View Article Online
View Journal

Food & Function

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: S. A. Petropoulos, A. Fernandes, L. Barros, A. Ciric, M. Sokovic and I. C. F. R. Ferreira, *Food Funct.*, 2017, DOI: 10.1039/C7FO01446E.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/food-function

View Article Online
DOI: 10.1039/C7FO01446E

Chemical composition, nutritional value and antimicrobial properties of *Abelmoschus esculentus* seed

Running Title: Chemical composition and antimicrobial properties of okra seeds

Spyridon Petropoulos[a,*], Ângela Fernandes[b], Lillian Barros[b], Ana Ciric[c], Marina Sokovic[c], Isabel C.F.R. Ferreira[b,*]

[a]*University of Thessaly, Department of Agriculture, Crop Production and Rural Environment, 38446 N. Ionia, Magnissia, Greece.*

[b]*Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal*

[c]*University of Belgrade, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", Bulevar Despota Stefana 142, 11000 Belgrade, Serbia.*

*Corresponding authors: Spyridon A. Petropoulos. Postal address: University of Thessaly, School of Agricultural Sciences, Fytokou Street, 38446, N. Ionia, Magnissia, Greece. Email address: fangio57gr@gmail.com; Tel: +30-2421093196; Fax: +30-2421093155.

Isabel C.F.R. Ferreira. Postal address: Polytechnic Institute of Bragança, Campus de Santa Apolónia, 1172, 5300-253 Bragança, Portugal. Email address: iferreira@ipb.pt; Tel: +351-273303219; Fax: +351-273325405.

View Article Online
DOI: 10.1039/C7FO01446E

ABSTRACT

Okra is a vegetable crop usually used for its immature pods. Harvest stage (fruit size) depends on consumers' preferences and fruit that do not meet market requirements are being disposed of. Considering the short time interval from fruit set to harvest stage, the present study evaluates nutritional value, chemical composition, antioxidant and antimicrobial properties of okra seeds from genotypes cultivated under Mediterranean conditions, as an alternative end-use product. For this purpose, seeds from four okra cultivars and local landraces commonly cultivated in the Mediterranean basin, as well as seeds from four commercial cultivars from North America were collected at maturity stage. A significant variation between the studied okra genotypes was observed for all the evaluated parameters. Okra seeds of cv. "Silver Queen" were a significant source of proteins and minerals, such as Ca, K, Fe and Zn. Seeds of all the genotypes contained significant amounts of gamma-tocopherols, liposoluble pigments, and linoleic and palmitic acid. Total phenols content differed between the studied genotypes and correlated with EC_{50} values of Reducing Power assay. Seed extracts exhibited significant antibacterial properties, especially against *Listeria monocytogenes*, *Salmonella enteritidis* and *S. typhimurium*, while fungistatic and fungicidal properties were better than ketoconazole in a genotype dependent manner. Antifungal properties of seeds were noticed towards all tested fungi, where *Aspergillus versicolor* and *Caldosporium cladosporioides* were the most sensitive species. Moreover, two of the tested genotypes ("Boyati" and "Clemson Spineless") exhibited higher fungistatic and fungicidal properties than ketoconazole. In conclusion, okra seeds could be considered as innovative okra products and could be proposed for alternative end-uses in the food and pharmaceutical industry, especially for functional foods with antimicrobial and bioactive properties.

View Article Online
DOI: 10.1039/C7FO01446E

Keywords: *Abelmoschus esculentus*; antimicrobial properties; antioxidant activity; gamma-tocopherol; local landraces; nutritional value

1. Introduction

Okra or lady's finger (*Abelmoschus esculentus* L. Moench.) is a vegetable crop commonly used for its edible immature fruit, which belongs to Malvaceae family. Its center of origin is disputed and according to the literature, both North Asia and East Africa are considered as centers of origin due to great genetic diversity that these regions exhibit.(1) However, not only fruit but other plant parts can be also used, including leaves, flowers, stems and especially seeds which are a rich oil and protein source.(2–4) According to Çalişir et al.(5) mature okra seeds can be used as a coffee substitute in Turkey, while Adelakun et al.(6) have suggested the use of seed flour in the food industry as a rich protein source. Seeds extracts and fomentation have also been traditionally used for therapeutical purposes against abscess and bronchitis.(7)
Seeds represent about 17% of mature pods weight and their extracts are a good source of polyphenolic compounds, mainly catechins and quercetine derivatives.(8) They are also a good source of fatty acids, especially linoleic and palmitic acid which represent more than 70% of total fatty acids.(9) In Greece there are various cultivars and local landraces that are cultivated for their pods; however, the potential of using their seeds as an oil source was examined by Camciuc et al.(3), while Anwar et al.(10) studied the use of okra seeds as an alternative source of biofuels.
Okra seeds have high nutritional value and considered a rich protein source with high quality proteins and amino acids,(3,6) and have been suggested as an alternative source of these macromolecules.(11) Moreover, Camciuc et al.(3) who studied chemical composition of five Greek okra cultivars, including cv. "Veloudo", "Pylaea" and "Boyati" that are also evaluated in our study, have reported different seed protein content, ranging between 23.81 and 25.47%.

View Article Online
DOI: 10.1039/C7FO01446E

Seeds and seeds oil are rich in unsaturated fatty acids, especially linoleic acid; however, its content shows great variation in the literature which indicates that is dependent on genotype and growing conditions.[3,12] Liao et al.[13] who studied antioxidant activity of okra plant parts, have reported that seeds ethanolic extracts showed significant antioxidant potential, while Khomsug et al.[14] have correlated antioxidant properties with phenolics content which contributed significantly to antioxidant potency of seed extracts. Moreover, Singh[15] evaluated antimicrobial properties of seed extracts from okra (*Abelmoschus esculentus*), pumpkin (*Cucurbita pepo*) and pointed gourd (*Trichosanthes dioica*) and reported that okra seeds showed the largest inhibition zones against *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas fluorescens*.

Okra is considered a minor crop in the Mediterranean basin due to marketing options which are limited to fresh consumption and to a lesser extent to frozen and air-dried pods. The aim of the present study was to evaluate chemical composition, nutritional value, antioxidant and antimicrobial properties of seeds from okra genotypes cultivated under Mediterranean conditions, in order to propose innovative end-uses of okra products that will increase market and food industry interest. Moreover, a comparison of genotypes commonly cultivated in the Mediterranean basin with foreign genotypes was attempted, in order to help towards selecting the most promising okra genotypes, regarding their chemical composition and nutritional properties.

2. Materials and Methods

2.1 Plant Material

Seeds of four Greek okra (*Abelmoschus esculentus* L.) genotypes [three cultivars registered in the national catalogue of vegetable crops (cvs. “Boyati”, “Pylaea” and “Veloudo”) obtained from Greek Gene bank (Agricultural Research Center of Macedonia and Thraki, National

View Article Online
DOI: 10.1039/C7FO01446E

Agricultural Research Foundation, Thessaloniki, Greece) and one local landrace ("Lasithi") from the seed collection of Laboratory of Vegetables Production, University of Thessaly, Greece] were evaluated. In addition, seeds of four commercial cultivars from North America [cvs. "Choppee" (heirloom variety), "Clemson Spineless" (commercial cultivar), "Dwarf Long Green" (variety) and "Silver Queen" (heirloom variety)] were obtained from seed companies. Seeds from each genotype were put in seed trays containing peat on April 14th, 2016 and transferred in a nursery on heated seed beds (20 °C), while young seedlings (stage of 3 true leaves) were transplanted in the experimental field of the University of Thessaly in Velestino, Greece on May 11th, 2016 at a plant density of 25000 plants ha^{-1} (0.8 m between rows and 0.5 within each row). For each genotype, 18 plants were used (144 plants in total). Plants were irrigated at regular intervals depending on climate conditions, while fertilization was applied with irrigation water. Training of plants was provided with plastic strings twisted around the stems and attached to horizontal wires above each row.

Pods were harvested at maturity stage (e.g. at snapping point and before pods were fully open) from the first three nods of each plant in order to avoid variation in chemical composition due to pod position on plant. After harvest, seeds were extracted from each pod and ground with an electric ball mill (PX-MFC 90 D, Kinematica AG, Switzerland). Grinded seeds of each genotype were combined in batch samples, lyophilized and stored in deep-freezing conditions (-80 °C) until further analysis.

