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Research Article

HPLC Analysis of Water-Soluble Vitamins (B2, B3, B6, B12, and C) and Fat-Soluble Vitamins (E, K, D, A, and β-Carotene) of Okra (*Abelmoschus esculentus*)

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Okra is consumed as a vegetable by populations in Africa and Asia and particularly in Egypt. In this study, we investigated some nutritional components of okra grown in four different geographical locations of Egypt. A comparative analysis of water-soluble vitamins (B2, B3, B6, B12, and C) and fat-soluble vitamins (E, K, D, A, and β-carotene) in okra pods was carried out. Results of principal component analysis (PCA) showed three clusters of varieties. The first cluster included the Dakahlia (D) and Kafr El-Sheikh (K) varieties. The second and the third clusters separated out the Suez (S) and Mansoura (M) varieties independently. The S pod showed the highest contents of vitamins B6 (49.81 μg/100 g) and E (1.47 mg/100 g) but contained the lowest contents of vitamins B3 (1.42 μg/100 g) and B12 (undetected). The K pod showed the lowest vitamin C content (11.60 mg/100 g). The M pod showed the highest contents of vitamins B3 (22.70 μg/100 g), B12 (91.20 μg/100 g), C (27.14 mg/100 g), and K (0.21 mg/100 g). The D pod showed the lowest contents of vitamins E (0.15 mg/100 g), K (0.05 mg/100 g), and B6 (11.50 μg/100 g). These findings could help develop meal planning at the community level by incorporating okra varieties with high vitamin content.

1. Introduction

Cultivated okra (*Abelmoschus esculentus* L.), also known as "Lady's Finger" or Bamia, belongs to the Malvaceae family and is predominantly grown in the tropics and subtropics where the climates are relatively warm. Okra grows best in hot weather (temperatures above 26°C), especially in regions with warm nights (>20°C) [1]. In many areas, it is eaten once a week because of its high vitamin content. In general, vitamins play a very important role in maintaining health [2], contributing to a healthy immune system and providing all the nutrients essential for good health. Diets that do not contain adequate amounts of vitamins often result in vitamin deficiency-related diseases, including blindness and mental retardation, depending on the particular vitamin that is lacking. For example, nicotinamide is essential for carbohydrate metabolism and for nonredox adenosine diphosphate-ribose transfer reactions involved in DNA repair, while pyridoxine plays an essential role in amino acid transamination and riboflavin functions as a coenzyme for a wide variety of respiratory enzymes [3].

The contents of water-soluble vitamins (B-group), including thiamin, riboflavin, pyridoxine, nicotinic acid, pantothenic acid, biotin, cyanocobalamin, and folic acid, can be increased in food either in their phosphorylated forms, free forms, or attached to proteins. The numerous varieties of B vitamins available present a challenge for their detailed analysis. However, by using high-performance liquid chromatography (HPLC), 40% higher vitamin B contents can be detected on an average than that by using classical microbiological methods [4].

Vitamin C is regarded as one of the most suitable dietetic antioxidant agents since it is naturally available in great quantities in vegetable foods [5, 6]. Several studies have shown

Table 1: Geographic locations of okra samples used for determination of vitamin contents.

Origin	Code	Latitude	Longitude
Dakahlia	D	31.053103	31.580615
Mansoura	M	31.042536	31.380014
Kafer El-Sheikh	K	31.347304	30.80246
Suez	S	29.984721	32.524309

the health benefits of vitamin C [7]. Fat-soluble vitamins contain phytochemicals, which are shown to have antioxidant, antibacterial, antifungal, antiviral, and anticarcinogenic properties [8]. High concentrations of antioxidants, including α-tocopherol, are associated with a reduction in the risk of disorders related to free radical accumulation such as atherosclerosis, cancer, cataracts, and cell damage [9].

The objective of this study was to determine the vitamin contents of some okra varieties grown in Egypt in order to provide further authentic information about this important food source.

2. Material and Methods

2.1. Chemicals and Reagents. HPLC-grade solvents were used for analysis. Analytical reagent-grade acetonitrile and methanol were obtained from Lab-Scan (Tedia Company, USA). The water used for HPLC and sampling was prepared with a Millipore Simplicity instrument (Millipore, Molsheim, France). All vitamin standards were of chromatography grade and were purchased from Sigma Chemical Co. (Poole, Dorset).

