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THE NUTRITIONAL VALUE OF A HALOPHYTIC PLANT: DISTICHLIS PALMERI (VASEY)

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THE NUTRITIONAL VALUE OF A HALOPHYTIC PLANT: *DISTICHLIS PALMERI* (VASEY)

by

Susana Bojorquez de Yensen

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ABSTRACT

A proximal analysis was done on the grain of Distichlis palmeri, a halophytic grass. It was found to have 8.7% protein, 8.4% fiber, 1.8% fat. 1.6% ash, 64.4% starch, and 3.5% sugars. The gross energy was 3.9 Kcal/g. The mineral analysis showed 0.2% phosphorus, 0.3% sodium, 0.008% magnesium, 0.003% calcium, 20 ppm iron, 23.1 ppm zinc, and 4.2 ppm copper. The phytate content was 0.46%.

An amino acid profile of the grain was also run. The first three limiting amino acids were found to be methionine, lysine and threonine. Two feeding trials were made using mice as the laboratory animal. In the first experiment D. palmeri flour was fed as the sole source of protein and carbohydrates, using whole egg and lactalbumin as the control proteins. The corrected PER obtained for D. palmeri protein was 1.31 and the total body weight gain was 6.4 g compared to 17.9 g for whole egg and 12.7 g for lactalbumin. In the second experiment five diets were made: 1) just D. palmeri; 2) D. palmeri plus methionine; 3) D. palmeri plus methionine and lysine; 4) D. palmeri plus methionine, lysine and threonine; 5) whole egg as the control protein. The PER and the weight gains for the five diets were: 1) 1.29 $\pm$ 0.06, 7.1 $\pm$ 0.33 g. 2) 1.44 $\pm$ 0.09, 8.3 $\pm$ 0.60 g; 3) 1.53 $\pm$ 0.06, 7.2 $\pm$ 0.34 g;4) 1.90 $\pm$ 0.05, 12.3 $\pm$ 0.55 g; 5) 2.55 $\pm$ 0.07, 17.1 $\pm$ 0.40 g.

PROXIMAL ANALYSIS

Introduction

One of the ancient dreams of mankind has been the use of seawater for irrigation of forage and/or food crops. The development of conventional crops capable of growing in saline water is expensive, and time consuming. Epstein (1977), and others, however, have succesfully crossed commercial tomatoes with salt tolerant varieties of tomatoes, resulting in a cherry-tomato-sized fruit that can tolerate 70% full-strength seawater in the climate at Bodega Bay, California.

Although these successes show promise with conventional crops, another strategy is to find wild species that are already highly salt tolerant, i.e. halophytes and then examine their potential as a crop. Such halophytes could well be the solution to the salinity problem.

The word halophyte comes from the Greek words for salt, halos, and plant, phyte. As its name implies, these "salt-loving" plants can tolerate, and in some cases even grow better at high salinities. Most, however, can still grow in fresh water. Recent work with halophytes suggest that a wide variety of plants (approximately 5,000 species), from around the world can grow well in salinities up to that of seawater (Yensen, 1982). The study of halophytes as potential crops is fairly new; the usefulness of these plants for human and/or animal consumption is still to be determined.

It has been shown that certain halophytes, i.e. Distichlis

spicata, can be used for forage for cattle (Gonzales, 1979). North of Mexico City at ex-lago Texcoco, 10,000 hectares are under cultivation as forage. This success story had an accidental beginning as the Distichlis planting was originally done to stop erosion and dust storms (Benavides, per. comm.).

Other forage halophytes have been used for some time i.e. Atriplex spp has been used in Pakistan, Chile, Mexico, Australia, and Egypt (Gallagher, 1979). Glenn et al. (1980) have conducted feeding trials with mice and chicks using leaves of the halophytes Atriplex lentiformis, A. barclayana, and Salicornia europaea. They and others have shown that by grinding and soaking the foliage, the sodium content could be reduced from 105 ppt to 11 ppt. Ground, de-salinized A. barclayana leaves and stems were incorporated at up to 15% of the diet of mice and chickens. Growth was supported but with somewhat lower weight gains at the higher salt levels. It is obvious from the data that the salt content can be a problem in the use of halophytes.

Grains and berries, on the other hand, frequently have the salt excluded and can be used directly as foods. This is due to the exclusion of the salts at the root level, partitioning of the salts into vacuoles, and/or excretion of the salts through salt glands. The latter is the principal way in which Distichlis spp, a halophytic grass, gets rid of the salts.

The search for low-salt, edible products among the 5,000 species of halophytes has just began. Searching the ethnobotanical literature is a good way to look for this type of plants. One such plant was used

by the Seri Indians in Sonora, Mexico. They employed "eelgrass", Zostera marina, for food (Felger, 1973). Another halophyte grain crop is that of the Cucapa Indians from the Colorado River Delta. Vasey (1889) describes the visit of Dr. Edward Palmer to the Colorado River Delta where Palmer discovered Cucapas harvesting a halophytic grain crop, Indian Wheat. In this paper Vasey provided the original description and named the plant after Palmer: Uniola palmeri. Later this plant was placed by Fassett (unpubl.) in the genera Distichlis (Johnston, 1924). So today this plant is known as Distichlis palmeri.

Except for Palmer's notes, little has been published with respect to the nutritional value of the grain of D. palmeri. In his notes, Palmer describes the area where the Cucapas used to go in early spring to collect the seeds. He estimated that there were approximately 40,000 to 50,000 acres covered with this grass. He went further and described the harvesting of the seeds which was very painful, because the plants have rigid, pointed leaves.

