

These findings support the hypothesis that the principal cause for the observed vigor and higher yield in fresh weight of the tobacco plants that were treated with aldicarb is its effect on the carbohydrate (increase) and protein (decrease) content of the plant. Other things being equal, the higher concentration of water-soluble sugars in the treated plants may have developed a higher osmotic pressure that increases the absorption and/or retention of water in the plant tissue. This is reflected in the whole plant as an apparently higher yield and enhanced plant vigor.

In a biological system, such as the tobacco plant, the presence of a bioactive compound like aldicarb in its tissue could result in complex phenomena of interactions. Investigations regarding such interactions are very complicated and the interpretation is difficult. Further research would be necessary to elucidate the mechanisms involved in such complex phenomena.

**Registry No.** Aldicarb, 116-06-3; nicotine, 54-11-5; nitrate reductase, 9013-03-0; Fe, 7439-89-6; Mn, 7439-96-5; Zn, 7440-66-6; K, 7440-09-7; Cu, 7440-50-8; Na, 7440-23-5.

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## Chemical Composition of Seeds of Two Okra Cultivars

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Chemical compositions of the whole mature seeds of two okra varieties were investigated. The results of amino acid analyses indicated okra seed as a potential high-protein source which due to its high lysine level may serve as a supplement to cereal-based diets in which lysine is generally the first limiting amino acid. The most limiting amino acids in the Emerald variety were found to be valine (chemical score, 54.05), isoleucine (54.31), and threonine (60.0), while in the Ibtaira variety, tryptophan, isoleucine, and valine were the most limiting amino acids with chemical scores of 56.67, 57.41, and 67.03, respectively. The results of fatty acid analyses indicated okra seed oil is akin to other high oleic acid oils and thus is of low essential fatty acid content. The most predominant elements in okra seed were found to be K, Na, Mg, and Ca. The elements Fe, Zn, Mn, and Ni were also present in abundant amounts. Gossypol was found only as traces, while Halphen-positive cyclopropenoid compounds were detected in an amount equal to 2.5 times less than that present in crude cotton oil.

In response to the present deficit and the predicted world shortage of foods, considerable research is being directed toward expanding present supplies and exploring new sources. Okra [*Abelmoschus esculentus* (L) Moench], appears to have potential as a high-protein crop when

grown for its seed. Chemical and nutritional studies by Karakoltsidis and Constantimides (1975) on whole mature seeds of okra, Variety Emerald, have shown that the amino acid composition of okra was similar to that of soybeans and that the protein efficiency ratio (PER) was higher for okra. Martin et al. (1979) reported that a high-protein, high vegetable oil product can be prepared from okra seeds at the household level by using simple techniques. Savello et al. (1980) also reported on the nutritional composition of a seed meal prepared from an okra variety grown in

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Puerto Rico. The present study compares the amino acids, fatty acids, gossypol, fiber, starch, and mineral compositions of one of Iraqi okra variety, namely, Ibtaira, which is known for its very high yield when grown for seeds with that of Emerald variety, since such a knowledge will be of interest in evaluating the nutritional value of this local variety.

#### MATERIALS AND METHODS

**Seed Sample.** Seeds of both varieties were yield of plants grown under identical field conditions.

**Seed Flours.** Seeds were visually sorted for uniform size, color, and maturity. For flour preparation, about 600 seeds were ground to a fine powder in an electric mill. The powder thus obtained was used for all subsequent analyses.

**Amino Acid Analysis.** A sample (500 mg) was hydrolyzed with 300 mL of glass-distilled 6 N HCl at reflux temperature (105 °C) under a fine stream of high-purity N<sub>2</sub>. A trap, consisting of pyrogalol solution (Mondino and Bongiovanni, 1970), was used to free the N<sub>2</sub> from any traces of O<sub>2</sub>. The bubbling was maintained during the entire period of hydrolysis (22 h) and during the period required to cool the hydrolysate to room temperature. The hydrolysate thus obtained was filtered and evaporated to dryness on a rotary evaporator at 35–40 °C, and the residue was washed twice with a 2-mL portion of deionized distilled water, dissolved in 25 mL of citrate buffer (pH 2.2), kept overnight at 4 °C, and filtered. The volume of the filtrate was adjusted to 25 mL. Finally 1 mL was transferred to a 5-mL volumetric flask and the volume was made up to the mark. A 0.1-mL portion was used for amino acid analysis using a Beckman Model 120C amino acid analyzer following the procedure described by the manufacturer.

