intake may account for this observation. In either case, both were destroyed or reduced with heat, as indicated by the increase in food intake in the heated bean group. The presence of a food intake depressing factor was probably the principal cause of the low food intake because any unpleasant taste of the raw beans should have been masked by the sugar, which constituted 75% of the raw bean diet. Inevitably, the depression of food intake by this factor will result in growth depression and untimely death. Liener (1953) and Turner and Liener (1975) concluded that soybean hemagglutinin caused growth depression by reducing food intake. A similar food intake depressing effect of raw winged bean is possible since strong hemagglutinating activity has been found in the winged bean seed.

The main objective of this study is to demonstrate the biological activity of isolted WBTI and determine its relative contribution to the toxicity of raw winged beans on rats. The limitation of this approach is that one cannot observe the combined and possibly synergistic effects of all the antinutritional factors in the raw bean. Such effects can put sufficient stress on the rats, leading to death as opposed to the individual and separate effects of the isolated factors. Within this limitation, we conclude that WBTI was not primarily responsible for the toxicity of raw winged bean but it caused pancreatic and spleen hypertrophy and growth depression.

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Tocopherols of Winged Bean (*Psophocarpus tetragonolobus*) Oil

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Oil samples extracted from 27 varieties of winged bean seeds were analyzed directly for individual tocopherols after dissolution in the mobile phase by high-performance liquid chromatography (HPLC) and ultraviolet detection at 295 nm. γ-Tocopherol was found to be the dominant form of tocopherol with traces of α-, β-, and δ-tocopherols. The samples showed a low of 8 and a high of 130 mg of γ-tocopherol/100 g of oil while most of the samples fell in the range of 23–44 mg/100 g of oil. Less variation was observed in the oil content of the different varieties which averaged 14.7%. On the basis of published data on fatty acid composition of winged bean oil, the tocopherol to polyunsaturated fatty acid ratio was calculated to be 0.2 mg of *d*-α-tocopherol equiv/g of polyunsaturated fatty acids, a value similar to that of soybean and less than that of a number of vegetable oils. The nutritional and functional significance of the predominance of γ-tocopherol in winged bean oil is discussed.

The winged bean (*Psophocarpus tetragonolobus*) is considered to be a very promising source of protein and oil in the humid tropics where it is native, grows well, and tolerates a wide range of altitudes (National Academy of Sciences, 1975). The chemical composition of the mature seeds is very similar to that of soybean and therefore offers the same uses as soybean. The oil may be extracted and used for cooking while the defatted meal can be used as a protein source for humans and livestock. However, in its present limited use as a backyard vegetable crop, it is greatly underutilized as a source of protein and oil.

Effective utilization of winged bean oil for cooking and as a source of lipids and lipid-soluble nutrients requires information on its chemical composition and physical properties. The fatty acid composition of winged bean oil is similar to that of peanut oil (Ekpenyong and Borchers, 1980; Garcia et al., 1979; Cerny et al., 1971). Total unsaturated fatty acids account for 65% of the total, and the ratio of the unsaturates to saturates is about 2. As far as we know, there is only one report on vitamin E analysis of winged bean oil, given by Cerny et al. (1971), who obtained a value of 126 mg of total tocopherol/100 g of oil. Vegetable oils contain tocopherols as natural antioxidants and are rich sources of vitamine E in the human diet

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(Slover, 1971). Furthermore, the stability of vegetable oils during processing and storage depends largely on the relative amounts of unsaturated fatty acids and natural or added synthetic antioxidants.

Up to 1965, vitamin E is reported mostly as total tocopherols, with only 5% of the data given as individual tocopherols (Dicks, 1965). With the availability of methods of separation, the individual tocopherol content of foods has increasingly been reported (Slover, 1971; Parrish, 1980). These methods include paper, thin-layer, and gas chromatography. More recently, high-performance liquid chromatography (HPLC) had been employed (Van Niekerk, 1973; Fujitani, 1976; Hartman and Kayden, 1979; Carpenter, 1979) for direct determination of individual tocopherols and promises to be a quick and simple method for analysis of a large number of samples.

Considering that the most biologically active form of vitamin E is α-tocopherol, while the other isomers have much lower activities, reporting of individual forms is useful in assessing the effective vitamin E activity of various foods and feeds. In collaboration with the Philippine Council on Agriculture and Resources Research, we have been determining the chemical composition of winged bean varieties being tested in the Philippines. We are reporting here the tocopherol content in the oil and the oil content of 27 winged bean varieties.

