similar to the Cluster variety.

***α- and β-Acid Composition of Cascade Hops.*** The simplicity and rapidity of the direct extract method provided a means of investigating the consistency of homolog composition for a given hop variety grown in various locations. Six samples of 1974 crop Cascade hops grown in Washington State and two from Idaho were analyzed and the level of all homologs found to be consistent, cohumulone values ranging from 28.5 to 31.5% (Table III). In view of the fact that the α-acid level in the hops showed considerable variation, ranging from 4.4 to 7.1%, the homolog composition would appear to be much less sensitive to growing area or cultural practices than α- or β-acid contents.

Also, two samples of Cascade hops grown under identical conditions, with the exception of one being virus-free and the other virus-infected, were also analyzed. The homolog composition for both of these hops fell within the range found for commercially grown Cascade hops, while the α- and β-acid levels of the virus-infected hops were somewhat lower (Table III). Homolog percentages therefore do not appear to be significantly affected by virus expression whereas the α- and β-acids are known to be decreased.

The results obtained indicate that, at least for Cascade hops, the homolog composition of both α- and β-acid fractions is not significantly affected by location, cultural practice, or disease and is, therefore, a genetically fixed characteristic of the hop variety. This finding supports the value of the facile direct-extraction NMR homolog analysis method as a technique for evaluation of experimental varieties in hop breeding programs and for establishing the varietal authenticity of commercial hop samples.

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# Okra Seeds: A New Protein Source

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Okra seed was investigated for the first time for its potential as a seed protein. Chemical and nutritional studies were carried out to evaluate the seed and compare it to other seed proteins such as soya, cottonseed, etc. One variety (Emerald) of okra seeds was used throughout the study. All determinations were carried out on the whole seed including the seed coat and endosperm. The amino acid composition of okra seed was found to be similar to that of soybeans, yet the PER value was higher for okra seed. Rats fed on zein as a source of protein failed to grow. However, when okra or casein replaced zein, the rate of growth and recovery was about the same.

Oilseeds together with legume seeds are the most promising type of crops for protein production. Animal and fish products provide about one-third of the total dietary protein, whereas plant proteins account for 50–75% of the total needs. Cereal grains, oilseeds, and pulses are the three groups of plants which supply most of the protein in the world (Dimler, 1971). Though certain plant proteins are low in some essential amino acids (Watt and Merril, 1963) they are the main source of protein intake in many parts of the world where availability of animal protein is not adequate. So far among seed bearing plants, soybeans and cottonseeds only have been utilized to an appreciable extent for protein isolates and concentrates production.

Okra (*Hibiscus esculentus L.*) (Gobo, Combo, Gumbo, Bamya, or Ocra) is of African origin and was introduced into the United States and West Indies under the Spanish name, gumbo. It is one of the botanical species cultivated for its pod for more than 2000 years. It grows in many parts of the world, India, Malaysia, the Philippines, the Middle East, the Mediterranean region, Central, East, and West Africa, Central America, and in general throughout the tropics (Cooke, 1958).

Okra has been used as a vegetable for its green pods, in the fresh state, canned, or frozen, and no attempt has been made to use its seeds as a source of protein. It belongs in the Malvaceae or Mallow family, as does cotton. Yields as high as 2000 lb of seed per acre have been reported in Louisiana (Clopton et al., 1948; Miller and Wilson, 1949). An okra pod 9 in. long can bear up to 100 seeds. The okra plant grows in soils of medium fertility, well-drained sandy loam, and in a wide range of altitudes and rainfall. It grows both in dry and wet seasons. A plant that is constantly cropped can bear pods and seeds until killed by frost (Tindall, 1968; Spartsis, 1972).

Edwards and Miller (1947) analyzed samples of okra seed meal from which the oil had been extracted with hexane and found the following composition: crude protein, 13.56%; fat, 1.92%; carbohydrate, 31.50%; crude fiber, 8.14%; moisture, 6.69%; ash, 8.19%; CaO, 0.37%; $P_2O_5$,

