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CHEMICAL COMPOSITION OF TURKISH OKRA SEEDS (*Hibiscus esculenta* L.) AND THE TOTAL PHENOLIC CONTENT OF OKRA SEEDS FLOUR

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ABSTRACT

Okra is a common vegetable in most regions of Turkey and available all year-round, with a peak season during the summer months. In this study, first the chemical composition and physical properties of mature okra seeds were investigated. Then, the unroasted okra seed flour (OSF)and roasted okra seeds flour (ROSF) (40 min at 160^{0}C) were analyzed for total phenolic contents and 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity. The results of flour samples showed that the values of phenolics in OSF (157.80 mg GAE 100 g^{-1} flour) and ROSF (232.19 mg GAE 100 g^{-1} flour). The concentration that provided 50% radical scavenging (IC_{50}) was determined as 360.25 ± 2.01 mg ml^{-1} and 452.39 ± 12.27 mg ml^{-1} for roasted and unroasted okra seed flour, respectively. Regarding physical and chemical composition, okra seeds and their flour could be recommended for a good source of protein, fat, mineral and phenolic acid, which can be help remove the stigma of "starvation food" for people and promote them as a healthy food source.

Keywords: Okra seed, *Hibiscus esculenta* L., Antioxidant activity, Total phenolic content, DPPH

1. INTRODUCTION

The genus *Hibiscus* L. consists of approximately 200 species in the tropic and subtropic regions of the world [1]. The fruit is a greenish capsule up to 20 cm long, slightly curved, six-chambered pod of fibrous texture, containing numerous seeds [2]. Its Turkish name is known as “Bamya”. Fruits of some species are used as foods; their seeds are roasted, ground and used as coffee substitutes in Turkey (*H. esculenta* L.) [3].

The richest nutrition value part of the okra plant is the dried seed. The oil of the okra seeds is edible and the residual meal after oil extraction is significantly rich in protein. It was reported that the okra seeds cultivated in Greece contain between 15.9% to 20.7% oil [4]. The okra seed oil mainly consisted of linoleic acid (up to 47.4%), which is essential for human nutrition [5]. In the literature, a few study also investigated the potential of okra seeds as a source of oil and protein [6, 7]. And also, some other studies were stated that K, Na, Mg and Ca were carried out to be the main elements, with Fe, Zn, Mn and Ni to be also present [8-10].

Antioxidants are significant group of compounds that eliminate reactive free radical intermediates generated during oxidation reactions. Free radicals lead to the oxidation of biomolecules (e.g., protein, amino acids, lipid and DNA) which cause cell injury and death [11]. Their injurious effects can be declined by natural antioxidants available in foods. For this reason, the necessity to determine alternative natural and safe sources of food antioxidants arose and search of natural antioxidants, especially of plant origin, has notably increased recently.

Although extensive information is available on oil and protein content of okra seed, the antioxidant properties haven't been studied as much as oil and protein content. In recent times, polyphenolic

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content of okra seeds has been studied by a few researchers [12-17]. They reported that it is required to investigate the proximate ingredients of its flour as affected by pre-treatments for the optimal utilization of okra seed.

The objective of this study is to determinate some chemical and physical properties of mature okra seeds and the antioxidant activities of okra seed flour cultivated in Turkey, because okra plant is an important vegetable traded in fresh or processed forms. Consequently, it can be offered that the products from okra seeds could be put into alternative uses in Turkey instead of implanting or production.

2. MATERIALS AND METHODS

2.1. Materials

Okra samples were handpicked in autumn in Bilecik province located in Turkey. The mature okra is 18 cm long, slightly curved, fibrous texture, containing many seeds. First, the greenish capsule was removed and only the seeds were used to analyze. The seed samples were dried at room temperature in the laboratory and packed in thick gauge polythene bags. The samples in the polythene bags held at room temperature until analyzed.

2.2. Determination of Some Physical Characteristics

100 g of the mature okra were separately divided into seeds and the greenish capsules to determine the weight percent. The weight of the seeds and the greenish capsules, and also the number of seeds were found as 28.74 g, 71.26 g and 223 grains, respectively. The initial moisture content of the seed was determined as 11.65% dry basis. To determine the average size of the okra seed, 100 seeds were randomly picked and their three principal dimensions were measured by an accuracy of ± 0.01 mm. Each seed was weighed to obtain the mass with a precision balance reading to 0.001 g. Geometric mean diameter (D_g), sphericity (Ø), seed volume (V) and B values were found using the following formulae:

$$D_g = (LWT)^{0.333} \quad (1)$$

$$Ø = (LWT)^{0.333}/L \quad (2)$$

$$V = \pi B^2 L^2/(6(2L - B)) \quad (3)$$

Where L, W and T refers length (mm), width (mm) and thickness (mm) of the seed, respectively.

$$B = (WT)^{0.5} \quad (4)$$

The surface area($S=\pi D_g^2$) of the seeds was determined by assuming spherical surface for the samples and the expression given in equation [13].