Seed collections for all the tested genotypes were deposited in the Laboratory of Vegetable Production, University of Thessaly, Greece.

2.2 Chemical composition

2.2.1 Nutritional value

View Article Online
DOI: 10.1039/C7FO01446E

All samples were analysed in terms of macronutrients (moisture, proteins, fat, carbohydrates and ash), according to the AOAC procedures[(16)]. Protein was estimated using the macro-Kjeldahl method (Nx6.25); fat was estimated using a Soxhlet extraction with petroleum ether; ash was determined by sample incineration at 600 ± 15 ºC. Total carbohydrates were calculated by difference and the energetic value was calculated following the equation: Energy (kcal) = 4x (g protein + g carbohydrate) + 9x (g fat). For water content evaluation, samples of seeds were oven dried at 72 °C to a constant weight (approximately for 48 hours) and results were expressed as percentage (%) of moisture content.

For mineral composition analysis, samples of ground seeds were dried in a forced-air oven at 72 °C to constant weight, ground to powder, subjected to dry ashing and extracted with 1 N HCl to determine the mineral. Ca, Mg, Fe, Mn, Zn, and Cu content were determined by atomic absorption spectrophotometry (Perkin Elmer 1100B, Waltham, MA), and Na and K content by flame photometry (Sherwood Model 410, Cambridge, UK).

2.2.2 Free sugars

Dried sample powder (1.0 g) was spiked with the internal standard (IS 5 mg/mL, Sigma-Aldrich, St. Louis, MO, USA), and was extracted with 40 mL of 80% aqueous ethanol at 80 °C for 30 min. The resulting suspension was centrifuged at 15.000 *g* for 10 min. The supernatant was concentrated at 60 °C under reduced pressure and defatted three times with 10 mL of ethyl ether, successively. After concentration at 40 °C, the solid residues were dissolved in water to a final volume of 5 mL, filtered through a 0.22 μm disposable LC filter disk, transferred into an injection vial and determined by HPLC coupled to a RI detector (Knauer, Smartline system 1000, Berlin, Germany) using the internal standard (IS, melezitose, Sigma-Aldrich, St. Louis, MO, USA) method, as previously described by Barros et al.[(17)]

2.2.3 Organic acids

View Article Online
DOI: 10.1039/C7FO01446E

Samples (~1.5 g) were extracted by stirring with 25 mL of meta-phosphoric acid (25 °C at 150 rpm) for 25 min and subsequently centrifuged at 15.000 *g* and filtered through a 0.22 μm disposable LC filter disk, transferred into an injection vial prior to chromatographic analysis. The organic acids content was determined by ultra-fast liquid chromatography (UFLC) (Shimadzu 20A series UFLC, Shimadzu Corporation, Kyoto, Japan) coupled to a Photodiode array detector (PDA, using 215 and 240 nm as preference wavelenghts) following a procedure previously described by Barros et al.[17]

2.2.4 Tocopherols

The dry samples (500 mg) were added with butylated hydroxytoluene (BHT) solution in hexane (10 mg/mL; 100 μL) and IS solution in hexane (tocol; 50 μg/mL; 400 μL), followed by the addition of methanol (4 mL) and vortexed for 1 min. Subsequently, hexane (4 mL) was added and again vortex mixed for 1 min. Saturated NaCl aqueous solution (2 mL) was added, the mixture was homogenized (1 min), centrifuged (5 min, 4000 *g*) and the clear upper layer was carefully transferred to a vial. The sample was re-extracted twice with *n*-hexane. The combined extracts were taken to dryness under a nitrogen stream, redissolved in 2 mL of *n*-hexane, dehydrated with anhydrous sodium sulfate and filtered through 0.2 μm nylon filters and transferred into a dark injection vial. Tocopherols content was determined following a procedure previously described by Barros et al.,[18] using a HPLC system (Knauer, Smartline system 1000, Berlin, Germany) coupled to a fluorescence detector (FP-2020; Jasco, Easton, USA) programmed for excitation at 290 nm and emission at 330 nm, using the IS (tocol, Matreya, Pleasant Gap, PA, USA) method for quantification.

2.2.5 Chlorophylls content

The liposoluble pigments were extracted using a procedures previously described by Nagata & Yamashita,[19] were the dried powder (150 mg) was extracted with a mixture of acetone-hexane (10 mL, 4:6, v/v) by vigorously shaking for 1 min in a vortex. Then the extract was

View Article Online
DOI: 10.1039/C7FO01446E

filtered through Whatman No. 4 filter paper and the absorbance was measured at 453, 505, 645 and 663 nm. Pigment contents were calculated according to the following equation: beta-carotene (mg/100 mL) $= 0.216 \times A_{663} - 1.220 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$; lycopene (mg/100mL) $= -0.0458 \times A_{663} + 0.204 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$; chlorophyll a (mg/100 mL) $= 0.999 \times A_{663} - 0.0989 \times A_{645}$; chlorophyll b (mg/100mL) $= -0.328 \times A_{663} + 1.77 \times A_{645}$.

2.2.6 Fatty acids

Fatty acids obtained after Soxhlet extraction were methylated with 5 ml of methanol:sulfuric acid 95%:toluene 2:1:1 (v/v/v) for, at least, 12 h in a bath at 50 °C and 160 rpm; were added 3 mL of deionised water to obtain phase separation; the fatty acids methyl esters (FAME) were recovered by shaking in a vortex with 3 mL of diethyl ether, and the upper phase was transferred to a vial with Teflon, which was previously added with anhydrous sodium sulphate, to eliminate the water. Then the sample was filtered through a 0.2 µm Whatman nylon filter. The fatty acids were identified using a GC-FID (DANI1000, Contone, Switzerland) operating in the conditions described by Barros et al.[18] Fatty acid methyl esters (FAME reference standard mixture 37, Sigma-Aldrich, St. Louis, MO, USA) was used in order to identify and quantify the fatty acids present in the samples.

2.3 Antioxidant activity

Antioxidant activity and bioactive compounds were assessed according to methods previously reported by Roriz et al.[20] The lyophilized samples (1 g) were extracted by maceration (25 ºC at 150 rpm) with 30 mL of methanol/water (80:20, *v/v*) for 1 h and afterwards filtered through a Whatman No. 4 paper. The samples were re-extracted with an additional portion of methanol/water and the extracts were evaporated in a rotary evaporator (Büchi R-210, Flawil, Switzerland) to remove the methanol and the aqueous portion was frozen and lyophilized. A

View Article Online
DOI: 10.1039/C7FO01446E

final concentration of 50 mg/mL dissolved in methanol/water (80:20, *v/v*) was obtained and then further diluted to different working solutions.

Total phenols were determined based on procedures previously described by Wolfe et al.,[21] where an aliquot of the okra seeds extract solution (0.5 mL, 2.5 mg/mL) was mixed with *Folin-Ciocalteu* reagent (2.5 mL, 1:10 v/v diluted in water) and sodium carbonate (2 mL, 75 g/L). The tubes were shaken and allowed to stand, for color development, for 30 min at 40 °C. Absorbance was measured at 765 nm (AnalytikJena 200 spectrophotometer, Jena, Germany) and gallic acid (0.8 – 0.05 mM) was used as a reference compound to obtain the calibration curve (y = 1.8072 - 0.0211; R^2 = 0.999). The results were expressed as mg of gallic acid equivalents (GAEs) per gram of extract.

The antioxidant activity was evaluated by DPPH radical-scavenging activity, reducing power, inhibition of β-carotene bleaching in the presence of linoleic acid radicals and inhibition of lipid peroxidation using TBARS in brain homogenates.[22] The results were expressed in EC_{50} values (sample concentration providing 50% of antioxidant activity or 0.5 of absorbance in the reducing power assay) for antioxidant activity and Trolox was used as a positive control.