The Indians preferred to cut the grain when it was a little green due to the easy separation from the spikelets (Vasey, 1889), and for its quality, which they considered better or sweeter (Kelly, 1974). They made giant piles of green seeds and set fires between them or spread the seed in the sun to dry. Still, much of the harvesting was done by gathering the seeds from the seashore, where the high tides had deposited the mature seeds as beach drift. The dried material was then taken to a dirt threshing floor where the Indians would walk upon the seed heads to separate the grain from the chaff. Then, with the help of

the wind they would separate the seeds from the chaff. Kelly (1974), in his Cocopa Ethnography mentions D. palmeri as a staple food and sometimes as the only food the Indians would have in early Spring. They stored the grain in wicker baskets which were placed on stilts to keep rodents and other animals out.

The grain was roasted or ground on a metate to make a flour. This was then used to make bread, mush (atole), and to condiment their soups (Vasey, 1889; Castettler and Bell, 1951; Kelly, 1974; Williams, 1975, and Yensen and Yensen, 1984).

Today, unfortunately, it is very hard to find these seeds. From the 40,000 to 50,000 acres described by Palmer and others, there is almost nothing left. Only recently have a few plants been rediscovered along the tidal channels of the estuaries (Yensen and Yensen, 1983). The devastation of these once vast fields is due primarily to the changing river channels, and upstream diversion for irrigation. The river channeling and deltaic changes also serve to block the tidal waters from reaching the former fields. The rareity of plants and accessible collection sites (most of the area is covered with deep mud and patches of quicksand), has made it difficult to collect sufficient material for analysis. Hence, with the small amount of initial material, this study began with a proximal analysis of the seeds.

Materials and Methods

Seed Collection and Cleaning

The seeds were collected on the Colorado River Delta. Collection was done by sweeping up seed-laden beach-drift into small piles using long-handled brooms. The seeds, including sand, rocks, crab carapaces, etc. were then passed over a screening device that excluded sticks, rocks, sand, etc., and a more or less "clean" sample of seeds and chaff was obtained. The samples were then transported to Tucson, Arizona for further cleaning.

The Indians threshed their seeds by beating them with sticks and/or stamping on them with their feet (Vasey, 1889). For the present studies, a hammer mill was used to separate the seeds from the husks. This was followed by passing the milled material through a "clipper" seed separator, which removed extraneous sand, rocks, seeds, and husks at once. The seeds were then brought to the laboratory where they were washed and dryed with the help of fans. The final separation of the seeds from little rocks was done by hand, a rather tedious step. And, finally they were stored either at room temperature or in the refrigerator, to prevent any insect or rodent attack.

Seed Analysis

Once the seeds were clean, they were initially ground by using a Wiley mill (mesh 30). It was later found that a Weber mill was more effective (mesh 24), and that a more homogeneous sample was obtained. The latter flour was used for the preparation of the mice diets (see

next chapter).

The nitrogen determination was done by the micro-Kjeldahl procedure. A sample od 200 mg (mesh 30) was put in a 30 ml flask with 5 ml of the digestion solution (sulfuric and phosphoric acid 3:1 by volume). Approximately 100 mg of the catalyst was also added. The catalyst was a mixture of 0.05 g of Henger's Selenium, 0.15 g of copper sulfate (anhydrous), and 0.05 g of sodium sulfate (anhydrous). The mix was then placed in a digestor until the liquid became clear (2-3 hrs.). This was then transferred to a 25 ml volumetric flask and aliquots were taken to determine the nitrogen content by titration with hydrochloric acid using modified methyl red as indicator. The indicator was made by mixing 0.125 g of methyl red and 0.0825 g of methylene red in 100 ml of ethanol. The protein conversion factor for mg of protein (PCF) was:

$$PCF = \frac{N\ HCl \times 0.014\ g\ nitrogen \times 6.25\ g\ protein \times 1000\ mg}{1\ meq.\ wt \times 1\ g\ nitrogen}$$

The formula for the percent protein then was:

$$\% \text{ Protein} = \frac{ml\ of\ titration \times prot.\ conversion\ factor \times dilution}{sample\ weight\ (mg)}$$

Crude fat was determined by weighing 2 g of the flour in a Goldfisch beaker previously dried at 100°C for 2 hours. The sample was then placed in the oven overnight at 100°C. The extraction was done

with hexane for 4 hours in a Goldfisch apparatus (AOAC, 1960).

For the acid detergent fiber determination 2 g of the dry flour was measured into a 600 ml beaker. One hundred ml of cold 2% hexadecyltrimethylammonium bromide dissolved in 1N sulfuric acid was added. Two ml of decahydronapthalene was added as an antifoamant and to facilitate the removal of pigments. The beaker was then heated to boiling (10 to 12 min to bring to a boil) and refluxed for 60 min from the onset of boiling. The fiber was then filtered on a dry, tared sintered glass crucible using light suction and the later washed with hot deionized water (90-100^{o}C). The material was also washed with acetone until washing appeared clear. The crucible was then placed in the oven at 100^{o}C for 2 hr. The acid detergent fiber value was determined by subtraction of the dry crucible weight (Van Soest, 1963).

Ash determination was done by weighing 5 g of the flour into an acid-washed, tared crucible. The crucible was placed into a muffle oven at 550^{o}C overnight. The ash value was calculated by subtraction of the original sample (AOAC, 1960). An acid washed crucible was used with the purpose of saving the ashes for a later determination of minerals.

Moisture content was determined by weighing 2 g of the material in a tared crucible and placing it in an oven at 100^{o}C overnight. The moisture value was then determined by subtraction of the original weight (AOAC, 1960).

