A Progress Report
to the Minnesota Legislature
and Department of Agriculture

Project Title: A Minnesota Amaranth Feasibility Study

December 1, 1989

Submitted by

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Consultant's Report prepared for the Agriculture Department

Pursuant to 1988 Laws, Chapter 688, Article 21, Section 16

PREFACE

"The potentiality of man to solve problems has not yet been exhausted, and the potentiality of the resources latent in the earth to be brought into the horizon of usefulness is still beyond the power of man to conceive. The key to the situation is not the earth, but the minds of men determined to realize their own potential in act."

by J.C. Malin (1953)

1989 Article from the U.S. National Research Council Report entitled "Lost Crops of the Incas"

This picture appears in the 415 p. book just published and appeared in the Des Moines Register, Agribusiness Section, Sunday, November 5, 1989.

This is the species Amaranthus caudatus which is interfertile with the current U.S. cultivars of the species A. hypochondriacus.

Associated Press

Inca treasures

Luis Sumar Kalinowski holds a sample of an unusual-looking grain called kiwicha, a kind of amaranth, that is grown in Peru. He is one of four members on the government's National Research Council telling the world where to find the lost treasures of the Incas — not gold, but 31 different crops that could enrich the diets of people around the globe.

CAPSULE SUMMARY

1. Grain amaranth can be grown and mechanically harvested in Minnesota.

2. Grain amaranth is as nutritious as oats and can be used to fortify our major cereals and legumes.

3. Grain amaranth is suitable as a sole source of energy and protein for animals.

4. Grain amaranth contains high amounts of dietary fiber, calcium and iron.

5. This project discovered cholesterol-lowering agents in grain amaranth.

6. The cholesterol-lowering agents in amaranth are rare forms of Vitamin E which are capable of naturally regulating serum cholesterol.

7. The kinds of cholesterol-lowering agents in amaranth are being developed by Malaysian palm oil interests as the NEXT MAJOR CARDIOVASCULAR MEDICINE!

8. We have produced extruded amaranth products, including "Rice-Crispie-like" and "Cheerio-like" ones, made with various combinations of soybeans, wheat, sugar beet fiber and amaranth. These products will be nutritionally superior and potentially important for our domestic food supply and cardiovascular health. Extruded, texturized vegetable proteins (with soybean as the main ingredient) are important Asian market niches.

9. Malaysia is forecast to triple its palm oil production in the next decade, a development that will have important market consequences for soybean oil and the Vitamin E forms found in amaranth and other cereals.

10. Cereal markets are currently strong and are expected to remain so in the 1990s. Formulations that contain fiber and cholesterol-lowering properties are and will be important.

11. Montana is leading the nation in developing amaranth varieties and is rapidly developing the capability to dominate domestic production. If Minnesota hopes to gain a share of domestic amaranth production, further varietal development and testing will be needed.

TABLE OF CONTENTS

INSTITUTE FOR THE DEVELOPMENT OF AMARANTH PRODUCTS, INC.
A Minnesota non-profit organization dedicated to
the creation of new products
from an ancient North American crop.
P.O. Box 125, Bricelyn, MN 56014 (507) 653-4372

Friday, December 1, 1989

The Honorable Stephen G. Wenzel
Chair, House Agriculture Committee
House of Representatives
487 State Office Building
St. Paul, MN 55155

The Honorable Charles R. Davis, Chairman
Senate Agriculture Committee
Minnesota Senate
G-24 State Capitol
St. Paul, MN 55155

Mr. Ralph Groschen,
Marketing Director,
Minnesota Department of Agriculture
90 West Plato Boulevard
St. Paul, MN 55107

Dear Sirs:

As project coordinator for the Minnesota amaranth feasibility study, which was authorized by your committees in 1988, I submit to you a progress report on our activities and findings:

1. Grain amaranths (Amaranthus sp.) are an ancient North American crop which can be grown and effectively machine harvested in Minnesota. Tests at the University of Minnesota experiment stations suggest that it can be grown as far North as Grand Rapids. See Table 1. Realized seed yields with 3-10 acre production fields in Faribault and Freeborn Counties were 1000-1200 lbs/acre of clean grain. These were the actual combined yields NOT projected yields! Previous Minnesota yield ranges for early amaranth varieties were from 300 to 3800 lbs/acre during 1977-1982. See also "Growing Grain Amaranth as a Speciality Crop," a Minnesota Extension Service fact sheet (Appendix A) and the 1989 amaranth variety trial report (Appendix B) report, i.e., 1988 data, from the Experiment Station.

Table 1. 1989 seed yield of amaranth lines at Univ. of Minnesota Experiment Stations and farmer-cooperator fields. (Unpublished data from Dr. Dan Putnam, U of MN, 1989).

Line	Rosemont	Albert Lea	Morris	Grand Rapids
	lbs/acre			
A200D	1260	----	----	----
A2248	180	----	----	----
K266	----	1306	----	----
K283	----	1166	----	----
K343	1430	1031	1071	492
K432	----	1570	1171	1051
K433	----	1641	----	----
K436	----	877	----	----
MT-3	1730	1076	----	----
PI 477914	1824	----	----	----
LSD 5%	430	349	641	137

Nationwide Amaranth Production and Crop Improvement Status

The Montana Agricultural Experiment Station is currently testing and releasing amaranth varieties (See Appendices C,D). They suggest that it is possible to select amaranth varieties with early dry-down (to avoid the grain shattering associated with waiting for a killing frost) and with yields as high as 5341 kg/hectare or 4773 lb/acre. This translates to a yield potential of about 80 bushels/acre, assuming that amaranth's test weight is about 60 lbs/bushel. We doubt that these yields will hold up under typical production conditions but they do demonstrate the genetic potential of the crop!

The Montana Agricultural Experiment Station is also conducting a marketing feasibility study, has prepared a extension videotape for producers, and have produced a survey map of Montana soils and areas suitable to amaranth growing.

In 1989, the Crop Improvement Associations of eight states (including Minnesota) and the American Organization of Seed Certification Agencies, are drafting seed certification requirements for amaranth varieties. This work is anticipating the need to prevent cross pollination in seed production fields and to insure a reliable and identifiable seed source for producers. Seed certification would also generate new revenue for the Minnesota seed industry.

2. Grain amaranth was the co-staple crop of corn or maize in old Mexico, the area where corn was originally domesticated from wild weeds. Nutritionally, it is roughly the equal of oats in oil and protein yet it will complement other cereals, including maize, wheat, and sorghum. For instance, blends of 80% wheat and 20% amaranth form a nutritionally COMPLETE PROTEIN for human consumption. Amaranth supplies the usually scarce and vital amino acid called lysine.

3. Grain amaranth varieties are well suited for animal nutrition, too. I have been a junior author to studies at the USDA Meat Animal Center in Nebraska and the USDA Regional Plant Introduction Station in Iowa which show that amaranth is sufficient as a sole source of energy and protein for animal nutrition. See Appendix E. The presence of heat-labile antinutritional factors, which are common in legumes, needs to be more completely understood.

4. Grain amaranth will be marketed as a cereal and as an industrial product. Its FIBER, MINERAL CONTENT, and CHOLESTEROL-LOWERING abilities will greatly influence its acceptance and demand.

a). Total dietary fiber, mostly insoluble, in amaranth is very high (Table 2)

b). Mineral content of amaranth, especially its calcium at levels comparable to milk, could be very useful American diets for young people and individuals who are prone to osteoporosis. However, calcium in cereals is typically "tied up" by substances called phytates. Bioavailability of amaranth calcium in certain food applications is poorly understood and merits further investigation.

Table 2. Comparison of common cereal grain and grain amaranth.

	Iron	Calcium	Protein	Calories	Fiber (Crude)	Total Dietary Fiber
Amaranth	3-22	25-389	13-19%	366	4-14%	16-17%
Corn	2	10	9-13%	352	2-3%	-
Wheat	12	48	12-14%	343	1-2%	7%
Oats	4	50	14-16%	384	2%	6- 9%
Rice	3	10	8%	353	1%	2- 4%
Barley	3	16-34	12%	353	2-3%	6%

Compiled from USDA Agricultural Handbook No. 8, Washington, D.C.; Singhal and Kulkarni (1988); Saunders and Becker (1988); FAO, 1970; Pedersen et al., 1988; American Amaranth Inc. lab reports, 1988.

Iron and Calcium listed as mg/100mg.

Calories are listed as per 100 g.

5. This study resulted in the DISCOVERY of CHOLESTEROL-LOWERING AGENTS in AMARANTH. This discovery was made in cooperation with the USDA Cereals Crop Research Unit in Madison, WI and Dr. Asaf Qureshi, codiscoverers of cholesterol inhibitors in barley (Appendix F). Similar agents are also present in barley, oats, and palm oil. All of these agents are forms of Vitamin E and are therefore natural products which have existed in the food chain for a long time. Simply stated, we have been processing them out of our cereals. Because of their presence in minute quantities, the detection of the rare forms of Vitamin E has been very difficult.

The cholesterol-lowering agents in amaranth are at sufficiently high levels that it may be possible to extract them for treating cardiovascular disease (See Dr. Qureshi's letter to IDAP, Appendix G).

Due the possible economic and medical impact of these discoveries, this feasibility study will include a preliminary survey of amaranth cholesterol inhibitors (rare forms of Vitamin E). Fifty samples, including 1989 varieties grown at our University experiment stations, have been tested as of late November.

MARKET PROSPECTS AND INTELLIGENCE: A major domestic brewer is touting its barley flours for their high Vitamin E relatives or cholesterol-lowering agents. This company is likely planning to concentrate the agents from barley oil and market them as a NEW GENERATION of CARDIOVASCULAR MEDICINES. These agents, which are potent in quantities as low as 100 parts per million, decrease the action of the key cholesterol-producing enzyme (HMG CoA reductase) in the body!

The competition. Internationally, Malaysia produces palm oil in large quantities and markets it to the U.S. The soybean lobby has promoted labeled of palm and coconut oil as "tropical oils" due to undesirably HIGH levels of saturated fats. Ironically, some evidence suggests that palm oil also contains the cholesterol-lowering, Vitamin E forms which may, in fact, counter-balance the negative effects of saturated fats. The Palm Oil Research Institute of Malaysia (PORIM) is rapidly moving toward concentrating and marketing the Vitamin E relatives as a CARDIOVASCULAR MEDICINE, too (Appendix H).

By the year 2000, Malaysia and Indonesia will triple their production of palm oil from 6.3 million metric tons in 1986/87 to nearly 18 million metric tons. Domestic soybean oil production may come under even greater international competition because palm oil can be produced for $215/metric ton while U.S. soybean oil costs are estimated at $390/metric ton. (See Appendix I)

6. Research by Dr. William Breene at the University of Minnesota suggests that amaranth CAN BE EXTRUDED into many new products, either as a blend with current commodities such as Minnesota corn or soybeans or as 100% amaranth products (See Appendix J). Due to lack of genetic improvement or breeding of the crop, formulations with 10-30% amaranth appear more economically reasonable.

a). Key products which need further development for Asian and domestic markets are:

i). TEXTURIZED VEGETABLE PROTEINS with a unique identity.

ii). NEW MULTI-GRAIN CEREAL products including breakfast cereals, pastas, croutons, confections, and snacks.

MARKET INTELLIGENCE AND PROSPECTS

A. There is currently is $6.3 billion market for cereals. High-fiber, cholesterol-lowering amaranth is a future contender for fortifying oat, wheat, soybean and corn-based formulations. Unlike any known popped cereal, it can be to added to cereal or bread products and still retain some of its cholesterol-lowering agents. However, like other forms of Vitamin E, severe processing can reduce their presence to negligible amounts.

B. As a superior source of protein and possibly minerals, amaranth would be a valuable addition to our major staple crops. If amaranth bran could be processed to fully release its calcium (in the same manner as wheat is currently processed), this calcium could be help growing children and people prone to osteoporosis.

C. It may be counter-productive for commodity groups to consider new uses for their crops alone. Blending of grains and legumes to produce new and unique products could provide additional market niches. The current oat bran craze emphasizes how powerfully the addition of oat bran to other cereals improves their nutrition and selling power. In a parallel development, psyllium gum (or Metamucil, *Plantago ovata*) is being imported from India to provide the latest cholesterol-lowering effect to boost Kellogg's profit line and market share (See Wall Street journal article on psyllium, Appendix K). Kellogg's advertising budget for its psyllium promotion is $40 million! The use of amaranth bran (with increased cholesterol inhibitors) should produce a similar cholesterol-lowering effect plus allow Minnesota farm production of the grain.

7. Amaranth has minute (1-3 micron) starch particles. Starch chemists have sought to mill or process other starches such as corn starch (average size = 10 microns) to such tiny granules for BIODEGRADABLE PLASTICS. A Purdue University starch-plastics team suggests that "the smaller the starch granules, the better would be the dispersion and as such, should improve properties relative to current starch-plastic." See letter from Purdue starch chemist in Appendix IX.

Amaranth starch has been suggested as a replacement for talc in aerosols and cosmetics. This tiny granule starch has also been suggested for freezing-rethawing applications, such as reheatable and microwavable gravies and sauces for restaurant and home microwave applications.

8. Other facets of this feasibility study and related IDAP research include:

A. Scientific literature reviews and analyses of these topics: proteins, lipids/oils, antinutritional factors, and pigments.

B. Preliminary studies on rapid cycling of grain amaranth: a plant breeding technique to decrease the time needed to complete a generation. Current evidence suggests that five generations per year are feasible although there appear to be genes in wild amaranths for even faster flowering.

C. Identification of "protein fingerprints" or Plant Variety Protection standards for grain amaranths. The use of reversed-phase, high performance liquid chromatography (RP-HPLC) to identify and patent genotypes is being cooperatively developed by IDAP, Inc. and the Plant Protein Unit, USDA Northern Regional Research Labatory, Peoria, IL.

D. An amaranth processing feasibility study is being conducted in cooperation with American Amaranth, Inc. Three areas have been identified as critical to developing processing: assessment of existing processing capabilities and technologies, potential future needs, and capital requirements for a pilot amaranth plant. (Appendix L).

E. Limited variety evaluation trials and preliminary forage evaluation is being conducted by Dr. Dan Putnam at the University of Minnesota (Appendix M).

F. IDAP, Inc. has contracted Dr. Gordon Rose, Professor Emeritus of Economics (retired), to investigate the production economics of grain amaranth in Minnesota. He is developing computerized enterprise budgets and surveying growers to develop production scenarios based on experience and not projections.

G. The University of Minnesota's Center for Alternative Crop and Animal Products is planning to host the 4TH NATIONAL SYMPOSIUM/CONFERENCE ON AMARANTHS in 1990. IDAP and the Amaranth Institute (a 120 member, national non-profit organization) are co-hosting the conference.

RECOMMENDATIONS

1. Develop and release AMARANTH varieties for Minnesota farmers. Secure funding of a part- or full-time USDA-ARS researcher for amaranth breeding and improvement or provide a state-sponsored position.

2. Continue development and marketing of TEXTURIZED VEGETABLE PROTEIN products for the Asian market, estimated to be 4500 metric tons for Korea alone. (They like their instant noodles and dumplings!) This would allow greater consumption of soybeans, the major ingredient in TVP products. Continue development of "RICE-CRISPIE-" and "CHEERIO-LIKE" cereal/amaranth products using extrusion technology.

3. Test the effectiveness of AMARANTH's cholesterol-lowering properties relative to oats, barley, and palm oil in animal and human studies. Both the Mayo Clinic and the University of Minnesota Health Center could be involved. Texas A & M University has already released evidence of such effects in test animals.

4. Develop the technology to process amaranth in Minnesota. Once amaranth is milled into starch and protein/oil fractions, further oil extraction would yield the cholesterol inhibitors and another minor byproduct called squalene (a valuable cosmetics ingredient which the Japanese now manufacture and monopolize). The Japanese began commercial extraction of squalene from whale and shark liver oil in 1987. Nippon Petrochemicals built a 1000 metric ton squalene extraction plant valued at 300 million yen.

5. Apply seed coating technologies to amaranth to assure stand establishment. (Because amaranth is so similar to a tiny vegetable seed, the horticultural, seed coating technologies to insure vegetable seed emergence should be applied.) Conduct further research on amaranth response to fertility in crop rotations, ridge tillage, and low-input agriculture.

This is a progress report on the feasibility study. Our final report to the Minnesota Department of Agriculture will be submitted by approximately February 1, 1989. As former curator of the United States' collection of amaranth at Regional Plant Introduction Station at Ames, Iowa during the last four years, I was amazed and continued to be astounded by our lack of knowledge concerning future crop plants.

Best wishes to you in the holiday season.