2.4 Antimicrobial properties

Four Gram (+) bacteria [*Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240), *Staphylococcus aureus* (ATCC 6538), and *Listeria monocytogenes* (NCTC 7973)], and Gram (-) bacteria [*Escherichia coli* (ATCC 35210), *Enterobacter cloacae* (human isolate), *Salmonella enteritidis* (clinical isolate) and *Salmonella typhimurium* (ATCC 13311)], were used for testing antibacterial activity of okra seeds extracts, while seven fungi [*Aspergillus fumigatus* (ATCC 1022), *Aspergillus versicolor* (ATCC 11730), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus niger* (ATCC 6275), *Cladosporium cladosporioides* (ATCC 11278), *Penicillium funiculosum* (ATCC 36839), and *Penicillium verrucosum* var. *cyclopium*] were used to test antifungal activity. The microorganisms were obtained from the

View Article Online
DOI: 10.1039/C7FO01446E

Mycological laboratory, Department of Plant Physiology, Institute for biological research "Sinisa Stanković", University of Belgrade, Serbia. The antimicrobial assay was carried out by a microdilution method (CLSI, 2009),[23] following a procedure previously described.[24] The concentrations that completely inhibited bacterial growth were defined as the lowest concentrations without visible growth, at the binocular microscope (MICs) and were determined by the colorimetric microbial viability assay based on reduction of INT ((p-iodonitrotetrazolium violet) and by reinoculation of 10 µl of medium with inoculum and testing of extracts in fresh clean medium. After 24 hours, growth of microorganisms is observed, and absences of growth were considered as an MBC. The MBC and MFC were also calculated, indicating 99.5% killing of the original inoculum. Streptomycin (Sigma-Aldrich S6501), Ampicillin (Sigma-Aldrich A9393), Ketoconazole (Sigma-Aldrich K1754) and Bifonazole (Sigma-Aldrich B3563) were used as positive controls and 5% DMSO was used as a negative control.

2.5 Statistical analysis

The experiment was laid out according to Randomized Complete Blocks design with three replications. Three samples were analyzed for each genotype, while all the assays were carried out in triplicate. Results were expressed as mean values and standard deviations (SD), and analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $p = 0.05$. The analysis was carried out using Statgraphics 5.1.plus (Statpoint Technologies, Inc., VA, USA). Principal Component Analysis (PCA) was performed in order to examine the contribution of the recorded variables to total diversity between the studied genotypes and to classify them according to their chemical composition and nutritional value by using statistical program Statgraphics 5.1.plus (Statpoint Technologies, Inc., VA, USA). The results of PCA analysis are presented as Supplementary Material.

View Article Online
DOI: 10.1039/C7FO01446E

3. Results and Discussion

Proximate composition of okra seeds is presented on a dry weight basis in **Table 1**. Water content differed significantly between the studied genotypes ranging between 6.5 ("cv. Pylaea") and 11.5% (cv. "Silver Queen"). Seeds were a rich proteins source (378-407 g kg^{-1} d.w.), while significant amount of fat and carbohydrates were observed, contributing significantly to total energetic value. Protein content of the studied genotypes was within the range already reported in the literature;(6,25) however, other studies have reported significantly lower protein values,(5,11,26) especially the study of Camciuc et al.(3) who cultivated the same genotypes as in the present study (namely cv. "Pylaea", "Veloudo" and "Boyati") in France and reported protein content between 23.28 and 24.67%. The contradictory results could be probably attributed to different genotypes and/or growing conditions, since in many countries, okra plants are rain-fed and no irrigation is applied, which is essential during fruit development and maturation, not only for yield but also for quality.(27) In addition, Savello et al.(28) observed a significant variation in okra seed meals from whole or sieved seeds, with a higher content in sieved seed meals comparing to whole seed (32.50 and 21.10%, respectively), which suggests that protein is located in seed endosperm rather in seed coat. Therefore, seed size is important for nutritional value, since larger seeds contain a higher amount of endosperm comparing to small seeds, and consequently a higher ratio of endosperm to total seed weight.

Mineral composition of seeds is presented in **Table 2**. The tested genotypes showed significant variation in macro and micro-minerals content, with cv. "Boyati" having the highest content in Ca, Fe and Zn, while "Lasithi", "Veloudo" and "Choppee" had the highest content in Mg, Mn and K, respectively. Ca and K contents were significantly higher than those reported in the literature,(11,28,29) while Na and Mn were significantly lower. These differences could be attributed to different methodologies and analytical equipment used in

View Article Online
DOI: 10.1039/C7FO01446E

the abovementioned studies, as well as to genotype variation. Despite the differences, okra seeds should be considered as rich mineral sources, especially cv. "Boyati" which is an excellent source of Ca, Fe and Zn, whereas Na content is considerably low.

Sugar and organic acid composition is described in **Table 3**. The main detected sugars were raffinose and sucrose, with significant differences in their contribution to total sugar content between the tested genotypes. The highest raffinose and sucrose contents were observed for cv. "Boyati" and "Veloudo", respectively. The organic acid profile of genotype "Clemson Spineless" is provided in **Figure 1**. Citric acid was the most abundant organic acid for most of the genotypes, followed by oxalic and quinic acid, while total organic acids content was the highest for cv. "Pylaea" and "Boyati". These organic acids could be considered as important secondary antioxidants, classified as synergists for promoting the activity of the primary antioxidants.[30,31] To the best of our knowledge, this is the first report regarding sugar and organic acids composition of okra seeds, since so far only reports about total soluble sugars are available.[6,32]

Tocopherols and pigments contents are presented in **Table 4**. The tocopherol profile of "Pylaea" genotype is presented in **Figure 2.** The only detected tocopherols vitamers were gamma- and alpha-tocopherols, with cv. "Pylaea" having the highest content of both gamma- and total tocopherols (17.8 and 26.5 mg 100 g^{-1} d.w). Alpha-tocopherol has been detected in similar amounts (30.4 mg 100 g^{-1} of dry seeds) by Karakoltsidis & Constantinides.[11] András et al.[33] have also detected alpha- and gamma-tocopherols as the main tocopherol vitamers in okra seed extracts and seed oils obtained from Greece. However, they reported higher amounts than those in our study which could be explained by the different solvents they used for seed extraction. Liposoluble pigments content was also affected by genotype with significant differences being observed between the studied okra seeds (**Table 4)** with beta-

View Article Online
DOI: 10.1039/C7FO01446E

carotene being positively correlated with chlorophyll a content, with highest content being observed in cv. "Boyati" and "Veloudo".

Fatty acids composition is presented in **Table 5**. Twenty four individual fatty acids were detected, with significant differences in fatty acids profile between the tested genotypes. The most abundant fatty acid was linoleic acid (40.69-48.01%), followed by palmitic (27.1-28.50%) and oleic acid (16.98-25.58%). Polyunsaturated fatty acids (PUFA) accounted for almost 50% of total fatty acids, while the ratio of polyunsaturated: saturated fatty acids (PUFA/SFA) ranged between 1.24 and 1.42 and was higher than 0.45, as suggested by Guil et al.[(34)] for higher nutritional value. However, n-3 fatty acids content was very low comparing to n-6 fatty acids, which is typical for most of vegetables and vegetable oils, thus resulting in very high n-6/n-3 ratios (283-597). Despite the high values of n-6/n-3 ratios though, the low content in high homologues of n-6 fatty acids (fatty acids with >18 carbon atoms) is beneficial for human health, since they are considered as precursors of inflammatory responses.[(34)] Similar results have been reported in the study of Berry (9) and Savello et al.[(28)], while Al-Wandawi[(29)] have detected high amounts of oleic instead of linoleic acid in seeds of two okra varieties.

