.

Raffinose-family oligosaccharides

The RFO content of pods was low in most accessions, ranging between 125.5 and 1229.7 mg kg^{-1} FW, although in the landrace Cp 5128 it reached 4871 mg kg^{-1} FW (Fig. 5). RFOs, which are ubiquitous in legume seeds, cause flatulence in humans due to the lack of α-1,6-galactosidase in the intestinal mucosa. Their presence in considerable amounts in cowpea seeds (up to 53 g kg^{-1} dry weight)[36,37] poses a constraint to cowpea dietary consumption.[8] However, based on the results of the present study, cowpea pods contain substantially lower levels of RFOs than seeds; therefore, similar to nitrates, RFOs are not considered an important antinutritional constituent in cowpea pods. This is another feature that supports the use of this novel food in our diet.

Grouping of accessions according to cluster analysis

The grouping of accessions/varieties using cluster analysis (supporting information, supplementary Table S2) showed that accessions Cp 4877 and Cp 5647 were classified in one group due to their higher TSSC and TA values. Furthermore, accessions Vg52, BGE038478, BGE038479, AUA1 and AUA8 exhibited high antioxidant activity, while accessions Cp 4877, Cp 5556, Cp 5647, AUA20 showed high glucose and fructose contents. However, when classification was based on dietary characteristics (chlorophyll, phenolics and protein content), accessions in groups did not necessarily have similar levels in the above-mentioned dietary factors, as there were no similar responses of the accessions in all those dietary factors. No grouping was possible when both nitrates and RFOs were selected as classification variables, as accessions showed completely different trends for those two characteristics. Overall, cluster analysis did not indicate differences in pod quality and dietary characteristics between the tested accessions that might be related to the country of origin or the subspecies (*unguiculata* or *sesquipedalis*).

In Africa, accessions of ssp. *unguiculata* are mainly cultivated for the production of dry seeds, and ssp. *sesquipedalis* is preferred for the long, succulent pods; therefore, African ssp. *unguiculata* accessions do not produce fresh pods of high quality.[38] By contrast, the present study indicates that in cowpea accessions from southern Europe fresh pods of the ssp. *unquiculata* are of comparable or superior quality and dietary properties to those of ssp. *sesquipedalis*. This is possibly because ssp. *unguiculata* accessions in southern Europe were selected both for the production of seeds and fresh pods. Thus, as small pods of the ssp. *unguiculata* are preferred to the longer pods of ssp. *sesquipedalis* for postharvest handling, packaging and subsequent use by consumers, they can be considered as a novel legume vegetable of high potential to enter the market.

CONCLUSION

Most of the accessions/varieties tested in this study produced fresh pods of high to superior quality. No considerable differences in pod quality and dietary value were observed among the accessions of ssp. *unguiculata* and ssp. *sesquipedalis*. Similarly, the country of origin had no significant effect on the quality and dietary properties of the pods. Cowpea pods were proved to be rich in

