makes it more difficult to detect the 50-g bolus by barium analysis, presumably because the large animals have larger intestinal systems and eat more, thus diluting the barium. However, apparently some animals had not retained the bolus. In only one case was the barium content less after treatment than before treatment. This is an indication that the bolus was not retained. If the acceptance or rejection criteria is arbitrarily set at 1.5 times the pretreatment level, we could also conclude that the bolus was present in only seven of the ten aminals 2 weeks after treatment.

Although the vegetation on which animals are grazing will affect the background levels of barium detected in the feces, this method will be useful in future bolus retention studies provided that base-line data are collected to determine the background levels and statistical techniques can be developed as criteria to accept or reject a level of barium as indicative of the presence of the bolus.

LITERATURE CITED

Chamberlain, W. F. *Southwest. Entomol.* **1977**, *2*, 11.
Chaudhry, F. M.; Wallace, A.; Mueller, R. T. *Commun. Soil Sci. Plant Anal.* **1977**, *8*, 795.
Miller, J. A.; Knapp, F. W.; Miller, R. W.; Pitts, C. W. *Southwest. Entomol.* **1979**, *4*, 195.
Smith, K. A. *Plant Soil* **1971**, *34*, 369.

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Evaluation of Food Potential, Some Toxicological Aspects, and Preparation of a Protein Isolate from the Aerial Part of Amaranth (Pigweed)

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Amaranthus spp. (pigweed, water hemp, etc.) grows profusely in cropped fields in the midwest and is considered a troublesome weed. Vegetative parts of the plants are high in protein, calcium, potassium, iron, and ascorbic acid, indicating a high food potential, but also may contain appreciable amounts of nitrate and oxalate. Although the leaves and tender tops of these plants have been used for human consumption on three continents for centuries with no apparent problems, the potential for toxic effects cannot be ignored. Several fractions taken from fresh, cooked, and oven-dried plants were analyzed for nutrient content and also for levels of nitrate and oxalate. Results indicate that any nitrate and soluble oxalate are removed by extraction into the cooking water. The resulting food appears to be of good quality as determined by chemical analyses.

In recent years a number of articles have appeared in scientific as well as in the popular press extolling the virtues of amaranth as a potential crop for feeding a protein-hungry world (Cole, 1979; Marx, 1977; Olatunbosun, 1976; "Proceedings of the First Amaranth Seminar", 1977; "Proceedings of the Second Amaranth Conference", 1980; NAS, 1975). For centuries both vegetative and grain varieties of amaranth have been a dietary staple for humans in many tropical and subtropical countries spread over three continents. Because the leaf tissue wilts rapidly soon after harvest, amaranth greens are usually obtained from garden plots or from fence rows rather than from markets. Consequently, it is the poor, rural people in these countries, rather than the urban dwellers, who use these plants as potherbs.

Varieties of *Amaranthus* spp. known popularly as pigweed, redroot, water hemp., etc. grow profusely in the cultivated fields in Midwestern United States, especially in fields which are summer fallowed. While descriptions of these species may be found in lists of edible plants (Hall, 1973; McPherson and McPherson, 1977), farmers in this area consider them to be troublesome weeds.

For decades we have been cognizant that the vegetative parts of these plants may contain high levels of nitrate and that they have been implicated in numerous cases of livestock poisoning (Duckworth, 1975; Osweiler et al., 1969). In addition to high nitrate levels, usually there is a high level of oxalate which is linked also to livestock problems (Marshall et al., 1967) and problems in humans as well (Valyasevi and Dhanamitta, 1974; Hodgkinson, 1977). Therefore, *Amaranthus* spp. remains an enigma. The present study was undertaken with three main objectives: (1) to determine the levels of nitrate and oxalate present in varieties of amaranth found in the Midwest; (2) to make compositional analyses of the aerial parts of the common varieties of amaranth native to the Midwest; (3) to evaluate these plants as potential sources for a protein isolate which might have possible use for diet enrichment. A procedure for the preparation of such of a protein isolate from the total aerial parts of five common varieties of amaranth is given.