Total phenolic content was the highest in cv. "Dwarf Long Green" and "Silver Queen", while cv. "Veloudo" showed the lowest content **(Table 6**). The values reported in this study are between those reported for okra seed extracts by Khomsug et al.[(14)] (142.8 mg GAE 100 g^{-1} extract) and Hu et al.[(35)] (2810 mg GAE 100 g^{-1} extract), indicating the great variation in the literature which could be attributed to genotype and growing conditions differences. Antioxidant properties were evaluated with four different assays, however no consistent results were observed for the studied genotypes, while total phenolic compounds content seems to be correlated only with the results of reducing power assay with cv. "Dwarf Long Green" and "Silver Queen" having the lowest EC_{50} values (**Table 6**). Shui & Peng[(36)] and Hu

View Article Online
DOI: 10.1039/C7FO01446E

et al.[35] have also correlated antioxidant properties with phenolic compounds content and quercetin and quercetin derivatives in particular, while Mohammad et al.[37] and Khomsug et al.[14] have also suggested that okra seeds have good reducing power and radical scavenging properties, respectively. However, the tested assays that estimate the elimination of free radicals (DPPH and beta-carotene inhibition) showed various genotype responses, which indicates that probably other tests have to incorporated for more consistent results, since plant mechanisms against oxidative stress differ between plant species.(38)

In vitro antimicrobial properties of the studied okra genotypes against various bacteria strains are presented in **Tables 7** and **8**. The bacteriostatic activity ranged from 100 to 1000 μg mL^{-1}, whereas positive controls of streptomycin and ampicillin presented MIC values ranging from 40 to 400 μg mL^{-1} (**Table 7**). Bactericidal activity values oscillated between 150 and 1500 μg mL^{-1}, whereas for positive controls MBC values ranged between 100 and 750 μg mL^{-1}. The various genotypes showed lower inhibition effects than streptomycin, except for the case of *Salmonella enteritidis* where seeds extracts of "Silver Queen" showed better results. In addition, inhibition effect of ampicillin was comparable or lower than seed extracts against most of the tested bacteria, especially against *Listeria monocytogenes*, *S. enteritidis* and *S. typhimurium*. Fungistatic and fungicidal properties are presented in **Table 8**, with MIC values ranging between 60 and 750 μg mL^{-1} for seed extracts, while for positive controls (ketoconazole and bifonazole) values ranged between 50 and 1500 μg mL^{-1}). Seeds extracts of genotypes "Boyati" and "Clemson Spineless" showed better fungistatic and fungicidal properties than ketoconazole. Bifonazole was more effective than all seed extracts with only exception of "Boyati" and "Clemson Spineless" genotypes against *A. versicolor.* Moreover, cv. "Boyati" showed significant fungistatic and fungicidal properties against *A. versicolor*, *Cladosporium cladosporioides* and fungistatic properties against *P. funiculosum*, while "Lasithi" had significant fungistatic properties against *A. fumigatus*. Nwaiwu et al.[39] and

View Article Online
DOI: 10.1039/C7FO01446E

Singh[15] have previously reported antimicrobial properties of okra seeds against various bacteria. These antimicrobial properties could be attributed to the presence of high levels of organic acids found in this matrix, namely citric acid that is naturally occurring antimicrobial substances of biotic origin, oxalic acid for which there is a growing evidence of its resistance against pathogens and quinic acid which may indicate their effectiveness against the growth of microorganisms.[40–42]

Moreover, to the best of our knowledge, this is the first report regarding fungicidal and fungistatic properties of okra seed extracts.

4. Conclusion

Nutritional properties and chemical composition of the studied okra seeds showed a significant genotypical variation, while PCA analysis revealed distinct groups between the tested genotypes. However, in any case okra seeds could be considered as rich sources of protein and minerals, while they also contain significant amounts of bioactive compounds which contribute to antioxidant and antimicrobial properties. Therefore, considering that market standards in the Mediterranean basin require fruit of small size, fruit that do not meet these standards could be harvested at full maturity for their seeds, allowing for innovative okra products that could find uses in the food and pharmaceutical industry. An essential quality feature that has to be examined in future research is the content in anti-nutrient factors, since okra seeds have been reported to contain anti-nutrients such as trypsin, hemagglutinin, tannins, phytates and oxalates. Therefore, screening of local landraces may reveal genotypes that contain less amounts of these compounds which could be very important for the food industry.

Conflicts of interest

View Article Online
DOI: 10.1039/C7FO01446E

All authors declare that they do not have any conflict of interest for publishing this research work.

Acknowledgment

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Program PT2020 for financial support to CIMO (UID/AGR/00690/2013), A. Fernandes grant (SFRH/BPD/114753/2016) and L. Barros contract. The authors are also grateful to the Serbian Ministry of Education, Science and Technological Development, grant number 173032 for financial support.

References

1. Kumar S, Dagnoko S, Haougui A, Ratnadass A, Pasternak D, Kouame C. Okra (Abelmoschus spp.) in West and Central Africa: Potential and progress on its improvement. African J Agric Res [Internet]. 2010;5(25):3590–8. Available from: http://www.academicjournals.org/AJAR
2. Adetuyi F, Osagie A. Nutrient, antinutrient, mineral and zinc bioavailability of okra Abelmoschus esculentus (L) Moench variety. Am J Food Nutr [Internet]. 2011;1(2):49–54. Available from: http://www.scihub.org/AJFN/PDF/2011/2/AJFN-1-2-49-54.pdf
3. Camciuc M, Deplagne M, Vilarem G, Gaset A. Okra - Abelmoschus esculentus L. (Moench.) a crop with economic potential for set aside acreage in France. Ind Crops Prod. 1998;7(2–3):257–64.
4. Gemede HF, Ratta N, Haki GD, Woldegiorgis AZ. Nutritional Quality and Health Benefits of Okra (Abelmoschus esculentus): A Review. Food Sci Qual Manag. 2014;33:87–97.

View Article Online
DOI: 10.1039/C7FO01446E

5. Çalişir S, Özcan M, Haciseferoğullari H, Yildiz MU. A study on some physico-chemical properties of Turkey okra (Hibiscus esculenta L.) seeds. J Food Eng. 2005;68(1):73–8.

6. Adelakun OE, Oyelade OJ, Ade-Omowaye BIO, Adeyemi IA, Van de Venter M, Koekemoer TC. Influence of pre-treatment on yield chemical and antioxidant properties of a Nigerian okra seed (Abelmoschus esculentus Moench) flour. Food Chem Toxicol [Internet]. Elsevier Ltd; 2009;47(3):657–61. Available from: http://dx.doi.org/10.1016/j.fct.2008.12.023

7. Güne S, Savran A, Yavuz M, Ko M. Ethnopharmacological survey of medicinal plants in Karaisali and its surrounding (Adana-Turkey). J Herb Med. 2017;8:68–75.

8. Arapitsas P. Identification and quantification of polyphenolic compounds from okra seeds and skins. Food Chem. 2008;110(4):1041–5.

9. Berry SK. The fatty acid composition and cyclopropene fatty acid content of the maturing okra (Hibiscus esculentus L.) fruits. Pertanika. 1980;3(2):82–6.

10. Anwar F, Rashid U, Ashraf M, Nadeem M. Okra (Hibiscus esculentus) seed oil for biodiesel production. Appl Energy [Internet]. Elsevier Ltd; 2010;87(3):779–85. Available from: http://dx.doi.org/10.1016/j.apenergy.2009.09.020

11. Karakoltsidis P a, Constantinides SM. Okra seeds: a new protein source. J Agric Food Chem. 1975;23(6):1204–7.

12. Jarret RL, Wang ML, Levy IJ. Seed oil and fatty acid content in okra (Abelmoschus esculentus) and related species. J Agric Food Chem. 2011;59:4019–24.

13. Liao H, Dong W, Shi X, Liu H, Yuan K. Analysis and comparison of the active components and antioxidant activities of extracts from Abelmoschus esculentus L. Pharmacogn Mag. 2012;8(30):156–61.

14. Khomsug P, Thongjaroenbuangam W, Pakdeenarong N, Suttajit M, Chantiratikul P.

View Article Online
DOI: 10.1039/C7FO01446E

Antioxidative Activities and Phenolic Content of Extracts from Okra (Abelmoschus esculentus L.) [Internet]. Vol. 5, Research Journal of Biologi Sciences. 2010. Available from: http://docsdrive.com/pdfs/medwelljournals/rjbsci/2010/310-313.pdf

15. Singh K. Phytochemical determination and antibacterial activity of Trichosanthes dioica Roxb (patal), Cucurbita maxima (pumpkin) and Abelmoschus esculentus Moench (okra) plant seeds. National Institute of Technology, India; 2012.

16. AOAC. Official methods of analysis of AOAC International. 20th ed. Horwitz W, Latimer G, editors. Gaithersburg: MD: AOAC International; 2016.

