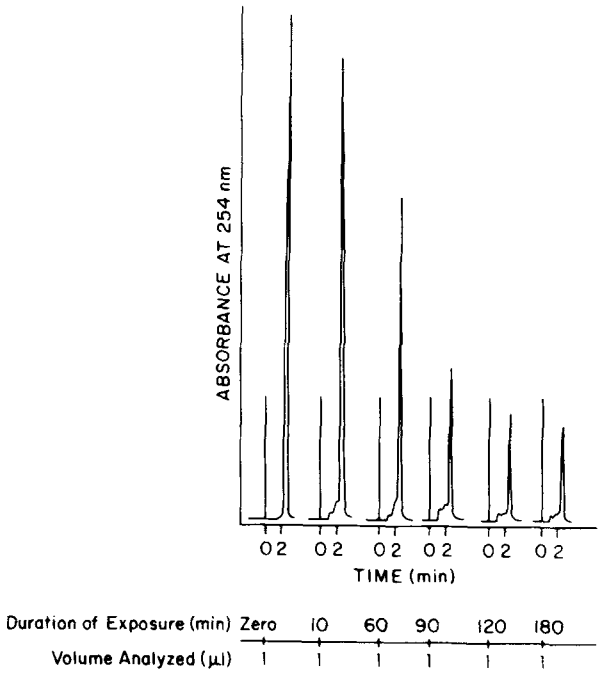


**FIG. 3. Time course degradation profile of gossypol as a thin film upon exposure to ultraviolet irradiation. See Figure 1 for explanation.**

degradation products (R.T. = 2.17, 2.40 min) remained almost unchanged (Fig. 3).

In all cases, as the exposure time increased, gossypol and its degradation product levels decreased as deduced by the area under each peak. This was attributed to the decomposition of gossypol and its degradation products to compound(s) that did not absorb at 254 nm. This can take place only by the breakdown of the aromatic structure, which in turn means the decomposition of the binaphthalene skeleton of the gossypol molecule.

This study demonstrates that gossypol is sensitive to degradation by ultraviolet light. Therefore, as a potential male contraceptive agent, it should be protected from light when formulated as a drug.

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# A Comparison of the Fatty Acids and Sterols of Seeds of Weedy and Vegetable Species of *Amaranthus* spp.

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## ABSTRACT

The seeds of weedy and vegetable species of *Amaranthus* were analyzed for sterols and fatty acids. The major sterol was spinasterol, which ranged from 46 to 54% by weight of the total sterol mixture. $\Delta^{-7}$ stigmasterol occurred in the next higher amount with lesser amounts of $\Delta^{-7}$ ergosterol, stigmasterol and 24-methylene-cycloartenol. There was little difference in the sterol composition of the vegetable species compared to the weedy species. The fatty acid compositions of the species were essentially all the same. Linoleic acid was present in the greatest amount, with lesser amounts of oleic, palmitic, stearic, myristic, linolenic, arachidic and lignoceric acids.

## INTRODUCTION

The amaranth plant (*Amaranthus* spp.) has been a major food source for ancient civilizations in the tropical highlands of Central America and Asia. The consumption of amaranths as food was displaced by larger seed grains, such as maize, and as a result amaranths have become relatively unimportant in human diets (1). Amaranths are fast growing plants found in tropical and temperate areas of the world and are considered pioneers of early successional stages of vegetation development. Most of the *Amaranthus* species, including *A. dubius* and *A. spinosus*, are considered weeds (5). Recently some vegetable species of amaranth plants that were used for food by ancient cultures have received attention because of the high levels of protein in their leaves and lysine in their seeds. For example, one of the vegetable amaranths *(A. edulis)* is reported to contain 6.2 g lysine/100 g protein (3), which is of considerable importance since lysine is one of the critical amino acids frequently deficient in plant protein (1). Seeds of amaranths contain 13% protein and 63% starch. The level of starch is equivalent to that of premium priced waxy maize (3,6).

Because of their high protein and lysine content the vegetable species of amaranths are receiving a great deal of attention in developing countries of the world as a means to combat protein malnutrition. Since protein rich foods of animal origin are either unavailable or expensive to purchase,

it is frequently suggested that protein deficiencies could be overcome to some extent by consuming protein rich vegetables. The seed yield of amaranths is high; a harvest of about 1018 kg per hectare of *A. hypochondriacus,* cultivated in India, has been reported (9). In developing countries the amaranth grain is first parched, milled, and the flour used to prepare a dough which is made into pancakes, cooked for gruel or made into confections. The grain also is powdered and used as a drink (1). Thus seeds are an important edible part of the amaranth. This study compares the fatty acids and sterols of 3 weedy and 3 vegetable species of amaranth seeds to determine if antinutritional fatty acids or sterols are present in the grains. These factors would limit their use as a source of protein. For example, in winged bean *(Psophocarpus tetragonolobus)* seed, behenic acid, which is a potential antinutritional compound (4), comprised 13.5% of the total fatty acids. In other plant parts, which also are eaten, the level of behenic acid varied from 0.1% to 6.9% (2). Therefore, consumption of winged bean seed should be discouraged until nutritional studies are complete.

