First Report of the Characterization of the Threatened Plant Species *Amaranthus pumilus* (Seabeach Amaranth)

Massimo F. Marcone†

Department of Food Science, Ontario Agricultural College, University of Guelph, Guelph, Ontario, Canada N1G 2W1

This paper reports the first ever investigation of the chemical components/composition of the seeds of *Amaranthus pumilus* (a threatened amaranth species) and compares the results to those of the more commonly cultivated *Amaranthus hypochondriacus* (Plainsman). This study clearly revealed that much genetic diversity exists between these species, indicating that potential breeding possibilities for the improvement of more commonly cultivated amaranth lines do exist. *A. pumilus* offers a much larger and more desirable seed size and weight (2−3-fold higher), permitting greater biomass production, in addition to lower levels (half) of free carbohydrate for improved value/performance in diabetic-type diets. In addition to the higher edible oil content found in *A. pumilus*, its lower saturated/unsaturated ratio (one of the lowest thus far measured) makes it potentially a better source of nutritional oil. In addition to the 2-fold-higher quantity of vitamin E found in *A. pumilus*, the higher levels of squalene also found may one day serve as a renewable crop source of this compound and may diminish the world's dependence upon marine animals. Considering the imminent danger posed to the survival of the seabeach amaranth in its native environment, it is hoped this study will raise public awareness of the importance of this species and thereby protect it from reaching extinction.

Keywords: *Amaranthus pumilus; seabeach amaranth; characterization; seeds*

INTRODUCTION

The cultivation and utilization of grain amaranth had been largely forgotten for centuries after its rise/peak and subsequent fall during the Aztec period, but its rediscovery, promotion, and dramatic comeback began in the mid 1970s and continues to grow strongly to the present day (Lehmann, 1996). Information concerning the nutritional attributes and benefits associated with the consumption of grain amaranth abound, especially when incorporated into bakery goods, breakfast cereals, and infant food formulas, etc. (Lehmann, 1996; Hozova et al., 1997), whereas other studies have demonstrated a variety of important/unique nutraceutical applications for grain amaranth (Lehmann, 1996; Chaturvedi et al., 1997). Such applications include the incorporation of amaranth grain as an adjunct in the human diet to lower blood glucose response in non-insulin-dependent diabetics (Chaturvedi et al., 1997) as well as for making such diets more nutritious in terms of a more balanced amino acid pattern and providing high amounts of calcium and iron (Chaturvedi et al., 1997). Other nutraceutical applications for amaranth grain include its use as a therapeutic adjunct to lower blood serum cholesterol levels in susceptible individuals, with its hypocholesterolemic effect being largely attributed to its high content of dietary fiber, tocotrienols, squalene, and isoprenoid compounds (Lehmann, 1996; Chaturvedi et al., 1993; Dunz et al., 1992). In addition, its high quantity of squalene has found potential use in the cosmetic industry as a lucrative cosmeutical hydrocarbon for use as a skin penetrant and lubricant (Lehmann, 1996; Lyon et al., 1987; Rayas-Duarte and Joeb, 1992).

The development of grain amaranth with enhanced characteristics for the above applications has been aided by the fact that there is a great deal of genetic variation available within the species (Chan and Sun, 1997). Although several *Amaranthus* species do exist, a few wild species, including *Amaranthus brownii* and *Amaranthus pumilus*, are quickly being extirpated from their native habitats and, therefore, may reduce the overall level of biodiversity available to plant breeders for the amelioration of present amaranth cultivars (Weakley et al., 1996). The latter species, that is, *A. pumilus* (seabeach amaranth) (a halophyte having no apparent close relatives), is an annual plant endemic to the barrier island beaches of the Atlantic coast, occurring historically in 31 counties in 9 states from Massachusetts to South Carolina (Weakley et al., 1996; Figure 1A,B). Over the past decade, the species has been completely extirpated from 6 of the 9 states of its original range. By 1993, with fewer than 55 remaining populations left in the wild (or ∼1000 individuals known), this plant species was given a global rank of G2 or "globally imperiled" and received federal protection as a threatened plant under the U.S. Endangered Species Act (Weakley et al., 1996). By all current accounts, the most recent hurricanes of 1999, that is, Hurricanes Dennis and Floyd, have almost completely destroyed all remaining populations and put the survival of the species in imminent danger.