MATERIALS AND METHODS

Winged Bean. All 27 varieties, except 1, were supplied to us by Dr. Ponciano A. Batugal, Director of International Programs, Philippine Council on Agriculture and Resources Research. The varieties were grown in 1979 in central Philippines. The Chimbu variety (Tpt-2) was obtained from the University of Florida and was part of a 1979 crop.

Sample Preparation. The seeds were ground in a Wiley mill and extracted at room temperature 3 times with *n*-hexane (1:10, sample:solvent) for 3 h in the first extraction and 1 h for the next two. The extract was filtered and dried with anhydrous Na_2SO_4, and the solvent was evaporated in a rotary vacuum evaporator at 40 °C. The weight of the oil was taken accurately to calculate the oil content of the seeds. The oil samples were stored frozen until ready for analysis.

High-Performance Liquid Chromatography. The chromatographic separation was done on a Beckman 332 HPLC by using a μ-Porasil column (4.1 mm × 25 cm; Waters Associates) with a mobile phase of 1.5% 2-propanol in hexane (HPLC grade) at a flow rate of 1.8 mL/min. Twenty-microliter samples were injected for each determination by using a 20-μL vacuo-loop injection valve. The tocopherols were detected by their absorbance at 295 nm with a UV–visible detector, Model 1SS-40. The solvents were degassed every day before starting the analysis. The column was regenerated every day by pumping 2-propanol, methanol, 2-propanol, and the mobile phase in sequence, each wash lasting for 30 min.

α-Tocopherol and γ-tocopherol standards (Eastman Organic Chemical, Rochester, NY) made up in the mobile phase were run in concentration ranges from 0.10 to 10 μg/mL. Since β- and δ-tocopherol standards were not available from commercial sources, the retention times were established by using soybean oil prepared fresh and comparing with the data of Carpenter (1979).

A standard addition technique was used to determine if the oil interfered with the direct determination of tocopherol. Winged bean oil samples were analyzed before and after addition of known concentrations of α- and γ-tocopherols, and recoveries were calculated. Recoveries

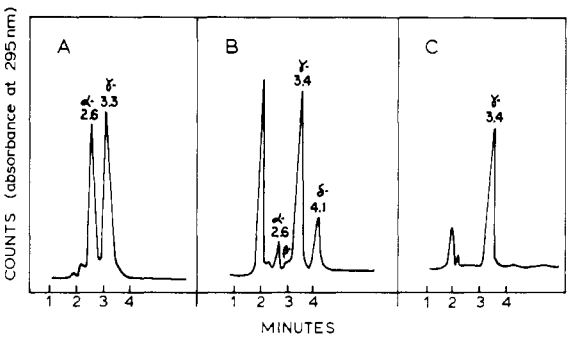

Figure 1. HPLC chromatogram of (A) a mixture of α- and γ-tocopherol standards, (B) soybean oil, and (C) winged bean oil. The number above the corresponding peak refers to the retention times.

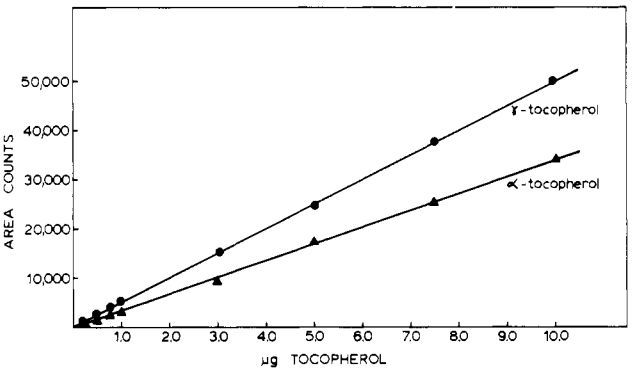

Figure 2. Calibration curves for α- and γ-tocopherols.

were 102.0% for α-tocopherol and 97.9% for γ-tocopherol; therefore, no internal standards were used.

RESULTS AND DISCUSSION

Chromatograms of a mixture of standards, soybean oil, and a representative sample of winged bean oil are shown in parts A, B, and C of Figure 1. α-and γ-Tocopherols were eluted consistently at 2.6 and 3.3 min, respectively, under the conditions used (Figure 1A). Because we were unable to obtain β- and δ-tocopherol standards, freshly extracted soybean oil was chromatographed and compared with the data of Carpenter (1979), who also used the μ-Porasil column and the same mobile phase and flow rate. A peak that came out after γ-tocopherol with a retention time of 4.1 min was assumed to be δ-tocopherol (Figure 1B). A small peak that eluted just before γ-tocopherol and would correspond to β-tocopherol remained unresolved and did not register counts. In some winged bean samples where this peak was more pronounced but did not register count, it was assigned to β-tocopherol. On the basis of these data, the only major form of tocopherol in winged bean oil was γ-tocopherol (Figure 1C). Only traces of the other forms were discernible in the chromatograms.