## EXPERIMENTAL

### Sample Preparation

Seeds used for this study were obtained from the Rodale Research Center, Kutztown, Pennsylvania, and included weedy species, *A. tricolor* (78S-113), *A. retroflexus* (78S-73), *A. hybridus* (81S-394) and vegetable species, *A. tricolor* (79W-294), *A. dubius* (78S-223), and *A. cruentus* (78S-40). Seeds were separated from the floral sepals and 7 g of each species was ground in a mortar and pestle. Known amounts of heptadecanoic acid (17:0) and cholesterol were added to each ground sample as internal standards (2). Lipids were extracted by refluxing the samples in $CHCl_3$-MeOH (2:1, v/v) in a Soxhlet apparatus for 24 hr. The crude lipid extract was flash evaporated in a water bath at 40 C and the residue was resuspended in 20 ml of $CHCl_3$. The $CHCl_3$ was evaporated and the total lipid weight determined. Lipids were then saponified with 10 ml 70% Et/OH/KOH for 30 min, and extracted with 20 ml ether, methylated with 10 ml of borontrichloride in methanol (10%, w/v) by boiling for 5 min. The methylated fatty acids in methanol were partitioned into 60 ml of hexane and separated from sterols by column chromatography (7,8). Fatty acid methyl esters and sterols were then identified by gas chromatography.

### Gas Chromatography

A Varian gas chromatograph (Varian Associates Incorporation, Maryland) Model 3700 with a flame ionization detector (FID) was used. The operating conditions were: for fatty acid methyl esters: column 18 cm × 3.4 mm i.d., 15% Hi Eff 1 BP on Gas Chrom P, (Applied Science Labs) 20 psi and 165 C, detector 205 C, and flash heater, 205 C, carrier gas helium; for sterols:column 18 cm × 3.4 mm i.d., 3% SE-30 on Gas Chrom Q (Applied Science Labs) 20 psi and 244 C, detector 300 C, and flash heater 300 C, carrier gas helium.

Fatty acid methyl esters and sterols were identified and quantified by comparing their relative retention time (RRT) to known concentrations of authentic fatty acids and sterols (7,8).

## RESULTS AND DISCUSSION

Tables I and II summarize the types, amounts and relative percentages of sterols and fatty acids in weedy and vegetable species of amaranth seeds. Also included for comparison is the sterol and fatty acid composition of winged bean seeds (2). Basically there was no difference in the sterol composition of the 6 species of *Amaranthus* seeds (Table I). In all species, spinasterol occurred in the highest amount (.12-.19 mg/g dry weight or 46-54% total sterol) whereas there was considerably less $\Delta^{-7}$ stigmasterol (.03-.05 mg/g dry weight or 15-18% total sterol), $\Delta^{-7}$ ergosterol (.02-.05 mg/g dry weight or 12-15% total sterol), stigmasterol (.02-.03 mg/g dry weight or 11-13% total sterol), while 24-methylene cycloartenol occurred in the lowest amounts (.01-.03 mg/g dry weight or 5-11% total sterol).

In contrast, the sterol composition of winged bean seeds was very different. Although both plants contained stigmasterol, the level in winged bean seeds was much higher (.07 mg/g dry weight or 22% total sterol). Winged bean seed contained .02 mg/g dry weight or 9% campesterol and high levels of sitosterol (.21 mg/g dry weight of 69% total sterol) which were not detected in the amaranths. The 4 other sterols found, i.e., $\Delta^{-7}$ ergosterol, spinasterol, $\Delta^{-7}$ stigmasterol and 24-methylene cycloartenol, were not detected in winged bean seeds. Cholesterol was not detected in seeds of either plant.

The fatty acid composition of the amaranths and winged bean seeds are presented in Table II. As with the sterols, there were no consistent differences in fatty acid composition between any of the species. Linoleic acid was present in the greatest amount (20.01-23.43 mg/g dry weight or

**TABLE I**

**Sterol Composition of Weedy and Vegetable Species of Amaranths and Winged Bean Seeds**

| Sterols | Varieties | | | | | | |
|---|---|---|---|---|---|---|---|
| | *A. tricolor*[a] (78S-113) | *A. retroflexus*[a] (78S-73) | *A. hybridus*[a] (81S-394) | *A. tricolor*[b] (79W-294) | *A. dubius*[b] (78S-223) | *A. cruentus*[b] (78S-40) | Winged bean[c] |
| Stigmasterol | .03 (11)[d] | .03 (10) | .02 (11) | .03 (13) | .03 (12) | .03 (11) | .07 (22) |
| $\Delta^{-7}$ Ergosterol | .05 (14) | .05 (15) | .02 (12) | .04 (14) | .05 (15) | .04 (14) | – – |
| Spinasterol | .19 (52) | .18 (54) | .12 (53) | .14 (46) | .13 (48) | .17 (50) | – – |
| $\Delta^{-7}$ Stigmasterol | .05 (16) | .05 (16) | .03 (16) | .05 (16) | .04 (18) | .05 (15) | – – |
| 24-Methylene cycloartenol | .02 ( 7) | .01 ( 5) | .01 ( 8) | .03 (11) | .02 ( 7) | .03 (10) | – – |
| Campesterol | – – | – – | – – | – – | – – | – – | .02 ( 9) |
| Sitosterol | – – | – – | – – | – – | – – | – – | .21 (69) |
| Total sterol (mg/g dry) wt. | .36 | .35 | .22 | .30 | .28 | .34 | .31 |

[a]Weedy amaranths; [b]vegetable amaranths.