If not driven to extinction, the seabeach amaranth (*A. pumilus*) may potentially serve as a useful plant resource in future breeding programs. Favorable genetic traits include its tolerance to high soil salinity and

† E-mail mmarcone@foodsci.uoguelph.ca; telephone (519) 824-4120, ext. 8334; fax (519) 824-6631.

10.1021/jf991133a CCC: $19.00
Published on Web 01/25/2000

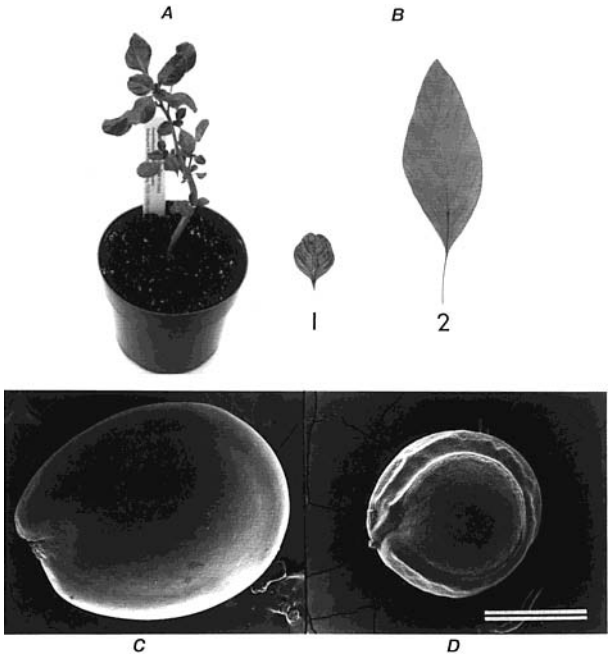

Figure 1. Photographs of (A) *A. pumilus* plant; (B) (1) *A. pumilus* leaf, (2) *A. hypochondriacus* leaf; (C) scanning electron micrographs of *A. pumilus* seed; and (D) *A. hypochondriacus* seed (bar represents 720 μm).

ability to grow in poor soil conditions containing nearly pure silica sand substrate, factors that may potentially expand the geographic range for the cultivation of amaranth grain (U.S. Fish and Wildlife Service, 1996). Other factors include its ability to reduce wind-induced erosion, and its large seed size (largest of all studied amaranth species) would make harvesting easier and permit greater biomass production (Weakley et al., 1996).

Considering the imminent danger posed to the survival of the seabeach amaranth in its native environment and the almost complete lack of any scientific literature about the species, this paper reports the first ever investigation of the chemical components/composition of its seed and compares them to those of the more commonly cultivated *Amaranthus hypochondriacus*. This study will identify possible chemical components that could be of special interest for future plant breeding programs and simultaneously attempt to raise public awareness to the importance of this threatened species and, therefore, protect it from reaching extinction.

MATERIALS AND METHODS

Materials. Seeds of the threatened plant species *A. pumilus* were obtained from the USDA (Ames, IA) PI553083 under provision 50CFR17.71(a) of the U.S. Endangered Species Act. *A. hypochondriacus* K343 was obtained from the Department of Food Science (University of Guelph) germplasm collection. Seeds of *A. pumilus* were propagated following a 30 day incubation period at 2 °C on a moistened blotter (0.2% potassium nitrate) to break seed dormancy. Specimens of both *A. pumilus* and *A. hypochondriacus* were grown in Promix B (Premier Horticulture Inc.) potting medium mixture and housed within the University of Guelph's research greenhouses under natural light condition (25/32 °C night/day temperatures). Mature seeds from both species were collected (~4 months from germination) and dried in a temperature-controlled chamber set at 37 °C with a relative humidity of 45%.

Colorimetric Determination. The color (L^*, a^*, and b^* values) of amaranth seeds and meals was determined using a Minolta Chroma Meter CR-200b (Minolta Camera Co. Ltd., Osaka, Japan).

Scanning Electron Microscopy. Seeds of both species were mounted on aluminum stubs and coated with gold/palladium (60/40) to a thickness of 25−30 nm using an Anatech Hummer VII sputter coater (Alexandria, VA). Following coating, seeds were viewed under a Hitachi S-570 scanning electron microscope with a high voltage setting of 10−20 kV.