2.3. Proximate Analysis

The following chemical properties; moisture, ash, cellulose, lignin, volatile matter, crude protein, crude oil were analyzed for seed samples. The protein content was determined by using Kjeldahl method as described [18] in which percent nitrogen (N) was multiplied by 6.25. Oil extraction was carried out by using solvent extraction technique (AOAC 920.39) [19]. Moisture (AOAC 945.21), ash (AOAC 923.03), lignin (ASTM D1106-96), volatile matters (ASTM E872-82) were determined by

standard method [20-23] and cellulose was calculated as the remaining value of the other contents. The values of analyses were the means of triplicate measurements.

2.4. Extraction of Oil

Solvent extraction is used to separate oil from okra seeds by using n-hexane on a water bath for 6 hours in the soxhlet extractor. The solvent was evaporated at 45 °C by using a rotary evaporator.

2.5. Determination of Fatty Acids by GC/MS

Methyl esters in the okra seed oil were analyzed by Varian GC/MS equipped with CP-3800 GC and Varian 1200L Quadropole Mass Selective Detector. The OOME's were discriminated on capillary column HP-5MS (30 m x 0.25 mm; film thickness 0.25 µm). A sample volume of 1.0 µL was driven into the column using the split mode (split ratio 1:100). The carrier gas used was helium at a flow rate of 1.2 mL min^{-1}. The column oven temperature was fixed from 40 °C to 280 °C at a linear rate of 5 °C min^{-1}, initial and final time was 2 and 5 min, respectively. The scanning mass range changed from 20 to 400 m z^{-1}.

2.6. Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) images (SE and BSE) of the sample surface were obtained using an EVO-50XVP (Carl Zeiss SMT Ltd.) Chemical composition was determined using the Genesis 4000EDX detector (EDAX Inc.).

2.7. Phenolic Extraction

The cleaned okra seeds were roasted in an oven (Leader Engineering GP180) at 160 °C for 40 min and were allowed to cool before the preparation of the flour [24]. Pretreated and control Okra seeds were grounded into a fine powder (250 µm) with a food processor (Waring Commercial 32BL79) and stored at -20 °C until analyzed within four weeks.

Roasted and unroasted okra seed flour samples were extracted by soxhlet extraction system with n-hexane for 6 hours. After extraction with n-hexane, the residue was transferred for phenolic extraction with 80% aqueous methanol.

The extracts were mixed, homogenized, and weighed (Ohaus PA214C) for both OSF and ROSF samples. The extraction procedure was carried out according to the following method in order to obtain a quantitative extraction: approximately 3.0 grams of each flour (OSF and ROSF) samples were extracted twice with 50 ml of MeOH 80% (4:1 of methanol and water) for 1 h, 40 ml for 30 min in a ultrasonic water bath (Bandelin RK 102 H). The extracts were gathered and filtered through Whatman No.1 and the supernatant evaporated to dryness under vacuum at 40 °C in a rotary evaporator. The phenolic extracts of flour samples were freeze-dried at-54 °C for 15 hours with a freeze-dryer (LabconcoFreezone 2.5), and stored in dark color bottle at 4 °C until analyzed. Each lyophilized extract (approximately 5 mg) was diluted with 5 mL methanol (80% v/v) before using.

These extracts were used for determination of both total antioxidant activities and total phenolic content of unroasted and roasted okra seed flour.

2.8. Total Phenolic Content

Total Phenolic Contents (TPC) of seed flour extracts from okra was determined according to Khomsugetal [25] with some slight modifications by using the Folin-Ciocalteau assay. The extract

solutions (0.5 mL) were mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and 2.0 mL of 7.5% Na_2CO_3 solution added to these mixtures. All the solutions were vortexed and allowed to stand in a dark place for 30 min. The absorbance of extracts and prepared blank were measured at 765 nm using UV-visible spectrophotometer (Jenway 7315). Quantification of TPC was based on a Gallic acid standard curve generated by preparing 0-100 mg mL^{-1} of Gallic acid. The TPC were expressed as milligrams of Gallic acid equivalents (GAE) 100 g^{-1} flour (unroasted and roasted). The samples were analyzed in triplicate.