[c]Bean, et al. (2)

[d]Quantities expressed as mg/g dry weight and (%) of total sterol.

TABLE II

**Fatty Acid Composition of Weedy and Vegetable Species of Amaranths and Winged Bean Seeds**

| Sterols | | Varieties | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *A. tricolor*[a] (78S-113) | *A. retroflexus*[a] (78S-73) | *A. hybridus*[a] (81S-394) | *A. tricolor*[b] (79W-294) | *A. dubius*[b] (78S-223) | *A. cruentus*[b] (78S-40) | Winged bean[c] |
| Myristic acid | 14:0 | 0.05 ( 1)[d] | 0.10 ( 1) | 0.09 ( 1) | 0.07 ( 1) | 0.10 ( 1) | 0.09 ( 1) | – – |
| Palmitic acid | 16:0 | 7.01 (18) | 9.43 (21) | 9.01 (21) | 10.01 (23) | 11.01 (25) | 8.91 (20) | 0.64 ( 9) |
| Stearic acid | 18:0 | 1.13 ( 4) | 2.00 ( 3) | 1.23 ( 2) | 1.71 ( 4) | 1.63 ( 3) | 1.73 ( 4) | 0.43 ( 6) |
| Oleic acid | 18:1 | 9.62 (25) | 8.98 (20) | 9.72 (23) | 8.93 (21) | 9.53 (21) | 9.66 (22) | 2.52 (26) |
| Linoleic acid | 18:2 | 20.01 (51) | 23.43 (51) | 21.01 (50) | 20.91 (48) | 21.01 (47) | 21.45 (50) | 1.81 (26) |
| Linolenic acid | 18:3 | 0.36 ( 1) | 0.48 ( 1) | 0.30 ( 1) | 0.29 ( 1) | 0.38 ( 1) | 0.41 ( 1) | 0.51 ( 7) |
| Arachidic acid | 20:0 | 0.26 ( 1) | 0.31 ( 1) | 0.30 ( 1) | 0.38 ( 1) | 0.29 ( 1) | 0.31 ( 1) | 0.12 ( 2) |
| Behenic acid | 22:0 | – – | – – | – – | – – | – – | – – | 0.96 (13) |
| Lignoceric acid | 24:0 | .20 ( 1) | .21 ( 1) | .26 ( 1) | .27 ( 1) | .21 ( 1) | .31 ( 1) | 0.06 ( 1) |
| Total mg fatty acid | | 38.91 | 44.94 | 41.92 | 42.47 | 44.16 | 42.87 | 7.05 |
| Ratio (sat/unsat) | | .29 | .37 | .35 | .41 | .43 | .36 | .45 |

[a]Weedy amaranths; [b]vegetable amaranths.

[c]Bean et al. (2).

[d]Quantities expressed as mg/g dry weight and (%) of total fatty acid.

47-51% of total fatty acids), with lesser amounts of oleic acids (8.93-9.72 mg/g dry weight or 20-25% of total fatty acids), and palmitic acid (7.01-11.01 mg/g dry weight or 18-25% of total fatty acids). The remaining fatty acids, and in particular linolenic acid, occurred in much lower amounts (0.29-0.48 mg/g dry weight or 1% of total fatty acids).

The 2 highest ratios of saturated to unsaturated fatty acids, i.e., .41 and .43, occurred in 2 of the vegetable species, *A. tricolor* (79W-294) and *A. dubius*, whereas the lowest ratio of saturated to unsaturated fatty acid was in the weedy species *A. tricolor* (78S-113). Winged bean seeds also had a high ratio of saturated to unsaturated fatty acids, i.e., .46; however, the total amount of fatty acids in winged bean seeds was much less than the amaranths. Winged bean seeds contained 7.05 mg fatty acids whereas the amaranths contained 38.91-44.94 mg fatty acids per g dry weight.

In conclusion, these studies indicate that there is no reason why consumption of amaranths should not be encouraged. We were unable to detect cholesterol in any of the species and in the fatty acid analyses, the level of fatty acids is high, and the ratio of saturated to unsaturated fatty acids was at a level that would be considered safe. No behenic acid was detected as in winged bean, nor any other fatty acids that would be considered unsafe for human consumption. There were no large differences among the 6 species tested in either their sterol or fatty acid composition.

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