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**Table I. Chemical Analysis of Okra Seeds**

| | Okra | Soya[a] |
|---|---|---|
| | Percent | |
| Protein | 20.58 ± 0.55[c] | 34.10 |
| Moisture | 7.92 ± 0.29 | 10.00 |
| Ash | 4.44 ± 0.12 | 4.70 |
| Lipids | 20.06 ± 1.17 | 17.70 |
| Gossypol | 0.0032 ± 0.0002 | |
| | mg per 100 g | |
| Calcium | 282.26 ± 33.23[c] | 226.00 |
| Iron | 10.26 ± 0.18 | 8.40 |
| Thiamine | 0.69 ± 0.02 | 1.10 |
| Riboflavine | 0.14 ± 0.01 | 0.31 |
| Niacin | 4.01 ± 0.40 | 2.20 |
| α-Tocopherol | 30.40 ± 2.77 | 11.00–16.00[b] |

[a] Watt and Merril (1963). [b] Green et al. (1955); Brown (1952). [c] $X$ = standard error = means ± standard error (five replicate runs).

3.47%; and thiamine, 4.72–5.78 μg/g. Analysis of the hulls gave: crude protein, 12.33%; ash, 3.83%; CaO, 0.27%; and $P_2O_5$, 0.76%.

In the present investigation mature dry whole okra seeds were used throughout for the mineral, vitamin, amino acids, fatty acids, gossypol, and nutritional evaluations of the okra seed. A preliminary report on okra seeds as a source of protein was presented by Karakoltsidis and Constantinides (1974).

## MATERIALS AND METHODS

The AOAC methods (1970) were used for moisture content, ash, fat, calcium, iron, thiamine, riboflavine, and niacin. The Kjeldahl method (AOAC, 1970) was used for the determination of total nitrogen in the dry okra seed. The sample sizes were 1.0–2.0 g. The protein content was obtained by multiplying percent N by 6.25, since this value applies for many protein fractions from seeds.

α-Tocopherol was extracted using the method described by Dilley and Crane (1963). The rapid method developed by Roughan (1967) was used for the quantitative determination of α-tocopherol. Gossypol was determined using the procedure developed by Storherr and Holley (1954).

The Technicon Model NC-1 amino acid autoanalyzer was used for the amino acid analysis. Well-ground okra seed flour (100 mg) was placed into 10-ml heavy-walled Pyrex test tubes supplied with an evacuation connector (Type K-896850-0010, Kontes Glass Co., Vineland, N.J.) to avoid losses of amino acid due to air oxidation. Soya samples were run along with the okra for comparison. Norleucine was used as an internal standard (Blackburn, 1968).

Fatty acid analysis was performed using a Varian Aerograph 1800 series gas chromatograph. The okra seed lipids were extracted and methylated as described by Dilley and Crane (1963) and Metcalfe et al. (1966). Injections of 1.0-μl aliquots of sample methyl esters were applied on the column with a 1-μl 7101-N Hamilton syringe under the following conditions: detector temperature, 250°; injection port temperature, 190°; and oven temperature, 195°. Nitrogen was used as the carrier gas at 20 ml/min; compressed air at 250 ml/min and hydrogen at 25 ml/min were supplied to the flame ionization detector. Two columns were used for the separation of fatty acid methyl esters. A polar column (⅛ in. i.d., 8 ft long and packed with Chromosorb W, 80–100 mesh, impregnated with 10% diethylene glycol succinate, DEGS, Analabs, Inc., North Haven, Conn.) and a nonpolar column (⅛ in. i.d., 8 ft long column, packed with Uriport, impregnated with 3% GC grade, methyl silicone gum, SE-30, Analabs, Inc., North Haven, Conn.).

Animal studies were carried out for the nutritional evaluation of okra seeds and soybean protein by measuring the protein efficiency ratio (PER). The okra seeds, variety Emerald, were purchased from Ferry-Morse Co., Inc. (Buffalo, N.Y.) and Joseph Harris Corp. (Rochester, N.Y.). Soybean, protein-edible 72%, was obtained from Nutritional Biochemical Corp. Weaning 22-day old male rats CD Strain (Charles River Laboratories, Wilmington, Mass.) were used. The animals were fed with the ANRC reference casein (prepared by the Sheffield Chemical Co., Norwich, N.Y.) diet for 3 days, after which they were divided into groups of 10. Grouping was done so that the average weight in each group was about the same. The basic diet was a modification of the AOAC (1970) PER diet with the following composition: crude protein (N × 6.25), 10%; starch (corn starch, dextrin, glucose, and sucrose in a 1:1:1:1 ratio), 72.8% (Stoewsand et al., 1965); lard, 5%; corn oil, 5%; cellulose (nonnutritive), 2%; mineral mix, 4% (Rogers and Harper, 1965); and vitamin mix, 1.2% (Campbell, 1960, 1963).