Proximate Analysis of Amaranth Seeds and Elemental (P, K, Mg, and Ca) Analysis. Proximate analysis was performed as prescribed in the official standard methods of the American Association of Cereal Chemists, Inc. (AACC, 1983) after seeds were ground using a standard seed mill. For elemental analysis, 0.250 g samples of oven-dried seed material were wet digested and subjected to atomic absorption analysis as described by Marcone and Yada (1997).

Fractionation of Amaranth Proteins Using an Osborne-Type Procedure. Petroleum ether defatted seed meal (1.0 g) was sequentially extracted at 22 °C for 1 h with 0.5 M NaCl, 70% ethanol, and 0.1 M NaOH using 3 × 30 mL of each solution. The suspension was centrifuged for 20 min at 10000*g*, and the supernatants were collected. SDS−PAGE was performed as described by Marcone and Yada (1997).

Trypsin Inhibitor Activity. Trypsin inhibitor activity determination was performed as described by Budin et al. (1996).

Amino Acid Analysis. Amino acid analysis on the seed and protein fractions was performed as described by Marcone and Yada (1997).

Free Carbohydrate Analysis. Defatted seed material was quantitatively extracted with distilled water (1:8) and liquid dried. Aliquots (0.5 mL) of STOX (oxime-internal standard reagent) were then added and incubated at 70 °C for 30 min prior to the addition of 0.5 mL of *N*-trimethylsilylimidazole (TMSI); 0.1 μL was injected onto a Hewlett-Packard GC model 5830A equipped with 6 ft × 0.25 in. glass column packed with 3% OV-17 on Chromosorb W(HP) 80/100 mesh. The operating conditions were as follows: the run temperature was started at 240 °C and ramped to 260 °C at 10 °C/ min. The initial temperature was held for 4 min, and the final temperature was held for 12 min. The injector port temperature was set to 270 °C, whereas the detector port temperature was at 280 °C. Standards were obtained through Sigma-Aldrich Co., Oakville, ON.

Fatty Acid and Triacylglyceride Analysis. Fatty acid analysis was performed exactly as described by Christie (1982). The official AOCS method (Ce 5b-89) (AOCS, 1994) for triacylglyceride analysis by HPLC was used (with slight modification).

Squalene and Vitamin E Analysis. Squalene and vitamin E were both determined on Soxhlet extracted (petroleum ether) oils by HPLC as described by Sun et al. (1995).

Statistical Analysis. Statistical analysis was performed using an SAS Statistical Analysis System package. Significant differences among treatments were determined by Duncan's multiple-range test ($p \leq 0.05$) (SAS, 1990).

RESULTS AND DISCUSSION

Seed Morphology/Physical Characteristic/Examination of Seed Material. Physical characterization and comparison of the seeds of *A. hypochondriacus* (AH) and *A. pumilus* (AP) indicate the existence of major distinguishable differences between them, in terms of shape, size, and overall color (Table 1 and Figure 1). Scanning electron microscopy revealed that AP seeds were of much larger size (1.6-fold) and possessed more of an elongated lenticular shape than those of AH (Figure 1C,D). These observed morphological differences were further seen in the almost 2.63-fold higher seed weight of AP, making these seeds the largest ever studied and, therefore, potentially more desirable due to higher biomass content. Colorimetric analysis re-

Table 1. Compositional Analysis of the Seeds of *A. hypochondriacus* K343 (AH) and *A. pumilus* (AP; Seabeach Amaranth) (Threatened Species)[a]

	AH	AP
Proximate Analysis (%)		
moisture	7.30a	11.60b
ash	3.32a	2.10b
protein	17.90a	10.22b
fat	7.87a	12.73b
carbohydrate (by difference)	63.61a	63.35a
Seed Characteristics		
color		
*L** (seed/seed meal)	66.57a/83.02b	25.57c/61.35d
*a** (seed/seed meal)	5.79a/1.71b	4.34a/2.42c
*b** (seed/seed meal)	27.25a/15.99b	2.41c/10.90d
av seed wt (mg)	0.8a	2.1b
av seed size (mm)	1.2a	1.9b
Mineral (ppm)		
phosphorus (P)	1700a	4500b
potassium (K)	6720a	4340b
calcium (Ca)	3270a	2250b
magnesium (Mg)	3000a	1450b
iron (Fe)	65.1a	241b
Free Cabohydrate (%)		
fructose	0.05a	0.01b
glucose	0.76a	0.05b
sucrose	1.63a	0.69b
Fatty Acid (%)		
palmitic (C16:0)	21.26a	9.12b
palmitoleic (C16:1)	4.11a	1.41b
stearic (C18:0)	3.39a	1.96b
oleic (C18:1)	22.70a	31.48b
linoleic (C18:2)	40.83a	48.31b
linolenic (C18:3)	7.72a	7.71a
S/U	0.327a	0.125b