2.9. Total Antioxidant Activity

The total antioxidant activity (TAA) was investigated using the stable free radical diphenylpicrylhydrazyl (DPPH) assay as reported by Adelakun et al. [24] and Liu et al. [26] with a little modification: 1 g of each okra seed flour extracts was mixed with 1 mL of DPPH solution (0.2 mM). Blank was made from methanol. The absorbance's samples were measured at 515 nm using a UV-visible spectrophotometer for triplicate measurements.

The percentage of remaining DPPH against the sample concentration was plotted to obtain the amount of antioxidant μg necessary to decrease free radicals by 50%. A smaller IC_{50} value corresponds to a higher antioxidant activity.

To determine the hydrogen donating ability of the extract a method based on the reduction of a methanolic solution of the coloured free radical DPPH to the nonradical form was used. DPPH radical was calculated by using the formula:

$$TAA\,(\%) = (A_{t0} - A_{t20}) / A_{t0} x\, 100 \quad (5)$$

Where A_{t0} is the absorbance of DPPH• in methanol solution without an antioxidant, and A_{t20} is the absorbance of DPPH• in the presence of an antioxidant.

3. RESULTS AND DISCUSSION

3.1. Dimensional and Proximate Analysis of Okra Seeds

The dimensional properties and proximate analysis of mature okra seeds are given in Table 1 and Table 2.

Table 1. Dimensional properties of okra seeds

Dimensional properties	Mean value
Length (mm)	5.29 ± 0.01
Width (mm)	4.32 ± 0.01
Thickness (mm)	4.53 ± 0.01
Geometric mean diameter (mm)	4.70 ± 0.03
Weight (g)	0.07 ± 0.01
Volume (mm^3)	61.72 ± 0.03
Surface area (mm^2)	69.21 ± 0.03
Sphericity	0.89 ± 0.10
Moisture%	11.65 ± 0.01

These data on the physical properties of the okra seed could be useful in the determination of the stability of the seed in storage and designing appropriate storage conditions for the seeds.

Table 2. Chemical composition of okra seeds

Sample	Moisture (%)	Ash (%) (dry basis)	Cellulose (%)	Lignin (%)	Volatile matter (%)	Crude protein[a] (%)	Crude oil (%)
Grinded seeds	9.95 ± 1.02	5.21 ± 1.09	48.67 ± 1.87	17.28 ± 0.94	79.35 ± 2.12	28.21 ± 1.55	22.62 ± 1.48

[a] N*6.25

In this study, the protein values were higher than reported protein values by Çalışır et al. [3] which was of 19% and within the range reported by Oyelade et al. [7] which was between 22% and 45% for okra seed. The fat contenting the seed coat as reported by Oyelade et al. [7] was 11%, endosperm was 34% and whole seed was 22%. Adelakun et al. [24] had reported that the ash content in the Nigerian okra seed was between 3.42-3.88%. The ash content value obtained in this study was higher than reported by Adelakun [24]. The findings of this work is consistent with the previous other studies that okra seed could serve as an alternative rich sources of oil and protein to both Turkey and other producing countries. It could be concluded that mature okra seeds are a promising source of protein and fat as reported by other researchers.

3.2. Fatty acid composition of okra seed oil

GC/MS chromatogram of okra seed oil methyl esters was given in Figure 1. The fatty acid composition of okra seed oil is reported as a relative percentage in Table 3.