## RESULTS AND DISCUSSION

Chemical analysis of the whole okra seed is presented and compared to soya in Table I. The whole dry okra seed contained 20.58% protein, 7.92% moisture, 4.44% ash, and 20.06% lipids. Compared to soya seeds the okra seed is lower in protein. In the rest of the nutrients okra seed is high in both minerals, iron and calcium, compared to soybean. Vitamin analysis showed that soybean is high in thiamine and riboflavine but low in niacin and α-tocopherol in comparison to okra seed. Vitamin E present in okra seed is contained in the oil to the extent of 0.30 mg/g of oil. Okra seed oil contains the same amount of vitamin E as crude cottonseed oil but more than double the amount of soybean oil. It was observed by Fisher (1945) that γ-tocopherol has about 3 times the antioxygenic activity of α-tocopherol and that the okra seed oil content of γ-tocopherol is higher than in other oilseeds. Since the tocopherols are antioxidants and γ-tocopherol is about three times as active as α-tocopherol, it might be expected that the oils having the highest content of γ-tocopherol, as in okra seed oil, would be very stable. Gossypol, a toxic phenolic compound present in cottonseed (Adams et al., 1960), was not found

**Table II. Fatty Acid Composition of Okra Seed Lipids[a]**

| Fatty acid[b] | Okra | Soybean | Cottonseed[c] | Sunflower[d] |
|---|---|---|---|---|
| 14 | | 0.10 | 0.10 | |
| 14:0 | 0.12 | 0.30 | 0.80–1.40 | |
| 15:0 | Traces | | | |
| 16:0 | 33.53 | 10.80 | 19.90–23.4 | 6.50 |
| 16:1 | 0.14 | 0.30 | 0.40–2.00 | |
| 17:0 | 0.24 | | | |
| 17:1 | Traces | | | |
| 18:0 | 3.81 | 3.20 | 3.10 | 5.30–7.60 |
| 18:1 | 29.29 | 24.00 | 25.70 | 17.10–26.00 |
| 18:2 | 31.48 | 54.40 | 48.50 | 60.40–71.10 |
| 18:3 | 1.42 | 6.50–9.50 | 0.10 | |
| 19:0 | Traces | | | |
| 19:1 | 0.09 | | | |
| 20:0 | | 0.10 | 1.30 | |
| 21:4 | Traces | | | |
| 22:0 | | 0.10 | | |
| 24:4 | 0.12 | | | |

[a] Fatty acids are expressed as numbers describing in order the chain length and number of double bonds. [b] Percent fatty acid basis. [c] Sreenivasan (1968). [d] Cummins et al. (1967); Earle et al. (1968).

**Table III. Amino Acid Composition[a]**

| | Okra seed | Soybean | Okra pod[b] | Casein (cow's)[b] | Whole egg[b] | FAO ref. protein[b] |
|---|---|---|---|---|---|---|
| Asp | 15.47 ± 4.07[d] | 17.00 ± 4.14[d] | 20.05 | 7.11 | 9.59 | |
| Thr | 4.38 ± 1.04 | 5.47 ± 0.80 | 2.33 | 4.65 | 5.11 | 2.80 |
| Ser | 6.71 ± 1.41 | 7.42 ± 1.17 | 2.71 | 6.02 | 7.62 | |
| Glu | 20.48 ± 4.02 | 21.05 ± 3.95 | 15.19 | 21.19 | 12.70 | |
| Pro | 6.06 ± 1.70 | 7.71 ± 1.90 | 8.90 | 11.54 | 4.15 | |
| Gly | 5.79 ± 1.05 | 4.32 ± 0.87 | 2.62 | 1.97 | 3.30 | |
| Ala | 5.89 ± 0.86 | 6.13 ± 1.03 | 3.05 | 3.07 | 5.91 | |
| Val | 6.40 ± 1.13 | 5.26 ± 1.22 | 3.14 | 6.72 | 6.83 | 4.20 |
| Cys | 1.54 ± 0.64 | 1.61 ± 0.16 | 1.00 | 0.36 | 2.43 | 2.00 |
| Met | 1.29 ± 0.42 | 1.25 ± 0.03 | 1.33 | 2.78 | 3.35 | 2.20 |
| Ile | 4.65 ± 0.65 | 6.46 ± 0.84 | 2.43 | 5.40 | 6.27 | 4.20 |
| Leu | 8.47 ± 1.05 | 9.35 ± 0.76 | 3.86 | 9.49 | 8.79 | 4.80 |
| Tyr | 3.60 ± 0.68 | 3.72 ± 0.22 | 1.43 | 5.81 | 4.15 | 4.80 |
| Phe | 4.70 ± 1.08 | 5.26 ± 0.33 | 2.33 | 5.23 | 5.71 | 2.80 |
| Lys | 8.04 ± 2.81 | 8.00 ± 2.35 | 3.33 | 8.80 | 6.96 | 4.20 |
| His | 2.99 ± 0.97 | 2.67 ± 0.65 | 1.81 | 2.91 | 2.43 | 2.40 |
| Arg | 12.46 ± 6.42 | 10.07 ± 1.08 | 3.62 | 3.74 | 6.08 | 2.00 |
| Trp | *c* | *c* | 0.57 | | 1.70 | 1.40 |