Sincerely,

J. W. Lehman

Dr. James W. Lehmann
Project Coordinator
Minnesota Amaranth Feasibility Project

MINNESOTA EXTENSION SERVICE

UNIVERSITY OF MINNESOTA

Center for Alternative Crops & Products

AG-FS-3458
1988

GROWING GRAIN AMARANTH AS A SPECIALTY CROP

Robert L. Myers and Daniel H. Putnam[1]

Amaranth, an ancient crop originating in the Americas, can be used as a high-protein grain or as a leafy ve\getable, and has potential as a forage crop. Grain amaranth species have been important in different parts of the world and at different times during the past few thousand years. The largest acreage ever grown was during the height of the Aztec civilization in Mexico during the 1400's. During the past two centuries grain amaranth has been grown in scattered locations, including Mexico, Central America, India, Nepal, China, and eastern Africa. Research on amaranth by U.S. agronomists began in the 1970's, so optimum production guidelines and uniform, adapted varieties have not yet been fully developed. Only a few thousand acres of amaranth are commercially grown in the United States, and markets for that small acreage are fragile. Growers are advised to begin with a few acres, and to have a contract or identify buyers before planting the crop.

Plant Description

The two species of grain amaranth commonly grown in the U.S. are *Amaranthus cruentus* and *Amaranthus hypochondriacus*. Grain amaranths are related to redroot pigweed *(Amaranthus retroflexus)*, but are different species with different characteristics. The grain amaranths have large, colorful seed heads and can produce over 1000 pounds of grain per acre in Minnesota, though a portion of this grain yield may be lost in harvesting.

Grain amaranth plants are about five to seven feet tall when mature, and are dicots (broad-leaf) plants with thick, tough stems similar to sunflower. The tiny, lens-shaped seeds are one twenty-fifth of an inch in diameter and usually pale-colored, while the seeds of the pigweed are dark-colored and lighter in weight.

The crop is reportedly drought-tolerant, similar to sorghum, provided there is sufficient moisture during the early growing period. Frost damage is not a problem because the crop is not sown until late May or early June. However, frost plays an important role in the harvest of the crop. Since amaranth is an annual crop native to the southern latitudes of North America, it does not mature completely in Minnesota's short growing season. A frost is necessary to terminate the crop's growth so that the plant material will be dry enough to harvest.

Varieties and Seed Source

Uniform varieties of grain amaranth have not yet been developed. Available material consists of selected lines that vary in their uniformity and degree of adaption to temperate latitudes. The Rodale Research Center in Pennsylvania and the USDA Plant Introduction Station at Ames, Iowa, are two locations where significant work has been done in developing amaranth varieties. A small number of commercial breeders are also working with the crop. While the Ames research group is not yet releasing its lines, Rodale has been distributing a number of lines, including some that have been grown successfully in Minnesota, R1041, R1011, K343, K283, and K266. University trials at Rosemount from 1977 to 1982 showed yields ranging from 300 to 3800 lbs/a for the 20 lines tested. Amaranth seed is also available commercially (see Table 1), but at prices as high as $25 per pound.

Table 1. Sources of grain amaranth seed.

Johnny's Selected Seeds, Albion, ME 04910
Mellinger's, Inc., 2310 W. South Range Road, North Lima, OH 44452
Plants of the Southwest, 1812 Second St., Sante Fe, NM 87501
Rodale Research Center, RD 1 Box 323, Kutztown, PA 19530
The Good Seed Company, P.O. Box 702-D4, Tonasket, WA 98855

Production

Planting

Planting should be done when the soil temperature is at least 65°F., and after early weed growth has been controlled by tillage or a contact herbicide. Seedlings are fragile, so it is important to have a fine, firm seedbed. Seedbed preparation can be done with a disk or spiketooth harrow, followed by cultipacking and planting, preferably using a planter with press wheels. Seeds should be planted no more than 1/2 inch deep, depending on soil texture and surface moisture at planting time. Crusting can be a serious problem, and although no solutions have been researched, rotary hoeing may be helpful. Poor emergence, as low as 50%, is not uncommon. Since seeds are shallow planted, there is potential for them to wash out on sloping ground.

An optimum plant population has not been established, but one-half to two pounds of seed per acre is considered suitable (approximately 600,000 seeds per pound). Row spacing should be based on the cultivator equipment available. A number of planter types have been used successfully to deal with the small seeds of amaranth. Approaches that have proven successful include: using a vegetable planter with a small plate appropriate for carrots or celery; installing special amaranth seed plates in a sugar beet planter; using the insecticide application box as a planter; or using a standard grain drill. Grain drills are not recommended due to problems in controlling seeding rate and depth, but one can be used if the amaranth seeds are diluted with a "carrier" like ground corn. A mixture suitable for drilling consists of one-half pound of amaranth with four and one-half pounds of ground corn. Set the drill for a seeding rate of five pounds per acre.

Fertilization

There is currently little data available on the fertilizer response of amaranth, although it appears to be intermediate between small grains and corn in fertility needs. Nitrogen will be the most limiting nutrient under most environments. Phosphorous and potassium should only be applied in soils that are especially deficient in these nutrients.

1. Graduate research assistant and assistant professor, respectively, Department of Agronomy and Plant Genetics, St. Paul, MN.

Weed Control

Since amaranth is not planted until early June, many weeds will already have emerged. These early weeds must be controlled by tillage or a contact herbicide prior to planting. Grain amaranths grow slowly during the first several weeks, so three or four cultivations may be needed during this period to control weeds (**no selective herbicides are labeled for use with amaranth**). Once the amaranth plant is about a foot tall it begins to grow rapidly and is competitive with weeds. Two species of weeds which are especially competitive with amaranth in Minnesota are lambsquarter and redroot pigweed. Fields with high populations of these weeds should not be used for grain amaranth production. Since grain amaranth seeds do not undergo dormancy, and since plant growth is not vigorous early in the season, it is unlikely that amaranth would be a weed problem in succeeding crops.

Insects and Diseases

Tarnished plant bug, flea beetle, and amaranth weevil, have been identified as potentially significant insect pests of amaranth. The insect most likely to affect yields is the tarnished plant bug, a sucking insect which often reaches high populations in the seed head during the critical seed fill stage. Flea beetles damage young leaf tissue. The adult amaranth weevil feeds on leaves, but the larval stage is more damaging because they bore into the central tissue of roots and occasionally stems, causing rotting and potentially lodging. No significant disease problems have been conclusively identified for grain amaranth. One possible problem is a damping-off fungus, which can kill seedlings.

Harvest

Management during harvest is a most critical stage in grain amaranth production. Without careful harvest techniques it is possible to lose the majority of the seed. Before harvesting can begin a killing frost must occur followed by a week of good drying weather (there are no approved desiccants for amaranth). If the stems and leaves are too wet, the seeds become sticky and adhere to the inside of the combine as well as the straw discharge. Shattering during the cutting process can also cause losses, so adjustments should be made to minimize shattering of the heads. When reel heads are used it may be helpful to remove several reel bats or raise the height of the reel. Row headers perform better at harvesting amaranth than reel heads for combining amaranth. A regular combine can be used if fitted with appropriately-sized separator screens.

Handling and Storage

Plans should be developed for handling and storage of the grain before harvest begins. It is important to clean the grain to remove plant and foreign material which will increase the chance of molding. Cleaning can be done using a 1/16 inch screen top, and a 1/23 inch screen, 22 x 22, or 24 x 24 wire mesh on the bottom. A gravity table can be used to separate out particles of the same size but of different weight, such as the dark pigweed seeds. Maximum moisture for storing the grain is approximately 11%. Small amounts of grain can be dried by blowing air across the amaranth; heated air may be necessary at certain times. The optimum way to store the grain after cleaning and drying is in wooden storage bins or in heavy duty (4 or 5 ply) paper bags. University studies at Rosemount, Minnesota showed average test weight of 63 pounds per bushel.

Potential Uses

Food Uses

Grain amaranth has been used for food by humans in a number of ways. The most common usage is to grind the grain into a flour for use in breads, noodles, pancakes, cereals, granola, cookies, or other flour-based products. The grain can be popped like popcorn or flaked like oatmeal. More than 40 products containing amaranth are currently on the market.

Nutritional Value

One of the reasons there has been recent interest in amaranth is because of it useful nutritional qualities. The grain has 12 to 17% protein, and is high in lysine, an amino acid other grain crops are low in. The grain is high in fiber and low in saturated fats, factors which contribute to its use by the health food market.

Forage Uses

Little is known about the production and utilization of amaranth as a forage. The leaves, stem and head are reportedly high in protein (15-24% on a dry matter basis). A relative of grain amaranth, redroot pigweed, has been shown to have 24% crude protein and 79% *in vitro* digestible dry matter. Vegetable amaranths, which are closely related, produced 30 to 60 tons/a of silage (80% moisture) on plots in Iowa. In areas where corn silage yields are low due to moisture limitations, grain amaranth may become a suitable silage alternative after further research.

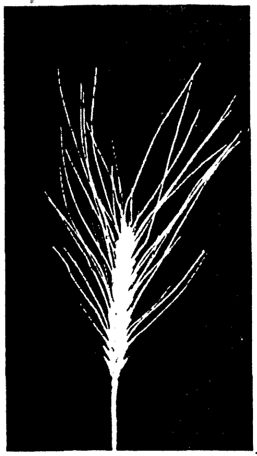
Processing and Marketing

Perhaps the greatest problem facing the development of amaranth as a crop is finding markets for the grain. The crop has only been grown commercially during the 1980's, and the markets are still very small. A farmer entering the market with grain from several hundred acres of amaranth could cause a surplus and drastically lower prices. For this reason amaranth should be grown only after identifying a market for the crop, and preferably after arranging a contract with a buyer.

Farmers that grow amaranth have marketed their crop in a number of ways. Some sell small bags of the whole grain or flour mail-order to consumers. Many of these purchasers are allergic to wheat products. Other growers sell to local or regional health food stores or restaurants. There are also a few middlemen who buy grain from the farmers and market it to the larger health food companies. Health food companies that have developed grain amaranth products include Health Valley Natural Foods, Arrow Mills, Walnut Acres, and Nu-World Amaranth.

For More Information ...

Additional information on producing grain amaranth is available in Minnesota Experiment Station Bulletin AD-SB-2949, "Amaranth, Quinoa, Ragi, Tef, and Niger: Tiny Seeds of Ancient History and Modern Interest" (1986), and the "Amaranth Grain Production Guide" published annually by the Rodale Research Center (RD 1, Box 323, Kutztown, PA, 19530).



Wheat

Grain Crops

AMARANTH

Amaranth is a grain crop developed by the Aztec and Mayan civilizations. It is currently grown in China and India, and on both the North and South American continents. And, it is drawing increased interest in Minnesota.

Amaranth has large seed heads ranging in color from yellow and green to brown, red and maroon. Plants range in height from three to nine feet, and produce very small, lens-shaped seeds. It is a drought-tolerant crop and grows best in warm, dry weather. It is widely adapted to many locations in the Midwest.

The crop is planted in late May or early June. Cultivation of wide rows is required in the absence of approved herbicides. Seed yields of 300 to 3800 pounds per acre have been reported in Minnesota. A killing frost followed by a week of drying weather is required before harvest can be accomplished by combine. Amaranth is combine-harvested, as a high-protein grain crop for human consumption.

Table 3. Seed yield of amaranth lines.

Line	Rosemount	Waseca	Lamberton	Rosemount
	1987	1987	1987	1988
	lbs/A			
K266	2024	787	708	—
K283	804	477	311	—
K343	1707	910	541	—
K432	—	—	—	1247
K433	—	—	—	895
Mt-3	—	—	—	1023
PI477914	1262	611	280	815
LSD 5%	268	247	155	297

One of the biggest constraints to successfully growing grain amaranth is in finding markets for the grain. Amaranth should not be grown without first identifying a market and then establishing a contract with a buyer for the grain. It is used in various flour-based products, or can be popped like popcorn or flaked like oatmeal. More than 40 products containing amaranth are currently on the market.

Additional information and seed sources are provided in the "Amaranth Grain Production Guide" available from the American Amaranth Institute, Box 216, Bricelyn, MN 56097. A fact sheet, "Growing Grain Amaranth as a Specialty Crop" (item no. AG-FS-3458), is available from the Center for Alternative Crops and Products, 305 Alderman Hall, University of Minnesota, St. Paul, MN 55108.

Table 4. Characteristics of amaranth lines.

Line	100% Bloom				Lodging				Height			Seed Wt.	Test Wt.
	Rosemount 1987	Lamberton 1987	Rosemount 1988	Aug	Rosemount 1987	Waseca 1987	Rosemount 1988	Aug	Lamberton 1987	Rosemount 1988	Avg	Rosemount 1988	Rosemount 1988
	days				score[1]				inches			g/1,000	lbs/bu
K266	73	76	76	75	3.4	4.6	5.7	4.6	60	66	63	.466	54.5
K283	65	67	72	68	1.8	2.3	2.0	2.3	56	62	53	.605	59.0
K343	82	78	74	78	2.3	2.4	4.0	2.9	61	70	66	.657	59.5
K432	—	—	75	75	—	—	2.7	2.7	—	52	52	.530	58.5
K433	—	—	74	74	—	—	1.5	1.5	—	53	53	.526	59.0
Mt-3	—	—	74	74	—	—	3.0	3.0	—	73	73	.606	55.5
PI477914	77	73	74	75	4.1	4.3	4.0	4.1	60	76	68	.525	57.0

[1] 1-9, 1 = Erect, 9 = Horizontal

ANNUAL CANARYGRASS

Annual canarygrass is grown as a cash grain crop. It is used for feeding caged and wild birds. Kittson County is the North American production and processing center for the crop.

RECOMMENDED VARIETIES

Alden—Medium yield, medium maturity and height. Poor lodging resistance. Medium size seed of medium test weight. May outyield Keet in favorable environments for canarygrass. Developed cooperatively by Minnesota Agricultural Experiment Station and Minn-Dak Growers Association. Released in 1973.

Elias—High yield, medium maturity and height. Fair lodging resistance. Medium size seed of very high test weight. Released by Minnesota Agricultural Experiment Station in 1983.

Keet—High yield, early, medium height. Fair lodging resistance. Medium size seed of high test weight. Released by Minnesota Agricultural Experiment Station in 1979.

REGISTRATION OF MONTANA-3 GRAIN AMARANTH GERMPLASM

MONTANA-3 (MT-3) (Reg. no. GP-1) (PI 515959) NSSL 230601 grain amaranth (*Amaranthus cruentus* L.) germplasm was released in March 1988, by the Montana Agricultural Experiment Station for breeding and experimental purposes.

MT-3 grain amaranth is a selection from RRC-1041 developed by the New Crops Department of the Rodale Research Center (RRC) at Kutztown, PA, from a single plant selection RRC-78S-1015 (2). Kauffman and Reider (3) in-

dicated that RRC-1015 originated from a single plant selection, RRC-A-369, and is a Mexican grain type of *A. cruentus* L., that has a main central panicle, usually with thick, erect to drooping, finger-like branches. The main stalk is relatively thin, especially at high planting densities. It has varying degrees of side branching at low densities. The seed color is white, the flower color is light green. RRC-1015 yielded 1880 kg ha^{-1} grain in 1978 and 781 kg ha^{-1} grain in 1979 in RRC studies (2).

Kauffman (2) described RRC-1041 as a white seeded cultivar with an exceptional yield potential in an unfavorable year. However, he mentioned that in 1979 there was a severe problem with black-seeded segregates. This problem still is not completely solved in MT-3. Kauffman also recommended reselection for uniformity of RRC-1041. Grain yields of RRC-1041 at Rodal were 1252 kg ha^{-1} in 1979 and 1236 kg ha^{-1} in 1980. The grain yield of RRC-1041 in 1982, in a preliminary nonreplicated field trial at Huntley, MT [911 m elevation, 1779 average accumulative maize growing degree days (1) from June 1 to September 15] was 1879 kg ha^{-1} (4). The plants produced in 1982 were not completely true to type. They had red and marbled flower color segregates and plant height differences. During the 1985 to 1987 growing seasons we selected for uniform height and light-green flower type by roguing before anthesis, and for large panicles by mass selection. In 1987, in a replicated grain yield trial at Huntley, MT, the mean yields of MT-3, RRC-K283, RRC-K266, and RRC-K343 were 5341, 3034, 2714, and 2483 kg ha^{-1}, respectively; MT-3 exceeded ($P < 0.05$) the yields of the three RRC cultivars. This trial received 90 kg N ha^{-1} and 34 kg P_2O_5 ha^{-1}, which were incorporated prior to planting. Residual soil nitrate was 112 kg NO_3-N ha^{-1}. Sprinkle irrigation of 3 cm after planting was followed by three flood irrigation applications about 3 wk apart of 10 cm each.

The Montana Cooperative Extension Service has distributed small seed samples of MT-3 to farmers and the seed trade for testing in different environments within the state.

Small seed samples of MT-3 are available on request and will be provided upon agreement to make appropriate recognition of their source a matter of record when the germplasm contributes to the development of a new cultivar. Seed can be requested from Jurgen R. Schaeffer, Department of Plant and Soil Science, Montana State University, Bozeman, MT 59717-0002.

JURGEN SCHULZ-SCHAEFFER,* GILBERT F. STALLKNECHT, DONALD E. BALDRIDGE, AND RONALD A. LARSON (5).