MATERIALS AND METHODS

Dried samples of various fractions of mature *A. edulis* plants, a grain amaranth, grown at the University of Nebraska by using seed obtained from Nigeria, were made available to us. These samples were analyzed for the usual food constituents as well as for nitrate and oxalate content. All other studies were conducted on wild plants harvested at Lincoln, NE, or Waseca, MN, during the summer of 1980. These are *A. caudatus* (an ornamental variety), *A. retroflexus* (pigweed; redroot), *A. hybridus* (both red stem and green stem varieties), and *A. graecizans* (prostrate pigweed). The plants of upright habit were harvested when 1–2 m tall; stems were cut at 10 cm above the roots. The prostrate variety was harvested when the runners were at least 1 m long.

Total nitrogen was estimated according to the Kjeldahl procedure 46-12 approved by the American Association of Cereal Chemists in 1976 (AACC, 1969). Nitrate nitrogen

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Table I. Analysis of Aerial Parts of *A. retroflexus*

		crude protein ($N \times 6.25$), %		nitrate as NO_3^-, %		corrected[a] protein, %		oxalic acid, %		ascorbic acid, mg/100 g
		fresh basis	dry basis	fresh basis	dry basis	fresh basis	dry basis	fresh basis	dry basis	fresh basis
A. retroflexus:[b]	leaves	3.38	22.4	0.11	0.76	3.14	21.0	0.80	5.36	160
	stems	4.41	14.7	0.95	3.17	2.61	8.7	0.79	2.66	

[a] Corrected protein = (Kjeldahl nitrogen − nitrate nitrogen) × 6.25. [b] Rough pigweed; redroot.

Table II. Compositional Analyses of *Amaranthus* Species

	%, dry basis										
	crude protein ($N \times 6.25$)	ash	crude fiber	ether extract	nitrate as NO_3^-	Ca	Mg	P	Fe	Zn	K
A. edulis: bottom stem	8.09	16.6	45.4	0.93	4.12	0.52	0.13	0.20	0.025	0.005	4.83
middle stem	8.45	15.8	43.8	1.02	4.68	0.62	0.24	0.14	0.021	0.005	4.58
top stem	9.02	13.0	39.7	0.96	3.66	0.83	0.38	0.21	0.024	0.003	4.22
seed head	19.9	11.7	21.5	2.65	1.90	0.91	0.41	0.43	0.022	0.004	3.01
leaf	18.0	22.0	12.4	1.60	1.65	6.21	2.20	0.34	0.017	0.002	3.68
A. retroflexus (MN)[a]	26.8	21.5	15.1	2.41	4.42	3.04	1.70	0.52	0.041	0.008	5.43
A. retroflexus (NE)[a]	21.1	20.4	13.5	3.02	2.51	3.00	1.40	0.33	0.046	0.005	5.63

[a] Entire aerial portion.

was determined by the Orion nitrate electrode, Model 92-07. An oven-dried sample was extracted with boiling water on a steam table for 1 h, cooled, and diluted with phosphate buffer, pH 7, to a final ionic strength of 0.1 M prior to nitrate measurement. This procedure gives very reproducible results which are in good agreement with chemical methods in which nitrate is reduced to ammonia with Devarda's Alloy in an alkaline solution. Since total nitrogen by Kjeldahl includes nitrate nitrogen, corrected crude protein = (total nitrogen − nitrate nitrogen) × 6.25.