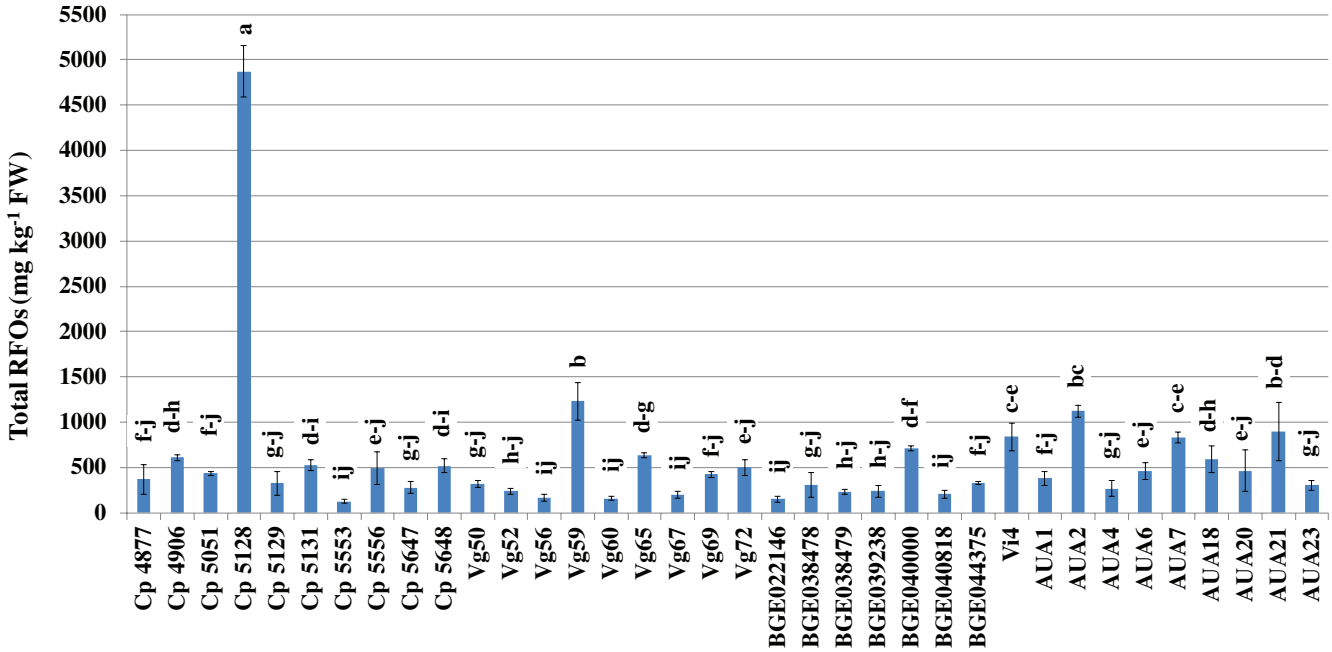

Figure 5. Content of RFOs (mg kg^{-1} FW) of cowpea fresh pods from 37 accessions/varieties originating from southern Europe and harvested at the suitable stage for consumption as vegetables. Bars are mean ± standard deviation ($n = 4$). Means followed by different letters do not differ significantly ($P < 0.05$, Tukey's HSD test).

proteins and phenolics, with high antioxidant activity compared to that reported for other vegetables, whereas their content in antinutritional factors such as RFOs and nitrates was very low.

Cowpea accessions grown in southern Europe could be introduced in the market as a novel legume vegetable and be considered as valuable genetic material for the production of cowpea fresh pods of even higher quality and dietary value. The production of cowpea fresh pods may be expected to increase in the future as a result of the awareness of consumers for healthy vegetables and improved preservation methods. Consequently, accessions from southern Europe may be exploited to produce new varieties with high yields and superior quality and dietary properties.

Last but not least, growing cowpea for green pods needs a much shorter growing season with fewer inputs than other vegetables, making the crop more sustainable and much more adapted to several abiotic stresses imposed by climate change.

ACKNOWLEDGEMENTS

This research study was funded by the 7th Framework Programme: 'EUROLEGUME: Enhancing of legumes growing in Europe through sustainable cropping for protein supply for food and feed' (Grant Agreement No. 613781). Seeds of the Portuguese accessions were kindly supplied by Dr Maria da Graça Mendonça Pereira of the Research Unit of Biotechnology and Genetic Resources, National Institute for Agrarian and Veterinarian Research, IP (INIAV), Portugal. The authors are indebted to Associate Professor Georgios Papadopoulos for his advice and assistance on multivariate statistical analyses.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Gómez C, *Cowpea: Post-harvest Operations. INPhO – Post-harvest Compendium*. Food and Agriculture Organization of the United Nations (FAO), Rome (2004).
2 Ehlers JD and Hall AE, Cowpea (*Vigna unguiculata* L. Walp.). *Field Crop Res* **53**:187–204 (1997).
3 Tosti N and Negri V, Efficiency of three PCR-based markers in assessing genetic variation among cowpea (*Vigna unguiculata* ssp. *unguiculata*) landraces. *Genome* **45**:656–660 (2002).
4 Food and Agriculture Organization (FAO), *FAOStat, World Production* (2013). [Online]. Available: http://faostat3.fao.org/browse/Q/QC/E [12 September 2016].
5 Rubatzky VE and Yamaguchi M, *World Vegetables: Principles, Production and Nutritive Values* (2nd edn). Springer – Science and Business Media, Dordrecht, p. 859 (1997).
6 Peksen A, Fresh pod yield and some pod characteristics of cowpea (*Vigna unquiculata* L. Walp.) genotypes from Turkey. *Asian J Plant Sci* **3**:269–273 (2004).
7 Oluwatosin OB, Genotype × environment influence on cowpea (*Vigna unguiculata* [L.] Walp.) antinutritional factors. 1. Trypsin inhibitors, tannins, phytic acid and haemagglutinin. *J Sci Food Agric* **79**:265–272 (1999).
8 Gonçalves A, Goufo P, Barros A, Domínguez-Perles R, Trindade H, Rosa EAS *et al.*, Cowpea (*Vigna unguiculata* L. Walp), a renewed multipurpose crop for a more sustainable agri-food system: nutritional advantages and constraints. *J Sci Food Agric* **96**:2941–2951 (2016).
9 Lazaridi E, Ntatsi G, Fernández JA, Karapanos I, Carnide V, Savvas D *et al.*, Phenotypic diversity and evaluation of fresh pods of cowpea landraces from Southern Europe. *J Sci Food Agric*. https://doi.org/10.1002/jsfa.8249 (2017).
10 Arnon DI, Copper enzyme in isolated chloroplast polyphenoloxidase in *Beta vulgaris*. *Plant Physiol* **24**:1–15 (1949).
11 Lichtenthaler HK and Buschmann C, Chlorophylls and carotenoids: measurement and characterization by UV–VIS spectroscopy. *Curr Protoc Food Anal Chem* F4.3.1–F4.3.8 (2001).
12 Singleton VL and Rossi JA Jr, Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *Am J Enol Viticult* **16**:144–158 (1965).
13 Brand-Williams W, Cuvelier M and Berset C, Use of a free radical method to evaluate antioxidant activity. *Lebensm-Wiss Technol* **28**:25–30 (1995).