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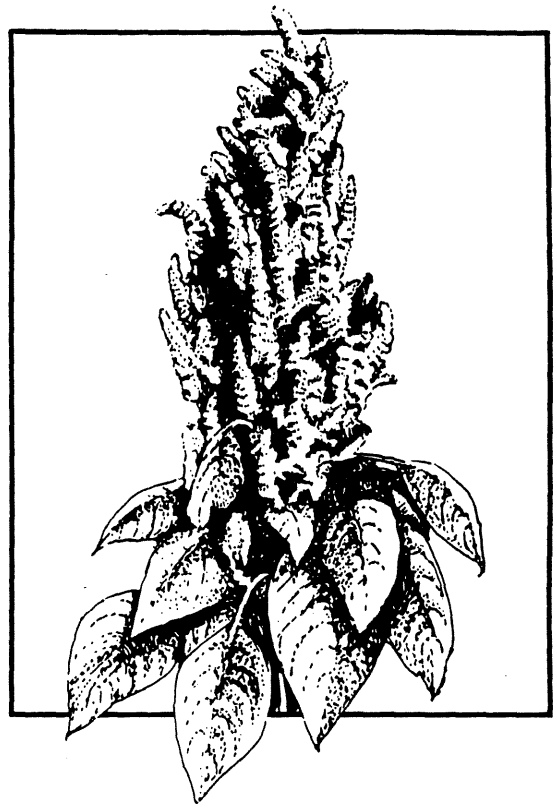
Grain Amaranth: A Way to Diversify Your Farming Enterprise

by Jurgen Schulz-Schaeffer, Professor of Agronomy and Genetics; Donald E. Baldridge, Professor of Extension Agronomy; Gilbert F. Stallknecht, Associate Professor of Agronomy; and Ronald A. Larson, Research Associate.

Grain amaranth is a specialty crop that requires special planting, growing, harvesting and marketing consideration. It is utilized as a specialty product due to its unique amino acid composition and high protein content.

Grain amaranth originated in Central and South America and is one of a few broadleaved, non-grass plants that produce significant amounts of edible grain. These plants are sometimes called pseudo-cereals to distinguish them from the grain-producing grasses like wheat, rice and maize. The type which seems best adapted to Montana, *Amaranthus cruentus,* is native to Mexico and Guatemala. Many agronomic plants are classified into either C_3 or C_4 plants based on their biochemical mechanism to convert CO_2 into the organic compounds which are used for plant growth. Grain amaranth is classified as a C_4 plant, similarly to corn, sorghum, and millet. C_4 plants utilize CO_2 more efficiently for plant growth. Grain amaranth, requires less than half the moisture needed to produce a crop of barley or wheat, C_3 plants. While variety development is in its infancy, a newly developed germplasm, Montana-3 (MT-3), has a yield potential under irrigation of 4,800 lbs/acre. MT-3 yields are higher than other germplasms presently available. On dryland, grain amaranth is capable of yielding 200 to 1,300 lbs/acre.



Planting

The planting date for sweet corn in your area can be used to determine the optimal amaranth planting date which is approximately one week after sweet corn planting. Amaranth requires a fine seedbed which must be firmed to provide the extremely small seed with good soil contact to provide moisture. The seed requires a soil temperature of 65° to 75° F for maximum speed of germination. If given optimal conditions of temperature and soil moisture, seed germination will occur in four to six days. Soil crusting can be a problem to plant establishment due to the small seed size (1,000 seeds weigh less than 1 gram). A seeding rate of 1 to 2 lbs/acre is recommended for stand establishment under general cropping practices. Amaranth can be planted with a variety of planters. Sugar beet or corn planters like the John Deere 71 Flexi-planter[1] have been adapted with a specialized vegetable crop celery plate. Growers in Montana have successfully used the John Deere LZ[1] hoe drill. On a standard grain drill box the drill speed should be decreased with the reducer sprocket. While amaranth seeds should ideally be planted ¼ inch deep, plant depths of close to ½ inch (which does delay and lower seedling emergence) may be more realistic for adequate seed and soil contact. Equipping the drill with packer wheels will improve the soil and seed contact. Seeding depth control can be achieved by equipping the planter with depth bands. If the amaranth crop is to be grown under irrigation, it is recommended that the crop be irrigated immediately after planting. If crusting occurs, a second irrigation could be applied. Soil and moisture conditions are critical for seed germination and plant establishment. Once the plant has emerged and commenced growth, the moisture requirements become less stringent.

Weed Control

Cultivation several weeks in advance of planting may promote germination of summer annual weeds which then can be controlled by cultivation immediately before planting. Also, application of a non-specific herbicide such as Roundup[1] or Gramaxon[1] can be applied to control weeds which have emerged at planting time. A single cultivation when the plants have grown 1 to 1½ feet is usually sufficient to control weeds for the rest of the growing season. Plant growth is generally quite rapid and the crop canopy over the rows inhibits weed growth. Hand removal of weeds in extremely weedy fields may be necessary since weed seeds in the harvested crop can be a significant problem. Pigweed is a close relative of grain amaranth and easily hybridizes with it. Pigweed plants in the vicinity of a grain amaranth field should be eliminated before flowering time.

Diseases and Insects

Diseases of economic importance are presently not a problem in grain amaranth. Poorly drained soils can lead to damping-off and stalk-rot caused by *Fusarium* and *Bacterium*. Farmers should check plants on a weekly basis, and if disease symptoms are noticed, plant samples should be sent to the Extension Plant Pathologist at Montana State University for identification.

Insects which can cause serious damage to grain amaranth are the tarnished plant bug [*Lygus lineolarius* (Palisot de Beauvois)] and the amaranth weevil [*Contrachelus seniculus* Lec.]. The tarnished plant bugs feed on the succulent plant and floral parts and on the immature grain, resulting in lower yields and grain quality. The amaranth weevil larvae bore into the roots and stems of the plants increasing susceptibility to diseases and lodging. European corn borer larvae [*Ostrinia nubilalis* (Hubner)] damage has been observed in the floral parts of some amaranth lines. The injured flowering parts dry up resulting in loss of seed production. Spray programs for insect control, if required, should be planned with your farm chemical dealer and in accordance with the pesticide label.

Fertilization and Irrigation

Applications of 80 lbs N/acre and 30 lbs P_2O_5/acre to soil containing 100 lbs/acre residual soil nitrate resulted in high yields at Huntley, Montana, in 1987. Rates of higher than 120 lbs N/acre are not considered economical. Irrigation increases production but the water requirement for amaranth is only 42-47% that required for wheat. Three flood irrigation applications spaced about 3 weeks apart and application of 2 to 3 inches each time resulted in good yields in Montana.

Harvest and Cleaning

In Montana, grain amaranth plants mature in mid-September. Amaranth stems and leaves remain high in moisture content until they are partially killed by frost and dry up. Lines with advanced genetic dry-down have been developed in Montana and this trait is presently being incorporated into breeding material. Advanced dry-down is a desirable characteristic in grain amaranth. In cereal grains like wheat, maize, and oats, the entire plant dries down at the same time the seedhead matures. In contrast to cereal grains, the stems and leaves of the amaranth plant often exhibit lush, green growth at the time of seed maturity. Thus, these plant parts are still high in moisture at seed harvest, which can result in much of the seed adhering to the moist plant residue during combining. These seeds are thus lost in the harvest process. The lack of advanced dry-down at maturity limits amaranth production with presently available cultivars to areas with killing frost, unless swathing with subsequent combining is possible.

Grain cleaning can be achieved by using either a clover or alfalfa screen for seed separation. However, the quality of seed cleaning will depend on the moisture content of the plant at harvest time. Generally the combine will be operated at a slower ground speed as compared to cereal grain harvest. The cylinder clearance and speed and fan adjustments must be specifically set for optimum threshing success. Excess chaff and plant residues will not only interfere with grain drying but can also increase the possibilities of grain molds and insect attraction which can impart undesirable flavors in the flour. Cleaning of the small-seeded amaranth grain must be done carefully so that loss of grain will be kept to a minimum. Dirt particles can be removed with an alfalfa scarifier or alfalfa rubber clay rollers.

Grain Drying and Storage

Amaranth grain must be dried to 10-12% moisture for prolonged storage and good product shelf life. The Rodale Research Center, Kutztown, Pennsylvania, suggests that amaranth should not be grown unless provisions can be made for drying the grain. Grain dryers must be modified to dry the small amaranth seed. A five-mesh nylon sheet placed over the perforated floor of the dryer has been used successfully to dry the amaranth grain. Dry storage conditions are required to maintain a good quality grade of grain.

Marketing

Marketing of grain amaranth in the United States is in its early development stages. The main consumer of grain amaranth is the human specialty food industry, but in spite of unfavorable price levels of grain amaranth for livestock feed, large broiler farms are inquiring about possible suppliers. The American Amaranth Institute, Bricelyn, Minnesota, is presently in the process of developing guidelines and standards for this new crop.

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[1] *Product names are used in this report solely to provide specific information. Mention of product names does not constitute a guarantee of the product by the Montana State University Extension Service, nor does it imply an endorsement over comparable products that are not named.*

FILE UNDER: FIELD CROPS
D-3 (Alternate Crop) MT8808 AG
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Appendix E

NUTRITION REPORTS INTERNATIONAL

UTILIZATION OF FOUR CULTIVARS OF GRAIN AMARANTH FOR GROWTH IN RATS

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Abstract

Growing Sprague-Dawley male rats were used in two experiments to determine the nutritional adequacy of four cultivars of grain amaranth for growth. The amaranth cultivars (16.8, 16.0, 16.4 and 16.1% protein) were fed as the sole source of protein and energy or diluted with maize to provide 46.1% amaranth in the diet. All cultivars were fed in the ground, unheated form. A diet containing maize as the sole source of protein (10.3%) and energy was used as a control and a 16% protein maize-soybean meal diet was fed as a reference diet. Rats were fed _ad libitum_ for 14 days in individual wire-bottom cages in a light- and temperature-controlled room. In each experiment (5 rats fed each of 10 diets in Exp. 1 and 10 rats fed each of 6 diets in Exp. 2), body weight gain, feed intake and gain to feed ratio were recorded for each rat. Three cultivars (_Amaranthus hypochondriacus_ 1024, _A. hypochondriacus_ 1046 and _A. hypochondriacus_ K188) produced weight gain and feed utilization significantly greater ($P<0.01$) than maize; two cultivars (_A. hypochondriacus_ 1046 and K 188) produced weight gain not significantly different from that obtained with the maize-soy reference diet, although daily feed intake was greater with the reference diet. One cultivar (_A. cruentus_ 1011) allowed normal growth during week 1, followed by steady weight loss and decreased feed intake during week 2. Refeeding for 7 days of half the rats fed 1011 with maize-soy reference diet resulted in rapid weight recovery and absence of gross pathology of liver, kidney, stomach, spleen, adrenal, and testes at slaughter. The nature of the toxic factor present in _A. cruentus_ 1011 is unknown, but ingestion seems not associated with permanent organ damage after 2 weeks of feeding. We conclude that three of the four amaranth cultivars tested promote growth of rats superior to that obtained with maize and comparable to that obtained with a 16% maize-soy diet.

INTRODUCTION

Several species of the genus _Amaranthus_ have been used as a human food in many areas of the world for centuries (1), but little effort has been made to characterize their nutritional qualities until recently (2,3,4,5,6,7). Germplasm collections of grain amaranth exist at Rodale Organic Gardening and Farming Research Center, Kutztown, Pennsylvania, and at the USDA Plant Introduction Station, Ames, Iowa.

The purpose of the present work was to test the nutritional adequacy of four cultivars of grain amaranth produced at the USDA Plant Introduction Station for the growth of laboratory rats fed the ground unheated seed of each cultivar as the sole or partial source of protein and energy in the diet.

MATERIALS AND METHODS

Animals and Housing

Growing male Sprague-Dawley rats were used in two 14-day experiments. Animals were caged individually in wire-floored stainless steel cages and fed the experimental diets *ad libitum* from porcelain jars equipped with lids and screens to minimize feed wastage. Drinking water from the central water line was provided by an individual nipple waterer attached to each cage. The environmentally controlled room was kept at 22 ± 2°C and illuminated with fluorescent lights on a 12 hr light and 12 hr dark cycle. Body weight of each rat was recorded at 0, 7 and 14 days, and feed consumption was recorded during the 14-day experimental period in each experiment. Daily weight gain, feed consumption and gain to feed ratio were recorded for each rat.

The composition of the experimental diets is shown in Table I.

Table I. Composition of Diets

Ingredient	Reference	Maize Control	Amaranth[a]	Maize-Amaranth[a]
Maize	75.1	96.1		50.0
Amaranth[a]			96.1	46.1
Soybean meal	21.0			
Vitamin premix[b]	0.2	0.2	0.2	0.2
Choline chloride	0.2	0.2	0.2	0.2
Trace element premix[c]	0.2	0.2	0.2	0.2
Salt (iodized)	0.4	0.4	0.4	0.4
Dicalcium phosphate	2.4	2.4	2.4	2.4
Limestone	0.5	0.5	0.5	0.5
Total, %	100.0	100.0	100.0	100.0
Dry matter, %	94.2	93.2	92.9-93.5[d]	93.0-93.4[e]
Crude protein, %	16.3	10.3	16.1-16.8[f]	12.5-12.7[g]

[a] Four cultivars were tested: Amaranth 1011, 1024, 1046 and K188; see text for taxonomic details.
[b] Supplied the following vitamins (units/kg of diet): vitamin A, 5,280 IU; vitamin D_3, 704 IU; 2-ambo-alpha tocopheryl acetate 35.2 mg; menadione sodium bisulfate 3.52 mg; vitamin B_{12} 26.4 mcg; riboflavin 5.28 mg; niacin 28.16 mg; d-pantothenic acid 21.12 mg; biotin 88 mcg; thiamine 2.2 mg.
[c] Supplied the following minerals elements (mg/kg of diet): Cu, 10 (as $CuSO_4 \cdot 5H_2O$); Fe, 160 (as $FeSO_4 \cdot 7H_2O$); Mn, 20 (as MnO); Zn, 100 (as ZnO); $CaCO_3$ used as carrier.
[d] Values for diets containing Amaranth cultivars 1011, 1024, 1046 and K188 were 93.5, 93.0, 93.4 and 92.9%, respectively.
[e] Values for diets containing maize plus Amaranth cultivars 1011, 1024, 1046 and K188 were 93.0, 93.2, 93.4 and 93.4%, respectively.
[f] Values for diets containing Amaranth cultivars 1011, 1024, 1046 and K188 were 16.8, 16.0, 16.4 and 16.1%, respectively.
[g] Values for diets containing maize plus Amaranth cultivars 1011, 1024, 1046 and K188 were 12.7, 12.6, 12.7 and 12.5%, respectively.

Four amaranth cultivars were tested as the sole source of protein and energy or diluted with maize to provide 46.1% of the diet. Diets diluted with maize were included in experiment 1 to test the possibility that the amino acid mixture created by a blend of maize and amaranth would be adequate to support growth equal to that produced by a maize-soybean meal mixture (reference diet). All cultivars were fed ground and uncooked. A diet containing maize as the sole source of protein and energy was used as a control and a 16% protein maize-soybean meal diet was used as a reference diet. The four amaranth cultivars were:

(1) *Amaranthus cruentus* (1011), a standard Mexican grain type.
(2) *Amaranthus hypochondriacus* (1024 or R103), a golden seeded grain type grown for flour in Kansas.
(3) *Amaranthus hypochondriacus* (1046), a Nepalese grain type.
(4) *Amaranthus hypochondriacus* (K188), an experimental grain type grown by the Committee for Agricultural Development, Iowa State University.

The feeding period of eight rats fed *A. cruentus* 1011 in Exp. 2 was extended an additional 7 days beyond the 14-day feeding period. Four rats were continued on the *A. cruentus* 1011 diet, whereas four were fed the maize-soybean meal reference diet for 7 days. Final body weight and weights of liver, heart, stomach, kidneys, adrenals, testes, and spleen were recorded for each rat at slaughter.

Statistical Analysis

The data were subjected to general linear models least-squares means analysis of variance (8) adjusted by covariance to constant initial weight in a split-plot design with diet and time (week) as main effects. Diet, time and diet x time interaction were tested. Individual animal was the experimental unit.