[a] Grams of amino acid/16 g of nitrogen. [b] FAO (1970). [c] Tryptophan was not determined because of destruction during acid hydrolysis. [d] $X$ = standard error = mean ± standard error (five replicate runs for okra and three for soybeans).

**Table IV. Average Weight Gains, Protein Intakes, and Protein Efficiency Ratios (PER[a]) of Rats Fed Casein, Soybean Protein, and Okra Seed Flour (4-Week Period)**

| | Weight gain, g/28 days[a] | Protein intake, g/28 days[a] | PER[a] | Adjusted PER[b] |
|---|---|---|---|---|
| Okra[c] | 126.30 ± 4.33ab[d] | 36.76 ± 1.29ab | 3.45 ± 0.08b | 2.52 |
| Soybean protein | 100.20 ± 4.05c | 44.09 ± 1.60c | 2.27 ± 0.05a | 1.66 |
| Casein | 139.40 ± 6.24a | 40.72 ± 1.64cb | 3.42 ± 0.04b | 2.50 |

[a] $X$ = standard error = mean ± standard error. [b] PER values have been adjusted on the basis of a PER of 2.5 for casein. [c] Okra seeds powdered and sieved. [d] A common roman letter after the number indicates no significant difference ($P < 0.05$).

in significant amounts in okra seeds. The amount found in okra is 0.0032%. Much higher levels of 0.015–0.020% of free gossypol can cause toxicity symptoms and death to animals (Berardi and Goldblatt, 1969). The fatty acid analysis showed that the ratio of saturated to unsaturated fatty acids was 1:2, with a ratio of 1:6 for soybeans. The Emerald variety was found to contain 33.53% palmitic, 3.81% stearic, 29.29% oleic, 31.48% linoleic, and 1.42% linolenic acid. Insignificant amounts of 14:0, 15:0, 17:0, 19:0, 16:1, 17:1, 19:1, 21:4, and 24:4 fatty acids were detectable in the chromatography chart, as presented in Table II, not found previously by chemical methods (Crossley and Hilditch, 1951; Chisholm and Hopkins, 1957; Clopton et al., 1948; Hussain and Dollear, 1950; Jamieson, 1943).

The presence of linolenic acid was not reported before in okra seed oil. Since soybean oil usually contains 6–9% of linolenic acid a mixture with okra seed oil will lower the percentage of this acid. Also, since soybean oil products normally contain only about 11% palmitic acid, their effectiveness as a shortening has limitations (Wolf and Cowan, 1971). Therefore, soybean oil products could be improved by mixing with okra seed oil which contains 35% palmitic acid. Soybean oil is used in large quantities in the production of margarine that is prepared with a blend of hydrogenated oils. Usually, the oils that are hydrogenated are a blend of soybean and cottonseed with soybean content as high as 80% (Wolf and Cowan, 1971). Cottonseed oil could be successfully replaced by the okra seed oil, which has similar fatty acid composition.

