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Chemical composition, nutritional value and antioxidant properties of Mediterranean okra genotypes in relation to harvest stage

Spyridon Petropoulos[a,*], Ângela Fernandes[b], Lillian Barros[b], Isabel C.F.R. Ferreira[b,*]

[a] Universityof Thessaly, Department of Agriculture, Crop Production and Rural Environment, 38446 N. Ionia, Magnissia, Greece
[b] Centrode Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

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ABSTRACT

The aim of the present study was to determine the effect of fruit size on nutritional value, chemical composition and antioxidant properties of Mediterranean okra genotypes. For this purpose, pods from four okra cultivars and local landraces commonly cultivated in Greece, as well as pods from four commercial cultivars from North America were collected at two sizes (3–5 and > 7 cm). Significant differences were observed between the studied genotypes for both nutritional value and chemical composition parameters. Small fruit had a higher nutritional value, whereas chemical composition differed in a genotype dependent manner with most of the studied cultivars showing better results when harvested in small size. In conclusion, fruit size has a genotype dependent impact on chemical composition and nutritional value of okra pods and the common practice of harvesting okra fruit while they still have a small size helps to increase nutritional value for most of the studied genotypes.

1. Introduction

Okra (*Abelmoschus esculentus* L. Moench.) is a vegetable native to the tropical and subtropical regions of the world, that belongs to Malvaceae family and is usually consumed for its edible immature fruit in fresh or dried form, although its leaves and seeds are also edible with less common use (Adetuyi & Osagie, 2011; Camciuc, Deplagne, Vilarem, & Gaset, 1998). Apart from edible use, extracts from okra fruit have been used for various applications in the food and pharmaceutical industry as emulsifiers, drug tablet formulations or blood plasma replacement, due to their high content in biopolymers, such as polysaccharides (mainly pectins), and bioactive compounds such as ascorbic acid and beta-carotene (Adetuyi & Osagie, 2011; Arlai, Nakkong, Samjamin, & Sitthipaisarnkun, 2012; Ghori, Alba, Smith, Conway, & Kontogiorgos, 2014).

Its origins are believed to come from East and/or South Africa, India or South-East Asia (Siemonsma, 1982). Nowadays, it is widespread throughout the world, while in Europe its cultivation and consumption is more common around the Mediterranean basin, and especially Cyprus, Egypt, Greece, and Turkey where it is a basic ingredient in many local and traditional dishes (Çalişir, Özcan, Haciseferoğullari, & Yildiz, 2005). In Greece and Turkey, they are considered as minor vegetable crops, however they are important vegetable species and the small immature fruit are very popular in various summer dishes (Çalişir et al., 2005).

The nutritional value of immature pods is highly appreciated from consumers, since okra fruit are considered a rich source of dietary fibers, carbohydrates, vitamins and minerals (Adetuyi & Osagie, 2011; Gemede, Ratta, Haki, & Woldegiorgis, 2014); however, high variation in proximate and chemical composition has been reported between various okra accessions (Gemede, Haki, Beyene, Woldegiorgis, & Rakshit, 2016) and different growing conditions (Makhadmeh & Ereifej, 2004), while harvest stage may also affect chemical composition of immature pods (Piloo & Kabir, 2011). Okra fruit also present significant antioxidant properties, mostly due to their high content in vitamin C, carotenoids and flavonoids (Gemede et al., 2014), as well as therapeutic properties against diabetes, hyperlipidaemia, microbes, ulcers and neurodegenerative diseases (Atawodi et al., 2009; Kamalesh, Subrata, Asraf, & Pranabesh, 2016; Mishra, Kumar, & Rizvi, 2013). Apart from nutrients and beneficial health compounds, okra pods may also contain anti-nutrients such as phytates, oxalates and saponins which affect bioavailability of minerals (Ca, Fe and Zn) and limit nutritional value of the fruit (Adetuyi & Osagie, 2011; Gemede et al., 2016).

There are many Greek cultivars and local landraces that according to Koutsos, Koutsika-Sotiriou, Gouli-vavdinoudi, and Tertivanidis

* Corresponding authors at: University of Thessaly, School of Agricultural Sciences, Fytokou Street, 38446 N. Ionia, Magnissia, Greece (S.A. Petropoulos). Polytechnic Institute of Bragança, Campus de Santa Apolónia, 1172, 5300-253 Bragança, Portugal (I.C.F.R. Ferreira).
E-mail addresses: fangio57gr@gmail.com (S. Petropoulos), iferreira@ipb.pt (I.C.F.R. Ferreira).

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(2000) are closely related with Turkish cultivars that were imported from immigrants and were artificially selected and adapted to specific agro-climatic conditions throughout the years. In the past few years, there used to be many Greek okra cultivars, including cv. "Pylaea", "Boyati", "Veloudo", "Lasithi", "Pentagoni" and so forth; however nowadays the only Greek cultivar that its seeds are commercially available is cv. "Pylaea", while for the rest of the cultivars only small amounts are available, mainly from seeds kept from growers own production from year to year. In addition, Koutsos et al. (2000) have reported that cv. "Boyati" and "Veloudo" probably have the same origin and separated after cultivation at different regions and growers selection.