17. Barros L, Pereira E, Calhelha RC, Dueñas M, Carvalho AM, Santos-Buelga C, et al. Bioactivity and chemical characterization in hydrophilic and lipophilic compounds of Chenopodium ambrosioides L. J Funct Foods. 2013;5(4):1732–40.

18. Barros L, Pereira C, Ferreira ICFR. Optimized analysis of organic acids in edible mushrooms from Portugal by ultra fast liquid chromatography and photodiode array detection. Food Anal Methods. 2013;6(1):309–16.

19. Nagata M, Yamashita I. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. Nippon Shokuhin Kogyo Gakkaishi. 1992;39(10):925–8.

20. Roriz CL, Barros L, Carvalho AM, Santos-Buelga C, Ferreira ICFR. Pterospartum tridentatum, Gomphrena globosa and Cymbopogon citratus: A phytochemical study focused on antioxidant compounds. Food Res Int [Internet]. Elsevier Ltd; 2014;62:684–93. Available from: http://dx.doi.org/10.1016/j.foodres.2014.04.036

21. Wolfe K, Xianzhong W, Liu RH. Antioxidant activity of apple peels. J Agric Food Chem. 2003;51:609–14.

22. Barros L, Oliveira S, Carvalho AM, Ferreira ICFR. In vitro antioxidant properties and characterization in nutrients and phytochemicals of six medicinal plants from the

View Article Online
DOI: 10.1039/C7FO01446E

Portuguese folk medicine. Ind Crops Prod [Internet]. Elsevier B.V.; 2010;32(3):572–9. Available from: http://dx.doi.org/10.1016/j.indcrop.2010.07.012

23. Tsukatani T, Suenaga H, Shiga M, Noguchi K, Ishiyama M, Ezoe T, et al. Comparison of the WST-8 colorimetric method and the CLSI broth microdilution method for susceptibility testing against drug-resistant bacteria. J Microbiol Methods [Internet]. Elsevier B.V.; 2012;90(3):160–6. Available from: http://dx.doi.org/10.1016/j.mimet.2012.05.001

24. Soković M, Van Griensven LJLD. Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, Agaricus bisporus. Eur J Plant Pathol. 2006;116(3):211–24.

25. Ogungbenle HN, Arekemase EF. Nutritional evaluation of nigerian dried okra (Abelmoschus esculentus) seeds. Pakistan J Sci Ind Res Ser B Biol Sci. 2014;57(3):129–35.

26. Rao PU. Chemical composition and biological evaluation of okra (Hibiscus esculentus) seeds and their kernels. Qual Plant. 1985;35:389–96.

27. Home PG, Panda RK, Kar S. Effect of method and scheduling of irrigation on water and nitrogen use efficiencies of okra (Abelmoschus esculentus). Agric Water Manag. 2002;55(2):159–70.

28. Savello PA, Martin FM, Hill JM. Nutritional composition of okra seed meal. J Agric Food Chem. 1980;1163–6.

29. Al-Wandawi H. Chemical composition of seeds of two okra cultivars. J Agric Food Chem. 1983;(1979):1355–8.

30. Maieves HA, López-Froilán R, Morales P, Pérez-Rodrígueza ML, Hoffmann RR, Cámara M, et al. Antioxidant phytochemicals of Hovenia dulcis Thunb. peduncles in different maturity stages. J Funct Foods. 2015;18(B):1117–24.

View Article Online
DOI: 10.1039/C7FO01446E

31. Martínez-Esplá A, García-Pastor M, Zapata P, Guillén F, Serrano M, Valero D, et al. reharvest application of oxalic acid improves quality and phytochemical content of artichoke (Cynara scolymus L.) at harvest and during storage. Food Chem. 2017;230:343–9.

32. Adelakun OE, Ade-Omowaye BIO, Adeyemi IA, Van de Venter M. Mineral composition and the functional attributes of Nigerian okra seed (Abelmoschus esculentus Moench) flour. Food Res Int [Internet]. Elsevier Ltd; 2012;47(2):348–52. Available from: http://dx.doi.org/10.1016/j.fct.2009.01.036

33. András CD, Simándi B, Örsi F, Lambrou C, Missopolinou-Tatala D, Panayiotou C, et al. Supercritical carbon dioxide extraction of okra (Hibiscus esculentus L) seeds. J Sci Food Agric. 2005;85(8):1415–9.

34. Guil JL, Torija ME, Giménez JJ, Rodriguez I. Identification of fatty acids in edible wild plants by gas chromatography. J Chromatogr A,. 1996;719:229–35.

35. Hu L, Yu W, Li Y, Prasad N, Tang Z. Antioxidant activity of extract and its major constituents from okra seeds on rat hepatocytes injured by carbon tetracholride. Biomed Res Int. 2014;2014.

36. Shui G, Peng LL. An improved method for the analysis of major antioxidants of Hibiscus esculentus. J Chromatogr A. 2004;1048:17–24.

37. Mohammad B, Ebrahimzadeh A, Nabavi F, Nabavi M. Antihypoxic and antioxidant activity of Hibiscus esculentus seeds. Animals. 2010;61(1):30–6.

38. Chen S, Shen X, Cheng S, Li P, Du J, Chang Y, et al. Evaluation of garlic cultivars for polyphenolic content and antioxidant properties. PLoS One. 2013;8(11).

39. Nwaiwu N, Mshelia F, Raufu I. Antimicrobial activities of crude extracts of Moringa oleifera, Hibiscus sabdariffa and Hibiscus esculentus seeds against some Enterobacteria. J Appl Phytotechnology Environ Sanit. 2012;1:11–6.

View Article Online
DOI: 10.1039/C7FO01446E

40. Kim SA, Rhee MS. Synergistic antimicrobial activity of caprylic acid in combination with citric acid against both Escherichia coli O157:H7 and indigenous microflora in carrot juice. Food Microbiol. 2015;49:166–72.

41. Duangjai A, Suphrom N, Wungrath J, Ontawong A, Nuengchamnong N, Yosboonruang A. Comparison of antioxidant, antimicrobial activities and chemical profiles of three coffee (Coffea arabica L.) pulp aqueous extracts. Integr Med Res 2016;5(4):324–31.

42. Deng J, Bi Y, Zhang Z, Xie D, Ge Y, Li W, et al. Postharvest oxalic acid treatment induces resistance against pink rot by priming in muskmelon (Cucumis melo L.) fruit. Postharvest Biol Technol. 2015;106:53–61.

View Article Online
DOI: 10.1039/C7FO01446E

Figure captions

Figure 1. Individual profile of organic acids of genotype "Clemson Spineless". 1- oxalic acid, 2- quinic acid and 3- citric acid.

Figure 2. Individual profile of tocopherols of "Pylaea" genotype. 1- α-tocopherol, 2- γ-tocopherol, 3- Tocol, IS.

View Article Online
DOI: 10.1039/C7FO01446E

Table 1. Water content (%), nutritional value (g/100 g dw) and energetic value (kcal/100 g dw) of seeds of the studied okra genotypes (mean ± SD).

Genotype	Water content (%)	Fat	Proteins	Ash	Carbohydrates	Energy
Lasithi	11.3±0.7	26.0±0.2d	39.4±0.7bc	4.83±0.07d	29.7±0.7bc	510.9±0.7d
Pylaea	6.5±0.2	27.2±0.2c	37.4±0.5e	4.8±0.1d	30.6±0.1ab	516.9±0.6c
Boyati	10.2±0.5	27.6±0.1b	37.8±0.1e	5.0±0.1cd	29.5±0.1c	517.8±0.9c
Veloudo	8.0±0.3	28.89±0.01a	39.11±0.04cd	5.24±0.04bc	26.76±0.01e	523.5±0.1a
Choppee	10.8±0.6	26.1±0.1d	39.88±0.03b	5.5±0.1ab	28.49±0.01d	508.5±0.8e
Dwarf Long Green	8.6±0.5	26.0±0.1d	39.1±0.1cd	5.70±0.03a	29.13±0.03cd	507.3±0.3e
Silver Queen	11.5±0.7	28.6±0.3a	40.7±0.3a	5.5±0.3ab	25.3±0.7f	520.8±0.2b
Clemson Spineless	7.1±0.6	24.77±0.03e	38.50±0.04d	5.5±0.2ab	31.3±0.2a	501.9±0.7f

Different Latin letters in the same column indicate significant differences between the studied genotypes according to Tukey's HSD test (p=0.05).