Methods described by the Association of Official Analytical Chemists (AOAC, 1980) were used for determination of ash (7.009), crude fiber (7.065), ether extract (7.056), calcium, magnesium, iron, zinc, and potassium (3.007), ascorbic acid (43.056), oxalate (33.035), and phosphorus solubilization (3.005). Phosphorus was determined by the molybdenum blue method (Fiske and Subbarow, 1925) adapted to a final volume of 10 mL. Amino acid analyses of the protein isolate were carried out as follows: Ground samples were hydrolyzed in 6 N HCl for 24 h at 110 °C. The hydrolysate was filtered and then evaporated to dryness. The residue was taken up in sodium citrate buffer, pH 2.2. Analyses were made with a Beckman 120 C amino acid analyzer equipped with a system AA computing integrator. Basic amino acids were fractionated on PA-35 resin by using citrate buffer, pH 5.28, while the acidic and neutral amino acids were fractionated on Bio-Rad A-6 resin with citrate buffers of pH 3.28 and 4.25. α-Amino-β-guanidinopropionic acid was used as an internal standard for the basic amino acids, while norleucine was the internal standard for acidic and neutral amino acids. Reproducibility was within 2% of mean values.

Aerial parts of amaranth plants were chopped and boiled with 7 parts w/w of distilled water for 15 min. After being cooled, the mixture was filtered. The residue was dried at 50 °C. Protein and nitrate contents of the filtrate and residue were determined.

RESULTS AND DISCUSSION

Table I shows typical average levels of protein, ascorbic acid, nitrate, and oxalate in *A. retroflexus* harvested during June and July at Lincoln, NE. The leaves contain high levels of protein and ascorbic acid which enhance the food value. The oxalate content of the leafy material is elevated but is well below the values reported by Marshall et al. (1967), and it is not out of line with values reported for other leafy plants such as spinach and chard (Hodgkinson, 1977; der Marderosian et al., 1980). Therefore, only a few samples were checked to determine the range of oxalate concentration. The stems contain the greater amount of nitrate. Nitrate levels in feeds in excess of 1.0% NO_3^-, on a dry matter basis, are considered toxic to ruminants (Buck et al., 1973; Emerick, 1974). No toxic level for nitrate intake has been set for human adults, but concern has been expressed that small children might experience problems when eating canned spinach which might be high in nitrate (Simon, 1966).

Table II compares the compositional analyses for various fractions of mature *A. edulis* plants with the entire aerial portions of *A. retroflexus* plants harvested in Minnesota and Nebraska. No green plants of *A. edulis* were available. It is seen that all varieties of *Amaranthus* tested have high protein and calcium contents. The mineral content compares well with that of other leafy vegetables (Paul and Southgate, 1978). As with most forage crops, the nitrate content is higher in the stem than in the leafy material.

Preparation of a Protein Isolate. Since vegetable amaranths are used as potherbs in most developing countries, the extent to which any crude protein may be extracted by boiling water was investigated. Only a negligible amount of crude protein (about 5% after correcting for nitrate) was extracted (Table III). In this study, the cooking water was poured off and the amaranth was dried without washing. When cooler water was used for extraction, a greater amount of nitrogen was found in the extract. Essentially all of the nitrate was removed in either case. Extracton of protein by cool water suggested that, perhaps, a useful amount of protein could be isolated. Procedures similar to those used for alfalfa (Pirie, 1966; Free and Satterlee, 1975; Edwards et al., 1978; Hood and Brunner, 1975) were investigated by using *Amaranthus* leaves as the starting material. A small quantity of protein could be obtained in this manner. When the entire plant was used as the starting material, only a negligible amount of liquid could be expelled by pressure. A procedure which permitted isolation of a considerable amount of protein from several varieties of amaranth and yielded a final product having a light color, no bitter taste, and no odor

Table III. Protein and Nitrate Extracted from Amaranth Greens by Boiling Water

plant spp.	protein[a] in aerial parts before extraction, % (dry basis)	protein[a] in postboiling residue, % (dry basis)	nitrate in extract, g of NO_3^-/ 100 g of sample (dry basis)	nitrate in residue, g of NO_3^-/ 100 g of sample (dry basis)
A. retroflexus	22.9	22.1	1.59	0.15
A. hybridus	26.9	25.9	2.90	0.27

[a] Corrected protein.