Gross energy was measured by using the Parr Bomb Calorimeter (Oxygen Bomb Calorimetry amd Combustion Methods, 1968). One g of the sample was weighed into a metal crucible. The bomb head was then placed

on the support stand and 10 cm of platinum fuse wire were attached to the electrodes. The crucible was then placed into the loop holder and the fuse wire was adjusted so that it was close to the surface but not touching it. Approximately 1 ml of deionized water was then placed in the bomb cylinder. The bomb head was then placed into the cylinder carefully trying not to disturb the sample. The calorimeter bucket was filled with 2000 g of deionized water. The bomb was then filled with oxygen and the ignition buttom was pressed. The temperature then was carefully read. After final temperature reading, the bomb was turned off, and the sample was removed from the calorimeter. The sample was put into a 100 ml beaker, washing the interior of the bomb cylinder with deionized water. The washings were titrated with standarized 0.0725 N sodium hydroxide using methyl red as indicator. The gross energy or gross heat of combustion was then calculated as follows:

$$Hg = tW - e^1 - e^2 / m$$

Hg = gross heat of combustion in cal/g

t = net corrected temperature rise in degrees F.

W = energy equivalent of the calorimeter in cal/degrees F.

e^1 = correction for heat of formation of nitric acid in calories (1 cal/ml 0.0725 N NaOH).

e^2 = correction for heat of combustion of the fuse wire

m = mass of the sample in grams.

Carbohydrate determination was done by following the anthrone method of Clegg (1956). A sample of 200 mg was weighed into a 50 ml centrifuge tube and 5 ml of deionized water was added. Twenty five ml of hot 80 % ethanol was then added and the mix was stirred throughly and allowed to stand for 5 min. Then it was centrifuged at 10,000 rpm for 20 min. The supernatant was saved for the determination of sugars. The extraction was repeated with 30 ml of hot ethanol, stirred well and allowed to sit for 5 min. The mix was centrifuged again. After saving the supernatants the pellet was dried in an oven at 60^{o}C for an hour to evaporate all residual ethanol. Five ml of water was then added and stirred vigorously with the "super-mixer". Then. 6.5 ml of 52% perchloric acid was added and stirred again. The tube was then placed in a shaker for an hour or longer until the pellet was completely broken up. Twenty ml of water was added and the mixture was centrifuged again. The supernatant was decanted into a 100 ml volumetric flask. The procedure was repeated for 30 more min or until the pellet was well dispersed. All the materials (solids, acid and water) were placed in a 100 ml volumetric flask. Several ml were then filtered through ± 1 Whatman paper, discarding the first 5 ml of the filtrate. One ml of the filtrate was then placed into a 10 ml volumetric flask and brought up to the mark with water. Aliquots of 1 ml were then put into 15 ml screw cap test tubes and run in triplicate. To each tube, 1 ml of water were added followed by 10 ml of the anthrone reagent. The tube was then capped and inverted several times to mix the contents. Then, they were placed in a boiling water bath for exactly 12 min. The tubes were then

placed in iced water to stop the reaction. Once cooled the absorbance was read in an spectophotometer at 630 nm. A blank was made by using 2 ml of water and then adding the reagent and following the procedure mention above.

For the sugar determination the supernatants were placed in a flask and then concentrated on a rotary evaporatorr. Five ml of water were then added to solubilize the residue and then diluted to 1:50 with water. A 1 ml aliquote was then placed in a 50 ml volumetric flask and brought to volume. One ml aliquots were taken and 1 ml of water and the anthrone reagent were added. The rest of the determination was run using the starch determination. A standard curve was prepared by dissolving 20 mg of glucose in 100 ml of water and taking aliquots to make the proper dilutions. The values for the absorbance as well as the concentrations were then plotted and a linear regresion curve was drawn. From this curve it was posible to calculate the quantity of sugars and starch in the sample.

The mineral analysis was run on the ashes (from the ash determination), by first washing the ashes with concentrated nitric acid (5 ml). The acid was later slowly evaporated and the sample was put in the muffle oven at 550^{o}C overnight. The ashes were then dissolved in 10 ml of 5% hydrochloric acid. From this solution, dilutions were made for analysis by atomic absorption spectrophotometer, Hitashi Model No. 180-70. Standards were also run to determine the range of sensibility of the apparatus. Analyses were made for the following minerals: Na, Zn, Fe, Cu, Mg, and Ca.

The gluten determination was run by using the method described in the Modern Cereal Chemistry book (Kent-Jones, 1947). For this purpose, 20 g of the sample were made into a normal dough with the requisited amount of water. First, the dough was allowed to stand in a bowl of tap water for one hour at room temperature. Then the dough was knead by hand under a gentle stream of tap water and washed for 10 min. The weight of the remaining dough was recorded after drying in an oven at 100^{o}C for 24 hours.

Results and Discusion

Whenever a "new" or non-conventional plant is analysed, it is typically compared to another as a basis for evaluating its nutritional potential. Therefore, the grain of this halophytic grass, Distichlis palmeri, was compared to some of the grains that it resembles the most... wheat, oats, barley and brown rice. The composition of these grains is given in various tables for comparison with the D. palmeri grain.

All analyses were done on homogeneous samples and the data are reported on a dry weight basis. Table 1 shows the proximal analysis comparison of D. palmeri seeds, wheat (hard red winter variety), oat, barley, and brown rice. The seeds of D. palmeri were found to have 8.7% protein, a rather low percentage if compared to wheat (13.7%), oat (13.2%), and barley (13.0%), but close to brown rice (9.5%). It should be mentioned that this is a wild variety that has a potential for improvement through breeding experiments. Because the wide varietion in D. palmeri found along the coast of the Gulf of California it could be crossed and ideally produce a higher protein content. Another way could be the breeding of D. palmeri with D. spicata, which has a higher protein content, 11.4% dry weight (Yensen, 1984).