2.2. Sample Preparation and Drying. Okra pods were collected from four different geographical locations in Egypt as follows: Suez (S pod) adjacent to the Eastern Desert, Mansoura (M pod) adjacent to the River Nile, Kafr El-Sheikh (K pod) adjacent to the Mediterranean Sea, and Dakahlia (D pod) adjacent to the Manzala Lake. Table 1 shows detailed information on the geographic location of the four Egyptian okra pods analyzed. After washing with tap water and draining, the fruits were sliced into 10 mm thick transverse slices using a fruit slicer. Subsequently, they were dried under direct sunlight in the dry season with an overall maximum daytime air temperature of approximately 37°C and a minimum night temperature of approximately 20°C. The pods were weighed at various intervals over the entire drying period until obtaining a constant weight. The dried slices of okra were milled into a coarse powder by using a laboratory mill (D-6072, Germany) and stored in polyethylene bags at room temperature.

2.3. Determination of Water-Soluble Vitamins

2.3.1. Okra Vitamin B Analyses. The vitamin B group was extracted according to a previously described method [10]. In brief, okra powder (2 g) was placed in 25 mL of H_2SO_4 (0.1 N) solution and incubated for 30 min at 121°C. Then, the contents were cooled and adjusted to pH 4.5 with 2.5 M sodium acetate, and 50 mg Takadiastase enzyme was added. The preparation was stored at 35°C overnight. The mixture was then filtered through a Whatman No. 4 filter, and the filtrate was diluted with 50 mL of pure water and filtered again through a micropore filter (0.45 μm). Twenty microliters of the filtrate was injected into the HPLC system. Quantification of vitamin B content was accomplished by comparison to vitamin B standards. Standard stock solutions for thiamine, riboflavin, niacin, pyridoxine, and cobalamin were prepared as reported previously [11, 12]. Chromatographic separation was achieved on a reversed phase- (RP-) HPLC column (Agilent ZORBAX Eclipse Plus C18; 250 × 4.6 mm i.d., 5 μm) through the isocratic delivery mobile phase (A/B 33/67; A: MeOH, B: 0.023 M H_3PO_4, pH = 3.54) at a flow rate of 0.5 mL/min. Ultraviolet (UV) absorbance was recorded at 270 nm at room temperature [13].

2.3.2. Okra Ascorbic Acid Analyses. Vitamin C was extracted according to a modification of a published method [14]. The okra powder (10 g) was blended and homogenized with an extracting solution containing metaphosphoric acid (0.3 M) and acetic acid (1.4 M). The mixture was placed in a conical flask and agitated at 10,000 rpm for 15 min. The mixture was then filtered through a Whatman No. 4 filter, and samples were extracted in triplicate. The ascorbic acid standard was prepared by dissolving 100 mg of L-ascorbic acid in a metaphosphoric acid (0.3 M)/acetic acid (1.4 M) solution at a final concentration of 0.1 mg/mL. The calibration line was converted to a linear range based on four measured concentration levels.

Quantification of ascorbic acid content was performed on an Agilent HPLC system. Chromatographic separation was achieved on an RP-HPLC column through isocratic delivery of a mobile phase (A/B 33/67; A: 0.1 M potassium acetate, pH = 4.9, B: acetonitrile: water [50 : 50]) at a flow rate of 1 mL/min. UV absorbance was recorded at 254 nm at room temperature.

2.4. Determination of Fat-Soluble Vitamins

2.4.1. Okra Vitamin D, E, K, A, and β-Carotene Analyses. In 10 g okra powder, 1 g of pyrogallic acid, 70 mL ethanol, and 30 mL (50%) KOH were added, stirred, and refluxed for 40 min using a water bath at 50 ± 2°C [15, 16]. Extracts were obtained three times using various ether concentrations (50 mL, 30 mL, and 20 mL). Double-distilled water was used to neutralize the extract, which was dehydrated using anhydrous sodium sulfate. Further, the extract was concentrated to approximately 5 mL by using a water bath (50 ± 2°C), diluted to 10 mL by using methanol, filtered using a 0.45 μm membrane, and finally subjected to HPLC analysis.