Table IV. Protein Content of Purified Isolates from Aerial Parts of Amaranth

	protein in aerial part of plants, % (dry basis)	protein in purified isolate, %	av recovery of original protein in isolate, %
A. caudatus	23.2	61.0-71.0	24
A. retroflexus[a]	21.2	72.5-75.0	35
A. hybridus[b]	22.4	78.1-79.6	30
A. hybridus[c]	22.1	73.7-75.7	36
A. graecizans[d]	21.3	71.2-71.6	32

[a] Pigweed; redroot. [b] Green stem. [c] Reddish stem. [d] Prostrate amaranth.

is outlined in Figure 1. Protein yields of the products obtained by this method are shown in Table IV. Approximately one-third of the protein in the plant is obtained in the protein isolate. In Table V the amino acid composition of the protein isolate is compared with that of shell corn, soybean meal, and wheat. The protein in the isolates would appear to be of good quality due to a lysine content higher than that of corn and wheat and comparing well with soy protein. However, the availability of the lysine in the isolates was not determined. *A. hybridus* yielded an isolate with the greatest lysine content.

Table VI compares the proximate analysis of the press cake with that of the starting material for the various varieties. These data indicate that this residue has potential for use as livestock feed by itself or in combination with the chloroplast fraction, a green paste which comprises about 6–10% of the original sample on a dry basis and contains about 38% crude protein. The *deproteinized juice* contains soluble carbohydrates and most of the nitrate and soluble oxalate and may be useful as a fertilizer.

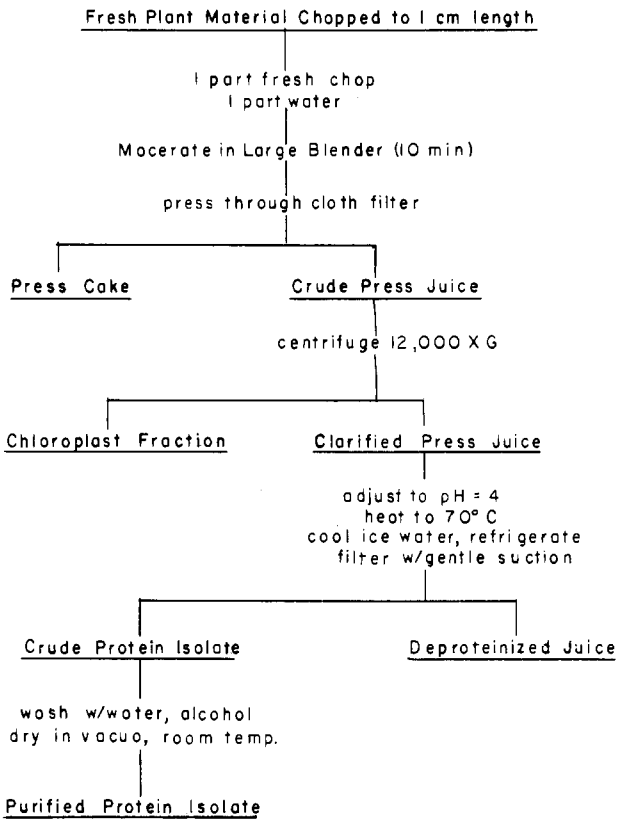

Figure 1. Preparation of protein isolate.

The analytical data presented here indicate that the several varieties of amaranth have a good nutritive potential. As long as they are used as potherbs and the cooking water is discarded, there will be no danger from

Table V. Partial Amino Acid Content of Purified Protein Isolates of Amaranth

	mg of amino acid/g of nitrogen							
	A. retroflexus	*A. hybridus* green	*A. hybridus* red	*A. graecizans*	*A. caudatus*	corn[d]	soybean meal[d] (defatted)	wheat[d]
lysine[c]	391	427	407	412	412	169	320	179
histidine[c]	124	121	118	132	131	170	159	143
arginine[c]	332	341	341	331	329	269	443	288
aspartic acid	452	545	597	452	597	392	719	308
threonine[c]	191	222	246	187	234	225	267	183
serine	199	250	269	197	260	311	347	187
glutamic acid	516	616	667	514	608	1184	1157	1866
proline	200	232	260	193	294	559	351	621
glycine	221	252	284	211	283	231	278	245
alanine	255	291	317	247	301	471	284	308
valine[c]	300	350	384	290	374	303	320	275
isoleucine[c]	303	341	382	284	359	230	302	204
leucine[c]	471	536	603	457	577	783	489	417
tyrosine	169	217	227	167	237	239	237	187
phenylalanine[c]	236	278	301	233	308	305	313	282
cystine		66[a]				100	100	159
methionine[c]	(110)[b]	126[a] (97)[b]	(151)[b]	(107)[b]	(116)[b]	120	80	94