The percentage of fat (1.8%) is comparable with that of wheat (1.9%), barley (1.9%), and brown rice (1.9%). The study of the composition of this fat, however, remains to be studied.

With respect to fiber, D. palmeri has more fiber (8.4%) than wheat (2.6%), barley (6.0%), and brown rice (1.0%), but a little less

Table 1. Proximal analysis of Distichlis palmeri, hard red winter wheat, oat. barley. and brown rice grain.

	Distichlis palmeri	Wheat[1]	oat[1]	barley[1]	brown rice[1]
% Protein	8.7	13.7	13.2	13.0	9.5
% Fiber	8.4	2.6	11.9	6.0	1.0
% Fat	1.8	1.9	5.1	1.9	1.9
% Ash	1.6	1.9	3.8	3.4	1.2
% Carbohydrate	79.5	79.9	66.0	75.7	86.5
% Starch	69.6	65.5	-	72.0	-
% Sugar	3.8	-	-	-	-
Gross Energy Kcal/g	3.9	4.1	4.7[2]	4.5[3]	4.1

1 Reference: National Academy of Sciences, 1971.
2 Oat grain, rolled.
3 Barley grain, ground.

than oat (11.9%). This high value of fiber could be of benefit to people who need increased amounts of fiber in their diets. More studies are needed to determine the nature of the fiber.

The high content of carbohydrates (75.6%) makes this seed a good source of starch, and suitable for bread making (Table 1). When compared to the other grains, D. palmeri grain has a little more carbohydrate than oat (66%), and wheat (65.5%), a little less than brown rice (86.5%), and almost the same quantity as barley (75.7%). Still, there is a need for an in-depth study on the characterization of the starch and sugars present in these seeds.

The Cucapa Indians used to make bread with the flour of D. palmeri, therefore an attempt was made to make bread in the laboratory and find out if the seeds contained gluten present. The flour dough was found to be very elastic, like a mixture of wheat flour with corn flour. Oddly, however, when the gluten test was performed in Tucson as well as at Kansas State University, it was found that no gluten was present in the flour of D. palmeri grain. These test are not conclusive, however, since more detailed studies are needed to really establish if this halophytic grain has gluten. It is proposed that it is the kind of starch present in the flour that gives the dough its plasticity. More test should be run, then, to evaluate the quality of the starch as well as its composition. Several other studies have concluded that pentosans, a non-starch polysaccharide, as well as gluten, can give dough plasticity (Hosney, 1969 and Hosney, et al, 1984). The characterization of the carbohydrate present in this fascinating grain

is open for future research.

The low ash value (1.6%), is also of great interest as the plant normally grows in seawater. Apparently, the seed does not accumulates salts. The plant does excrets the salts through bicellular salt glands on leaves, as evidenced by salt crystals that can be seen on the leaves. Compared to other grains, D. palmeri grain has much less ash content than wheat (1.9%), oats (3.8%) and barley (3.4%). This is particularly noteworthy if it is taken into consideration that these other plants grow in fresh water. Brown rice, however, has a little lower ash content (1.2%) than D. palmeri grain.

The values for P, Na, Zn, Fe, Cu, Mg, and Ca in D. palmeri, are shown in Table 2. They are all lower than those for wheat, oat, barley, and brown rice, with the exception the Na, Mg and Ca contents. From a mineral composition standpoint D. palmeri grain most closely ressembles brown rice.

It is interesting that even though it has a high value for Na (0.3%), D. palmeri grain does not taste salty. The analysed grain was taken from plants growing in waters with 40 ppt of salt, therefore it is clear that a low uptake of salts is not critical to the production of salt-free grain. It should also be taken into consideration that this plant can be grown inland with water salinities less than 10 ppt. In that case, the accumulation of salts will even be less of a problem. It should be kept in mind, however, that conventional crops do not grow well with waters above 5 ppt, a salinity that is almost ideal for many halophytes, including D. palmeri.

Table 2. Mineral comparison of *D. palmeri* grain, hard red winter wheat grain, oat, barley, and brown rice.

Mineral	*D. palmeri*	Wheat[1]	oat[1]	barley[1]	brown rice[1]
P (%)	0.2	0.43	0.37	0.45	0.28
Na (%)	0.3	0.10	0.08	0.07	0.04
Mg (%)	0.008	0.12	0.18	0.15	0.10
Ca (%)	0.003	0.05	0.10	0.08	0.05
Cu (ppm)	4.20	6.60	9.30	6.50	3.90
Fe (ppm)	20.00	40.00	80.00	90.00	30.00
Zn (ppm)	23.10	53.40	-	-	-

1 Reference: National Academy of Sciences, 1971.

PROTEIN QUALITY

Introduction

As with every non-conventional crop, a protein evaluation is necessary to help evaluate the potential use of a "new" plant for animal or human consumption. For example Amaranthus spp., a salt tolerant plant, that was used by the Aztec Indians in Mexico. They mixed the seeds with honey and/or syrup to make an idol which they ate as a part of their rituals. After the Spaniards conquered Mexico, however, this plant was banned because of the religious implications that its use was ligated to (Cole, 1979). But Amaranthus seeds are still in use in Mexico, India and other countries. They have an unusually high content of lysine, which makes its protein even better than that of wheat (Smith et al, 1969). Another example of protein crops is poppy seeds which have been found to have a high quality protein (Eklund, 1975). Likewise, Thompson, et. al. (1978), has also evaluated the protein of Buffalo Gourd, (Cucurbita foetidissima, HBK), another wild plant that grows in the Northern part of Mexico and Arizona. Much of Buffalo Gourd's value is due to its resistance to drought, its high content of protein (33% crude protein) and its starch. These characteristics make this plant a suitable one for desertic areas where water is a problem.