	AH	AP
Triacylglycerol (%)		
mono-, diglycerides	1.16	
LnLnLn		
LLL	8.26a	14.18b
OLL + OOLn	13.43a	26.81b
PLL + PLnO	21.40a	12.20b
OOL + PoOO	10.35a	18.42b
POL	17.78a	11.51b
PPL + MPO	10.46a	2.59b
OOO + MSO + OOP + PSL + POP	11.45a	12.97a
PPO + PPP + OOS + POS + SOS + SSO	5.77a	1.31b
LM/MM	4.80a	6.00b
Squalene (% in Oil)		
squalene	9.26a	10.34b
squalene (% in seed)	0.73a	1.32b
Tocopherols (μg/g of Seed)		
α-tocopherol	18.37a	34.95b
δ-tocopherol	32.90a	65.43b
γ-tocopherol	9.18a	22.05b
total tocopherol (per g of seed)	60.45a	122.43b
Protein (%)		
albumins	17.73a	22.08b
globulin	29.58a	20.10b
protamines	1.20a	1.60a
glutelins[b] (contains globulin fraction)	51.49a	56.21a
nitrogen solubility index (%)	26.06a	29.26b
globulin/albumin ratio	1.67a	0.91b
Trypsin Inhibitor Activity/mg		
TUI/mg	3.5a	3.6a

	whole seed	globulin	whole seed	globulin		whole seed	globulin	whole seed	globulin
aspartic + asparagine	8.1	7.8	9.6	8.2	isoleucine	3.5	4.0	4.3	3.4
threonine	3.4	2.9	4.0	2.8	leucine	5.8	6.3	7.0	5.6
serine	8.3	5.6	7.1	5.7	tyrosine	2.6	3.3	2.6	3.0
glutamic + glutamine	17.3	20.9	16.5	23.3	phenylalanine	4.0	5.2	4.9	4.8
glycine	14.0	9.8	11.0	9.4	lysine	7.8	7.1	7.0	6.8
alanine	7.2	5.8	7.3	6.3	histidine	3.5	3.8	3.6	3.1
valine	5.1	5.2	6.2	4.8	arginine	7.4	9.0	6.5	10.2
methionine[c]	2.0	3.3	2.3	2.7					

[a] Abbreviations: M, myristic; S, stearic; P, palmitic; O, oleic; Po, palmitoleic; L, linoleic; Ln, linolenic; S, saturated; U, unsaturated; LM, low melting; MM, medium melting. Values in each category row with the same letter are not significantly different ($p \geq 0.05$). [b] On dry seed weight basis. [c] By oxidation.

vealed that AP seeds were sufficiently darker (lower *L** values) as well as more reddish brown in color than those of its counterpart (AH). Although pale seeds are typically more desirable (Lehman, 1996), examination of AP seed meal upon mechanical grinding clearly revealed that the darker pigments of AP seeds were confined to its seed coat due to the substantially lighter overall meal color.

Carbohydrate. Carbohydrate was the single most abundant component found in the seeds of both AP and AH (Table 1). Although almost identical amounts of total carbohydrate were found in each species, differences in their content of free carbohydrate, namely, sucrose, glucose, and fructose, were noted (Table 1). Levels of sucrose found in AP were more consistent with those normally observed in common cereals such as wheat, rye, and millet (Saunders and Becker, 1984), whereas the amount of total soluble sugar found in AH fell well within the expected range reported for 63 studied accessions of amaranth (Saunders and Becker, 1984). The lower values of soluble sugars found in AP would be more favorable for the formulation of diabetic-type diets in which lower glycemic indexes are required.