The results of the amino acid analyses of okra seed and soybean seed are presented in Table III. During hydrolysis, some methionine is converted to methionine sulfoxide. The methionine values in the present study reflect the methionine content only, and do not include the oxidation product. The most limiting amino acids in okra seed protein based on analytical evidence appeared to be the sulfur-containing amino acids. However, since cystine and cysteine are unstable during acid hydrolysis (Blackburn, 1968) especially in the presence of carbohydrates (Lugg, 1933; Halwer and Nutting, 1946) the cystine value is likely to be low. No attempt was made to estimate cystine by oxidizing it to cysteic acid (Schram et al., 1954) because both okra seed and soybeans were analyzed for their amino acid composition under identical conditions. Okra and soybeans contain the same amount of lysine, a promising factor for the future utilization of okra seed as a supplement food mixture. Most of the amino acid levels in okra proteins are equal to or exceed the levels in egg, casein, and the FAO reference protein with one exception, the sulfur amino acids as it is seen in Table III. Therefore, okra proteins can be blended with other seed proteins such as sesame or wheat flour to provide a good mixture of the essential amino acids especially the sulfur amino acids.

Results of the animal feeding tests comparing okra seed flour, soybean protein, and casein are presented in Table IV. The PER of okra was found to be similar to that of casein, 3.4. Soya gave a PER of 2.3. It is difficult to explain this finding, since the biological value of a protein can be influenced by a number of factors that affect the amino acid availability (Liener, 1972). Furthermore, unknown

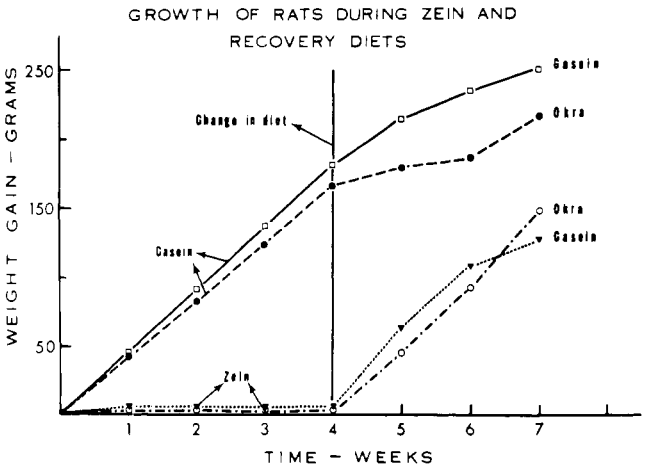


**Figure 1.** Recovery of growth by okra proteins or casein following a 4-week period on a zein diet. Two groups of 20 rats were fed zein and casein for 4 weeks. The animals on the zein diet were then fed okra or casein. One group of animals on the casein diet continued to eat casein whereas another group was fed okra. Growth curves indicate the average weight gain of each group of ten rats.

growth factors may be present in okra seed as has been suggested for soybean meal by other workers (Wilcox et al., 1961a,b; Griffith and Young, 1966). The casein and okra fed groups gained significantly more weight than the soya fed animals. Furthermore, the growth rate of the casein and okra fed animals was the same for the period of 4 weeks. Okra seed flour was heated at 130° for 3 hr and fed to rats in the same way as above. There was no difference in the PER value between heated and nonheated okra flour, indicating the absence of antinutritional factors.

Okra seed protein was tested for its ability to cause a recovery of growth after rats were fed zein for a period of 4 weeks, at which time no growth was observed. Two-month-old rats weighing about 250 g were given a diet consisting of 10% zein plus the other ingredients as in the above PER study. After 4 weeks on this diet, zein was replaced by okra flour for one group of rats and casein for another group. Figure 1 shows the recovery of growth in the rats. The rate of growth of the okra fed rats is similar to the casein fed rats and furthermore this rate of recovery in growth is similar to the original rate of growth of animals that were continuously fed casein. Okra protein appeared to be equal to casein in respect to inducing growth to the animals following a period on a poor protein diet, such as zein.

In order to test the baking characteristics of okra flour, wheat flour was mixed with okra flour in different proportions and bread was prepared. Preliminary studies showed that wheat flour can be mixed with 5% okra flour and a highly acceptable loaf of bread can be produced having a distinct yet acceptable flavor. Ziemba (1966) reported that soya flour added to wheat flour at a level of 5% did not affect the baking characteristics of wheat flour.

The above study shows that okra seeds meet all the requirements of a rich protein food or feed and should be explored further. Many technological problems still remain unsolved such as dehulling, milling, and utilization of the okra flour. Toxicological studies should also be performed. The technology and solutions found for other seed proteins may be adapted and applied to this new protein source.

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