Table 2. Mineral composition (mg/100 g dw) of seeds of the studied okra genotypes (mean ± SD).

Genotype	Ca	Mg	K	Na	Fe	Zn	Mn
Lasithi	1239.3±38.2c	600.0±16.4a	3066.7±115.5b	13.3±2.3b	6.98±0.27b	7.37±0.47ab	0.73±0.12ab
Pylaea	810.7±35.2de	484.0±12.2b	1866.7±230.9c	12.0±2.0b	6.49±0.01c	6.37±0.06d	0.77±0.12ab
Boyati	3052.0±31.7a	509.3±30.6ab	2066.7±305.5c	12.7±3.1b	7.51±0.25a	7.78±0.12a	0.55±0.10b
Veloudo	2112.7±113.5b	474.7±62.8b	2066.7±416.3c	13.3±2.3b	7.33±0.12ab	6.75±0.11c	1.01±0.10a
Choppee	864.7±14.2d	428.0±26.5b	4000.0±200.0a	15.3±2.3ab	5.85±0.11d	6.81±0.16bc	0.66±0.09ab
Dwarf Long Green	904.0±48.9d	474.7±15.3b	2133.3±305.5c	20.0±2.0a	6.07±0.18cd	6.22±0.26d	0.53±0.05b
Silver Queen	681.3±52.1e	467.3±26.6b	2133.3±305.5c	16.0±2.0ab	7.52±0.16a	7.75±0.01a	0.79±0.14ab
Clemson Spineless	1309.3±60.9c	507.3±66.5ab	1800.0±200.0c	14.0±2.0ab	5.83±0.06d	6.44±0.12c	0.92±0.10ab

Different Latin letters in the same column indicate significant differences between the studied genotypes according to Tukey's HSD test ($p=0.05$).

Table 3. Composition of seeds in sugars and organic acids (g/100 g dw) of the studied okra genotypes (mean ± SD).

Genotype	Sucrose	Raffinose	Total Sugars	Oxalic acid	Quinic acid	Citric acid	Total organic acids
Lasithi	2.86±0.03b	4.05±0.01f	6.91±0.04e	0.43±0.02d	0.141±0.002c	0.351±0.004g	0.92±0.03f
Pylaea	2.34±0.03e	6.30±0.03b	8.639±0.005a	0.440±0.001d	0.25±0.01a	0.894±0.007b	1.59±0.02a
Boyati	2.28±0.04ef	6.45±0.02a	8.74±0.06a	0.569±0.001a	0.183±0.006b	0.813±0.009c	1.57±0.02a
Veloudo	3.26±0.04a	4.62±0.06e	7.9±0.1c	0.293±0.001f	0.102±0.002d	0.479±0.003f	0.874±0.005g
Choppee	2.625±0.002d	3.89±0.05g	6.51±0.05f	0.463±0.001c	0.013±0.001f	0.58±0.02e	1.05±0.02d
Dwarf Long Green	2.23±0.08f	6.13±0.07c	8.4±0.2b	0.351±0.002e	0.089±0.005e	0.663±0.005d	1.10±0.01c
Silver Queen	2.778±0.001c	3.53±0.06h	6.30±0.06g	0.490±0.001b	0.174±0.006b	0.340±0.004g	1.00±0.01e
Clemson Spineless	1.95±0.01g	5.56±0.03d	7.50±0.02d	0.272±0.002g	0.024±0.002f	0.952±0.007a	1.25±0.01b

Different Latin letters in the same column indicate significant differences between the studied genotypes according to Tukey's HSD test (p=0.05).

Table 4. Composition of seeds in tocopherols and pigments (mg/100 g dw) of the studied okra genotypes (mean ± SD).

Genotype	α-Tocopherol	γ-Tocopherol	Total Tocopherols	β-carotene	Chlorophyll a	Chlorophyll b	Total Chlorophylls
Lasithi	9.20±0.04a	13.17±0.07f	22.4±0.1e	0.09±0.01de	4.6±0.4de	6.0±0.5ab	6.43±0.43ab
Pylaea	8.70±0.05b	17.83±0.01a	26.53±0.04a	0.16±0.03bc	0.9±0.2bc	3.3±0.7c	4.16±0.48cd
Boyati	8.68±0.04b	14.40±0.05e	23.08±0.01c	0.37±0.02a	2.1±0.1a	1.3±0.1e	3.37±0.03d
Veloudo	7.03±0.04e	15.13±0.05c	22.15±0.01f	0.35±0.03a	1.9±0.2a	1.4±0.1d	3.39±0.04d
Choppee	7.83±0.02d	14.78±0.01d	22.61±0.02d	0.19±0.02b	1.1±0.1b	2.5±0.3cd	3.54±0.18cd
Dwarf Long Green	8.35±0.02c	11.53±0.02h	19.88±0.04g	0.13±0.02bcd	0.8±0.1bcd	3.7±0.7c	4.43±0.55c
Silver Queen	9.19±0.08a	16.70±0.07b	25.9±0.2b	0.08±0.01e	0.41±0.02e	6.9±0.4a	7.32±0.38a
Clemson Spineless	6.633±0.02f	11.80±0.01g	18.44±0.01h	0.11±0.01cde	0.56±0.04cde	5.0±0.3b	5.55±0.31b

Different Latin letters in the same column indicate significant differences between the studied genotypes according to Tukey's HSD test (p=0.05).

Table 5. Fatty acids composition (%) of seeds of the studied okra genotypes (mean ± SD).