Minerals. Although the total mineral (ash) contents in both studied species were higher than those observed in conventional grains, that of AP was found to be substantially lower than that of AH (Table 1) (Saunders and Becker, 1984). This result would be consistent with the fact that the larger seed size previously noted for AP would possess more endo- and perisperm and less seed coat/bran, that is, where higher levels of minerals are usually located. Both AP and AH are much richer sources of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and iron (Fe) than more common cereal grains (Saunders and Becker, 1984). AP was found to be a much richer (3.7 and 2.6 times higher) source of iron and phosphorus, respectively, as compared to AH.

Protein. After carbohydrate, the second most abundant component found within each seed meal was protein. Major differences were noted in their overall percentages, that is, with AH containing significantly higher quantities than AP (Table 1). In addition to the differences in total protein content, differences were also noted in the composition of proteins in each species as evidenced by a slight difference in the electrophoretic fingerprint patterns of their respective total seed proteins (Figure 2). To further determine both the types of proteins responsible for those observed differences and their overall quantities, a modified Osborne protein

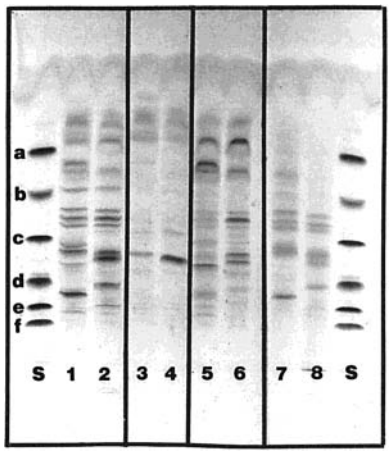

Figure 2. SDS−PAGE (reducing) (1 μL of 1 mg mL^{-1} protein solutions) applied to Homogeneous 20 PhastGels (Pharmacia LKB): (lane S) standards [(a) α-lactalbumin, 14400 Da; (b) soybean trypsin inhibitor, 20100 Da; (c) carbonic anhydrase, 30000 Da; (d) ovalbumin, 43000 Da; (e) bovine serum albumin, 67000 Da; (f) phosphorylase *b*, 94000 Da]; (lane 1) total *A. hypochondriacus* seed proteins; (lane 2) total *A. pumilus* seed proteins; (lane 3) albumins of *A. hypochondriacus*; (lane 4) albumins of *A. pumilus*; (lane 5) globulins of *A. hypochondriacus*; (lane 6) globulins of *A. pumilus*; (lane 7) glutelins of *A. hypochondriacus*; (lane 8) glutelins of *A. pumilus*.

fractionation scheme was employed to classify them in terms of their selective solubility in various types of solutions. As a first step, aqueous soluble proteins, that is, albumins and globulins, were quantitatively extracted together from each species, revealing that AH contained slightly higher levels of this particular fraction than AP (i.e., 47.31 and 42.18%, respectively). Further separation of both these proteins (in this particular fraction) by dialysis revealed significant differences in their globulin-to-albumin ratios. Electrophoretic fingerprinting of both their crude albumin and globulin fractions separately revealed that the previously observed differences in the composition of their total seed proteins were the result of differences in the composition in both their albumin and globulin fractions (Figure 2). The next sequential extraction of the seed meals of each species, that is, with organic solvents, revealed extremely low levels of prolamine-like proteins. Although the last/final extraction of the meals with an alkali solution for the isolation of glutelins from each species indicated very high levels of this particular type of protein, examination of their electrophoretic patterns revealed that they were substantially contaminated with their respective globulins (Figure 2). It had been demonstrated by some researchers that the recovery of salt-soluble proteins, that is, globulins, from various plant origins was substantially reduced when the meal was defatted prior to extraction (Byers et al., 1983; Fu and Sapirstein, 1996) and subsequently appeared in the glutelin fraction. It may, therefore, be concluded that, although 47.31 and 42.18% of the total albumin plus globulin fraction were determined for AH and AP, respectively, these values would represent vast underestimations of these particular proteins, which may be as high as 85% (Fu and Sapirstein, 1996). Although this is true, a few distinct/unique subunit bands were noted in the glutelin fraction from each species. Differences were also observable in these unique bands (between species), indicating further differences in the types of glutelin-like proteins between them. The amino acid compositions also showed slight differences of total seed proteins between species (particularly in the globulin fraction) but overall revealed that they were comparable to those observed for other amaranth species (Saunders and Becker, 1984).