[a] Values from J. R. Brunner (by performic oxidation). [b] For comparison only (HCl hydrolysate). [c] Essential amino acids. [d] FAO (1970); Paul and Southgate (1978).

Table VI. Comparison of Proximate Analysis of Press Cake with the Original Plant Material

	%, dry basis					
	nitrate as NO_3^-	ash	crude protein	cor crude protein[a]	ether extract	crude fiber
A. caudatus: plant	1.71		23.2	20.0		
press cake	n[b]	14.0	17.4		1.21	22.4
A. retroflexus: plant	2.98	22.2	21.2	15.6	1.58	13.1
press cake	n	14.6	11.4		1.14	20.0
A. hybridus (green): plant	1.89	16.5	22.4	18.8	1.33	24.6
press cake	n	18.0	14.3		1.40	27.5
A. hybridus (red): plant	1.98	19.0	22.1	18.4	1.73	16.7
press cake	n	16.1	15.1		1.23	20.9
A. graecizans: plant	2.29	24.3	22.3	18.1	1.65	14.5
press cake	n	23.6	17.4		1.26	22.1

[a] Corrected protein = (Kjeldahl nitrogen − nitrate nitrogen) × 6.25. [b] Negligible (below the limit of detection).

nitrate. If the leaves are consumed as salad greens, in the manner in which salads are consumed in the United States, the danger from nitrate intoxication is minimal because of the relatively small amount of leaves consumed in relation to the total diet. However, if the raw leaves were to comprise a major part of the diet, the nitrate might pose a problem.

SUMMARY

Results of this study confirm previous reports that about 40% of the total oxalate is in the soluble form (der Marderosian et al., 1980). Oxalate, too, could be a problem if large amounts of raw leaves were consumed. However, as in the case of nitrate, soluble oxalate is extracted into the cooking water. The remaining oxalate, which probably is not solubilized in the gut, would be eliminated without appreciable amounts being absorbed.

The protein isolates, obtained from the different varieties of amaranth, appear to be of high nutritive value. The partial amino acid composition shows the presence of quality protein. The press cake contains no nitrate or soluble oxalate. This material should be an excellent feed for ruminants. It would be necessary to dry it to ensure safe storage unless it was mixed with a source of fermentable sugar and ensiled. Any insoluble oxalate, which might be solubilized in the rumen, would be tolerated by ruminants in good condition.

Developing countries in which many persons presently utilize vegetative amaranth as a major part of their diet could utilize such a protein isolate to good advantage for increasing protein intake. If the lysine is found to be available, the protein isolate could supplement the low lysine content of corn in those countries in which corn is a dietary staple. Most important, though, would be the more efficient use of a limited cropping area. The protein isolate could be used as a supplement to the human diet, and the fibrous press cake together with the chloroplast material could be used as a ruminant feed since ruminants could utilize the residual fiber. Thus, the same crop could feed both humans and animals. The combined deproteinized juice and washings, containing all the nitrate and soluble oxalate, might best be used for irrigation in countries with limited water resources. At present it has not been determined whether or not the alcohol washings could be omitted if the color of the protein isolate was of little importance. Previous authors have reported that water washing of leaf protein concentrates appeared to remove a rat growth inhibitor(s) (Cheeke and Bronson, 1980). In the present method, the additional water used may remove any such materials along with nitrate and soluble oxalate. This will be investigated.