**Tryptophan.** Due to its destruction during acid hydrolysis, tryptophan was determined by hot-alkaline hydrolysis. A sample (100 mg) was mixed with 3 g of Ba(OH)<sub>2</sub>·8H<sub>2</sub>O followed by addition of 6 mL of distilled water, and mixed thoroughly. Hydrolysis and quantitation were carried out following the method reported by Kehayoglou and Manoussopoulos (1977).

**Oil Extraction and Fatty Acid Analysis.** Lipids were obtained by extraction of okra seed flour with redistilled hexane (1:20 w/v) in a Soxhlet extractor for 4 h. After extraction, solvent was removed by using a rotary evaporator and the oil kept in dark under high-purity N<sub>2</sub> gas until required for analysis.

**Fatty Acid Analysis.** About 25 mg of total lipids was transesterified by using methanol-HCl reagent (procedure A). In procedure B, about 150 mg of lipids was saponified by refluxing with 5 mL of 0.5 N methanolic NaOH (in the dark and under a fine stream of high-purity nitrogen gas) for 5 min. Then esterification was achieved by adding 5 mL of BF<sub>3</sub>-methanol complex reagent and the mixture was refluxed for 4 min. The fatty acid esters were then recovered by adding 5 mL of 2% aqueous NaCl solution and 10 mL of peroxide free diethyl ether. The fatty acid esters prepared by each of the above methods were determined on a Packard Model 419 gas chromatograph using an FID and a glass column (20.1 × 2 mm) packed with 3% SE-30 on Diatomite C (100–120 mesh). Detector and injector temperature was 120 °C and oven temperature 100–220 °C, 4 °C/min. Fatty acid esters were identified by comparing their retention times with those of known references, and the individual fatty acids were quantified by the method of triangulation.

**Gossypol.** Total gossypol was determined by using a procedure developed by Pons et al. (1958), while free gossypol was determined by using two different procedures

Table I. Chemical Composition of Okra Seeds

	% <sup>a</sup> (dry weight basis) for variety	
	Emerald	Ibtaira
protein ( <i>N</i> × 6.25)	21.82 <sup>y</sup> ± 1.33 <sup>d</sup>	17.66 <sup>y</sup> ± 0.22 <sup>d</sup>
moisture	6.96 <sup>y</sup> ± 0.38	6.84 <sup>x</sup> ± 0.16
ash	4.33 <sup>y</sup> ± 0.02	4.62 <sup>x</sup> ± 0.04
lipids	14.70 <sup>y</sup> ± 0.57	16.65 <sup>x</sup> ± 0.78
crude fiber	27.30 <sup>y</sup> ± 0.28	27.20 <sup>x</sup> ± 0.14
starch <sup>b</sup>	4.75 <sup>y</sup> ± 0.14	5.00 <sup>x</sup> ± 0.28
total carbohydrate <sup>c</sup>	24.89 ± 0.65	27.14 <sup>x</sup> ± 0.90

<sup>a</sup> Mean ± SD. <sup>b</sup> Values expressed as percent of defatted and sugar-free flour. <sup>c</sup> Total carbohydrate is determined as 100 - (moisture + protein + lipids + crude fiber + ash). <sup>d</sup> Means followed by same letter on a horizontal line are significantly different according to an L.S.D. test (*P* = 0.05).

(Storherr and Helley, 1954; Schramm and Benedict, 1958). Cyclopropenoid compounds were determined by using the Halpen test (Association of Official Analytical Chemists, 1975).