RESULTS AND DISCUSSION

Body weight curves of rats used in Exp. 1 and 2 are shown in Figures 1 and 2, respectively. Daily weight gain, feed intake and gain to feed ratio are summarized in Table II for Exp. 1 and in Table III for Exp. 2. Body weight curves (Figures 1 and 2) of rats fed *A. hypochondriacus* 1046 and K188 were similar to that of rats fed the maize-soy reference diet in both experiments, whereas the body weight curve of rats fed *A. hypochondriacus* 1024 was significantly less ($P<0.01$), although rats fed all three of these cultivars as the sole source of protein and energy had higher ($P<0.01$) body weight gain than rats fed maize. Body weight of rats fed *A. cruentus* 1011 increased during the first week and then declined to day 14 in both experiments, suggesting the presence of a toxic factor in this cultivar. *A. cruentus* 1011 is a standard Mexican cultivar used as a human food, and we are aware of no published evidence of toxicity. Bressani *et al*. (5) obtained satisfactory growth of rats fed *A. cruentus* 1011 that had been

Table II. Effect of Amaranth Cultivar on Daily Weight Gain, Feed Consumption and Gain-to-Feed Ratio of Growing Rats, Exp. 1[a]

Diet	No. of rats	Daily gain, g	Daily gain, g	Gain-to-feed ratio
Reference	5	6.1^{b}	19.8^{b}	.311^{b}
Maize control	5	1.7^{c}	18.2^{c}	.098^{c}
Amaranth 1011	5	2.6^{d}	13.2^{d}	.198^{d}
Amaranth 1024	5	5.2^{e}	16.2^{e}	.322^{b}
Amaranth 1046	5	5.8^{bf}	17.4^{ce}	.334^{b}
Amaranth K188	5	5.6^{b}	16.6^{e}	.336^{b}
Maize-A. 1011	5	3.5^{g}	16.2^{e}	.216^{d}
Maize-A. 1024	5	4.4^{h}	18.3^{c}	.244^{e}
Maize-A. 1046	5	5.0^{e}	18.9^{bc}	.266^{e}
Maize-A. K188	5	3.9^{h}	17.8^{ce}	.219^{d}
Standard deviation		.6	1.5	.031
Probability		Diet<.01, Time<.05, DietxTime<.01	Diet,Time, DietxTime<.01	Diet,Time, DietxTime <.01

[a] Initial body weight 86 grams, 14 day experiment.
[bcdefgh] Means in the same column without a common superscript differ.

soaked in water for 20 min and then passed through a double-drum drier at 2 rev·min^{-1} using steam at 4.2 kg cm^{-2} pressure to heat the drums. Garcia et al. (9) reported greater food intake, weight gain and protein efficiency ratio in rats fed heated compared with unheated treated samples of three species of Amaranthus (*A. caudatus*, *A. cruentus* and *A. hypochondriacus*). It seems possible that a heat labile factor may be present in *A. cruentus* 1011 which, under normal food preparation for humans, would be destroyed on cooking. Koeppe et al. (6) reported the presence of trypsin inhibitor in *A. hypochondriacus*; extrusion reduced inhibitor activity. The presence of nitrates, oxalates, saponins, phenolics, trypsin inhibitors and other toxic compounds has been cited (6,10,11,12) as a potential deterrent to the acceptability of grain amaranth as a food and feed resource. However, the body weight loss of rats fed *A. cruentus* 1011 was reversed when animals were transferred for 7 days to the reference diet (Table IV), and no gross pathological signs were noted in liver, heart, stomach, kidney, adrenal, testes or spleen when animals were necropsied following euthanasia after 7 days of refeeding on the reference diet after the original 14-day feeding of *A. cruentus* 1011. Feed intake of rats fed the reference diet was greater in both experiments than that of rats fed maize or any of the amaranth diets. This reduced feed consumption resulted in a tendency for an increase in gain to feed ratio in rats fed *A. hypochondriacus* 1046 and K188 compared with maize-soy-fed rats, but the difference did not reach statistical significance (P>0.05).

Replacement of the amaranth-containing diets with 50% maize in Exp. 1 (Table II) resulted in lower (P<0.01) daily weight gain and gain to feed ratio in all comparisons except for *A. cruentus* 1011, in which weight gain and gain to feed ratio were improved (P<0.01), presumably

Table III. Effect of Amaranth Cultivar on Daily Weight Gain, Feed Consumption and Gain to Feed Ratio of Growing Rats, Exp. 2[a] (Ten Rats Per Diet)

Trait	Maize-soy Reference	Maize Control	Amaranth cultivar 1011	1024	1046	K188	SD	Probability
Daily gain, g	6.6^{b}	1.6^{c}	3.4^{d}	5.4^{e}	6.3^{b}	5.8^{b}	.3	<0.01
Daily feed, g	20.1^{b}	17.4^{c}	12.2^{d}	17.3^{c}	18.1^{c}	17.6^{c}	2.5	<0.01
Gain to feed ratio	.341^{b}	.106^{c}	.137^{c}	.327^{b}	.366^{b}	.351^{b}	.026	<0.01

[a] Initial body weight 75.7 grams, 14-day experiment.
[bcde] Means without a common superscript differ.

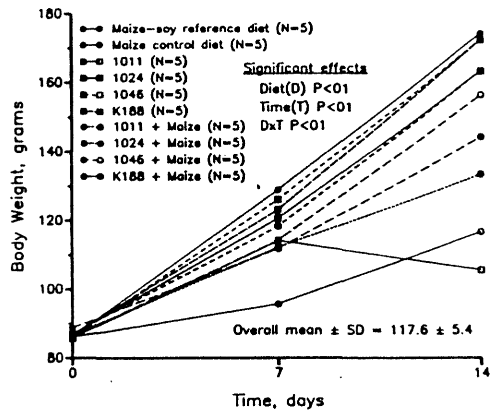

Figure 1. Body weight curves of growing rats fed maize or four cultivars of _Amaranthus_ sp alone or diluted with maize.

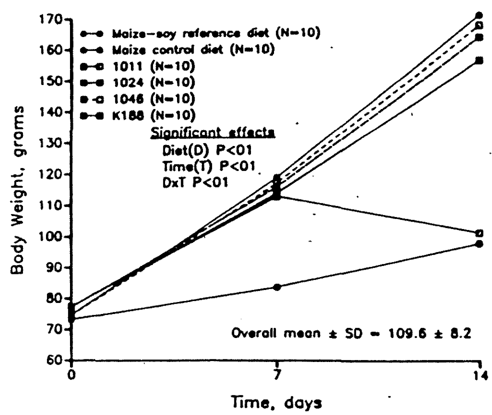

Figure 2. Body weight curves of growing rats fed maize or four cultivars of _Amaranthus_ sp

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Table IV. Effect of Transfer to an Adequate Diet on Subsequent Weight Gain, Feed Consumption and Organ Weights of Rats Fed Amaranthus, _A. cruentus_ 1011

	Rats continued on 1011 for 7 days beyond 14-day Expt.	Rats transferred to refererence diet for 7 days beyond 14-day Expt.	SD	Prob.
No. of rats	4	4		
Initial wt, g	105	99	9.4	NS
Final wt, g	95	170	8.6	<0.01
Daily gain, g	-1.5	10.1	1.3	<0.01
Daily feed, g	6.9	22.1	2.2	<0.01
Gain to feed ratio	-.262	.460	.210	<0.01
Organ weights				
Liver, g	4.56	8.96	.80	<0.01
Liver, % of body wt	4.81	5.27	.36	NS
Heart, g	.49	.73	.05	<0.01
Heart, % of body wt	.52	.43	.05	<0.05
Stomach, g	1.13	1.56	.26	<0.05
Stomach, % of body wt	1.20	.91	.17	<0.05
Kidney, g	.99	1.30	.18	<0.05
Kidney, % of body wt	1.05	.76	.10	<0.01
Adrenal, mg	35.5	28.3	5.2	NS
Adrenal, % of body wt	.037	.017	.004	<0.01
Testes, g	1.26	1.99	.13	<0.01
Testes, % of body wt	1.34	1.17	.12	NS
Spleen, g	.17	.50	.05	<0.01
Spleen, % of body wt	.17	.30	.04	<0.01

due to the dilution of whatever toxic factor may be present in this cultivar.

The high protein content of all four cultivars of Amaranthus (16.0 to 16.8%) places this feed resource in a protein range similar to that of some wheat varieties, but the superior amino acid balance of amaranth (1,13,14) relative to maize and the cereal grains offers the possibility of providing the entire protein and energy requirements of growing animals from a single feed resource. The lysine content of _A. hypochondriacus_ and _A. cruentus_ has been reported (13,14) to be 5.5 and 5.1 g/16 g of N, respectively, a value two to three times that present in cereal grains. This attribute has important implications for on-farm use in developed and developing countries for livestock or direct use after harvest as the sole or major staple food in human diets without the need for protein supplementation.

Preliminary data (Pond, W. G., unpublished) provide evidence that _A. hypochondriacus_ 1046 is able to support reproduction of rats fed amaranth as the sole dietary source of protein and energy. Females fed _A. cruentus_ 1011 lost weight during the final trimester of gestation and produced smaller litters whose individual pups were smaller at birth compared with litters from dams fed A. hypochondriacus 1046 or the reference diet. Long term studies are needed with food animal species fed grain amaranth cultivars, including those used in these experiments, to ascertain their value in life-cycle feeding and to identify and characterize the factor(s) present in A. cruentus 1011 and other cultivars found to contain factors that interfere with normal growth or reproduction.

ACKNOWLEDGMENTS

The authors thank Bruce Larsen and associates for diet mixing and for animal care and feeding; Jeff Waechter and associates for feed analysis; Lei-Hwa Yen for technical assistance and statistical analysis of the data; and Sherry Hansen for stenographic work.

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The Structure of an Inhibitor of Cholesterol Biosynthesis Isolated from Barley*

(Received for publication, March 12, 1986)

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Purification of the oily, nonpolar fraction of high protein barley (*Hordeum vulgare* L.) flour by high pressure liquid chromatography yielded 10 major components, two (I, II) of which were potent inhibitors of cholesterogenesis *in vivo* and *in vitro*. The addition of purified inhibitor I (2.5–20 ppm) to chick diets significantly decreased hepatic cholesterogenesis and serum total and low density lipoprotein cholesterol and concomitantly increased lipogenic activity. The high resolution mass spectrometric analysis and measurement of different peaks of inhibitor I gave a molecular ion at *m/e* 424 ($C_{29}H_{44}O_2$) and main peaks at *m/e* 205, 203, and 165 corresponding to $C_{13}H_{17}O_2$, $C_{13}H_{15}O_2$, and $C_{10}H_{13}O_2$ moieties, respectively, which are characteristic of *d*-α-tocotrienol. This identification was confirmed against synthetic samples. The tocotrienols are widely distributed in the plant kingdom and differ from tocopherols (vitamin E) only in three double bonds in the isoprenoid chain which appear to be essential for the inhibition of cholesterogenesis.

It is well established that 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA)[1] reductase (EC 1.1.1.34) i the first rate-limiting enzyme in the biosynthetic pathways for cholesterol and other isoprenoids (1). There is strong evidence that cholesterol and mevalonic acid (or post-mevalonate, nonsterol products) independently exert feedback regulation on mevalonate biosynthesis (1).

Populations that consume diets rich in cereal grains tend to have a low incidence of cardiovascular disease (2–4). Our studies show that of the cereal grains, barley is the most effective in lowering blood cholesterol levels of experimental animals (5–10). β-D-Glucans, the principal fiber component of barley endosperm might influence cholesterol excretion (11). However, a major cholesterol-suppressive action of barley is at the level of HMG-CoA reductase (5–10). High protein barley flour (HPBF), the commercial pearling fraction consisting of the aleurone, subaleurone, and germ, is the richest source of the cholesterol-suppressive factors (12, 13). Sequential extraction of HPBF with petroleum ether, ethyl acetate, methanol, and water remove all cholesterol-suppressive activity from HPBF; each of the solvent fractions contains HMG-CoA reductase-suppressive material (14). In this report, we describe the isolation of two cholesterol-suppressive metabolites, cholesterol inhibitors I and II, from the petroleum ether-soluble fraction (PESF) of HPBF. The determination of the structure of cholesterol inhibitor I and the results of studies *in vivo* and *in vitro* confirming its cholesterol-suppressive action are reported.

EXPERIMENTAL PROCEDURES

Chemicals—Sources of chemicals, substrates, labeled substrates, enzymes, and diagnostic kits were identified previously (12–16). Chemicals and solvents were of analytical grade. HPBF was contributed by the Minnesota Grain Pearling Co., East Grand Forks, MN, and *d*-α-tocotrienol by Dr. Shiro Urano, Tokyo Metropolitan Institute of Gerontology, Japan (17). The PESF of HPBF was prepared as previously described (14); 100 g of HPBF yields 3.5 g of PESF-HPBF.

Response of Chick and Rat Hepatocytes to PESF-HPBF—Hepatocytes were isolated from livers of fasted (48 h) refed (72 h), 8-week-old white Leghorn pullets and 6-week-old Sprague Dawley male rats (15, 16). The hepatocytes (30 mg of protein) suspended in Krebs-Henseleit buffer (pH 6.8) were incubated with PESF-HPBF (0–10 mg) and 10 μg of Tween 80 in a volume of 1 ml. After 15 min incubation at 37 (rat) or 42 °C (chicken), the incubation mixture was centrifuged at 5000 × *g* for 2 min at 4 °C. The sedimented cells were suspended in 0.4 ml of homogenizing buffer (0.1 M potassium phosphate, pH 7.4, 4 mM $MgCl_2$, 1 mM EDTA, and 2 mM dithiothreitol) and homogenized with a Polytron homogenizer. Following preliminary centrifugation, the 100,000 × *g* supernatants (cytosolic fraction) and precipitates (microsomal fraction) were held at −20 °C prior to assay for enzymatic activities. Protein concentrations were determined by a modification of the biuret method using bovine serum albumin as the standard (18).

Fractionation of Cholesterol-suppressive Factors by HPLC—The PESF-HPBF was fractionated by semipreparative HPLC. A 50-μl aliquot of PESF-HPBF (20 mg of PESF-HPBF in methanol: petroleum ether, 2:1) was eluted through a C_{18} reverse phase column (30 cm × 10 mm inner diameter, 10 μm particle size) with HPLC grade methanol at a flow rate of 1 ml/min. Ten fractions (Fig. 1, detecting wavelength, 205 nm) were collected; pooled fractions were taken to dryness under nitrogen at 5 °C; the dried fractions and Tween 80 were dissolved in sufficient Krebs-Henseleit buffer to provide 2 mg of HPLC fraction and 10 μg of Tween 80/ml. These

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‡ To whom reprint requests should be sent.

[1] The abbreviations used are: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HPBF, high protein barley flour; PESF, petroleum ether-soluble fraction; HPLC, high pressure liquid chromatography; LDL, low density lipoprotein; HDL, high density lipoprotein.

18 Wood Circle
Madison, WI 53705
(608) 262-4481

August 29, 1989

Dr. James W. Lehmann
IDAP, Inc.
P.O. Box 125
Bricelyn, MN 56014

Dear Dr. Lehmann:

Please forgive me for my tardiness in replying to your letter. Enclosed please find the statement on the presence of tocotrienols in amaranth seeds. I am also enclosing some of my work on the effect of tocotrienols in different animal models and human subjects.

I am very much interested in carrying out analysis of tocotrienols and tocopherols for any number of samples. A reasonable charge in my opinion would be about $15/sample. I have recently established an HPLC unit which is especially used for the analyses of tocotrienols and tocopherols from different varieties of oats and barley.

If you have further questions please feel free to call me at (608) 262-4481.

With regards.

Sincerely,

Asaf A. Qureshi

Asaf A. Qureshi, Ph.D.

AAQ:dks

Enclosure

received
9-1

TOCOTRIENOL AND TOCOPHEROLS OF AMARANTH SEEDS

We have recently reported the isolation of α-tocotrienol as a cholesterol inhibitor from barley and oats. Apart from α-tocotrienol barley, oats, wheat and rice are very rich sources of α-tocopherol as well as γ and δ-tocotrienols. The total concentration of these tocotrienols varies from 35 ppm-65 ppm in these cereals. Wheat contains mainly 85 ppm α-tocopherol, barley and oats consist of α and γ-tocotrienols. Rice contain τ-tocotrienols as the major component. All these tocotrienols and tocopherols are found in the aleurone or sub-aleurone layers of the endosperm of the seed and most of the time these are associated in the bran fraction of the seeds removed during processing. The petroleum ether soluble fraction of these brans (oily component of the seed) yields 700-1100 ppm tocotrienols and tocopherols in the crude oils.

Our most recent studies in genetically hypercholesterolemic swine, quails and human subjects indicates that apart from α-tocotrienol the remaining other tocotrienols (except β) are very potent cholesterol inhibitors for lowering not only the total serum cholesterol level but specifically lowers the LDL-cholesterol levels in animal models and human subjects.

Recently, a great deal of interest has been generated in rice brans oil due to its high content of γ-tocotrienol. Our recent high performance liquid chromatography of the petroleum ether soluble fraction of Amaranth seeds indicates very high concentration of not only α- and β-tocopherols (69 ppm), but also shows a very high content of β- and γ- (50-64 ppm) and δ-tocotrienols (182 ppm). In this respect, these seeds are unique due to its very high content of δ-tocotrienol and it might be a very useful commercially viable source of γ-tocotrienol, which can be used for the control of cardiovascular disease in humans. The present data on the tocotrienol and tocopherol levels were obtained by using only 2 varieties of amaranth seeds. These findings strongly warrant carrying out a systematic and complete analysis of tocopherols and tocotrienols in different varieties of amaranth seeds.