In the present case of D. palmeri grain, we find that there is the precedent for its use as a human food. In the past, the Cucapa Indians used to make bread and atole (mush) with the seeds. Although

this clearly demonstrates it to be edible, a qualification of its protein is still necessary to evaluate its potential for future uses.

Materials and Methods

The seeds were first ground in a Weber mill (mesh 024). Twenty five ml of 6 N hydrochloric acid were added to a 50 mg of the sample contained in a flask, covered with an inverted beaker, and placed in an autoclave at 121°C (15 psi) for 16 hours (the analysis was done in duplicate). The autoclaved samples were then placed in a rotoevaporator to extract the acid and to wash the samples with water. Once washed the samples were suspended in sodium citrate buffer (pH 2.2), and filtered. To one of the samples 100 mg of sodium thioglycollate was added. The amino acid analysis was carried out in a two column Beckman 121 Automatic Amino Acid Analyser (Spackman et. al., 1958). The aminogram was done in duplicate. And, to one of the samples, sodium thioglycollate was added.

Acidic hydrolysis typically destroys all tryptophane present, so in the present study, two other methods were used to determine tryptophane. The first method was a colorimetric analysis based on the Hopkins-Cole method (Vollmer, 1972). Duplicate 100 mg samples were hydrolyzed in 10 ml of 4N sodium hydroxide in covered, calibrated, centrifuge tubes which had been placed in an autoclave overnight at 121°C (15 psi). The pH was then adjusted to 7 using sulfuric acid, water and phosphate buffer (pH 7). Then the sample was centrifuged at 2500 rpm for 20 min. From here, two 2 ml aliquots of supernatant were drawn and placed in 30 ml beakers in an ice bath, under the hood. Four

ml of acetic-iron-glyoxylic solution were then added with agitation followed by 4 ml of concentrated sulfuric acid. Once cooled, the beaker was allowed to stand at room temperature for 20 min. The samples were then read spectophotometrically at 545 nm. A standard curve was made to determine the tryptophane concentration in the samples.

The second method used for the tryptophan determination was a microbiological method (Greene and Black, 1944),which was done at the Center for the Coordination of the Research (CCI) at the University of Sonora in Mexico. The test was done in duplicate, and 1 g of the ground sample (024 mesh) were weighed into 125 ml EM flasks. Twenty five ml of 6N $Ba(OH)_2$ were added to each flask and then autoclaved for 7 hours at 121°C (15 psi). The pH was then adjusted to 7.0 with H_2SO_4 and the sample was centrifuged to separate the $BaSO_4$ precipitate. The supernatant was collected and the precipitate washed twice with deionized water. The supernatant and the washings were transferred to a 100 ml volumetric and brought to volume with deionized water. The samples were then filtrated using Whatman ±2 paper and aliquots were taken from this filtrate to make the proper dilutions for the tryptophane determination (the final TRP concentration should be about 2 ug/ml). The microorganism used was Lactobacillus arabinosus 17-5 which was previously prepared in Tryptophane Assay Medium, made accordingly with the instructions and autoclaved at 121°C (15 psi) for 15 min. The microorganism was incubated in 10 ml of the medium contained in test tubes, at 37°C for 16-24 hours. Then centrifuged and washed 2 times with the same medium. The final suspension of cells was used for the

inoculation of the assay tubes containing different concentrations of the sample. The incubation was at 37 ^{o}C for 72 hours and acid production was measured. The acid production was meeasured by titrating with 0.1 N NaOH using bromothymol blue as indicator. A standard curve was prepared in the same way by using different concentrations of trytophane in the media (0, 1, 2, 3, 4, 5, 6, 8 and 10 ug/ml).

For the feeding trials two experiments were conducted. In the first experiment, 3 diets were used. One using D. palmeri flour as the sole source of protein and carbohydrates; and the others were made with whole egg and lactalbumin as control proteins (table 3). The diets were formulated to supply all the nutrients in compliance with the NCR requirements for mice (National Academy of Sciences, 1972). The protein level for the 3 diets was approximately 7.5%. All diets were isocaloric and isonitrogeneous. Mice were used as the experimental animal due to the low protein content of the seeds (8.0% in the whole seed). Furthermore, mice eat less than rats in a daily basis, and at the time of the study, there was a shortage on D. palmeri seeds. Feed and water were supplied ad-libitum to Charles River CD-1 weanling mice for 3 weeks. There were 10 females and 10 males per treatment, housed one male and one female per cage. The feed intakes were measured twice a week, whereas the body weights were measured once a week. Feed and feces were collected for later analysis to determine the absorption of the nutrients.

In Experiment 2, five diets were prepared. The protein level was 7.5%, with the diets being isocaloric and isonitrogeneous. The 5

Table 3. Diet composition, in percentages, for experiment 1.