14 Benzie IFF and Strain JJ, The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. *Anal Biochem* **239**:70–76 (1996).
15 Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**:248–254 (1976).
16 Cataldo DA, Haroon M, Schrader LE and Youngs VL, Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Commun Soil Sci Plant Anal* **6**:71–80 (1975).
17 Piccaglia R and Galletti GC, Sugar and sugar alcohol determination in feedstuffs by HRGC, HPLC and enzymic analysis. *J Sci Food Agric* **45**:203–213 (1988).
18 Dekker RFH and Richards NG, Determination of starch in plant material. *J Sci Food Agric* **22**:441–444 (1971).
19 Barham D and Trinder P, An improved color reagent for the determination of blood glucose by oxidase system. *Analyst* **97**:142–145 (1972).
20 Omueti O, Ojomo OA, Ogunyanwo O and Olafare S, Biochemical components and other characteristics of maturing pods of vegetable cowpea (*Vigna unguiculata*). *Exp Agric* **22**:25–32 (1986).
21 Trail MA, Wahem IA and Bizri JN, Snap bean quality changed minimally when stored in low density polyolefin film package. *J Food Sci* **57**:977–979 (1992).
22 Culpepper CW, Effect of stage of maturity of the snap bean on its composition and use as a food product. *J Food Sci* **1**:357–376 (1936).
23 Passam HC, Karapanos IC and Alexopoulos AA, The biological basis of fruit quality, in *Breeding for Fruit Quality* (1st edn), ed. by Jenks MA and Bebeli PJ. Wiley, Chichester, pp. 5–38 (2011).
24 Sánchez-Mata MC, Cámara M and Dìez-Marquès C, Extending shelf-life and nutritive value of green beans (*Phaseolus vulgaris* L.), by controlled atmosphere storage: macronutrients. *Food Chem* **80**:309–315 (2003).
25 Shewfelt RL, Resurreccion AVA and Jordan JL, Quality characteristics of fresh snap beans in different price categories. *J Food Qual* **9**:77–88 (1986).
26 Hazra P, Chattopadhaya A, Dasgupta T, Kar N, Das PK and Som MG, Breeding strategy for improving plant type, pod yield and protein content in vegetable cowpea (*Vigna unguiculata*). *Acta Hortic* **752**:275–280 (2007).
27 USDA-ARS (United States Department of Agriculture, Agricultural Research Service), *USDA Nutrient Database for Standard Reference, Release 28.* USDA-ARS, Beltsville, MD (2016). [Online]. Available: https://ndb.nal.usda.gov/ndb/search [12 September 2016].
28 Olympios CM, *The Cultivation Technique of Field Vegetables*. A Stamoulis, Athens, pp. 571–599 (2015).
29 Cieślik E, Gręda A and Adamus W, Contents of polyphenols in fruit and vegetables. *Food Chem* **94**:135–142 (2006).
30 Aires A, Fernandes C, Carvalho R, Bennett RN, Saavedra MJ and Rosa EAS, Seasonal effects on bioactive compounds and antioxidant capacity of six economically important *Brassica* vegetables. *Molecules* **16**:6816–6832 (2011).
31 Rickman JC, Barrett DM and Bruhn CM, Nutritional comparison of fresh, frozen and canned fruits and vegetables. Part 1. Vitamins C and B and phenolic compounds. *J Sci Food Agric* **87**:930–944 (2007).
32 Berger M, Küchler T, Maaßen A, Busch-Stockfisch M and Steinhart H, Correlations of ingredients with sensory attributes in green beans and peas under different storage conditions. *Food Chem* **103**:875–884 (2007).
33 Ou B, Huang D, Hampsch-Woodill M, Flanagan JA and Deemer EK, Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. *J Agric Food Chem* **50**:3122–3128 (2002).
34 Santamaria P, Elia A, Serio F and Todaro E, A survey of nitrate and oxalate content in fresh vegetables. *J Sci Food Agric* **79**:1882–1888 (1999).
35 Siomos AS and Dogras C, Nitrates in vegetables produced in Greece. *J Veg Crop Prod* **5**:3–13 (1999).
36 Martín-Cabrejas MA, Díaz MF, Aguilera Y, Benítez V, Mollá E and Esteban RM, Influence of germination on the soluble carbohydrates and dietary fibre fractions in non-conventional legumes. *Food Chem* **107**:1045–1052 (2008).
37 Sosulski FW, Elkowicz L and Reichert RD, Oligosaccharides in eleven legumes and their air-classified protein and starch fractions. *J Food Sci* **47**:498–502 (1982).
38 Umaharan P, Ariyanayagam RP and Haque SQ, Genetic analysis of pod quality characteristics in vegetable cowpea (*Vigna unguiculata* L. Walp.). *Sci Hortic* **70**:281–292 (1997).