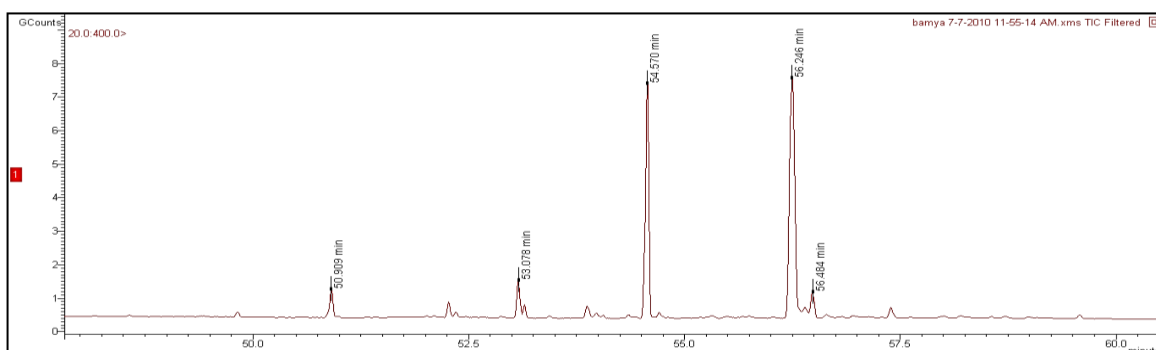


Figure1. GC/MS chromatogram of okra seed oil methyl esters

Table 3. Fatty acids (FA) composition (%) of okra seed oil methyl esters

Fatty acid	Relative percentage (%)
Palmitic acid	28.60 ± 1.44
Stearic acid	3.57 ± 0.87
Oleic acid	16.81 ± 1.32
Linoleic acid	49.54 ± 1.68
Linolenic acid	1.48 ± 0.06

This seed oil also represents a potential source of palmitic acid and important raw material for the cosmetic industry. Moreover, the linoleic acid could be utilized for producing dyes, resins and plastics.

3.3. Chemical Composition

Scanning Electron Microscopy (SEM) images and EDX analysis of the grinded okra seeds samples surface and EDX spectrum were given in the Figure 2 and Figure 3.

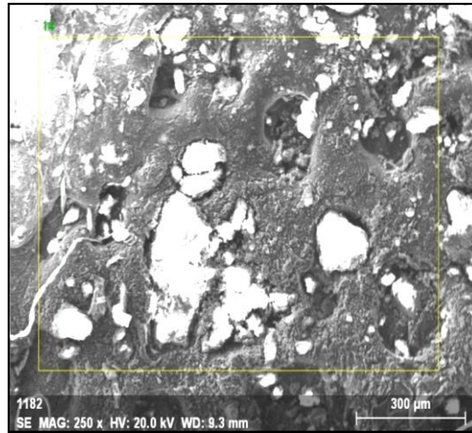
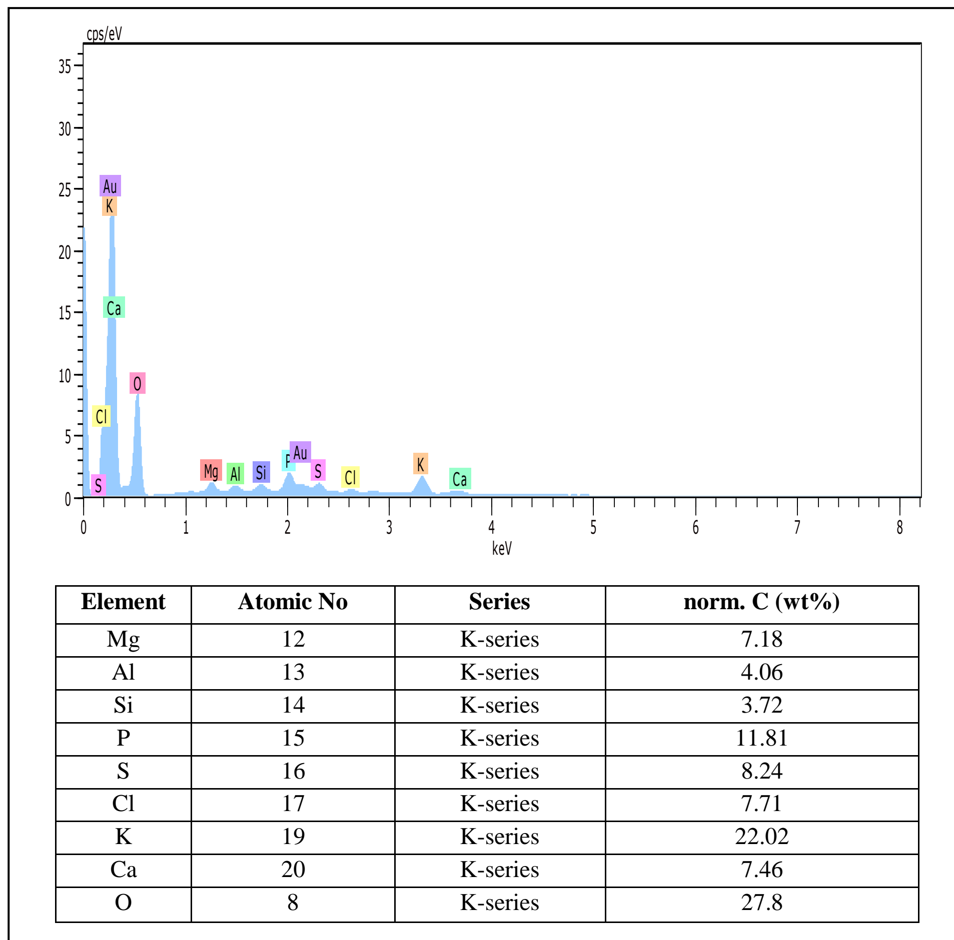


Figure 2. SEM of grinded okra seeds

Elemental spectrum was recorded in order to qualitatively characterize the samples. The weight percent of these elements have been given in the Figure 3. In the grinded okra seed samples; Mg, Al, Si, P, S, Cl, K, Ca, O are present. The EDX spectrum of grinded okra seed sample showing strong K signals.