Ingredients	Diet 1 Egg	Diet 2 *D.p.*	Diet 3 Lact.
Whole egg (46%)	16.30	--	--
Distichlis palmeri	--	92.90	--
Lactalbumin	--	--	9.93
Cerelose	54.34	--	78.85
Corn Oil	6.22	2.20	3.00
AIN Vitamin Mix	1.00	1.00	1.00
AIN Mineral Mix	3.50	3.50	3.50
Cr_2O_3	0.20	0.20	0.20
Choline Chloride	0.20	0.20	0.20
DL-Methionine	0.05	--	--
BHT	0.002	0.002	0.002
Cellulose	7.00	--	7.00
Bentonite	11.19	--	0.32

% protein	= 7.50
% fiber	= 7.00
% avail. phosp.	= 0.50
Egg Kcal/g	= 3.68
Lact. Kcal/g	= 3.95
D. p. Kcal/g	= 3.90

treatments were: whole egg (as the control protein), D. palmeri flour, D. palmeri flour plus DL-methionine, D. palmeri flour plus DL-methionine and lysine, and D. palmeri flour plus DL-methionine, lysine and threonine (table 4). These amino acids were the first 3 limiting in the protein of D. palmeri. Feed and water were supplied ad-libitum to Charles River CD-1 weanling mice for 3 weeks. The mice were housed one male and one female per cage for a total of 20 mice per treatment. The feed was measured twice a week and the body weights once a week. Feed and feces were collected by treatment. In both experiments the protein content of the diets was analysed prior to the feeding to assure that the right protein content was given.

Table 4. Diet composition, in percentages, of experiment 2.

Ingredients	Diet 1 Egg	Diet 2 *D.p.*	Diet 3 *D.p.* +MET	Diet 4 *D.p.* +MET +LYS	Diet 5 *D.p.* +MET +LYS +THR
Whole egg (46%)	16.30	--	--	--	--
D. palmeri (8.05%)	--	92.20	92.20	92.20	92.20
Cerelose	54.34	0.70	0.45	0.117	--
Corn Oil	6.22	2.20	2.20	2.20	2.20
AIN Vitamin Mix	1.00	1.00	1.00	1.00	1.00
AIN Mineral Mix	3.50	3.50	3.50	3.50	3.50
Cr_2O_3	0.20	0.20	0.2	0.20	0.20
DL-Methionine	0.05	--	0.255	0.255	0.255
Lysine	--	--	--	0.33	0.33
Threonine	--	--	--	--	0.11
BHT	0.002	0.002	0.002	0.002	0.002
Cellulose	7.00	--	--	--	--
Bentonite	11.19	--	--	--	--
Choline Chloride	0.20	0.20	0.20	0.20	0.20

Results and Discusion

A comparison of the amino acids in Distichlis palmeri grain and wheat (hard red winter variety), are given in g/16 g of N (Table 5). With some exceptions the amino acids are similar to the ones in wheat. Apparently D. palmeri grain has slightly more lysine than this variety of wheat but much less cystine. The values for tryptophane were not given for this particular variety of wheat, but other varieties average 1.4 g/16 g of N (Harvey, 1970). D. palmeri, in contrast had 4.05 g/16 g of N. It was a surprise to find such a large quantity of tryptophane in the seeds of D. palmeri. This value (4.05 g/16 g of N), was obtained using the colorimetric method (Vollmer, 1972). Hence the other method, microbiological, was done to assure that a mistake was not made. The value obtained through that method (4.02 g/16 g of N), was very close to that from the colorimetric analysis (4.05 g/16 g of N). Table 5 also shows the amino acid composition of whole egg. When compared to whole egg, D. palmeri amino acid composition becomes really deficient in most of the amino acids. It should be taken in consideration, however, that the seeds used for the experiments were part of a wild variety, and as such subjected to improvement through breeding programs.

Table 6 shows the essential amino acids of both D. palmeri and whole egg. Like other cereals, a deficiency was found in most of the amino acids when compared to whole egg. Table 6 also shows the requirements of the essential amino acids for human adults. The protein of D. palmeri full fill these requirements and in most of the cases almost doubling the amount of the essential amino acids. The exception,

Table 5. Amino acid comparison, in g/16g N, of *D. palmeri* grain, hard red winter wheat grain, and whole egg.

Amino acid	*D. palmeri*	Wheat	whole egg[1] (dried)
Lysine	3.1	2.8	7.2
Histidine	2.1	2.4	2.4
Arginine	4.3	5.0	6.5
Aspartic acid	5.5	5.4	10.9
Threonine	2.7	3.1	5.1
Serine	3.5	5.0	8.2
Glutamic acid	16.0	34.2	12.5
Proline	0.4	11.0	3.7
Glycine	3.1	4.5	3.4
Alanine	4.6	3.8	6.2
Cystine	0.01	2.8	2.3
Valine	4.7	4.7	5.9
Methionine	1.6	1.7	3.5
Isoleucine	3.1	3.8	6.0
Leucine	5.4	7.1	8.1
Tyrosine	2.6	3.2	3.4
Phenylalanine	3.2	5.0[2]	6.8[3]
Tryptophan	4.1	1.4[2]	1.5[3]

1 Reference: Harvey, 1970.
2 Average of several varieties.
3 Average for whole egg.

Table 6. Essential amino acid composition as g/16 g of N for whole egg and Distichlis palmeri grain compared to the requirements for human adults.

Amino acid	whole egg[1] (dried)	D. palmeri	Req. for humans[2]
Lysine	7.2	3.13	2.2
Histidine	2.4	2.09	0.0
Threonine	5.1	2.72	1.3
Valine	5.9	4.70	1.8
Methionine + Cystine	5.8	1.61	2.4
Isoleucine	6.0	3.08	1.8
Leucine	8.1	5.42	2.5
Phenylalanine + Tyrosine	10.3	5.8	2.5
Tryptophane	-	4.05	2.5

1 reference: Harvey, 1970.
2 reference: World Health Organization, 1973.

however, were the sulfur amino acids methionine and cystine. Although it could be possible that a mistake was done when the amino acid determination was done and consequentely obtaining lower values of these amino acids.