**Crude Fiber.** Crude fiber was determined by using the fibertec system Model 1010 Heat Extract, Tecator (Sweden) following the procedure described by the manufacturer.

**Starch.** One gram of defatted flour was extracted with 100 mL of 95% EtOH in a Soxhlet extractor for 4 h. The sugar-free residue thus obtained was dried overnight at 45–50 °C in a vacuum oven; then 100-mg samples were refluxed with 500 mL of 0.4 N H<sub>2</sub>SO<sub>4</sub> for 4 h, followed by cooling to room temperature and filtering.

The filtrate was up to 1 L. A portion (200 mL) was adjusted to pH 7.0 by addition of few milliliters of 50% KOH and left to stand in an ice bath for 15 min prior to centrifugation at 10 000 rpm for 15 min at (2 °C). The supernatant was decanted and reserved, while the precipitate was reextracted with 100-mL of distilled water. The supernatants were pooled and made up to a known volume. A 50-mL portion was evaporated at 45–50 °C. The residue was dissolved in 2 mL of distilled water, the flask was rinsed with another 2-mL portion of distilled water, and the final volume was adjusted to 5 mL.

Starch was determined as glucose by using anthrone reagent. Standard starch (potato starch) was treated similarly, and the resulting glucose showed excellent recovery (98.5–99.0%).

**Minerals.** The elements Ca, Mg, K, and Na were determined by using a 503 Perkin-Elmer atomic absorption spectrometer following the method described by the manufacturer. The elements Cu, Cr, Fe, Mn, Ni, Rb, Sr, and Zn were determined by the secondary absorption correction method (Jenkins, 1974) using a Philips 1140/00 X-ray spectrometer.

#### RESULTS

The results of chemical analyses of the major constituents of the whole mature seeds of a local okra cultivar, "Ibtaira", are presented and compared to the "Emerald" variety (Table I). The cultivars are similar in their crude fiber, ash, starch, total carbohydrate, gossypol, and moisture contents. Protein level, on the other hand, is slightly higher in Emerald, while Ibtaira shows a slightly higher lipid level. The results of the amino acid contents of the seeds of both cultivars are presented in Table II. The results reveal that with the exception of glutamic acid and arginine, which show higher levels in Emerald, and alanine, which is higher in Ibtaira, all other amino acids in both cultivars are either similar or slightly higher in Emerald. Fatty acid compositions of okra seed lipids are

Table II. Amino Acid Composition (Grams per 16 Grams of N) of Okra Seeds

amino acid	variety	
	Emerald	Ibtaira
Lys	7.24 <sup>y</sup> ± 0.09 <sup>a,b</sup>	8.90 <sup>y</sup> ± 0.28 <sup>a,b</sup>
His	1.78 <sup>y</sup> ± 0.17	1.83 <sup>x</sup> ± 0.13
Arg	11.04 <sup>y</sup> ± 0.10	10.16 <sup>y</sup> ± 0.23
Trp	0.96 <sup>y</sup> ± 0.11	0.85 <sup>x</sup> ± 0.03
Asp	11.82 <sup>y</sup> ± 0.48	13.17 <sup>x</sup> ± 0.27
Thr	3.02 <sup>y</sup> ± 0.10	3.49 <sup>x</sup> ± 0.27
Ser	5.25 <sup>y</sup> ± 0.30	6.35 <sup>x</sup> ± 0.21
Glu	22.08 <sup>y</sup> ± 0.82	20.74 <sup>x</sup> ± 0.51
Pro	3.83 <sup>y</sup> ± 0.28	4.18 <sup>x</sup> ± 0.26
Gly	6.13 <sup>y</sup> ± 0.21	6.66 <sup>x</sup> ± 0.24
Ala	5.51 <sup>y</sup> ± 0.04	6.66 <sup>y</sup> ± 0.34
Cys	2.45 <sup>y</sup> ± 0.14	2.53 <sup>x</sup> ± 0.07
Val	4.00 <sup>y</sup> ± 0.04	4.95 <sup>y</sup> ± 0.16
Met	1.66 <sup>y</sup> ± 0.13	1.85 <sup>x</sup> ± 0.13
Ile	3.15 <sup>y</sup> ± 0.14	3.32 <sup>x</sup> ± 0.16
Leu	6.68 <sup>y</sup> ± 0.16	7.03 <sup>x</sup> ± 0.14
Tyr	3.69 <sup>y</sup> ± 0.17	3.83 <sup>x</sup> ± 0.20
Phe	4.28 <sup>y</sup> ± 0.21	3.93 <sup>x</sup> ± 0.13