Element	Atomic No	Series	norm. C (wt%)
Mg	12	K-series	7.18
Al	13	K-series	4.06
Si	14	K-series	3.72
P	15	K-series	11.81
S	16	K-series	8.24
Cl	17	K-series	7.71
K	19	K-series	22.02
Ca	20	K-series	7.46
O	8	K-series	27.8

Figure 3. EDX results of grinded okra seed samples

As can be seen in Figure 3, phosphor is the highest value (11.84% wt.) element in the other, after K (22.02% wt.) element in the grinded okra seed sample. As can be seen in Figure 3 calcium is the highest value (16.14% wt.) element in the other, after K (52.24% wt.) element in the grinded okra skins sample. Calcium and phosphorus play important role in the skeletal structure of vertebrates. Phosphorus is also an important component of phospholipids, phosphoproteins and nucleic acids. Sodium, potassium and chloride perform major roles in the maintenance of osmotic pressure, water balance and membrane potentials. Magnesium lies on the border between the macro and microelements. It is primarily on intra cellular element, and it exerts regulatory and catalytic roles in numerous biochemical systems. Calcium plays a key regulatory role as a messenger in signal transduction, notably innerve and a messenger in signal transduction, notably in nerve and muscle cell. Silicon is found in the blood, muscles, skin, nerves, nails, hair, connective tissue and teeth. Insufficient silicon in the body may result in baldness or the graying of hair. Skin irritations and rashes may develop easily. N, O, and S are found extensively in protein in plant and animals the amino acid cysteine and methionine contain sulfur as do all polypeptides, proteins and enzymes which contain the amino acids [25]. The daily requirements of some essential elements for human being recommended by The National Academy of Sciences were given in Table 4 [27].

Table 4. The National Academy of Sciences recommendations for daily minerals intake

Groups / **Minerals**	**Ca** (mg day^{-1})	**P** (mg day^{-1})	K (mg day^{-1})	**Cl** (mg day^{-1})	**Mg** (mg day^{-1})
Men-Women	800	800	3000	500	350 - 300
Children	800	800	1500		250
Teenagers	1200	1200	3000		400
Infants	500	400			60-70
Pregnant Nursing Mothers	1200	1200	3000		450

The antioxidant activities of unroasted okra seed flour and roasted okra seed flour was evaluated by DPPH free radical scavenging assay decolorization assay. The model of scavenging the stable DPPH radical is widely used method to evaluate the free radical scavenging ability of various samples [15].

The results for total phenolic content in the okra seed and roasted okra seed flour are presented in Table5.

Table 5. Total phenolic content of OSF and ROSF samples

Okra Flour Sample	**TPC (mgGAE 100 g^{-1} flour)**	**IC_{50} (mg ml^{-1})**	**TAA %**
Unroasted (OSF)	157.80 ± 0.05	452.39 ± 12.27	56.06 ± 1.56
Roasted (ROSF)	232.19 ± 0.01	360.25 ± 2.01	70.39 ± 0.39

The results of okra seed flour samples showed that the values of phenolics in OSF (157.80 mg GAE 100 g^{-1} flour) and ROSF (232.19 mg GAE 100 g^{-1} flour) by reference to the standart curve ($y=9.465x+0.0515$, $r^2=0.9998$). The concentration that provided 50% radical scavenging (IC_{50}) was determined as 360.25 ± 2.01 mg ml^{-1} and 452.39 ± 12.27 mg ml^{-1} for roasted and unroasted okra seed flour, respectively.

The data present in Table 5 shown that pre-treatment like roasting can be positively affected to total phenolic content. It can be seen that roasted okra seed flour studied in this research had potential to contain antioxidant.

From this study, it can be deducted that okra seed and flour are a hopeful source of protein, fat, antioxidant and some essential elements for humankind. Pre-treatment by roasting was found to increase the total antioxidative activity and total phenolic contents. The consumption of okra seed flour is particularly important due to the ability to prevent against to chronic diseases and cancers.

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