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LITERATURE CITED

AACC "Approved Methods"; American Association of Cereal Chemists: St. Paul, MN, 1969 (update Oct 8, 1976).

AOAC "Official Methods of Analysis", 13th ed.; Association of Official Analytical Chemists: Washington, DC, 1980.

Buck, W. B.; Osweiler, G. D.; Van Geldern, G. A. "Clinical and Diagnostic Veterinary Toxicology"; Kendall/Hung: Dubuque, IA, 1973.

Cheeke, P. R.; Bronson, J. "Proceedings of the Second Amaranth Conference"; Rodale Press: Emmaus, PA, 1980; pp 5–11.

Cole, J. N. "Amaranth from the Past for the Future"; Rodale Press: Emmaus, PA, 1979.

der Marderosian, A. H.; Beutler, J.; Pfender, W.; Chambers, J.; Yoder, R.; Weinsteiger, E.; Senft, J. "Proceedings of the Second Amaranth Conference", Rodale Press: Emmaus, PA, 1980; pp 31–41.

Duckworth, R. H. *N.Z. Vet. J.* **1975**, *83*, 154–155.

Edwards, R. H.; deFremery, D.; MacKey, B. E.; Kohler, G. O. *Trans. ASAE* **1978**, *21*, 55–59.

Emerick, R. J. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1974**, *33*, 1183–1187.

FAO "Amino-Acid Content of Foods and Biological Data on Proteins"; Food and Agriculture Organization of the United Nations: Rome, 1970; Nutritional Studies No. 24, pp 38–42.

Fiske, C. H.; Subbarow, Y. *J. Biol. Chem.* **1925**, *66*, 375–400.

Free, B. L.; Satterlee, L. D. *J. Food Sci.* **1975**, *40*, 85–89.

Hall, A. "The Wild Food Trailguide"; Holt, Rinehart and Winston: New York, 1973; pp 82–83.

Hodgkinson, A. "Oxalic Acid in Biology and Medicine"; Academic Press: London, 1977; Chapters 3, 7.

Hood, L. L.; Brunner, J. R. *J. Food Sci.* **1975**, *40*, 1152–1155.

Marshall, V. L.; Buck, W. R.; Bell, C. L. *Am. J. Vet. Res.* **1967**, *28*, 888–889.

Marx, J. L. *Science (Washington, D.C.)* **1977**, *198*, 40.

McPherson, A.; McPherson, S. "Wild Food Plants of Indiana"; Indiana University Press: Bloomington, IN, 1977; p 84.

NAS "Underexploited Tropical Plants With Promising Economic Value"; National Academy of Sciences: Washington, DC, 1975; pp 14–19.

Olatunbosun, D. A. *Indian J. Nutr. Diet.* **1976**, *13*, 168–173.

Osweiler, G. D.; Buck, W. B.; Bicknell, E. J. *Am. J. Vet. Res.* **1969**, *30*, 557–566.

Paul, A. A.; Southgate, D. A. T. "McCance and Widdowson's The Composition of Foods", 4th ed.; Elsevier/North Holland Biomedical Press: New York, 1978; Section 2.
Pirie, N. W. *Science (Washington, D.C.)* **1966**, *152*, 1701–1705.
"Proceedings of the First Amaranth Seminar"; Rodale Press: Emmaus, PA, 1977.
"Proceedings of the Second Amaranth Conference"; Rodale Press: Emmaus, PA, 1980.
Simon, C. *Lancet* **1966**, *No. 7442*, 872.
Valyasevi, A.; Dhanamitta, S. *Am. J. Clin. Nutr.* **1974**, *27*, 877–882.