In the Mediterranean basin, okra fruit is harvested and consumed as soon as it reaches a length of 3–5 cm. However, in other markets of the world and especially in North America where different genotypes are cultivated, the acceptable fruit size is bigger (7–12 cm or larger, depending on the cultivar), provided that lignification of fruit walls and seed formation has not started. Considering the newly introduced Northern America cultivars in European markets where consumers are used to consume small-sized fruit, the aim of the present study was to evaluate chemical composition of okra fruit at two marketable sizes (3–5 and > 7 cm), as well as to compare the quality features of the main okra genotypes cultivated in Greece with genotypes of foreign origin.

2. Materials and methods

2.1. Plant material

Okra pods were collected from plants grown from seeds, including 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 Agricultural Research Foundation, Thessaloniki, Greece) and one local landrace ("Lasithi") from the seed collection of Laboratory of Vegetables Production, University of Thessaly, Greece]. In addition, seeds of four commercial cultivars from North America [cv. "Choppee" (heirloom variety; Southern Exposure Seed Exchange, VA, USA), "Clemson Spineless" (commercial cultivar; Park Seed Co., SC, USA), "Dwarf Long Green" (variety; Victory Seed Co., OR, USA) and "Silver Queen" (heirloom variety; Park Seed Co., SC, USA)] 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 beds (20 °C), while young seedlings (stage of 3 true leaves) were transplanted in the field on May 11th, 2016 in a plant density of 25,000 plants ha^{-1} (0.8 m between rows and 0.5 within each row). For each genotype and each treatment (fruit size), 18 plants were used (36 plants per genotype and 160 plants in total). Experiments were carried out at the experimental field of the University of Thessaly in Velestino, Greece.

Pods were harvested at two growth stages depending on their length, namely 3–5 cm and > 7 cm in order to simulate different market standards. For large-sized fruit, trial harvests took place in order to define the size were pods from each genotype lose marketability (inedible fruit due to thick pod walls and seed formation). In any case, only marketable fruit were considered for further analyses. The first pods were collected on June 22nd, 2016 (genotype "Lasithi") and harvests continued at regular intervals for one month period in order to avoid differences in chemical composition due to variability in growing conditions and position of pods on the plant. After each harvest, pods were cleaned with distilled water, dried with absorbent paper and cut into small pieces after discarding peduncle and calyx. Cut pods were put in sealed bags and stored in freezing conditions (−20 °C). When harvest for each treatment was completed, all fruit from the same treatment were put together in batch samples, freeze dried and stored in deep freezing conditions (−80 °C) until chemical analyses took place.

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

2.2. Chemical composition

2.2.1. Nutritional value

All pod samples were analysed in terms of macronutrients (moisture, proteins, fat, carbohydrates and ash), according to the AOAC procedures (AOAC, 2016). Crude protein content (Nx6.25) was estimated using the macro-Kjeldahl method; Soxhlet extraction with petroleum ether was used to determine the crude fat content; incineration at 600 ± 15 °C was used to measure ash content. Total carbohydrates were calculated by difference and the energetic value was calculated as following: Energy (kcal) = 4× (g protein + g carbohydrate) + 9× (g fat).

For mineral composition analysis, samples of fresh pods 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 1100 B, Waltham, MA), and Na and K content by flame photometry (Sherwood Model 410, Cambridge, UK).

2.2.2. Free sugars

Free sugars were 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, Pereira, Calhelha, et al. (2013). Mobile phase consisted of acetonitrile:water mixture (70:30 v/v, acetonitrile HPLC-grade, Lab-Scan, Lisbon, Portugal) and separation was achieved using a Eurospher 100-5 NH2 column (4.6 × 250 mm, 5 μm, Knauer). The results were recorded and processed using Clarity 2.4 software (DataApex, Prague, Czech Republic).

2.2.3. Organic acids

Organic acids were determined by ultra-fast liquid chromatography (UFLC) (Shimadzu 20 A series UFLC, Shimadzu Corporation, Kyoto, Japan) coupled to a diode-array detector (DAD) operating in the conditions described by Barros, Pereira, and Ferreira (2013). The compounds were identified and quantified by comparing the area of sample' peaks recorded at 215 nm with calibration curves obtained from commercial standards (Sigma-Aldrich, St. Louis, MO, USA). The results were recorded and processed using LabSolutions Multi LC-PDA software (Shimadzu Corporation, Kyoto, Japan).

2.2.4. Tocopherols

Tocopherols were determined following a procedure previously described by Barros, Pereira, Calhelha, et al. (2013), 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. Mobile phase consisted of a mixture of hexane:ethyl acetate (70:30, v/v, hexane and ethyl acetate HPLC-grade, Lab-Scan, Lisbon, Portugal), and chromatographic separation was performed using a Polyamide II column (250 × 4.6 mm, 5 μm; YMC, Kyoto, Japan). The results were recorded and processed using Clarity 2.4 software (DataApex, Prague, Czech Republic).

2.2.5. Carotenoids and chlorophyll

A fine dried powder (150 mg) of the lyophilized material was vigorously shaken with 10 mL of acetone–hexane mixture (4:6) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm (Nagata & Yamashita, 1992). Content of beta-carotene was calculated