<sup>a</sup> Means ± SD. <sup>b</sup> Means followed by same letter on a horizontal line are significantly different according to an L.S.D. test ( $P = 0.05$ ).

shown in Table III. Oleic acid, palmitic acid, and stearic acid are the major fatty acid constituents in both cultivars. In the Emerald variety, palmitic and stearic acid levels are in good agreement with data reported by Karakoltsidis and Constantinides (1975), while oleic acid is found in much higher levels in comparison with data reported by the same authors. Comparison of chemical score values of essential amino acids of okra seeds with those of wheat and soybean is shown in Table IV. Okra seed seems to be rich of lysine; thus, the chemical score for lysine was found to be close to that of soybean.

Elemental compositions of the seeds of both varieties are shown in Table V. The elements K, Na, Mg, and Ca are the major elemental constituents, while Fe, Mn, Zn, and Sr are present in the range of 2–10 mg/100 g of defatted seed flour. Other elements are found in the range of <1 to 2 mg/100 g of defatted seed flour. The Emerald variety, however, shows higher Ca, Na, and Mg levels, while K is higher in the Ibtaira cultivar.

## DISCUSSION

**Amino Acid Composition.** Amino acid composition and chemical scores for essential amino acids in whole mature okra seeds are presented in Tables II and IV, respectively. On the basis of analytical evidence, it was reported (Karakoltsidis and Constantinides, 1975) that the most limiting amino acids in whole mature okra seed were found to be the sulfur-containing amino acids, while iso-

leucine and valine were not limiting. Savello et al. (1980), on the other hand, reported the limiting amino acids in hulled okra seed meal. These were valine (amino acid score 50) isoleucine (69), and lysine (87). In the present study, the most limiting amino acids (based on analytical evidence) in the whole mature okra seed, variety Emerald were found to be valine (chemical score 54.05), isoleucine (54.31), and threonine (60.0). In the Ibtaira variety, tryptophan (chemical score 56.67), isoleucine (54.41), and valine (67.03) were found to be the most limiting amino acids. Okra seed seems to be rich of lysine. Thus, the chemical score for lysine is close to that of soybean. Therefore, okra seed may serve as a supplement to cereal-based diet in which lysine is generally the first limiting amino acid (Jansen, 1977).

**Fatty Acids.** The fatty acid composition determined by two different esterification procedures and expressed as percent fatty acids is shown in Table III. The Soxhlet procedure (using hexane as the extractant) was used because okra seed lipid contains a very minute (if any) amount of waxes, higher alcohols, lipoprotein, etc. that cannot be completely extracted with hexane (but rather with mixed solvent, chloroform-methanol, 2:1). The results (Table III) reveal that okra seed is very rich in monounsaturated fatty acids, namely, oleic acid ( $C_{18:1}$ ). In comparison to other high linoleic ( $C_{18:2}$ ) sources (see Sinclair, 1964), e.g., safflower (77%), sunflower (65%), corn (53%), soybean (51%), and cotton seed (45%), okra seed therefore may be considered as a poor source of essential fatty acids (EFA).

Finally, the fatty acid composition of okra seed oil in the present study correlates closely with previously published data (Sengupta et al., 1974; Karakoltsidis and Constantinides, 1975), and the only deviation is that in the present study: a higher percentage of oleic acid ( $C_{18:1}$ ) and a very low percentage of linoleic acid ( $C_{18:2}$ ) were observed.