The results for Experiment 1 are shown in Table 7 where the body weight gains, % of absorption (% Abs), and protein efficiency ratios (PER) from Experiment 1 are given. With D. palmeri as the sole source of protein, the animals did gain weight, but not much (6.4 g for D. palmeri, 12.7 g for lactalbumin, and 17.9 g for whole egg) . Still, life was supported and the animals did not appear sick. If the study had had wheat as a second control, it would be expected the results for Indian wheat to be very similar to commercial wheat because wheat also lacks of some of the essential amino acids (specifically, lysine and arginine). The percent absorption is also given in Table 7. The highest absorption was obtained when lactalbumin was fed, 86%, followed by whole egg, 80%, and D. palmeri grain, 74%. The absorption obtained for D. palmeri protein was similar to that for cereals, between 75% and 80% (Lloyd, 1978).

The second experiment was designed to test D. palmeri protein with the supplementation of the first three limiting amino acids. Table 8 shows the results of Experiment 2. There was a significant improvement in the body weights with the supplementation of methionine, lysine and threonine than without them (7.0 g for D. palmeri alone and 12.3 g for D. palmeri plus the three amino acids). There was also a significant difference in the body weight gains between the control diet

Table 7. Experiment 1. Total body weight gain (g), % Absorption and PER (corrected) of mice fed D. palmeri grain, lactalbumin, or whole egg.

Treatment	Total body wt. gain (g)	% Absorption	PER
Whole egg	17.9	80	2.50
Lactalbumin	12.7	86	2.02
D. palmeri	6.4	74	1.31

Table 8. Total body weight gains (g), % Absorption and PER from Experiment 2.

Treatment	Total body wt. gain[1] (g ± S.E.)	% Absorption	PER[1] (±S.E.)
Whole egg	17.1 ± 0.40[b]	82	2.55 ± 0.07[d]
D. palmeri	7.1 ± 0.33[a]	79	1.29 ± 0.06[a]
D. p. + MET	8.3 ± 0.60[a]	78	1.44 ± 0.09[ab]
D. p. + MET + LYS	7.2 ± 0.34[a]	78	1.53 ± 0.06[b]
D. p. + MET + LYS + THR	12.3 ± 0.55[c]	77	1.90 ± 0.05[c]

1 values with different letters are significantly different at the 0.005 % level.

and the diet supplemented with the 3 amino acids (17.3 g for whole egg and 12.3 g for D. palmeri plus the 3 amino acids). The same applies to the PER's. There was a significant difference between the diet not supplemented, 1.27, and the ones supplemented with either 2 or 3 amino acids (1.4 and 1.52 respectivelly). Still, there was a significant difference between the control diet and the one with D. palmeri plus the 3 amino acids (2.57 and 1.90 respectively). The percent absorption for D. palmeri was a little lower than the one with whole egg (79% and 82% respectively). Moreover, although the % absorption was a little lower in the supplemented diet than in the unsupplemented one, the difference was not too large (79% vs 77%), and probably not significant. The animals look healthy during the experiments and no mortalities were observed. After the experiment was finished some of the animals were sacrified and an autopsy was done to observe their internal organs. Nothing abnormal was observed in their organs. This could indicate that the seeds of D. palmeri may be used safely and that no dangerous anti-nutrients are present in the seeds. Still, it is suggested that another trial should be done to include the first 4 limiting amino acids. In this was the diet will be more comparable to whole egg, and hopefully the PER and weight gains will also improve. Although it is already known that the Cucapa Indians ate the seeds, more studies need to be done in order to fully evaluate the safety of consuming any wild plant.

PHYTATE DETERMINATION

Introduction

With the increasing interest in wild plants for food uses, a serious problem has to be faced: the lack of knowledge about the possible antinutritional factors that a wild plant may have. Even in domesticated crops, such as sorghum and wheat, antinutritional factors, such as phytates, are also present. Oberleas (1973), has stated that the majority of the phosphorus found in plant seeds is present as phytates [myo-inositol 1,3,4,5,6-hexakis (dihydrogen phosphate)$\frac{1}{4}$

Current interest about phytates in sorghum and wheat grains as well as in other plants is due to the chelation, by the phytates, of some essential minerals. It has been found that Fe, Ca, Zn, and Mn bind with the phytates present in the seed, thus making them unavailable for absorption, and consequently, lowering the utilization of these minerals by the body (Davies and Nightingale, 1975, Oberleas, 1973). Moreover, phytates form insoluble compounds with proteins at pH below their isoelectric points (Barre, 1956, Cosgrove, 1966). Because of this problem, it is important to know the phytate content of any new grain that is intended to be used as food.

Materials and Methods

There are several ways to determine the phytate content in a sample. One approach is to hydrolyze the inositol compound to form the free phosphate and inositol and to analyse the hydrolysate for inositol. The free inositol can then be analysed either by microbiological or by chemical methods (McKibbin, 1959, Eagle et. al., 1960). The other approach is to hydrolyze the phytate with an acid and determine the percentage of phosphorus. Because phytates have 28.18% phosphorus in their molecules, a simple equation will predict the percent of phytate in the sample, given that other phosphorus compounds are not likewise hydrolysed or, if so, their quantity known. The acid hydrolysis was used in this study and the phosphorus determination was done by two methods.