Palm Carotenoids, Tocopherols and Tocotrienols

In the following article, Barrie Tan of the Department of Chemistry, University of Massachusetts, Amherst, Massachusetts, identifies important minor components of palm oil, discusses their chemistry and relationship to vitamin A and E activities, and examines their commercial potential.

Oils from plant origin can be divided into seed oils and fruit oils. Although the number of oilseed sources is large, there are only two fruits for which oil production is of economic importance: oil palm (*Elaeis guineensis*) and olive (*Olea europaea, ssp. europaea*). Most of the olive harvest takes place in the Mediterranean region. Oil palms, meanwhile, are concentrated in southeast Asia.

The oil palm is a monoecious plant. The palm yields fruit for about 25 years, but has a life expectancy of at least 100 years. Each mature palm tree is capable of bearing about one bunch, containing 1,000–3,000 palm fruits, per month. The average weight of each bunch varies between 20–30 kg. The fig-like, ovoid-shaped fruit averages 2–5 cm in length and weighs 5–20 g.

Botanically, each fruit consists of a single seed, the kernel (containing the hard shell endocarp and the creamy endosperm within) and the succulent fibrous mesocarp. Palm oil is obtained from the mesocarp and palm kernel oil is from the endosperm. The predominant fatty acids in palm oil are palmitic, oleic, stearic and linoleic. Those of palm kernel oil are lauric, myristic, oleic and palmitic acids. The relative triglyceride composition, expressed in various permutations of saturated (S) and unsaturated (U) fatty acids, is SSS 6%, SSU 48%, SUU 43% and UUU 3%.

The fact that palm products possess relatively high levels of carotenoids and vitamin E is not new (1). However, the potential of extracting these natural substances has increased with the recent jump in palm oil production in southeast Asia.

PALM PLANTINGS

Malaysia and Indonesia have approximately 1.5 and 0.4 million hectares of palm plantations, respectively. Palm oil is approximately 12 to 15 times higher in terms of productivity (expressed in kilograms per hectare per year) than many seed oils (palm oil, 3,900 kilograms; sunflower, 589 kilograms; rapeseed, 409 kilograms; soybean oil, 319 kilograms; corn oil, 254 kilograms). However, oil palm trees only grow in the tropics (10 degrees latitude above and below the Equator).

The major palm-producing nations are Malaysia, Indonesia, Thailand, Nigeria, Ivory Coast, Colombia, Venezuela and Papua New Guinea. On the other hand, soybean crops are grown in such temperate regions as the U.S., Argentina and China, as well as tropic and subtropic regions in Brazil, India and parts of Indonesia (2). Unlike oilseeds, to get a high yield, oil palm can be commercially grown only on the global equatorial belt.

Malaysia produces 4.5 to 5.0 million metric tons (MT) of palm oil annually, mostly in west Malaysia. West and east Malaysia produced 4.12 million MT (90.7%) and 0.42 million MT (9.3%), respectively, in 1986/1987 (3). Indonesia produced 1.3 million MT in 1986/1987, mostly in western Kalimantan and northern Sumatra (3,4).

PRODUCTION CONCENTRATION

Oil palm trees were introduced to southeast Asia in the late 1800s, but serious commercial planting did not begin until the late 1950s. Today Malaysia processes more than 98% of its crude palm oil domestically and exports 90% of its processed oil. In the processing step, the carotenoids are completely bleached by acid-activated clay filters and vitamin E is concentrated in the palm fatty acid distillate (PFAD) fractions.

Because palm oil is derived from a perennial crop, a reliable supply is available throughout the year as opposed to most vegetable oils, which are from annual seasonal crops. As a result, the minor components may be recovered from palm oil raw materials continuously and in a relatively small geographical area.

Malaysia and Indonesia are continuing to enlarge palm oil production. Malaysia is the largest exporter and second largest producer of edible oils; Indonesia is the seventh largest exporter and sixth largest producer of edible oils. It is estimated that by the year 2000, global palm oil production will reach 18 million MT annually (5), with Malaysia accounting for ten million MT (6). There are indications that palm oil production will continue to be concentrated in southeast Asia.

PRODUCTION AND LABOR COSTS

It has been reported that the production of palm oil in southeast Asia has a clear cost advantage over that of seed oil in the U.S. and Europe (7). For example, the cost of production per MT is $750 for rapeseed oil in West Germany and $390 for soybean oil in the U.S.; the costs per MT for palm oil are $215 in Malaysia and $180 in Indonesia.

Labor cost per day for palm oil is higher for Malaysia ($4.40) and lowest for Indonesia ($1.50) (8). Thailand and the Ivory Coast maintain intermediate rates of $2.20 and $2.50, respectively. Such production and labor cost differentials, compared with the U.S. and the European Economic Community (EEC) countries, provide a clear impetus to consider the extraction of minor components of palm.

HEALTH-RELATED APPLICATIONS

It has been established that tocopherol components have antioxidant properties (9) for food applications and carotenoids can inhibit light-initiated flavor deterioration in soybean oil without affecting color quality (10). More recently, medically related findings have been made concerning palm oil's minor components. For instance, it has been reported that palm oil has antithrombotic effects in rats (11). Whether these properties are derived from the "major" oleic acid (40% of the oil) and linoleic acid (10% of the oil) content and/or from the "minor" tocopherols and tocotrienols has not been proven.

The normal blood plasma level of vitamin E (>80% as α-tocopherol, 10% as γ-tocopherol and 2% as β-tocopherol) is 0.7–1.6 mg/dl; less than 0.4 mg/dl is considered a deficiency. The daily requirement of α-tocopherol is 10–15 mg. In mammalian tissues, β-carotene is converted to retinal by the enzyme β-carotene 15,15'-dioxygenase (EC 1.13.11.21). Vitamin A activity comes from preformed retinol (75%) and carotene-derived (25%) sources. The normal blood plasma level of vitamin A is 40–80 μg/dl; less than 15 μg/dl is considered a deficiency. The normal plasma value of carotene is usually more than 50 μg/dl. The daily adult requirement of vitamin A is 1.5–1.8 mg, approximately tenfold lower than the recommended allowance for vitamin E.

When carotenoid-rich foods are estimated from those containing preformed vitamin A, it is the former that are associated with protective and anticancer effects (12,13). Epidemiologically, investigators strongly associate β-carotene with the prevention of lung cancer (14).

Nigerian children receiving red palm oil were reported to be in better health than those who did not (15,16). But the mechanism that connects palm oil and "better health" is unknown. Plasma from a healthy English subject showed a very different carotenoid profile to that of a healthy Nigerian subject (17). The large xanthophyll content from the English plasma in contrast to the larger carotene content from the Nigerian plasma is probably a reflection of a red palm oil diet in the latter. Detailed research on palm oil's minor components is needed but these possible health-related implications may stimulate interest in these nonsaponifiable components.

CAROTENOIDS

The name "carotene" is derived from carrot root (*Daucus carota*), which was isolated as a colored pigment in 1831. Interestingly, this pigment is related to the discovery of the technique of "chromatography" in 1906 by Russian botanist Michael Tswett. He separated carotenoid pigments from leaves on a chalk column into separate bands, and hence the words "chroma" for "color" and "graphy" meaning "writing."

Most of the approximately 500 known carotenoids are naturally occurring, typically pigmented (e.g., yellow-orange-red hues), and some 50 of these pigments possess vitamin A activity to varying degrees. Chemically, carotenoids are conjugated hydrocarbons that may be further classified as carotenes (without an oxygen molecule) and xanthophylls (with one or more oxygen molecules). The polyene backbone of the pigment is characteristically connected to any two of the seven 9-carbon recognized end-groups. A semi-systematic nomenclature includes two Greek letters indicating the structures of the two end-groups. Therefore the semi-systematic nomenclature of common names β-carotene and α-carotene are β,β-carotene and β,ε-carotene, respectively. In palm oil, only β, ε and ψ end-groups have been identified. An illustrative list of 40-carbon carotenoid structures (up to 8 isoprene units) is shown in Figure 1.

Palm oil carotenoids were analyzed by nonaqueous reverse-phase HPLC and UV-VIS diode array detection. Isocratic elution using a ternary solvent mixture (60% acetonitrile, 35% methanol and 5% methylene chloride) at 2 ml/min on a 25 cm C-18 column resulted in an analysis time of 22 minutes (18). Carotenes predominate in palm oil with minor amounts of xanthophylls, the latter mostly mono- and di-epoxy α- and β-carotene. β- and α-carotene are the dominant components found in a two-to-one ratio. Depending on the age and origin of the oil, an average of 550 ppm of α- and β-carotene alone in crude palm oil may be expected. Higher amounts of carotenoids in palm oil have been reported in the literature. Caution must be exercised to translate "total carotenes" obtained spectrophotometrically at a wavelength of about 450 nm into 'β-carotene.' False high values for β-carotene have been rigorously established from single wavelength spectrophotometric measurements compared with measurements that followed HPLC separation (19). Figure 1 shows some exemplary palm oil carotenoids, but a more complete list may be obtained elsewhere (18,20).

Isograms (i.e., wavelength vs. absorbance) or spectrochromatograms (i.e., wavelength vs. retention time vs. absorbance) can be generated using the HPLC diode array detector. An isogram of palm oil carotenoids is shown in Figure 2, which identifies the major β- and α-carotene as well as traces of ε-carotene and *cis*-β-carotene. Carrots and tomatoes are known to have high sources of carotenoids. Similar isogram plots of carrot oil carotenoids showed intermediate amounts of phytoene, phytofluene and ζ-carotene in addition to the major β- and α-carotene (21). In the case of tomato paste, the major carotenoid components were lycopene and phytoene with intermediate amounts of β-carotene and phytofluene (22). The following characteristics of palm oil carotenoids can be made from these comparisons:

- Palm oil contains the highest known concentration of agro-derived carotenoids.
- The preponderant palm oil pigments are β- and α-carotene. The major carotenoids in palm oil are the same as in carrot oil, but the latter contains at least three intermediate amounts of other carotenoids.
- Fewer *cis* isomers of carotenoids are identified in palm oil. Generally, *cis* carotenoids have decreased or no vitamin A activity.
- Palm oil has the highest carotene-derived vitamin A activity. This is primarily due to β-carotene and, secondarily, to α-carotene. For example, red palm oil

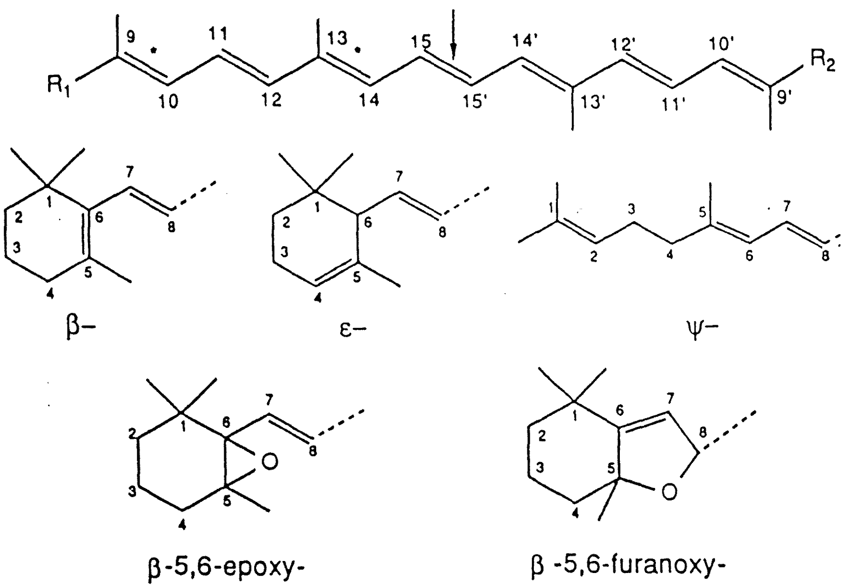


FIG. 1. Representative list of palm oil carotenoid structures [e.g., α-(β,ε), β-(β,β), ε-(ε,ε) carotene, phytoene (Ψ,Ψ; 3 conjugated double bonds), lycopene(Ψ,Ψ) and β-zeacarotene (β,Ψ)]. R_1 and R_2 contain one of the three (β, ε and Ψ) side groups. In β-carotene, cleavage to vitamin A occurs at 15,15′ (arrow) and *cis*-isomer usually occurs at position 9 or 13 (asterisks). Epoxy and furanoxy functions on the dominant β-ionone moiety are also found (18).

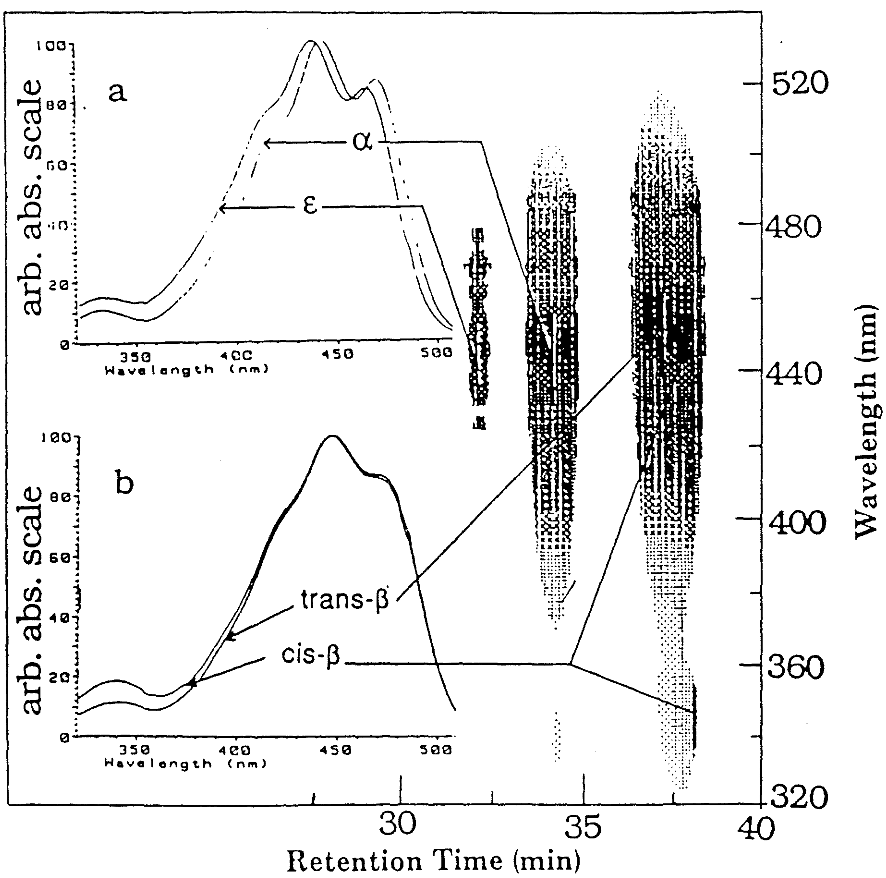


FIG. 2. HPLC isogram (wavelength vs absorbance) of major palm oil carotenoids was obtained using a UV-VIS diode-array detector. Spectral inserts include a) α-carotene (422, 444, 470 nm) and ε-carotene (418, 438, 466 nm), and b) all-*trans*-β-carotene (426, 449, 472 nm) and *cis*-β-carotene (possibly 9-mono*cis* isomer; 424, 446, 469 nm).

has 15-fold more vitamin A activity than carrots and 300-fold more than tomatoes (21).

TOCOPHEROLS AND TOCOTRIENOLS

Vitamin E was first reported in the early 1920s; at that time, it was associated with sterility and reproduction. The Greek meaning for "tocopherol" is "to bear offspring." The other vitamin E homolog—tocotrienol—was discovered in 1955. These compounds are fat-soluble and light yellow in color.

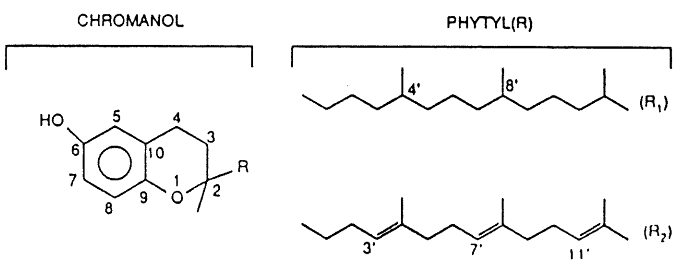
Chemically, tocopherols and tocotrienols are methyl-substituted chromanols with a three-isoprene moiety side chain (Fig. 3). The structures of these two homologs are readily distinguishable. Tocopherols have a saturated side chain with chiral carbons at positions 2, 4′ and 8′, and tocotrienols have an unsaturated (but unconjugated) side chain at positions 3′, 7′ and 11′. Because of their structures, there exist diastereoisomers of tocopherols and *cis-trans* isomers of tocotrienols (9). The chemical and IUPAC names of α-tocopherol are 2,5,7,8-tetramethyl-2-(4′,8′,12′- trimethyl tridecyl)-6-chromanol and 2R,4′R,8′R-α-tocopherol, respectively.