**Minerals.** On the basis of quantitative considerations, essential minerals can be classified into major and trace elements. Okra seeds seem to contain four major elements, namely, Na, K, Ca, and Mg, and five trace elements, namely, Fe, Cu, Mn, Zn, and Ni (Table V). The most predominant major element is found to be potassium. The seeds also contain abundant amounts of the elements Na, Mg, Ca, Fe, Zn, Mn, and Ni. The values reported for the elements Ca and Fe are in good agreement with those reported in the literature (Karakoltsidis and Constantinides, 1975). Finally, since most cereals are very deficient in some elements, in particular calcium; therefore, fortification of cereal flours with okra seed flour may improve the dietary saturation. Moreover, the most common toxic elements (lead, mercury, arsenic, and cadmium) were not detected in okra seed flour.

Table III. Fatty Acid Composition (Percent Fatty Acid Basis) of Okra Seed Lipids by Gas Chromatography of Methyl Esters Prepared by Two Different Procedures

fatty acid	variety			
	Emerald		Ibtaira	
	procedure <sup>a</sup> A	procedure <sup>a</sup> B	procedure <sup>a</sup> A	procedure <sup>a</sup> B
caprylic acid	0 <sup>c</sup> <sup>b,c</sup>	0 <sup>c</sup> <sup>b,c</sup>	0.01 <sup>c</sup> ± 0.001 <sup>b,c</sup>	0.02 <sup>c</sup> ± 0.003 <sup>b,c</sup>
capric acid	0.01 <sup>c</sup> ± 0.003	0.01 <sup>c</sup> ± 0.006	0.01 <sup>c</sup> ± 0.001	0.01 <sup>c</sup> ± 0.003
lauric acid	0.03 <sup>c</sup> ± 0.011	0.02 <sup>c</sup> ± 0.001	0.06 <sup>c</sup> ± 0.009	0.06 <sup>c</sup> ± 0.028
myristic acid	0.30 <sup>c</sup> ± 0.042	0.20 <sup>c</sup> ± 0.014	0.30 <sup>c</sup> ± 0.014	0.10 <sup>c</sup> ± 0.028
palmitic acid	39.14 <sup>c</sup> ± 0.226	42.19 <sup>c</sup> ± 0.552	32.91 <sup>c</sup> ± 0.549	35.74 <sup>c</sup> ± 1.075
stearic acid	4.19 <sup>c</sup> ± 0.269	3.37 <sup>c</sup> ± 0.212	3.46 <sup>c</sup> ± 0.127	4.50 <sup>c</sup> ± 0.410
oleic acid	55.92 <sup>c</sup> ± 1.315	53.70 <sup>c</sup> ± 0.849	62.87 ± 1.245	58.80 <sup>c</sup> ± 1.131
linoleic acid	0.10 <sup>c</sup> ± 0.014	0.17 <sup>c</sup> ± 0.014	0.09 <sup>c</sup> ± 0.014	0.30 <sup>c</sup> ± 0.028
arachidic acid	0.36 <sup>c</sup> ± 0.056	0.31 <sup>c</sup> ± 0.028	0.28 <sup>c</sup> ± 0.057	0.47 <sup>c</sup> ± 0.085

<sup>a</sup> See the text for details. <sup>b</sup> Mean ± SD. <sup>c</sup> Based on an L.S.D. test ( $P = 0.05$ ); no significant difference between values obtained by the two procedures or between the fatty acid levels in the two okra varieties investigated.

Table IV. Comparison of Chemical Score Values of Essential Amino Acids of Okra Seeds with Those of Wheat and Soybean<sup>a</sup>

essential amino acids (EAA)	chemical score			
	soybean <sup>b</sup> (var. Clark)	wheat	okra seed variety	
			Emerald	Ibtaira
leucine	98.99	77.50	75.06	79.10
isoleucine	85.00	74.20	54.31	57.41
cysteine and methionine	168.94	80.70	71.93	76.84
valine	65.81	74.30	54.05	67.03
tryptophan	74.00	60.00	64.00	56.67
phenylalanine	93.04	96.40	76.43	70.36
lysine	140.75	41.70	117.91	133.13
histidine	45.71	104.9	84.76	87.62
threonine	86.60	56.00	60.00	70.00
tyrosine				

<sup>a</sup> Data for EAA in the reference protein (whole egg) and EAA in wheat and the method used for calculation of the chemical score were reported by Osborne and Voogt (1978). <sup>b</sup> Al-Wandawi (1981).