	Genotypes							
	Lasithi	Pylaea	Boyati	Veloudo	Choppee	Dwarf Long Green	Silver Queen	Clemson Spineless
C6:0	0.004±0.001	0.001±0.001	0.006±0.001	0.010±0.001	0.0090±0.0001	0.003±0.001	0.003±0.001	0.003±0.001
C8:0	0.0040±0.0001	0.0020±0.0001	0.0030±0.0001	0.011±0.001	0.0020±0.0001	0.0020±0.0001	0.0010±0.0001	0.0020±0.0001
C10:0	0.0070±0.0001	0.0030±0.0001	0.0040±0.0001	0.027±0.001	0.0020±0.0001	0.0020±0.0001	0.0020±0.0001	0.0020±0.0001
C12:0	0.011±0.001	0.0060±0.0001	0.0060±0.0001	0.020±0.001	0.0080±0.0001	0.0060±0.0001	0.0090±0.0001	0.0075±0.0007
C13:0	0.019±0.001	0.024±0.001	0.026±0.001	0.024±0.001	0.016±0.001	0.016±0.001	0.020±0.001	0.014±0.001
C14:0	0.250±0.001	0.233±0.001	0.235±0.001	0.246±0.001	0.237±0.05	0.234±0.002	0.236±0.002	0.244±0.004
C14:1	0.0020±0.0001	0.0020±0.0001	0.0020±0.0001	0.0020±0.0001	0.0020±0.0001	0.0020±0.0001	0.0020±0.0001	0.0020±0.0001
C15:0	0.039±0.001	0.032±0.001	0.035±0.001	0.035±0.001	0.032±0.001	0.031±0.001	0.035±0.001	0.031±0.001
C16:0	27.56±0.05	27.65±0.14	28.50±0.03	27.47±0.08	27.1±0.1	28.16±0.01	26.95±0.08	28.11±0.06
C16:1	0.412±0.001	0.410±0.001	0.378±0.001	0.351±0.001	0.327±0.001	0.369±0.003	0.387±0.001	0.394±0.001
C17:0	0.196±0.006	0.185±0.002	0.203±0.001	0.193±0.001	0.189±0.001	0.167±0.001	0.209±0.001	0.159±0.001
C18:0	4.14±0.05	3.67±0.03	3.95±0.01	3.94±0.01	4.00±0.06	4.21±0.02	3.98±0.05	4.05±0.02
C18:1n9	21.96±0.09	20.98±0.02	16.98±0.01	20.40±0.03	25.58±0.08	21.08±0.03	20.4±0.2	21.47±0.04
C18:2n6	43.58±0.01	45.0±0.1	48.01±0.04	45.6±0.1	40.69±0.09	43.84±0.05	46.00±0.08	43.76±0.06
C18:3n3	0.242±0.001	0.218±0.001	0.221±0.008	0.208±0.001	0.231±0.001	0.264±0.001	0.232±0.002	0.244±0.001
C20:0	0.504±0.006	0.459±0.007	0.465±0.001	0.467±0.004	0.514±0.001	0.509±0.001	0.462±0.004	0.492±0.002
C20:1	0.057±0.004	0.069±0.001	0.047±0.004	0.059±0.001	0.074±0.001	0.067±0.001	0.055±0.006	0.051±0.003
C20:3n3+C21:0	0.073±0.001	0.116±0.007	0.064±0.001	0.059±0.001	0.068±0.001	0.082±0.001	0.088±0.005	0.080±0.001
C20:5n3	0.022±0.001	0.043±0.003	0.021±0.001	0.018±0.001	0.021±0.001	0.024±0.001	0.024±0.001	0.026±0.001
C22:0	0.743±0.001	0.685±0.001	0.690±0.006	0.66±0.01	0.774±0.001	0.757±0.003	0.695±0.009	0.709±0.006
C22:1n9	0.019±0.001	0.026±0.001	0.016±0.001	0.016±0.001	0.016±0.001	0.018±0.001	0.021±0.001	0.019±0.001
C23:0	0.051±0.001	0.041±0.001	0.044±0.001	0.044±0.001	0.034±0.001	0.038±0.001	0.048±0.003	0.030±0.001
C24:0	0.067±0.006	0.064±0.001	0.061±0.001	0.061±0.004	0.072±0.002	0.068±0.001	0.060±0.004	0.063±0.003
C24:1	0.041±0.004	0.043±0.002	0.031±0.001	0.035±0.001	0.039±0.001	0.052±0.005	0.035±0.003	0.032±0.003
Total SFA (% of total FA)	33.6±0.1c	33.1±0.1de	34.23±0.02a	33.2±0.1d	33.0±0.2e	34.20±0.02a	32.7±0.1f	33.9±0.1b
Total MUFA (% of total FA)	22.5±0.1b	21.48±0.02d	17.42±0.01f	20.83±0.03e	26.0±0.1a	21.54±0.03d	20.9±0.2e	21.94±0.04c
Total PUFA (% of total FA)	43.92±0.01f	45.4±0.1d	48.32±0.03a	45.9±0.1c	41.0±0.1g	44.21±0.05e	46.4±0.1b	44.1±0.1e
PUFA/SFA	1.31	1.37	1.41	1.38	1.24	1.29	1.42	1.30

n-6/n-3	461	283	565	597	462	414	414	413

Different Latin letters in the same row indicate significant differences between the studied genotypes according to Tukey's HSD test (p=0.05).

Table 6. Total phenolic compounds content (mg GAE/g extract) and antioxidant properties (EC_{50} values) of seeds of the studied okra genotypes (mean ± SD).

		Reducing power	Radical scavenging activity		Lipid peroxidation inhibition
Genotype	Folin-ciocalteu (mg GAE/g extract)	Ferricyanide/Prussian blue (EC_{50}; mg/mL)	DPPH scavenging activity (EC_{50}; mg/mL)	β-carotene/linoleate (EC_{50}; mg/mL)	TBARS (EC_{50}; mg/mL)
Lasithi	13.9±0.7e	4.66±0.02ab	6.7±0.1a	1.38±0.05f	0.34±0.01cd
Pylaea	14.3±0.3d	4.7±0.1a	6.16±0.03b	1.56±0.04d	0.33±0.02d
Boyati	13.5±0.3f	4.34±0.04c	6.07±0.03b	1.48±0.05e	0.36±0.02bcd
Veloudo	13.0±0.8g	4.57±0.05b	6.1±0.1b	1.83±0.05a	0.392±0.004a
Choppee	15.6±0.8b	3.9±0.1d	6.1±0.1b	1.88±0.01a	0.35±0.01bcd
Dwarf Long Green	16.5±0.8a	3.33±0.03g	6.0±0.1b	1.66±0.02c	0.38±0.04ab
Silver Queen	16.5±0.4a	3.66±0.01e	6.7±0.1a	1.74±0.04b	0.37±0.04abc
Clemson Spineless	15.06±0.07c	3.44±0.02f	6.6±0.1a	1.8±0.1b	0.353±0.004bcd

Different Latin letters in the same column indicate significant differences between the studied genotypes according to Tukey's HSD test (p=0.05).

Table 7. *In vitro* antibacterial activity of seed extracts (MIC and MBC in µg/ml) of the studied okra genotypes.

Genotypes		*Bacillus cereus*	*Micrococcus flavus*	*Staphylococcus aureus*	*Listeria monocytogenes*	*Escherichia coli*	*Enterobacter cloacae*	*Salmonella enteritidis*	*Salmonella typhimurium*
Lasithi	**MIC**	500	750	400	500	750	500	500	500
	MBC	750	1.000	750	1000	1000	750	750	750
Pylaea	**MIC**	500	500	400	400	500	500	250	500
	MBC	750	1.00	500	500	1000	750	500	1000
Boyati	**MIC**	250	500	500	250	500	500	500	250
	MBC	500	1000	1000	400	1000	750	1000	500
Veloudo	**MIC**	500	400	250	250	750	400	150	250
	MBC	750	750	500	500	1000	750	250	500
Choppee	**MIC**	400	1000	250	250	1000	500	150	400
	MBC	500	1500	500	500	1500	750	250	500
Dwarf Long Green	**MIC**	400	750	250	250	750	400	150	750
	MBC	500	1000	500	500	1000	500	250	1000
Silver Queen	**MIC**	400	1000	250	250	1000	400	100	250
	MBC	500	1500	500	500	1500	500	150	500
Clemson Spineless	**MIC**	500	500	1000	250	500	500	400	250
	MBC	750	1000	1500	500	1000	750	750	500
Streptomycin*	**MIC**	100	200	40	200	200	200	150	250
	MBC	200	300	100	300	300	300	300	500
Ampicillin*	**MIC**	250	250	250	400	400	250	300	400
	MBC	400	400	450	500	500	500	600	750

MIC: Minimum Inhibitory concentration; MBC: Minimum bactericidal concentration.
*Streptomycin and ampicillin were used as positive controls.

Table 8. *In vitro* antifungal activity of seed extracts (MIC and MFC in μg mL^{-1}) of the studied okra genotypes.

Genotypes		*Aspergillus fumigatus*	*Aspergillus versicolor*	*Aspergillus ochraceus*	*Aspergillus niger*	*Cladosporium cladosporioides*	*Penicillium funiculosum*	*Penicillium verrucosum* var. *cyclopium*
Lasithi	**MIC**	120	200	400	200	80	400	500
	MFC	250	250	750	250	120	500	750
Pylaea	**MIC**	500	200	500	750	250	400	500
	MFC	1000	250	1000	1000	500	750	750
Boyati	**MIC**	200	60	400	200	400	120	400
	MFC	250	120	750	250	600	250	500
Veloudo	**MIC**	250	250	400	500	250	500	500
	MFC	500	500	750	750	500	1000	1000
Choppee	**MIC**	200	250	400	200	120	250	250
	MFC	500	500	750	250	250	500	500
Dwarf Long Green	**MIC**	250	400	250	500	250	400	400
	MFC	500	500	500	750	500	500	500
Silver Queen	**MIC**	250	120	500	500	120	750	750
	MFC	500	250	750	750	250	1000	1000
Clemson Spineless	**MIC**	250	80	250	250	200	250	400
	MFC	500	120	500	500	250	500	500
Ketoconazole*	**MIC**	250	200	1500	200	750	200	200
	MFC	500	500	2000	500	150	500	300
Bifonazole*	**MIC**	150	100	150	150	50	200	100
	MFC	200	200	200	200	100	250	200

MIC: Minimum Inhibitory concentration; MFC: Minimal fungicidal concentration.
*Ketoconazole and bifonazole were used as positive controls.