RP-HPLC analysis was performed with the Agilent 1100 series HPLC system (Agilent; USA), including a diode array detector. The column was made of stainless steel. For β-carotene quantification, the Agilent TC-C18 column was used (5 μm, 4.6 × 250 mm) with an acetonitrile-methyl alcohol-ethyl acetate (88 : 10 : 2) solvent, and UV absorbance was recorded at 453 nm. For fat-soluble vitamins, the Agilent Eclipse XDB-C18 column was used (5 μm, 4. 6 × 150 mm),

Table 2: Water-soluble vitamin contents of okra pods.

Varieties	S	K	M	D
Riboflavin (vitamin B2) (μg/100 g DW)	ND	ND	ND	ND
Niacin (vitamin B3) (μg/100 g DW)	1.42 ± 0.42^{b}	7.21 ± 0.47^{b}	22.70 ± 8.69^{a}	7.65 ± 0.68^{b}
Pyridoxine (vitamin B6) (μg/100 g DW)	49.81 ± 4.02^{a}	17.90 ± 7.42^{c}	37.93 ± 6.29^{b}	11.50 ± 0.31^{c}
Cobalamin (vitamin B12) (μg/100 g DW)	ND	49.56 ± 18.55^{b}	91.20 ± 20.64^{a}	34.54 ± 3.69^{b}
Ascorbic acid (vitamin C) (mg/100 g DW)	12.51 ± 0.06^{c}	11.60 ± 0.21^{c}	27.14 ± 2.46^{a}	15.62 ± 0.05^{b}

Each value is presented as the mean ± standard deviation ($n = 3$). Data with different superscript letters in the same column of variety indicate a significant difference ($P < 0.05$) as analyzed using Duncan's multiple range test. Dry weight (DW) was not detected (ND). Riboflavin (B2), niacin (B3), pyridoxine (B6), and cobalamin (B12) are expressed in μg per 100 g. Ascorbic acid (C) is expressed in mg per 100 g.

Table 3: Fat-soluble vitamin contents of okra pods.

Varieties	S	K	M	D
Tocopherol (vitamin E) (mg/100 g DW)	1.47 ± 0.06^{a}	0.98 ± 0.09^{b}	0.76 ± 0.24^{c}	0.15 ± 0.02^{d}
Menaphthone (vitamin K3) (mg/100 g DW)	0.20 ± 0.02^{a}	0.11 ± 0.02^{b}	0.21 ± 0.01^{c}	0.05 ± 0.00^{d}
Retinol (vitamin A) (μg/100 g DW)	ND	ND	ND	ND
Cholecalciferol (vitamin D3) (μg/100 g DW)	ND	ND	ND	ND
β-Carotene (μg/100 g DW)	ND	ND	ND	ND

Each value is presented as the mean ± standard deviation ($n = 3$). Data with different superscript letters in the same column of variety indicate significant difference ($P < 0.05$) as analyzed using Duncan's multiple range test. Dry weight (DW) was not detected (ND). Tocopherol (E) and menaphthone (K3) are expressed in mg per 100 g. Retinol (A), cholecalciferol (D3), and β-carotene are expressed in μg per 100 g.

the solvent was methanol, and UV detection was recorded at 325 nm for vitamin A, 265 nm for vitamin D3, 290 nm for vitamin E, and 244 nm for vitamin K3. Separation of all vitamins was based on isocratic elution and the solvent flow rate was maintained at 1 mL/min. Twenty microliters of okra oil was directly injected into the HPLC column. Fat-soluble vitamins were identified by comparing their retention times with those of authentic standards. All procedures were carried out under subdued light conditions.

Standard solutions of vitamins were prepared by serial dilution to concentrations of 0.1, 1, 2, 5, and 10 mg per liter of vitamins D3, E, K3, A, and β-carotene, respectively. Standard solutions were prepared daily from a stock solution, which was stored in the dark at –20°C. Twenty microliters of standard solution was injected, and peak areas were determined to generate standard curves.

2.5. Statistical Analysis. Data from replications of all cultivars were subjected to analysis of variance (ANOVA) using SPSS 16.0 for Windows (SPSS Inc.; Chicago, IL, USA). The significance levels of differences between means were determined by using Duncan's new multiple range test; $P < 0.05$ was considered statistically significant. The correlations between all studied parameters were determined by principal components analysis (PCA) using XLSTAT software.