Trypsin Inhibitor Activity. Screening for antinutritional components such as trypsin inhibitors revealed no measurable difference in trypsin inhibitor activities between both species, with values falling well below those known to affect weight gain in laboratory animals. A value of 104.7 TUI/mg was noted for a raw soybean reference sample, which fell well within expected values.

Lipid and Lipid-Soluble Components. Results of proximate analysis revealed that AP was not only substantially higher in crude fat content than AH but also substantially higher than those previously reported for 21 studied *Amaranthus* accessions (29%) by Budin et al. (1996) (Table 1). Further differences were also observed in the fatty acid profiles of AP and its counterpart AH and those of the above-mentioned 21 *Amaranthus* species. AP was found to contain substantially less saturated fatty acids such as palmitic (C16:0) and stearic (C18:0) but significantly higher mono- and polyunsaturates such as oleic (C18:1) and linoleic (C18:2) (Table 1). These differences resulted in a much lower saturated and unsaturated (S/U) ratio for AP than for AH or those of other studied species and therefore would indicate that AP is potentially a better source of nutritional oil. Although fatty acid compositions and ratios are important indicators in determining whether an oil is edible and possesses nutritional and functional value, they are not the sole indicators to compare different oil qualities, and therefore oils were further analyzed for triacylglycerol (TAG) composition. Major differences were observed in the position of fatty acids in the triacylglycerol backbone between AP and AH as indicated by differences in the amounts of LLL, OOLn, and PLnO (Table 1). The differences in the distribution of low melting (LM) and medium melting (MM) TAGS in each species led to differences in their LM/MM ratios, which ultimately determine the oil's thermal behavior, stable polymorphism when crystallized, and functional properties. Although AP may potentially serve as a better source of nutritional oil, its higher LM/MM ratio would result in an overall lower melting range, oxidative stability, and functionality, if a plastic fat is required.

Further compositional analysis of the lipid material from both species revealed that AP was substantially higher in both vitamin E and squalene. Vitamin E profiles revealed that they both contained α-, δ-, and γ-tocopherols, although AP was substantially higher, that is, >2-fold higher, in total tocopherol content (Table 1). Comparison of total tocopherol content of AP with those found in the 21 accessions surveyed by Budin et al. (1996) quickly reveals that AP contains the highest amount of tocopherols of the amaranth species thus far studied. Although the low S/U and high LM/MM TAGS ratios previously determined for AP oil would indicate a lower oxidative stability as compared to AH, its higher content of tocopherols would help to stabilize the oil against oxidation during heating while serving as a much richer dietary source of vitamin E. AP was also found to be substantially higher in squalene content than AH (Table 1). From a comparison of the squalene content of AP with that found in the oils of the other amaranth varieties, that is, 6−8% (Sun et al., 1997), it is evident that AP contains the highest amounts of this important compound among all species so far investi-

gated. In light of the importance and high demand for squalene in cosmetics, skin penetrants, and oxidation-resistant industrial lubricants and the concern for marine animal protection (the primary industrial source of squalene), AP may one day serve as a particularly rich crop source of this compound.

Conclusion. Much genetic diversity was observed between both studied *Amaranthus* species, indicating that potential breeding possibilities for the improvement of commonly cultivated amaranth lines do exist. *A. pumilus* offers plant breeders a much larger and more desirable seed size and weight for improved biomass production in addition to lower levels of free carbohydrate for improved value/performance in diabetic-type diets. In addition to the higher edible oil content found in *A. pumilus*, its lower S/U ratio (one of the lowest so far measured) makes it potentially a better source of nutritional oil. Differences in both fatty acid and triacylglycerol composition offer great potential for *A. pumilus* use in plant breeding programs for the manipulation of oil composition for various food purposes. In addition to the 2-fold higher level of vitamin E offered by *A. pumilus*, higher levels of squalene may one day serve as a crop source of this compound and diminish the world's dependence upon marine animals.

LITERATURE CITED

AACC (American Association of Cereal Chemists). *Approved Methods of the American Association of Cereal Chemists*, 8th ed.; AACC; St. Paul, MN, 1983.

AOAC (Association of Official Analytical Chemists). Liquid Chromatographic Method for Determination of Triglycerides in Vegetable Oils in Terms of Their Partition Numbers: Summary of Collaborative Study. *J. AOAC Int.* **1994**, *77C4*, 954−957.