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Effect of Antioxidants on Stability of Astaxanthin Pigment in Crawfish Waste and Oil Extract

Huei-Mei Chen and Samuel P. Meyers*

A soy oil extraction method has been used to recover the carotenoid astaxanthin from heat-processed crawfish waste, *Procambarus clarkii*. Astaxanthin stability in comminuted crawfish meal and soy oil was determined as a function of time, temperature of storage, and antioxidant treatments by using ethoxyquin and Endox dry powder. Use of the Endox–oil mixture in the crawfish waste at a 0.05% level effectively protects the pigment component from degradation during frozen storage for as long as 16 weeks. Employment of ethoxyquin or Endox (dry powder) greatly inhibits autoxidative degradation of the isolated pigment during storage at 4 °C. The amount of oil-extractable astaxanthin from the waste after storage is affected by storage temperature, pretreatment of the waste via grinding, and its intensity of exposure to light or oxygen.

With increasing efforts in aquaculture and diet development, the value of crustacean meals for dietary formulations for shrimp and prawn species is being recognized. Such meals, in addition to their nutrient value, supply a source of carotenoid, notably that of astaxanthin (Meyers, 1977). The latter is the most prevalent carotenoid among crustacean groups (Lambertsen and Braekkan, 1971; Karrer and Jucker, 1950; Tanaka, 1978; Jangaard, 1975) and has been shown to elicit notable pigmentation in a variety of aquatic species when supplied as a dietary ingredient in the form of crustacean meal.

Among various sources of crustacean wastes, that from the Louisiana crawfish processing industry is especially noteworthy in view of the >10 million pounds of substrate available each year. Astaxanthin/astaxanthin ester and astacene, the oxidative product of astaxanthin, comprise the total carotenoids of heat-processed crawfish waste. A relatively high concentration, i.e., 153 μg/g, of astaxanthin/astaxanthin ester and astacene was reported in crawfish exoskeleton waste by using a polysolvent extraction system (Bligh, 1978; Meyers and Bligh, 1981).

Use of a soybean oil extraction procedure for recovery of astaxanthin from crustacean wastes, including red crab and crawfish, has been demonstrated (Spinelli and Mahnken, 1978; Chen, 1981). The quality of astaxanthin is affected by processing and storage conditions, i.e., heat treatment, length of period exposed to oxygen, and intensity of exposure to light. Our previous reports have documented the biochemical degradation of astaxanthin in dried crawfish meal and the effect of BHA and ethoxyquin (Bligh, 1978; Meyers and Bligh, 1981). Data reported here concern astaxanthin levels in the fresh (undried) comminuted crawfish meal prior to extraction with soybean oil and subsequent stability of the pigment in the oil.

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EXPERIMENTAL SECTION

Sample Collection. Heat-processed crawfish (*Procambarus clarkii*) waste, obtained from Seafood, Inc. (Henderson, LA), included the intact cephalothorax, abdominal exoskeleton, and viscera. Material was placed in double black polyethylene bags and frozen and held at −20 °C until used. The frozen intact waste was ground twice through a Hobart mixer (Model A-200) to a pastelike product, designated here as "comminuted crawfish" or "crawfish meal", prior to pigment analyses and antioxidant treatments.

Pigment Extraction. Soybean oil, ratio 1:1 (w/v), was added to calibrated weights of comminuted crawfish for total carotenoid extraction. This ratio was selected in view of its optimum efficiency in pigment extraction (Chen, 1981). The beaker containing the oil–crawfish blend was wrapped in aluminum foil to exclude light, with subsequent heating at 45–50 °C with continuous stirring, with final heating to 90 °C. A deep-red pigmented oil solution containing the dissolved carotenoids was recovered by centrifugation of the homogenate at 11000*g* for 10 min at 0 °C. A biphasic supernatant was observed, and the hypophase water was drained from epiphase pigmented oil in a separatory funnel. The total volume of the pigmented oil was recorded. The spectral characteristics of the carotenoid oil extract was analyzed spectrophotometrically (Beckman 25 spectrophotometer, Beckman Instruments, Inc.).

Pigment Quantitative Analysis. Astaxanthin and its ester have been identified as the major (90%) carotenoids in the exoskeleton of heat-processed crawfish waste (Bligh, 1978; Meyers and Bligh, 1981). Therefore, the term "astaxanthin" is used here to represent the total carotenoid

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