Paper and gas chromatography were used for vitamin E analyses in 1950s and 1960s, but HPLC techniques were utilized in the mid-1970s. Today, C-18 reverse-phase columns are used for separating α-tocopherol from its esters and other fat-soluble vitamins whereas silica and other modified normal-phase columns are used for the separation of tocopherol isomers and their tocotrienol counterparts (unpublished results). Further analytical and preparative work on vitamin E separation is under investigation. Despite isomeric identification compromise, there is some merit to using pulsed voltammetric techniques for vitamin E analyses (23).

Vegetable oils contain tocopherols, particularly the γ-isomer (highest in corn and soybean) and α-isomer (highest in sunflower and cottonseed) (23–25). With the exception of δ-tocopherol in soybean, β- and δ-isomers are not found in oil in significant amounts (Table 1). Most commercial oils are devoid of tocotrienols. Palm oil characteristically has the highest levels of γ- and α-tocotrienol, and the total tocotrienols represent about 83% of the palm oil vitamin E content. Coconut and sunflower oils have minor quantities of tocotrienols. Wheat germ oil is not considered a significant commercial oil but it has an unusually high concentration of tocopherols relative to other oils (24). The descending order of total tocopherols and tocotrienols in commercial oils is as follows: corn, soybean, palm (800–1100 ppm) > cottonseed, sunflower, rapeseed (550–800 ppm) > peanut, olive (150–350 ppm) > coconut, palm kernel (≤ 50 ppm).

More than 85% of palm oil vitamin E content consists of γ-tocotrienol, α-tocotrienol and α-tocopherol and, to a lesser extent, δ-tocotrienol (Table 2). Total vitamin E content is concentrated sixfold in the palm fatty acid distillate (PFAD) compared with the crude palm oil (CPO), while a 20-fold concentration is realized for the total sterols. This selective distribution allows the potential extraction of vitamin E isomers in the PFAD byproducts while significantly decreasing the sterols in the eventual refined oil. Findings show the following characteristics of palm oil tocopherols and tocotrienols:

- Palm oil has a high concentration of vitamin E and this content is comparable to that found in corn and soybean oil.
- The preponderant palm oil vitamin E components are γ- and α-tocotrienol and α-tocopherol; tocotrienols constitute 83% of total vitamin E content.
- PFAD byproducts have 4,000–6,000 ppm of vitamin E; relative distribution of the isomers is similar to that found in CPO.



Methyl Group	Tocopherol	Tocotrienol
5,7,8-trimethyl	αT1	αT3
5,8-dimethyl	βT1	βT3
7,8-dimethyl	γT1	γT3
8-methyl	δT1	δT3

FIG. 3. Vitamin E structures of tocopherols (T1; with R_1 phytyl chain) and (T3; with R_2 phytyl chain). Chiral positions of tocopherol are 2, 4′ and 8′, and the non-conjugated triene positions of tocotrienol are 3′, 7′ and 11′.

VITAMIN A AND E ACTIVITIES

To understand and assign vitamin A and E activities, it is necessary to distinguish (a) isomers of natural and synthetic origins, (b) structural requirements for activities, and (c) definitions of activities. Practically all nature-derived tocopherols have three chiral carbons and they are believed to be the d form (i.e., R confirmation). In contrast, the only synthetic form of vitamin

TABLE 1

Tocopherol and Tocotrienol Contents in Oils[a]

	Tocopherol (ppm)				Tocotrienol
Oils	α	β	γ	δ	(% of total)
Corn	223	32	790	26	0
Soybean	100	8	625	261	0
Palm	152	ND[b]	ND	ND	83[c]
Cottonseed	389	ND	387	ND	0
Sunflower	599	15	38	7	3.7[c]
Rapeseed	184	ND	380	12	0
Peanut	139	3	189	18	0
Olive	162	9	10	ND	0
Coconut	5	ND	ND	6	69[c]

[a]Data were obtained from ref. 23–25.
[b]ND indicated trace or absence of vitamin E isomer.
[c]Tocotrienols are found in palm (α, 205; γ, 439; δ, 94 ppm) sunflower (α, 25 ppm), and coconut (α, 5; β, 1; γ, 19 ppm).

has 15-fold more vitamin A activity than carrots and 300-fold more than tomatoes (21).

TOCOPHEROLS AND TOCOTRIENOLS

Vitamin E was first reported in the early 1920s; at that time, it was associated with sterility and reproduction. The Greek meaning for "tocopherol" is "to bear offspring." The other vitamin E homolog—tocotrienol—was discovered in 1955. These compounds are fat-soluble and light yellow in color.

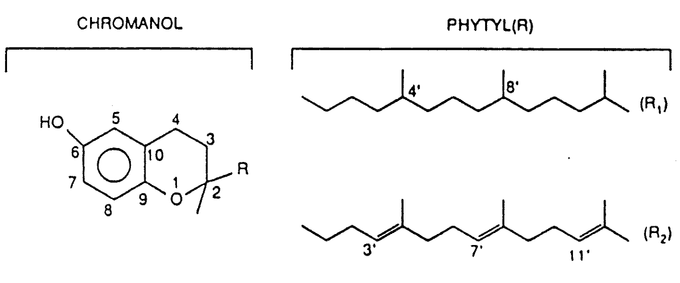
Chemically, tocopherols and tocotrienols are methyl-substituted chromanols with a three-isoprene moiety side chain (Fig. 3). The structures of these two homologs are readily distinguishable. Tocopherols have a saturated side chain with chiral carbons at positions 2, 4′ and 8′, and tocotrienols have an unsaturated (but unconjugated) side chain at positions 3′, 7′ and 11′. Because of their structures, there exist diastereoisomers of tocopherols and cis-trans isomers of tocotrienols (9). The chemical and IUPAC names of α-tocopherol are 2,5,7,8-tetramethyl-2-(4′,8′,12′- trimethyl tridecyl)-6-chromanol and 2R,4′R,8′R-α-tocopherol, respectively.

Paper and gas chromatography were used for vitamin E analyses in 1950s and 1960s, but HPLC techniques were utilized in the mid-1970s. Today, C-18 reverse-phase columns are used for separating α-tocopherol from its esters and other fat-soluble vitamins whereas silica and other modified normal-phase columns are used for the separation of tocopherol isomers and their tocotrienol counterparts (unpublished results). Further analytical and preparative work on vitamin E separation is under investigation. Despite isomeric identification compromise, there is some merit to using pulsed voltammetric techniques for vitamin E analyses (23).

Vegetable oils contain tocopherols, particularly the γ-isomer (highest in corn and soybean) and α-isomer (highest in sunflower and cottonseed) (23–25). With the exception of δ-tocopherol in soybean, β- and δ-isomers are not found in oil in significant amounts (Table 1). Most commercial oils are devoid of tocotrienols. Palm oil characteristically has the highest levels of γ- and α-tocotrienol, and the total tocotrienols represent about 83% of the palm oil vitamin E content. Coconut and sunflower oils have minor quantities of tocotrienols. Wheat germ oil is not considered a significant commercial oil but it has an unusually high concentration of tocopherols relative to other oils (24). The descending order of total tocopherols and tocotrienols in commercial oils is as follows: corn, soybean, palm (800–1100 ppm) > cottonseed, sunflower, rapeseed (550–800 ppm) > peanut, olive (150–350 ppm) > coconut, palm kernel (≤ 50 ppm).

More than 85% of palm oil vitamin E content consists of γ-tocotrienol, α-tocotrienol and α-tocopherol and, to a lesser extent, δ-tocotrienol (Table 2). Total vitamin E content is concentrated sixfold in the palm fatty acid distillate (PFAD) compared with the crude palm oil (CPO), while a 20-fold concentration is realized for the total sterols. This selective distribution allows the potential extraction of vitamin E isomers in the PFAD byproducts while significantly decreasing the sterols in the eventual refined oil. Findings show the following characteristics of palm oil tocopherols and tocotrienols:

- Palm oil has a high concentration of vitamin E and this content is comparable to that found in corn and soybean oil.
- The preponderant palm oil vitamin E components are γ- and α-tocotrienol and α-tocopherol; tocotrienols constitute 83% of total vitamin E content.
- PFAD byproducts have 4,000–6,000 ppm of vitamin E; relative distribution of the isomers is similar to that found in CPO.



Methyl Group	Tocopherol	Tocotrienol
5,7,8-trimethyl	αT1	αT3
5,8-dimethyl	βT1	βT3
7,8-dimethyl	γT1	γT3
8-methyl	δT1	δT3

FIG. 3. Vitamin E structures of tocopherols (T1; with R_1 phytyl chain) and (T3; with R_2 phytyl chain). Chiral positions of tocopherol are 2, 4′ and 8′, and the non-conjugated triene positions of tocotrienol are 3′, 7′ and 11′.

VITAMIN A AND E ACTIVITIES

To understand and assign vitamin A and E activities, it is necessary to distinguish (a) isomers of natural and synthetic origins, (b) structural requirements for activities, and (c) definitions of activities. Practically all nature-derived tocopherols have three chiral carbons and they are believed to be the d form (i.e., R confirmation). In contrast, the only synthetic form of vitamin

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[c]Tocotrienols are found in palm (α, 205; γ, 439; δ, 94 ppm) sunflower (α, 25 ppm), and coconut (α, 5; β, 1; γ, 19 ppm).

TABLE 2

Vitamin E and Sterol Isomers in Palm Oil Fractions

Oil fraction	Vitamin E[a] (%)					Sterols[b] (%)			
	αT1	αT3	γT3	δT3	γT1	CH	CM	ST	βS
CPO[c]	22	20	36	7	12	4	22	12	61
PFAD[d]	23	24	38	15	—	11	23	14	52

[a]T1 and T3 represent tocopherol and tocotrienol (Fig. 3).
[b]These sterols are: CH, cholesterol; CM, campesterol; ST, stigmasterol; βS, β-sitosterol.
[c]Total vitamin Es and sterols in crude palm oil (CPO) are 600–1000 ppm and 270–300 ppm, respectively.
[d]Total vitamin Es and sterols in palm fatty acid distillate (PFAD) are 4000–5000 ppm and ca. 6500 ppm, respectively.

E is the α-isomer of tocopheryl acetate but it is racemic (labeled with a prefix 'all-rac') at each of the three asymmetrical carbons (9). Therefore, there are four geometric/positional isomers from natural sources (α-, β-, γ- and δ-forms from the chromanol ring) and eight diastereoisomers of synthetic all-rac-α-tocopheryl acetate (from confirmations found in the isoprenoid side chain) (Fig. 3).

The situation with carotenoids is less complex. The type of nature-derived carotenoids is clearly a function of the sources (e.g., β-, α- and γ-carotene, β-cryptoxanthin; lutein). Natural carotenoids typically have a significant amount of xanthophylls (18,22). Synthetic carotenoids are limited to β-carotene, canthaxanthin, β-apo-8′-carotenal and its acid ester. Synthetic all-*trans*-β-carotene formulations frequently have impurities of α-carotene and detectable levels of *cis*-β-carotene (unpublished results).

In order to have vitamin E activity, the molecule must have a basic tocol structure—that is, a chromanol attached to three isoprene units at position 2. Other structural features that affect the vitamin E activity include the number of methyl groups on the chromanol (3, α-; 2, β- and γ-; 1, δ-designations) confirmation at C-2 (R or S), presence of double bonds on the side chain (tocopherol or tocotrienol), and the methyl confirmations at position 4′ and 8′ (R or S) of the isoprenoid side chain.

The presence of provitamin A activity requires the carotenoid molecule to have at least one β-ionone end-group and a highly unsaturated and conjugated isoprenoid backbone (Fig. 1). Other structural features that affect carotenoid-derived vitamin A activity include the degree of isomerization (*cis-trans* isomers of the polyene backbone) and formation of oxy-, hydroxy-, epoxy- and furanoxy- derivatives (xanthophylls).

The International Unit (IU) has been defined as the activity of 1 mg of dl-α-tocopheryl acetate in preventing fetal resorption in vitamin E-deprived female rats (9). The d-α-isomers and free alcohols have higher activities than dl-α-tocophenyl acetate. Beta-carotene is the best known carotenoid conversion to vitamin A. Ten IU of vitamin A activity is equivalent to 1 μg vitamin A retinol (or one retinol equivalent), 6 μg β-carotene and approximately 12 μg other β-ionone end-group carotenoids. Activity also may be defined as Retinol Equivalent (RE). In most domestic animals and man, 4 to 10 μg β-carotene is equivalent in activity to 1 μg retinol (26). Experimental data indicate that digestion and absorption in the intestines and deposition in tissues are responsible for inefficient and different conversion. A ratio of 6-to-1 is an acceptable mean.

Table 3 shows vitamin activities from palm oil carotenoids, tocopherols and tocotrienols. Carotenoids with *cis* isomers and oxygenated functions have less vitamin A activities than their parent compounds. Carotenoids that have one β-ionone ring will have less than or equal to 50% activity of β-carotene (2 β-ionone rings). The ringed ε-ionone and ψ (acyclic) side chains have no vitamin A activity. For example, α-carotene (β,ε-carotene) has half the activity of β-carotene, while lycopene (ψ,ψ-carotene) and α-zeacarotene (7′, 8′-dihydro-ε,ψ-carotene) are both vitamin A inactive (Fig. 1). Most vitamin A inactive palm oil carotenoids have ψ,ψ-side chains and they contribute only to a small extent to the total palm oil carotenoids (20).

Table 3 also shows that d-α-tocotrienol has about one-third the activity of d-α-tocopherol, and the activities of other tocotrienols are even smaller than that of d-α-tocotrienol. It is also important to note that all other isomers and their corresponding synthetic racemic mixtures have lesser activities than d-α- and dl-α-tocopherols. Tocopherols (as free alcohols) have antioxidant activities but their esters do not. The δ- and γ-tocopherols are better food antioxidants but α- and β-tocopherols are better *in vivo* antioxidants (24,27–29). It has been reported that there was coprotection of β-carotene and δ-tocopherol during singlet oxygen-initiated oxidation of methyl linoleate (30). Also, it has been suggested that a low concentration of β-carotene in membranes may retard the oxidative destruction of α-tocopherol (31).

ECONOMIC POTENTIAL

Vitamin E and carotenoids can be classified as food additives. Tocopherols, ascorbic acid esters, gallic acid esters, tert-butylhydroxyanisol (BHA) and di-tert-butylhydroxytoluene (BHT) are among the most important antioxidants. Tocopherols are used in retarding lipid oxidation in food products. Carotenoids are most important food colorants in yellow to red coloration. Carotenoids are also applied in animal pigmentation (e.g., poultry and fish) and fertility (e.g., cattle and sows). Examples of natural and synthetic carotenoids that are used (EEC number in parentheses) as food

TABLE 3

Vitamin A and E Activities from Palm Oil Carotenoid, Tocopherol and Tocotrienol Isomers

Carotenoid	Vitamin A activity (%)[a]	Tocopherol/ tocotrienol	Vitamin E activity (%)[c]
β-carotene[b]	100	d-α-tocopherol[b]	149
-9-*cis*[b]	38	-dl-α	110
-13-*cis*[b]	53	-dl-α, acetate	100
-5,6-epoxide[b]	21	-dl-α, succinate	89
-5,8-epoxide[b]	50	d-α-tocotrienol[b,d]	45
-5,8,5′,8′-diepoxide[b]	?	d-β-tocopherol[d]	75
-9-hydroxy	50–60	-dl-β	30
α-carotene[b]	50–54	d-β-tocotrienol	8
-9-*cis*	13	d-δ-tocopherol[d]	5
-5,6-epoxide[b]	25	-dl-δ	1
γ-carotene[b]	42–50	d-δ-tocotrienol[b]	?
β-zeacarotene[b]	20–40	d-γ-tocopherol[d]	15
lycopene[b]	inactive	-dl-γ	15
ε-carotene[b]	inactive	d-γ-tocotrienol[b]	1.5

Vit. E activity

[a]1 RE of vitamin A activity (or 1 μg retinol) is defined as 6μg β-carotene or about 12 μg of a carotenoid with one β-ionone end-group.
[b]Significant isomers found in palm oil.
[c]1 IU of vitamin E activity is defined as 1 mg synthetic dl-α-tocopheryl acetate.
[d]Unofficial value.

colorants include β-carotene (E160a), canthaxanthin (E161g), β-apo-8′-carotenol (E160e) and its ester (E160f), bixin (E160b), lycopene (E160d), astaxanthin and zeaxanthin.

If vitamin E (e.g., for atherosclerosis and thrombosis protection) and carotenoids (e.g., for cancer and radiation protection) were used in therapeutic drugs, their applications would greatly increase.

Recently, it was reported that oil palm leaves and leaflets contained large quantities of α-tocopherol and no tocotrienols (32,33). It was estimated that palm leaves and leaflets from frond pruning and oil palm replanting yield about 5 million tons (dry weight) annually. On a dry basis, α-tocopherol was found in unusually high concentrations in leaflets (0.32–0.56%) (33) and leaves (0.18–0.28%) (32). A trace amount of β-tocopherol (0.3% of α-tocopherol) was also reported in the leaves.