3. Results and Discussion

In this study, vitamin extraction was carried out by using 0.1 N H_2SO_4 and Takadiastase enzyme. Initially, hot acid hydrolysis was carried out. The enzyme was added after cooling, and the pH was adjusted to 4.5. Incubation at 35°C for over 12 h allowed the extraction of several vitamins simultaneously from the same sample. As previously reported, only one digestion method by using an acid and then the Takadiastase enzyme is sufficient for the detection of B vitamins [17]. The separation of vitamins using HPLC with fluorometric detectors has been applied. However, compared to UV detectors, fluorometric detectors are expensive, require sophisticated procedures, and are fragile. The use of HPLC coupled with UV detection for the study of water-soluble vitamins in food has been demonstrated to be a fast, simple, and reliable method [18]. Table 2 shows the water-soluble vitamin contents (B2, B3, B6, B12, and C) for different okra pods. The vitamin C content of the S pod was approximately 12.51 mg/100 g, while the M pod was rich in vitamin B3 (22.70 μg/100 g) and had the highest content of vitamin C (27.14 mg/100 g). The D pod showed the lowest content of vitamin B6 (11.50 μg/100 g) and a slightly high content of vitamin C (15.62 mg/100 g). No detectable quantities of vitamin B2 were observed. The M pod showed the highest vitamin B12 content (91.20 μg/100 g), whereas the S pod showed the lowest, in which no vitamin B12 was detectable. The K pod showed the lowest value of vitamin C (11.60 mg/100 g), but this value was nonetheless higher than those reported for vitamins B6, B12, and B2 (1.20, 0.13, and 1.13 mg/100 g) [8].

Table 3 shows important variations of the fat-soluble vitamin compositions in the studied okra samples. The M pod showed the highest content of vitamin K3 (0.21 mg/100 g), while the D pod had the lowest content (0.05 mg/100 g). The S pod had the highest vitamin E content (1.47 mg/100 g), followed by that in the K pod (0.98 mg/100 g), which was lower than the previously reported results for vitamins A, D, and K at 83.00, 0.07, and 1.00 mg/100 g, respectively [8]. The other pods showed no detectable quantities of vitamin A, β-carotene, and D3.

The β-carotene content was lower than that previously reported [19] (52.02 μg/100 g). The results showed significant

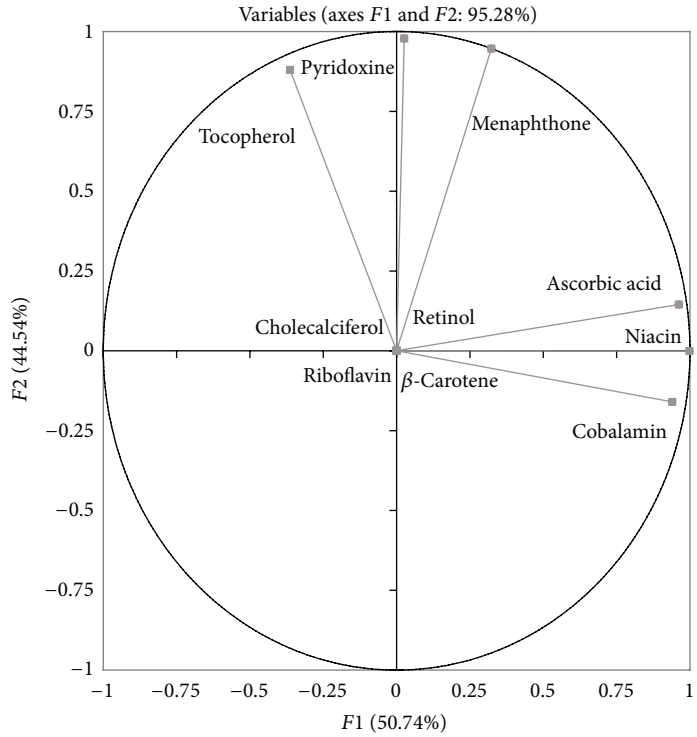

Figure 1: Plots of the principal component scores for vitamin contents of okra pods.