Figure 2 shows the calibration curves for α- and γ-tocopherols. γ-Tocopherol gave higher detector counts (absorption units) than α-tocopherol for the same concentrations at 295 nm. The area counts were proportional to tocopherol concentration up to 10 μg/mL. Higher concentrations of up to 100 μg/mL were also found to be linear but are not shown here. On the average, γ-tocopherol counts were higher than α-tocopherol counts by 50%. This is in agreement with the data of Carpenter (1979) but not with that of Abe et al. (1975), who showed that α-tocopherol had higher absorption than γ-tocopherol at the same concentration. Freed (1966) reported the extinction coefficients of tocopherols at their maximum absorption as follows: α-tocopherol (292 nm, $E^{1\%}_{1cm} = 72$) and γ-tocopherol (298 nm, $E^{1\%}_{1cm} = 92$). From these data,

Table I. Percent Oil and Tocopherol Content of Oil from Several Winged Bean Varieties[a]

variety	% oil	tocopherol, mg/100 g of oil			
		α	β	γ	δ
1, PI 7256A	14.76	T	ND	99 ± 0.7	ND
2, PI 7252	10.71	FT	ND	130 ± 1.4	ND
3, UPS 122	13.09	FT	FT	85 ± 2.1	ND
4, LG6 1202	14.05	FT	ND	121 ± 2.1	ND
5, UP5 31	15.71	T	T	89 ± 2.8	FT
6, UPS 121	16.66	T	ND	109 ± 0.7	ND
7, LG6 1201	13.33	T	ND	39 ± 0	ND
8, UPS 45	14.76	T	ND	32 ± 0	ND
9, UPS 32	16.43	T	ND	18 ± 0	ND
10, UPS 67	13.57	T	FT	37 ± 0	T
11, LG6 1203	13.81	T	T	44 ± 1.4	T
12, PI 7253A	15.48	T	FT	30 ± 0.7	ND
13, PI 7048	16.19	T	FT	23 ± 0	T
14, PI 7267	15.66	T	FF	29 ± 2.1	T
15, SJC 77-2	14.76	FT	ND	29 ± 1.4	ND
16, Batangas Long	13.65	FT	ND	39 ± 1.4	FT
17, Kade	12.70	FT	ND	40 ± 0	ND
18, LG6 1205	14.76	FT	FT	36 ± 0.7	ND
19, PI 7256	17.15	FT	FT	27 ± 0.7	ND
20, PI 7241	14.82	T	FT	33 ± 0.7	ND
21, LG6 1204	13.90	FT	ND	40 ± 1.4	ND
22, SJC 77-1	14.76	ND	ND	36 ± 0.7	ND
23, LBN C3	17.44	FT	ND	24 ± 0	ND
24, PI7 266	14.52	FT	ND	36 ± 0.7	ND
25, Chimbu	17.20	FT	ND	8 ± 1.4	ND
26, PI 7041	13.09	FT	ND	41 ± 1.4	ND
27, PI 7266	14.29	T	FT	24 ± 1.4	ND
av ± SD	14.71 ± 1.55			48 ± 33	

[a] Values for tocopherols are averages of three determinations. Trace (T) and faint trace (FT) peaks did not register counts in the detector. ND is not detected.

it would be expected that γ-tocopherol would have higher absorbance than α-tocopherol at 295 nm, which is 3 nm off from the absorption maxima of both tocopherols. This difference in absorption suggests the need to run individual standards of α- and γ-tocopherols for quantitation purposes.

The concentration of γ-tocopherol in winged bean oil samples fell within a wide range of values (Table I), with an average of 48 mg/100 g of oil; it was highest in variety PI 7252 (130 mg/100 g of oil) and lowest in the Chimbu (Tpt-2) variety (8 mg/100 g of oil). Most of the values, 74% of the samples, were within the range of 23–44 mg/100 g of oil. The highest value of 130 mg/100 g of oil was very close to that reported by Cerny et al. (1971) on total tocopherols of winged bean oil carried out on an unknown variety. It is interesting that the Chimbu variety which had been singled out as a high seed and tuber yielding variety (Lazaroff, 1981) had the lowest tocopherol content. However, it would be premature to make a definite conclusion since tocopherol content in seeds is affected by maturity and environmental temperature (Green, 1958) and seeds from different growing areas or grown in different seasons may vary in tocopherol content (Beringer and Saxena, 1968). Chimbu was the only variety that was not grown in the Philippines but was grown in Florida.