Table 4 shows the comparative recoveries of major components in palm products. From the large volume of leaf byproducts, recoveries of α-tocopherol and β-carotene can be exceedingly high provided economic extraction is achievable. Interestingly, these two palm components have the highest vitamin E and A activities, respectively (Table 3). The present annual carotenoid market is greater than $100 million and it is expected that natural carotenoids will be higher priced than synthetic carotenoids.

TABLE 4

Potential Annual Recoveries from Palm Minor Components

Item	Annual amounts (tons)	Major isomers
Vitamin Es		
PFAD[a,b]	800	γT3, αT3, αT1[a]
Pruning	26,000	αT1
Replanting	380	αT1
Carotenoids		
CPO[a,b]	4,450	β- and α-carotene
Pruning[c]	9,800	β-carotene
Replanting[c]	150	β-carotene

[a]See Table 2 and Figure 3 for explanation of abbreviations.
[b]The δT3 is of intermediate amount. The β- and α-carotene ratio is about 2:1.
[c]Rigorous analyses were not given (see ref. 35); β-carotene was the presumed major isomer.

The Palm Oil Research Institute of Malaysia (PORIM) and Japan's Ministry of International Trade and Industry (MITI) have completed a 5-year project on pilot plant extraction of vitamin E from PFAD. PORIM has claimed that a possible income of $26.4 million could be derived from vitamin E from PFAD. The nutritional and medical implications of the dominant δ- and α-tocotrienol from PFAD are not clear. However, with the exception of vitamin E isomers in PFAD, other palm products have largely α-tocopherol (d-form), β-carotene (mostly all-*trans*) and all-*trans*-α-carotene (Table 4). This is a distinct advantage in the light of purity requirements in drug formulation.

Malaysia and Indonesia account for 77% of the 8.52 million MT world palm oil production, and this 8.52 million MT represents 16% of total world oil production (34). In view of the dramatic oil production in southeast Asia and the large concentration of vitamin E and carotenoids in palm byproducts, the technical/commercial extraction of these chemicals is due.

ACKNOWLEDGMENT

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Psyllium article from Wall Street Journal
October 30, 1989

'Flea Seed' Helps Make Area in India A New Heartland

* * *

Focus on Blood Cholesterol Brings Attention to Crop, Called Psyllium in West

By ANTHONY SPAETH
Staff Reporter of THE WALL STREET JOURNAL

SIDHPUR, India—It is a peaceful time in this part of western India. The summer crop is harvested, winter sowing has yet to begin. Farmers in loose turbans and fancy earrings spend their afternoons laughing and gossiping at the markets.

One could imagine such a lull in the lives of the Arabs before the quadrupling of oil prices. For just as the Arabs were in the 1960s, the farmers of Sidhpur are on the brink of global power and fame. The Arabs had merely oil. These farmers may have a grip on the world's very heart. Or, at least, its heart disease.

That is because Sidhpur has a near-monopoly on the world's supply of flea seed, also known as flea wort or, in Western parlance, psyllium: a tiny, tasteless, obscure seed that, according to early research, may reduce cholesterol levels in the blood. Ever since the link to cholesterol was disclosed, Americans have begun scarfing up psyllium in their breakfast cereals. If further research proves the seed's benefits, this dusty farm district could become the epicenter of a health-food fad to rival all fads since cod-liver oil.

A Fanatic Following

"This seed's not grown anywhere else in India, or anywhere else in the world," says T.V. Krishnamurthy, a vice president of Procter & Gamble India Ltd., a major psyllium buyer and promoter. "The proper climatic conditions don't exist in many places in the world." Arvind Patel, a processor and exporter of the seed, raves: "If psyllium takes the place of oat bran, it will be huge."

Whether psyllium makes Sidhpur's fortune depends on cholesterol-fearing Americans, the U.S. Food and Drug Administration and, of course, the outcome of further research. Only one thing is certain here: Pysllium is likely to remain solely an export item from Sidhpur for a long time. Local farmers say it is as good a cash crop as mustard or fenugreek, a legume. But they have no desire to eat a bowl of psyllium each morning, and, perhaps, little need: lean, frugal vegetarians, the farmers are innocents in the clogged, treacherous world of cholesterol.

Psyllium is an annual herb, *Plantago ovata*, that has been used for centuries by folk doctors here, mainly as a laxative and anti-diarrheal. As such, the soluble fiber has an almost fanatic following in northern India. "I can assure you," attests a 25-year-old lawyer in New Delhi, with a meaningfully raised eyebrow, "from *personal experience*, it works." A prominent businessman in Bombay gives a similar testimonial: "I have been taking it daily since 1961." Folk doctors also prescribe it for kidney, bladder and urethra problems, duodenal ulcers and hemorrhoids. Some apply it to gouty joints.

Flea Wort for Breakfast

The plant has a hairy stem that produces flowers and diminutive seeds. It is the seed's colorlessness and size—1,000 of them weigh only 1.5 grams, or about as much as two paper clips—that explain the historical allusions to fleas. The translucent husk of the seed is removed, sifted and crushed; the seed itself is fed to animals. Some 90% of the crop, which was worth $26 million last year, is exported.

For decades, psyllium husk has been the main ingredient in such laxatives as Procter & Gamble Co.'s Metamucil, the top-selling brand in the U.S., and Ciba-Geigy Corp.'s Fiberall. But some time ago, researchers discovered that soluble fibers also lower cholesterol levels in the blood. Cincinnati-based P&G took an interest; it ordered two studies on psyllium and cholesterol.

One of the studies, done at the University of Minnesota, tested 75 people with raised cholesterol levels. After 16 weeks, the group that took three daily teaspoons of Metamucil saw a significant dip in their general cholesterol levels, and an even larger reduction in levels of low-density lipoproteins, the so-called bad cholesterol. In late 1987, P&G asked the FDA for approval to market Metamucil as the first non-prescription, cholesterol-lowering product in the U.S.

In April, the psyllium bandwagon got more crowded. General Mills Inc., the food giant, launched a breakfast cereal called Benefit, containing psyllium, oat, wheat and beet bran; the words, "reduce cholesterol" were prominently displayed on its package. In September, Kellogg Co. launched a competing psyllium-fortified cereal called Heartwise.

Suddenly, on television, in advertisements and on their cereal boxes, Americans were inundated with news about the obscure seed. The flood of claims and counter-claims worried consumers and actually hurt sales of the new cereals. This month, the Food and Drug Administration expressed concern that Americans might someday, in various forms, ingest too much psyllium.

'Flea Seed' Helps Make a Region In India a Veritable New Heartland

Continued From First Page

Currently, there is a lull in the psyllium war. The FDA has asked Kellogg and General Mills to show research that their cereals are safe. It also ordered P&G to produce more studies to buttress its claim that Metamucil can lower cholesterol. But the agency hasn't yanked psyllium off store shelves. If the FDA approves the new uses of psyllium, other companies are expected to rush to market with psyllium products.

"It's going to be a sensational thing," says Mr. Krishnamurthy of P&G in Bombay. Says psyllium exporter Mr. Patel: "I just got back yesterday from the U.S. In the newspapers, on the radio and TV, psyllium is everywhere."

But the news of the boom has yet to trickle down to the farmers. They only know of one use for the crop, as a laxative, and with psyllium prices currently languishing in the wake of a bumper crop, they think of the seed as a marginal crop, something to grow between summer wheat crops.

"Psyllium's not a good crop," complains Sooraji Jath, a 26-year-old farmer from the village of Lakshmipura. "You get a rain at the wrong time and the crop is ruined."

Even at the Basic Chemicals, Pharmaceuticals and Cosmetics Export Promotion Council, the government agency that promotes the seed, the psyllium boom is distant thunder. The staff brags about psyllium's hefty contribution to American regularity, without quite grasping the implications of the research on cholesterol. The council's annual report has psyllium on its last page, lumped with such unglamorous export items as sarsaparilla and *Nux vomica*, a plant that induces vomiting.

In one way, the psyllium middlemen—the buyers and exporters—are glad to keep news of the boom to themselves. They want psyllium prices low for their purchases next year.

But there's a catch. Sidhpur and adjacent districts are the only places in the world where psyllium is grown in large quantities. This is partly due to the particular demands of the crop. Psyllium needs sandy soil, dew during the first few weeks, and then total dryness when its seeds are maturing. Small crops are grown in Pakistan, France, Spain, Italy, Belgium and Brazil, but their quality can't compare to that of Indian psyllium.

Big buyers like Procter & Gamble say there are other spots on the globe, and in India, where the seed could be grown. "It's not a crop that can't be doubled or tripled," says Mr. Krishnamurthy. But no one has made a serious effort to transplant the crop.

In Sidhpur, it is almost time to sow this year's crop. Many farmers, too removed to glean psyllium's new sparkle in the West, have decided to plant mustard, fennel, cumin, fenugreek or castor-oil seeds.

Mr. Jath is thinking of passing up psyllium altogether this year in favor of a crop with a future such as cumin or fennel. "Maybe I'll plant castor-oil seeds." His brother, Parkhaji, whose head is swathed in a gorgeous crimson turban, nods vigorous assent.

So when next year's psyllium crop is harvested in March, it may be smaller than the 16,000 metric tons of the past few years—right at the crest of the psyllium boom.

And the world could experience its first psyllium shortage.

Amaranth Research Report

A production scale twin-screw extruder cooker (Buhler Miag Co.) was used to process approximately 100-lb. batches of whole amaranth grain (K-343) or amaranth flour (K-343, R-149) as such or in combination with other ingredients. This method of processing permits the continuous fabrication of a variety of food product types, e.g., breakfast cereals, crisp snacks, flat breads, confections, croutons, etc., with a minimal input of energy. The equipment allows for a wide variation in temperatures, pressures, shearing, expansion and size and shape of finished product.

The whole grain particles underwent popping (expansion) in the extruder and the individual particles were agglomerated to form larger ones, making them easier to handle. This process will be studied further to determine whether the heat employed is sufficient to inactivate any antinutritional factors that might be present in the grain (e.g., trypsin inhibitors) and whether other important changes might take place. The latter could include pregelatinization of starch or denaturation of protein, which could influence functional properties of amaranth flour in food formulations. The process could also alter the content of tocotrienals which are known to be present and which have cholesterol-lowering activity when consumed by humans. It might also affect the relative quantities of soluble and insoluble dietary fiber.

11-24-89

It was possible to manipulate operating conditions to produce puffed or expanded texturized products from 100% amaranth flour. However, the ease of producing such products was enhanced by the addition of other cereal ingredients at approximately 20% of the infeed material. Amaranth flour will probably be used in such products as an ingredient to boost nutritional value but these trials have demonstrated the possibility of making whole amaranth products. The added ingredients included up to 12% modified corn starch and up to 8% soft wheat flour. Sugar and/or salt were also added for flavor. Two basic product types were produced: a "salty" snack or crouton and a "sweet" cereal, the former as a solid piece and the latter using a so-called "cheerio" die. It was shown that other flavors such as cinammon/apple and cheese could be applied to modify flavor if desired.

Work is underway to determine whether and in what way extrusion processing has altered the viscosity and pasting characteristics and such chemical properties as tocotrienal, trypsin inhibitor and dietary fiber contents. A recently acquired Rapid Visco Analyzer is being used in the viscosity and pasting studies.

M. M. Greene 11/21/99

/AMARANTH

Appendix K.

Amaranth Processing Feasibility

Preliminary Report
November 1989
Compiled by American Amaranth, Inc.

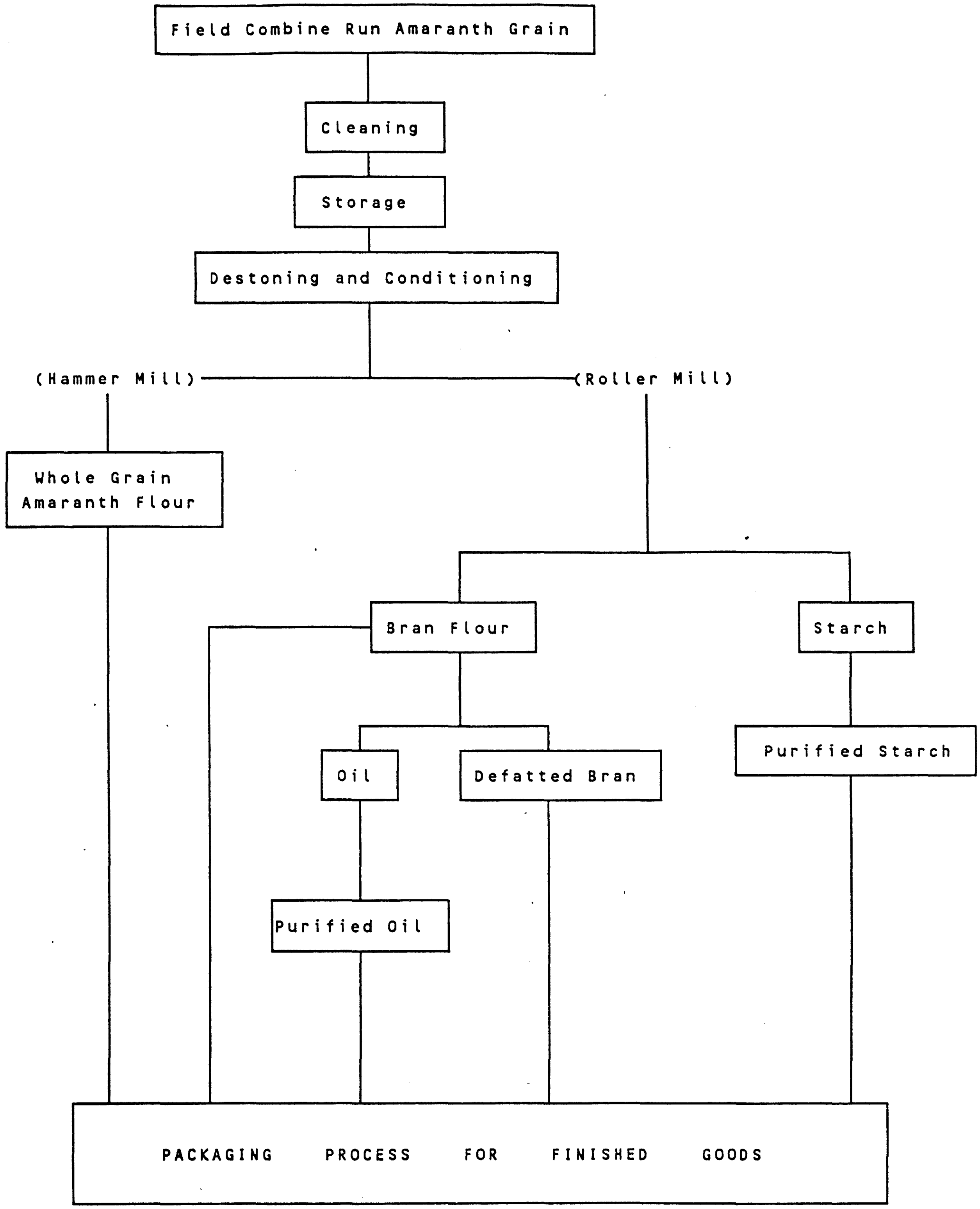
The feasibility of processing grain amaranth is currently being evaluated in three aspects:

1). Existing processing.
2). Potential future processing needs.
3). Capital requirements.

Existing processing of amaranth is currently done at various locations in the U.S.: Health Valley of California, Nu-World Amaranth of Illinois, J.R. Short and Company, U.S. Mills of Nebraska, Arrowhead Mills of Texas, Cross Seed of Kansas, and American Amaranth, Inc. of Minnesota. There are also other companies involved with amaranth processing on a lesser scale.

The above-mentioned processors can be divided into Basic Processing and/or Manufacturing. Basic Processing includes storage, cleaning, milling and popping. The majority of the finished products from processing would be in the form of ingredients sold to manufacturers. These ingredients would then be added to finished consumer products such as pasta, snack foods, baby food, and convenience entrees. Manufacturing would be defined as any company active as an assembler of a finished product by processing ingredients, mixing, and packaging consumer-ready products. The majority of the above listed companies are Manufacturers. <u>There is no state-of-the-art Basic Processing</u> of amaranth done in the United States at this time. This fact is especially significant in that the manufacturers are limited by the degree of quality and variety of amaranth ingredients available at this time. Companies such as <u>General Mills, General Foods, Proctor and Gamble and Continental Bakeries</u> are but a few of the many major companies requesting basic processed amaranth.

The need at this time is for a pilot processing plant that would fractionalize the amaranth grain. Fractionalization would include:



Field Combine Run Amaranth Grain
Cleaning
Storage
Destoning and Conditioning
(Hammer Mill)
(Roller Mill)
Whole Grain Amaranth Flour
Bran Flour
Starch
Oil
Defatted Bran
Purified Starch
Purified Oil
PACKAGING PROCESS FOR FINISHED GOODS

Finished products of Basic Processing would include:

- Whole grain
- Amaranth flour
- Amaranth bran
- Defatted bran
- Purified starch
- Oil and oil derivatives

A capacity of 10 million pounds per year would be the specific capacity of initial pilot plant processing.