Table V. Mineral Composition of Okra Seeds

element	mg/100 g of defatted seed flour for variety	
	Emerald	Ibtaira
calcium	375.5 <sup>y</sup> ± 10.04 <sup>a,b</sup>	268.8 <sup>y</sup> ± 3.68 <sup>a,b</sup>
copper	<1 <sup>y</sup>	<1 <sup>x</sup>
chromium	<1 <sup>y</sup>	<1 <sup>x</sup>
iron	9.80 <sup>y</sup> ± 0.96	7.30 <sup>x</sup> ± 0.45
magnesium	643.8 <sup>y</sup> ± 2.26	483.9 <sup>y</sup> ± 3.68
manganese	4.85 <sup>y</sup> ± 0.16	3.80 <sup>y</sup> ± 0.20
nickel	1.70 <sup>y</sup> ± 0.11	0.80 <sup>y</sup> ± 0.21
potassium	1309.00 <sup>y</sup> b 12.45	1591.40 <sup>x</sup> ± 8.20
rubidium	<1 <sup>y</sup>	<1 <sup>x</sup>
strontium	2.30 <sup>y</sup> ± 0.28	1.90 <sup>x</sup> ± 0.01
sodium	647.20 <sup>y</sup> ± 4.81	475.60 <sup>y</sup> ± 3.54
zinc	8.60 <sup>y</sup> ± 0.07	7.00 <sup>y</sup> ± 0.13

<sup>a</sup> Mean ± SD. <sup>b</sup> Means followed by same letter on a horizontal line are significantly different according to an L.S.D. test ( $P = 0.05$ ).

**Gossypol and Cyclopropenoid Compounds.** Gossypol, the phenolic compound found in cotton seed and known to cause undesirable physiological effects on non-ruminants such as poultry and swine (Pons, 1977), was found in okra seed only as traces. Cyclopropenoid compounds, which are found in seed lipids of the order Mal-

vales, were estimated in okra seed by using the Halpen test. These compounds were found in okra seed in an amount equal to half that present in cotton seed (determination of cyclopropenoid compounds in okra and cotton seed was carried out under identical conditions). It is worthwhile to mention that cotton seed oil contains concentrations of these cyclopropenoid compounds of 0.6-1.2% in crude oil and 0.1-0.5% in the processed oil (Mattson, 1973).

**Registry No.** Gossypol, 303-45-7; starch, 9005-25-8; Ca, 7440-70-2; Mg, 7439-95-4; K, 7440-09-7; Na, 7440-23-5; Cu, 7440-50-8; Cr, 7440-47-3; Fe, 7439-89-6; Mn, 7439-96-5; Ni, 7440-02-0; Rb, 7440-17-7; Sr, 7440-24-6; Zn, 7440-66-6.

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## COMMUNICATIONS

### Ranunculin: A Toxic Constituent of the Poisonous Range Plant Bur Buttercup (*Ceratocephalus testiculatus*)

Toxic ranunculin has been isolated from bur buttercup (*Ceratocephalus testiculatus*), a range plant poisonous to grazing sheep. Analyses of various growth stages of the plant revealed that the "early flower" stage contained the highest concentration of ranunculin.

Bur buttercup (*Ceratocephalus testiculatus*) is a small gray-green woolly, early appearing (March-May) annual weed. Introduced into the western United States, the plant

was first identified in Utah in 1932. It now grows in California, Colorado, Idaho, Nebraska, Nevada, Oregon, Utah, and Washington. Plants have been found on foot-