Published data on a number of vegetable oils show that most of the tocopherols are in the form of α-, γ-, and δ-tocopherols (Slover, 1971; McLaughlin and Weihrauch, 1979). Soybean, which has a chemical compositon very similar to that of winged bean, has predominantly γ-tocopherol (62% of total) followed by δ- and α-tocopherol. Our results with all the 27 varieties of winged bean revealed that forms of tocopherol other than γ-tocopherol were present in small or negligible amounts in oil. The average value of 48 mg of γ-tocopherol/100 g of oil was within the range reported in the literature for several vegetable oils. We did not attempt to analyze for tocotrienols, so their presence cannot be ruled out. However, very few vegetable oils had been reported to contain the unsaturated tocotrienols (Slover, 1971; McLaughlin and Weihrauch, 1979).

The predominance of γ-tocopherol in winged bean oil has significance in relation to its nutritional value and the stability of the oil to oxidative damage. Tocopherols are commonly used as antioxidants in vegetable oils. Current evidence indicates that γ-tocopherol is a better antioxidant than α-tocopherol. In vitro, γ-tocopherol had greater antioxidant activity than α-tocopherol since the latter was more easily oxidized by air (Ikeda and Fukuzumi, 1977). In aqueous media, at 0.05 and 0.25 mol of tocopherol/mol of linoleic acid, α-tocopherol exhibited prooxidant property while γ-tocopherol was antioxidant (Cillard and Cillard, 1980). Khafisov et al. (1975) concluded that γ-tocopherol was a better antioxidant than α-tocopherol in cottonseed oil. Therefore, γ-tocopherol should be an effective antioxidant during processing and storage of winged bean oil.

In a bioassay utilizing the reproductive performance of female rats, γ-tocopherol had an average biological activity of 10% relative to *d*-α-tocopherol (Bieri and McKenna, 1981). Winged bean oil had an average of 48 mg/100 g of oil, and according to the most recent nomenclature of expressing dietary values of vitamin E, this would be equal to 4.8 mg of *d*-α-tocopherol equiv/100 g of oil (National Research Council, 1980). To meet the recommended daily allowance of 10 mg of *d*-α-tocopherol/person would mean a consumption of about 200 g of winged bean oil if it was the only source of vitamin E in the diet. The recommended intake of vitamin E is also dependent on the presence of polyunsaturated fatty acids in the diet. Harris and Embree (1963) suggested the ratio of α-tocopherol to polyunsaturated fatty acids in the diet as 0.6 mg/g while Bieri and Evarts (1973) concluded that 0.4 mg/g was adequate for the U.S. diet. On the basis of the fatty acid composition of winged bean oil reported (Garcia et al.,

1979; Ekpenyong and Borchers, 1980), the average concentration of polyunsaturated fatty acid (linoleic plus linolenic) was 29 g/100 g of oil. The winged bean oil would have about 0.2 mg of d-α-tocopherol equiv/g of polyunsaturated fatty acid, a value lower than the recommended ratio for the U.S. diet. This ratio is similar to soybean oil but less than the oil obtained from peanut, corn, rice bran, palm, and cottonseed (Changbumrung et al., 1980).

Due to the limited amount of samples, only one determination of oil for each variety was carried out. The oil content did not show as much variation as γ-tocopherol among the varieties, with an average of 14.7%. This is slightly lower than those reported in the literature (Claydon, 1978; Ekpenyong and Borchers, 1980). A mild oil extraction procedure using hexane at room temperature (25 °C) (see Materials and Methods) was selected for this study to minimize oxidation losses of vitamin E. The AOAC (1970) procedure for fat determination in soybean flour requires extraction with petroleum ether (bp 35–45 °C) for 5 h, and Joslyn (1970) recommends extraction with anhydrous ether (bp 34.6 °C) for 16 h. Solvent extraction with hexane (bp 69 °C) is the preferred method for processing soybeans (Mustakas et al., 1981) and perhaps would be a method of choice for commercial extraction of winged bean oil in the future. Any one of these methods would have probably extracted a little more oil than the method used in our studies, but no actual data are available. Assuming that there was a small amount of oil that remained unextracted, there is no evidence in the literature that this would influence the composition of tocopherol (Parrish, 1980).

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