State-of-the-art processing is probably best accomplished through extrusion technology. This processing equipment would be used to manufacture cereal products, texturized vegetable protein (TVP), hard breads and snack products.

Capital requirements for the Pilot plant would be between $5-7.5 million.

The Pilot Plant would keep Minnesota the leader in amaranth processing technology for 5-10 years.

Currently, the weakest link in amaranth utilization within the food industry is the QUALITY and FRACTIONALIZATION of the grain relative to competitive cereal industries.

The capital requirements for amaranth processing are the responsibility of both government and private sectors.

Specialty crops such as amaranth could be a cornerstone of rural and state-wide economic development and recovery. Specialty crop processing requires less infrastructure that traditional crops yet it fits the small, rural community needs for job creation. Minnesota has an enormous economic resource in its rural transportation network and productive capacity of its land. <u>We must take advantage of the rich historical tradition and technologies that made Minneapolis-St. Paul and Duluth into centers of cereal trade and shipment.</u> In addition to adding processing industry, diversification into crops which complement or add value to our major crops is but one method of creating new wealth from the land.

Agriculture is our nation's largest industry. If we view research and development of agriculture from short-term gains only, then we will fall prey to the same R & D shortfall that is contributing to the nation's trade imbalances.

CAPITAL REQUIREMENTS ESTIMATE for

PILOT AMARANTH PROCESSING AND FRACTIONALIZATION

(in $1000's)

Grain cleaning equipment Conveyors, entoleter, aspirators, gravity tables, screening equipment, magnetic separators, disc separators.	$300
Grain storage Conveyors, bins	$120
Grain de-stoning and conditioning De-stoners, screening equipment, bins entoleter, and dust collectors	$100
Grain milling equipment Hammer mills, roller mills, stone mills	$300
Sifters, dust collectors, conveyors, storage	$200
Single-screw extruder, mixer, dryer	$100
Twin-screw extruder, mixer, dryer	$2500
Building construction	$2000
Oil extraction equipment	$ 300
Starch extraction equipment	$ 300
Packaging equipment	$ 100
Quality assurance equipment	$ 300
Office equipment	$ 180

PURDUE UNIVERSITY

LABORATORY OF
RENEWABLE RESOURCES
ENGINEERING

June 6, 1989

Mr. James W. Lehmann
P.O. Box 243
Bricelyn, MN 56014

Dear Mr. Lehmann:

Attached, are some materials on our work.

The smaller the starch granules, better would be the dispersion in the plastic matrix and as such should improve properties relative to current starch-plastic. However, compatibility will always be a problem and you need to address it.

Good luck on your research, and keep me posted. Best regards.

Sincerely,

Ramani Narayan

Enclosures

A. A. POTTER ENGINEERING CENTER, ROOM 216 • WEST LAFAYETTE, IN 47907 • (317) 494-7022

Appendix M.

A Preliminary Report by the University of Minnesota Agronomy Department on Amaranth Feasibility

AGRONOMIC STUDIES

Several agronomic studies were undertaken in 1989 to evaluate the production feasibility of grain amaranth in Minnesota. These studies were performed at many locations throughout the state, on Agricultural Experiment Stations, and on farmer-cooperator fields. Some of the research work is follow-up work of previous studies done in Minnesota, and other work was begun new this year. Where appropriate, data is included from previous years.

Variety Evaluation. A key aspect of crop adaptation to a region is a selection of varieties or lines which are appropriate for the climate and soils of that region. A number of lines are being developed by Rodale Research Center (Kutztown, PA), by private plant breeders, and by the Montana Agricultural Experiment Station. Selected lines were planted at Rosemount, Waseca, Lamberton, Albert Lea, Morris, and Grand Rapids, MN. Selected lines which were planted at Waseca and Lamberton sites were abandoned due to lack of spring moisture and poor stands. Varieties were planted in 30 inch rows in late May, and harvested after frost in October. Harvest was by combine at Albert Lea and Rosemount, and by hand at Morris and Grand Rapids. These results are provided in Table 1 (See progress report) and Table 2, and will be summarized, along with a narrative in the 1990 Minnesota Varietal Trials of Farm Crops (MR-24), so that our farmers will have immediate access to them.

Several of these lines are worth discussion. MT-3 is a germplasm release from Montana State Agricultural Experiment Station, and very similar to their recent variety release named AMONT, the first amaranth variety released in the United States. This a vigorous *Amaranthus cruentus* line which seemed to have reasonable lodging resistance under Minnesota conditions. Unfortunately, it is rather tall, and may provide difficulties in harvest. Rodale's K343 is the most widely grown line in the country, with a red flower color and fairly tall stature. Its yield has been medium to high in previous tests, and it is well adapted to Minnesota, but may be later maturing than desired. K432 is a semi-dwarf line which holds particular promise because of its ease of harvest and even, short height and earliness. Yields of this line (and the sister line K433) were significantly higher than other lines at Albert Lea in 1989. Unfortunately, both K343 and K432 were heavily lodged in Rosemount due to a heavy rain and wind in July. The dwarf stature did not seem to aid in lodging resistance in K432, as other lines, e.g., MT-3, stood better.

One interesting aspect of this year's study was the performance of these lines at Grand Rapids, especially K432. We had not expected much in the way of seed yield at this site, due to the shorter season and cooler night temperatures, but some varieties seem to perform adequately.

We conclude that amaranth lines now exist which are well adapted to Minnesota conditions, and that combine-harvested yields of 1000 to 1800 lbs/acre are feasible under normal conditions (yields up to 3800 lbs/acre have been measured in Minnesota). Modern breeding efforts on this crop span only 10-12 years in the U.S., and we anticipate further advances in yield potential, even maturity, lodging resistance, seed size and quality.

Stand Establishment

Three of seven locations planted in 1989 had to be abandoned because of lack of adequate stand (Lamberton, Waseca, and one site in Albert Lea). The same problems were experienced during the 1988 season, and have been reported from other regions (Nebraska and Kansas, Dr. Leon Weber, pers. communication). These stand failures were due largely to lack of timely rainfall to assure adequate germination after planting. Planting equipment (assurance of even depth, soil-seed contact) was also a factor in stand failure, and plant density is also a factor.

Because amaranth is planted late, moisture for adequate stands may continue to be a problem to be addressed. This may be addressed by: earlier planting, improved planting equipment, seed coatings, breeding for larger seed size, increased planting densities, or irrigation. Further research on the problem is needed.

Fertility

Two studies were initiated in 1989 to assess the nitrogen, potassium, and phosphorus fertilizer requirements of grain amaranth in Minnesota. Experiment 1, on nitrogen fertilizer/variety interactions, was planted at Lamberton and Rosemount, MN. in colloboration with Dr. Mike Schmidt of the Soil Science Dept., U. of MN, St. Paul. Soils were sampled to measure initial soil N levels. Two varieties, K432 and K343, were planted to measure the differential response of a tall, late line vs. a short, early line. This experiment was planted in Late May in both locations, but later abandoned because of lack of adequate stand at either location. This study will be initiated again in 1989, funds allowing.

Experiment 2, which is part of a long-term evaluation of N-P-K requirements of amaranth was planted at Lamberton and Rosemount in 1989. The Lamberton experiment was abandoned because of lack of stand. The Rosemount experiment produced an adequate stand, and the N data for this experiment is shown in Figure 1. Amaranth is very responsive to nitrogen fertilizer as shown in this figure, with yields more than doubling in previous years due to additions of N fertilizers. 1989 data was not so dramatic. However, amaranth is very susceptible to lodging, and higher N rates produce higher lodging rates in amaranth. Lodging was so severe in 1989 that all plots were heavily lodged.

It is evident that amaranth has a medium-to-high requirement for soil nitrogen. This could be supplied through nitrogenous fertilizers, manures, or through rotations with nitrogen-fixing crops. The requirement for N for high yields must be balanced with the need to prevent lodging which occurs at high N rates.

Plant Density

Yields of agronomic crops are often limited by lack of sufficient plant density, or reduced when plants are sown too thickly and lodging occurs. Plant density as a determinate of yield was examined in an experiment at Rosemount, MN in 1989. Two varieties of amaranth (K343 and K432) were seeded at 6 different rates of seeding with a cone-type experimental seeder. P and K fertilizers were applied according to soil test, and N was applied at 80 lbs/acre at planting.

The results are shown in Figure 2.

Forage Potential of Amaranth

Amaranth is primarily known for its use as a grain crop. However, several published reports indicated that amaranth forage may be superior in yield, digestibility, and crude protein production compared with other forages (Mugerwa and Bwabye, 1974; Pond and Lehmann, 1989). One lamb feeding study indicated that weight gain and feed utilization was equal for growing lambs fed alfalfa or amaranth as the sole forage. The potential for a new, summer annual forage for Minnesota, an emergency forage during drought conditions (amaranth is known for its drought resistance), or for multiple usage for grain amaranth, led to studies on the forage potential of amaranth. The objectives were to evaluate i) maturity and ii) variety effects on forage yield, forage quality, and ensiling characteristics. This was a cooperative project between the Department of Veterinary and Animal Sciences and Department of Agronomy and Plant Genetics (James STordahl, graduate student).

Two experiments were conducted at Rosemount and Albert Lea, MN. The complete analyses of these trials have not been completed as of this writing, but some data is provided in Table 3 for 1989, and in Figure 3 for 1988. The quality aspects of 1989 have not yet been completed. Similar to other forage species, quality is inversely related to yield, and time of harvest has a significant effect on both parameters (Figure 3). Early cutting of amaranth may be feasible, with the potential for regrowth. Certain of the lines showed greater promise than others for forage production. Ensiling amaranth in small-scale experiments appears successful, however the amaranth must be wilted to 30-40% dry matter for successful fermentation.

Nitrates and oxalates may be a nutritional problem under certain environmental conditions, such as drought or excessive nitrogen applications. Although this is known to be a potential problem, no published literature has shown a negative correlation between animal performance and nitrates or oxalates when amaranth forage is fed to ruminant animals.

Although these studies are incomplete, current evidence indicates that amaranth makes an acceptable, if not superior forage, and if harvested at the correct time, will provide a high-quality alternative, especially in drier areas. Final results of this study will be completed in early 1990, and will be published in an appropriate scientific journal.

Entomology

A study was planted in 1989 which would evaluate the impact of *Lygus* species on amaranth yield and characteristics. This is the most severe insect pest under Minnesota conditions, and our purpose was to evaluate the impact of this pest in Minnesota. This experiment had to be abandoned in 1989 because of very dry conditions. If amaranth is to expand in acreage, more evaluation of the impact of this pest, and potential controls should be conducted.

Table 2. Characteristics of amaranth lines, 1989.

Lines	Lodging Rosemount	Lodging G. Rapids	Shattering Rosemount	Height G. Rapids	Seed Weight	Test Weight
	--------score[1]-------		-score[2]--	-inches-	gm/1000	lbs/bu.
A200D	4.5	----	7.0	----	0.947	----
A2248	3.3	----	1.5	----	0.910	58.4
K266	7.0	1.0	7.5	42	0.907	59.7
K283	4.5	----	9.0	----	0.953	60.2
K343	7.0	5.0	7.3	60	1.017	59.9
K432	7.7	1.0	9.0	64	0.853	60.8
K433	5.7	----	8.0	----	0.927	55.7
K436	5.0	----	9.0	----	0.870	60.6
MT-3	4.0	----	9.5	----	1.037	60.0
PI477914	6.3	----	9.5	----	1.097	----

[1] 1 = erect, 10 = horizontal
[2] 1 = miminal seed loss, 10 = excessive seed loss

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Table 3. Forage yield of four amaranth varieties at four different harvest dates in Albert Lea, MN, 1989. Frost occurred Sept. 22.

	Harvest Date (Days After Planting)			
Variety	Aug. 9 (51)	Aug. 23 (65)	Sept. 6 (79)	Sept. 20 (93)
A5174	1.70 (V)[1]	2.70 (B)	4.34 (A)	4.96 (LF)
482049	1.63 (V)	2.64 (EF)	3.81 (A)	4.22 (S)
482051	1.65 (V)	3.12 (V)	3.59 (B)	4.80 (EF)
K343	1.69 (B)	3.05 (EF)	3.60 (A)	4.29 (S)

[1]Letter in parentheses is stage of growth: (V) Vegetative, (B) Bud, (EF) Early Flower, (A) Anthesis, (LF) Late Flower, (S) Seed.

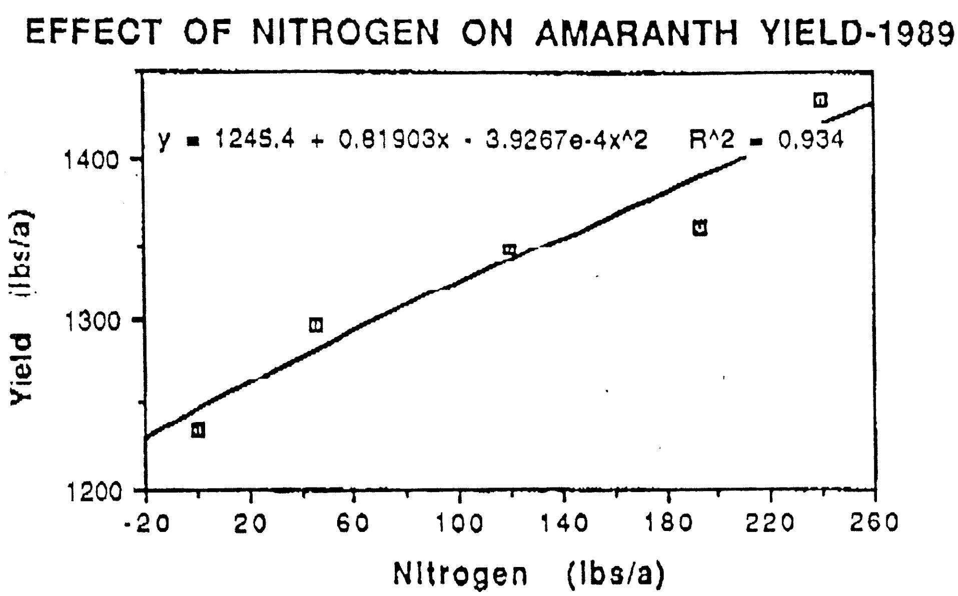

Figure 1. Nitrogen effects on amaranth yield, Rosemount, MN, 1989.

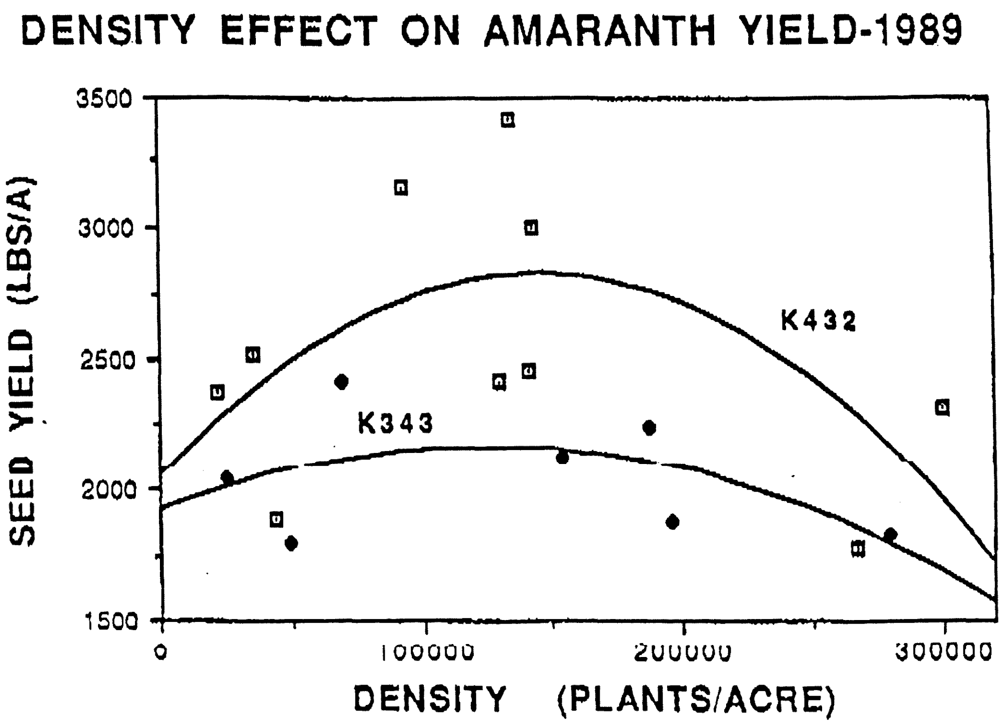

Figure 2. Density effect on amaranth yield, Rosemount, MN, 1989.