View Article Online
DOI: 10.1039/C7FO01446E

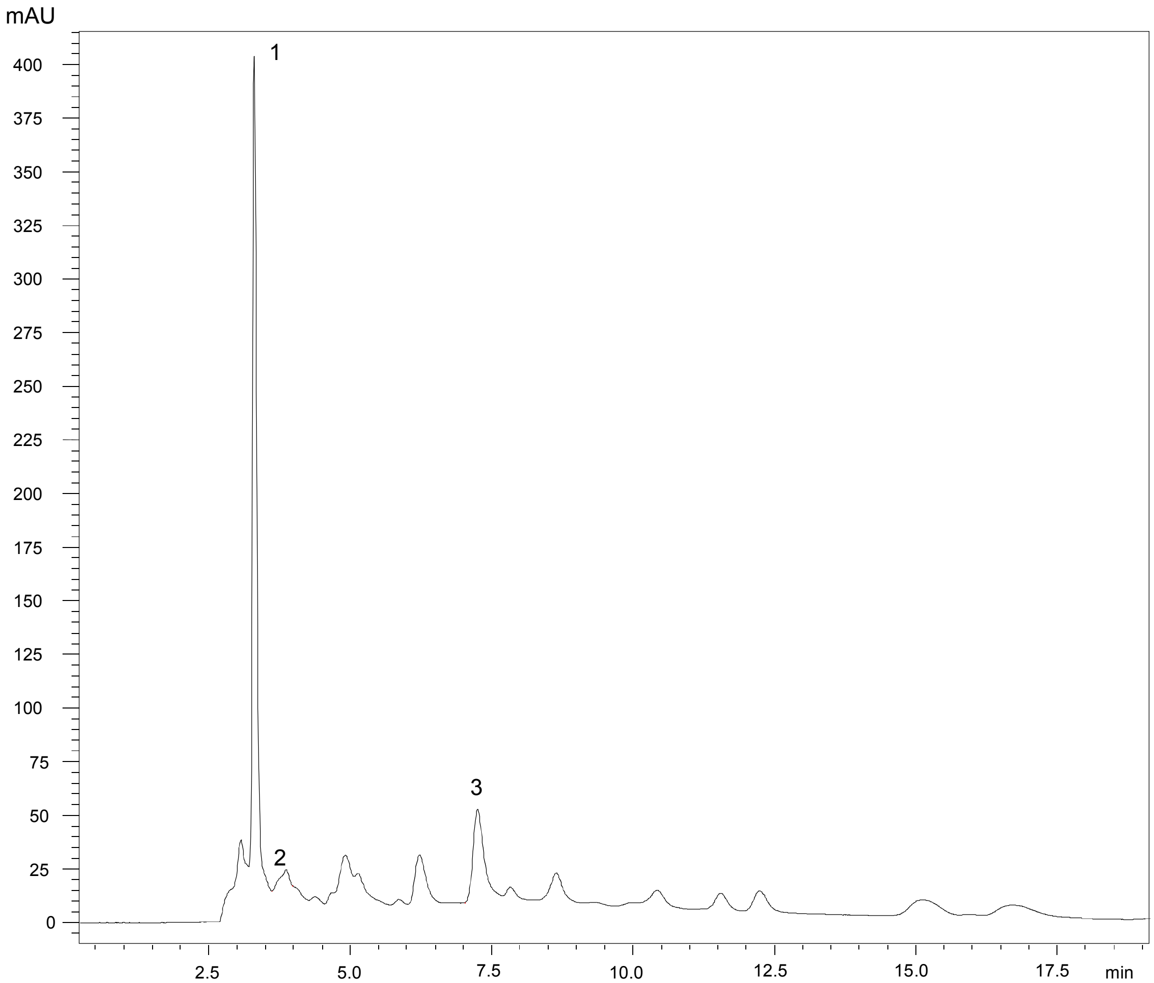

Figure 1. Individual profile of organic acids of genotype "Clemson Spineless". 1- oxalic acid, 2- quinic acid and 3- citric acid.

View Article Online
DOI: 10.1039/C7FO01446E

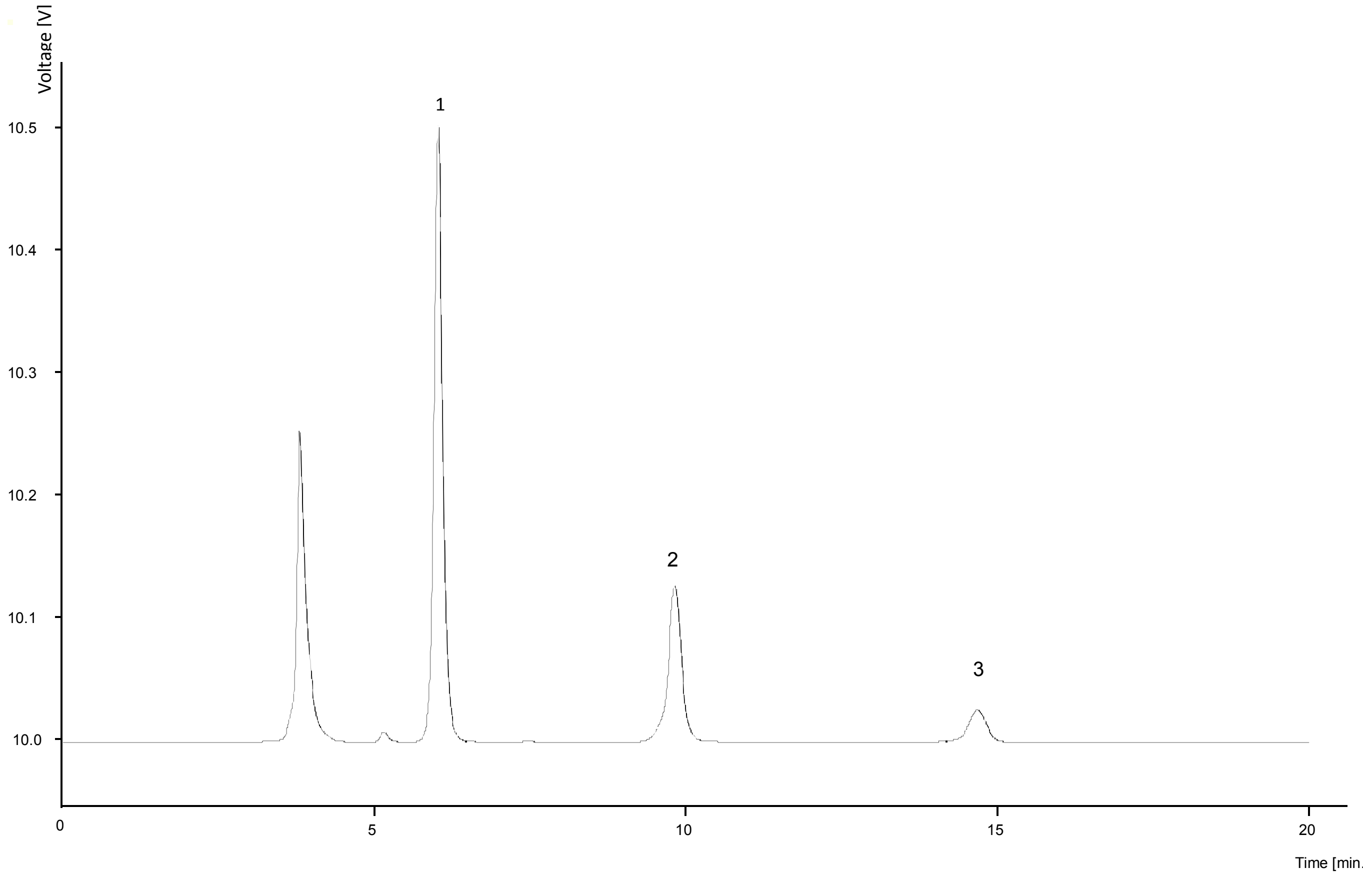

Figure 2. Individual profile of tocopherols of "Pylaea" genotype. 1- α-tocopherol, 2- γ-tocopherol, 3- Tocol, IS.