variations in the vitamin contents between the different okra varieties analyzed. Such variations can be explained by genetic factors and/or environmental factors, such as variations in soil composition among regions. The average contents of water-soluble vitamins across the different varieties were 9.75 μg/g for B3, 29.29 μg/g for vitamin B6, 43.83 μg/g for B12, and 16.72 mg/g for vitamin C. The average contents of fat-soluble vitamins were 0.84 mg/g for vitamin E and 0.14 mg/g for K3. Vitamins D3 and A and β-carotene were not detectable in any variety. These values are clearly lower than the daily requirements of vitamins for an adult (15–20 mg/day for B3, 2-3 mg/day for B6, 2.4 mg/day for B12, 60 mg/day for vitamin C, 8–10 mg/day for vitamin E, 0.08 mg/day for D3, 2-3 mg/day for B6, 0.8–1 mg/day for A, 2–7 mg/day for β-carotene, and 80 μg/day for K3).

3.1. Principal Component Analysis. A PCA was carried out on the vitamin content data obtained using HPLC analysis. Figures 1 and 2 present the plots of the PCA scores and correlation loadings, respectively. The score plot of PCA illustrates the large variability of the four okra varieties (S, M, K, and D) based on geographic location. The loadings represent the coefficients of the original variables that define each principal component [20]. The inertia percentages and variables correlated with axes PC1 and PC2 are displayed in Table 4. The first two axes retained 95.28% of the total inertia. PC1 explained 50.74% and PC2 explained 44.54% of the inertia, which positively correlated with pyridoxine, tocopherol, and menaphthone. Plots of the scores shown in Figure 1 indicate that the data cloud was mainly bidimensional. Figure 2 shows that the varieties formed three

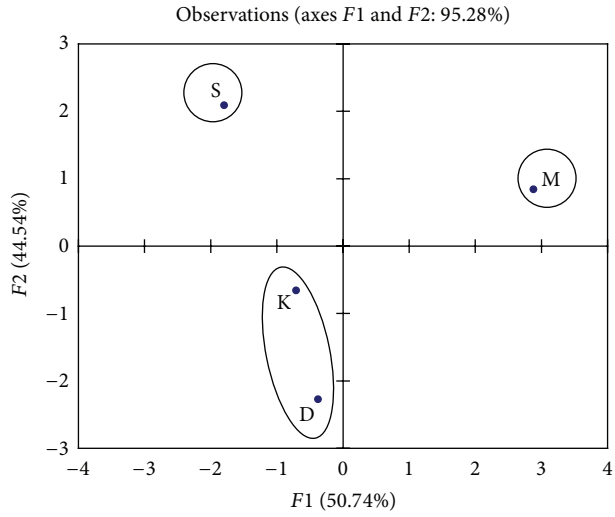

Figure 2: Plots of the x-loudings for vitamin contents of okra pods.

distinct clusters. The first cluster included the D and K varieties. The second and the third clusters separated out the S and M varieties independently.

4. Conclusion

This study focused on the quantification of okra vitamins commonly consumed in Egypt. The results were within the wide ranges of data reported in the literature. The vitamin contents of the okra varieties were significantly correlated

Table 4: Discriminate factors of principal components analysis based on vitamin contents of okra pods.

	*F*1	*F*2
Eigenvalue	3.04	2.67
Variability (%)	50.74	44.54
Cumulative (%)	50.74	95.28
Riboflavin	—	—
Niacin	+32.76	—
Pyridoxine	—	+35.81
Cobalamin	+29.02	—
Ascorbic acid	+30.42	—
Tocopherol	—	+28.94
Menaphthone	—	+33.5
Retinol	—	—
Cholecalciferol	—	—
β-Carotene	—	—

with geographical origin. The S pod variety had the highest contents of vitamins B6 and E and the lowest contents of vitamins B3 and B12. The K pod had the lowest vitamin C content. The M pod had the highest contents of vitamins B3, B12, and C, while the K and D pods had the lowest contents of vitamins E, K, and B6. To reduce quality loss during the drying process, some preventative measures should be taken. Some of these measures include shade drying to reduce photodegradation, slicing the vegetable into thin slices to reduce drying time, and the use of a predrying treatment, such as blanching, to reduce enzyme activities and loss of vitamins.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this study.

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