Budin, J. T.; Breene, W. M.; Putman, D. H. Some compositional properties of seeds and oils of eight *Amaranthus* species. *J. Am. Oil Chem. Soc.* **1996**, *73* (4), 475−481.

Byers, M.; Miflin, B. J.; Smith, S. J. A quantitative comparison of the extraction of protein fractions from wheat grain by different solvents and of polypeptide and amino acid composition of alcohol-soluble proteins. *J. Sci. Food Agric.* **1983**, *34*, 447−467.

Chan, K. F.; Sun, M. Genetic diversity and relationships detected by isozyme and rapid analysis of crop and wild species of *Amaranthus*. *Theor. Appl. Genet.* **1997**, *95* (5), 865−873.

Chaturvedi, A.; Sarojini, G.; Devi, N. L. Hypocholesterolemic effect of amaranth seeds (*Amaranthus exculantus*) [sic]. *Plant Foods Hum. Nutr.* **1993**, *44*, 63.

Chanturvedi, A.; Sarojini, G.; Nirmala, G.; Nirmalammay, N.; Satyanarayana, D. Glycemic Index of Amaranth Grain, Wheat and Rice in NIDDM Subjects. *Plant Foods Hum. Nutr.* **1997**, *50* (2), 171−178.

Christie, W. W. A simple procedure for rapid transmethylation of glycerolipids and cholesteryl esters. *Lipid Res.* **1982**, *23*, 1072−1075.

Dunz, R. A.; Lupton, J. R. Physiological effects of dietary amaranth (*Amaranthus cruentus*) on rats. *Cereal Foods World* **1992**, *37*, 489.

Fu, B. X.; Supirstein, H. D. Procedure for the isolating monomeric protein and polymoric glutellin of wheat flour. *Cereal Chem.* **1996**, *73*, 143−152.

Hozova, B.; Buchtova, B.; Dodok, L.; Zemanovic, J. Microbiological, nutritional and sensory aspects of stored amaranth biscuits and amaranth crackers. *Nahrung−Food* **1997**, *41* (3), 155−158.

Lehman, J. W. Case History of Grain Amaranth as an alternative crop. *Cereal Foods World* **1996**, *41* (5), 369−441.

Lyon, C. K.; Becker, R. Extraction and refining of oil from amaranth seed. *J. Am. Oil Chem. Soc.* **1987**, *64*, 233.

Marcone, M. F.; Yada, R. Y. Evidence for the Phosphorylation and Glycosylation of the Amaranth 11S Globulin (Amaranthin). *Food Biochem.* **1997**, *21*, 341−369.

Rayas-Duarte, P.; Joeb, R. Study on the squalene content in amaranth grains (Abstract). *Proceedings of the 6th Annual National Meeting of the Amaranth Institute*; North Dakota State University: Fargo, ND, 1992.

SAS Institute. *SAS User's Guide: Statistics*; Statistical Analysis System: Cary, NC, 1990.

Saunders, R. M.; Becker, R. *Amaranthus*: a potential food and feed resource. *Adv. Cereal Sci. Technol.* **1984**, *6*, 357.

Sun, H.; Wiesenborn, D.; Rayas-Duarte, P.; Mohamed, A.; Hagen, K. Bench-scale processing of amaranth seed for oil. *J. Am. Oil Chem. Soc.* **1995**, *72* (12), 1551−1555.

Sun, H.; Wiesenborn, D.; Tostenson, K.; Gillespie, J.; Rayas-Duarte, P. Fractionation of Squalene from Amaranth Seed Oil. *J. Assoc. Off. Anal. Chem.* **1997**, *74* (4), 413−418.

U.S. Fish and Wildlife Service. *Seabeach Amaranth Species Fact Sheet*; New York Field Office: Cortland, NY, 1996; pp 1−2.

Weakley, A.; Bucher, M.; Murdock, N. *Recovery Plan for Seabeach Amaranth (Amaranthus pumilus)*; U.S. Fish and Wildlife Service, Southeast Region: Atlanta, GA, 1996; pp 1−59.

Received for review October 18, 1999. Revised manuscript received December 3, 1999. Accepted December 3, 1999.

JF991133A