The first method used for the phytate determination was a modification of the Harland and Oberleas method (1977), done by Thorn (1981). Approximately 500 mg of the sample (mesh 30) were placed in a centrifuge tube with 10 ml of 1.2% hydrochloric acid. The analysis was done in duplicate. The tubes were placed in a water bath at 37^{o}C with agitation for 2 hours. The tubes were then centrifuged at 10,000 rpm for 20 min. The supernatant was filtered through Whatman ±1 filter paper. A 5 ml aliquot was taken from the filtrate and placed in a 25 ml volumetric flask and made up to volume. A 2 ml aliquot was then taken and passed through a column containing an anion exchange resin (Ag-1-x8), previously saturated with 0.7 M sodium chloride. The inorganic phosphorus was eluted with 15 ml of water followed with 30 ml

of 0.05 M sodium chloride and collected in a 50 ml volumetric flask that was later brought to volume with deionized water. The phytate was then eluted with 17 ml of 0.7 M sodium chloride and collected in a 30 ml micro-Kjeldahl flask. 0.5 ml of sulfuric acid and 3.0 ml of nitric acid were then added to the flask and it was placed in the digestor for 1.5 hours or until the orange fumes disappeared. After this, approximately 10 ml of deionized water were added and the flask was placed in a boiling water bath for 15 min. (to hydrolize pyrophosphates). The material was then transferred into a 25 ml volumetric flask and made up to the mark with deionized water.

To determine phosphorus, aliquots of the solutions were taken (8 ml from the inorganic phosphorus and 5 ml from the phytate), and transferred to a 10 ml volumetric tube. One ml of molybdate solution and 0.5 g of ferrous sulfate were added to the tube. The mix was then brought to volume with deionized water and left for 15 min. to develop a blue color. This solution was read in a Coleman Jr. Spectrophotometer at 660 nm. A standard curve was also prepared by making phosphorus dilutions (0.5, 1.0, 1.5, and 2.0 mcg P/ml). The percent phosphorus was then calculated with the following formula:

% P= Corr.Abs. x K x Dil. factor x 100 / sample weight (g).

K = Standard conc. (mg/ml) / Absorbance

The second method was a variation of the Doherty method (1982)

by Michael Kopplin (unpubl. data, 1983). Approximately 1 g of the ground sample (mesh 024 in the Weber mill), were placed in a centrifuge tube and 10 ml of 3 % trichloroacetic acid were added. The tube was stirred overnight at 37°C and then centrifuged at 12,000 rpm for 20 min. The residue was washed with deionized water twice. The residue was dissolved in 50 ml of deionized water (solution 1) and from this mix total phosphorus and phytates were analyzed. A 10 ml aliquot was taken from this mix and 4 ml of 20 mg Fe^{+3}/ml 5 % Na_2SO_4 in 1.2 % HCl were added. This mix was then heated at 100°C for 20 min. and centrifuged. The residue was resuspended in 5 ml 10% Na_2SO_4 in 1.2 % HCl heated at 100°C for 10 min. and centrifuged. The residue was transferred with small washings (to make a total of 5 ml) to a 30 ml Kjeldahl flask for phosphorus determination.

The phosphorus determination was done by adding to the Kjeldahl flask 0.25 ml of concentrated sulfuric acid and 1.5 ml of concentrated nitric acid. The flask was placed in the digestor for 2 hours. Fifteen ml of deionized water were then added and the mix was heated at 100°C for 15 min. The mix was then quantitatively transferred to a 50 ml volumetric flask and made up to volume. Three 1 ml aliquots were drawn from here to 10 ml volumetric tubes. One ml of molybdate solution plus 50 mg of ferrous sulfate were added and the solution was brought to volume with deionized water. The solution was left to develop a blue color for 15 min. and then measured its absorbance at 660 nm in a Spectronic 20. Total phosphorus was done by taking 10 ml from solution 1 and analyzed as described before. A standard curve for phosphorus was

made to determine the percentage of phosphorus. The equation used was the same as in the first method described for the phytate determination.

To assure a correct value for the percentage phosphorus a total phosphorus analysis was done with an acid hydrolysis. For this 2 g of the ground material were weighed into a 125 ml EM flask. Ten ml of concentrated nitric acid were added and the material was allowed to sit under a hood overnight. The material was then digested for 1 hour until heavy fumes came off. The heating was continued until the fumes subsided. Then 15 ml of perchloric acid were added, replacing the flask on the heat. The heating was continued until the liquid was clear and low in volume. The liquid was then transferred to a 100 ml volumetric flask. Total phosphorus was determined by pipeting triplicate 1 ml aliquots into 10 ml volumetric tubes. The determination was done as described before.

Results and Discusion

Phytate in foods and feedstuff have been found to have some antinutritional effects in laboratory animals and in humans (Davies and Nightingale, 1975, Cosgrove, 1966, Oberleas, 1973). Moreover it is estimated that 40-80% of the phytate phosphorus in cereals is available to man (McCance and Widdowson, 1935). Still, more research has yet to be done to fully evaluate the nutritional implications that phytates could have in humans. Furthermore, it was reported that the reduced availability of essential minerals by phytates or phytate-protein complexes depend on several factors, one of them being the concentration of phytic acid in foodstuffs (Rackis and Anderson, 1977).

The results show that D. palmeri has a low content of phytates (0.46%), whereas wheat and other cereals have a higher content. The percentages for phosphorus, phytates, and for total phosphorus (method III) are given in Table 9. The data show a great discrepancy between the results, in method I the % phytates was 0.11 and in method II it was 0.46. The percentage of phosphorus was also different for both methods, 0.02 and 0.16 for methods I and II respectively. These discrepancies were the reasoning behind getting the total phosphorus content of the sample (method III). An approach to this difference in values could be that the acid hydrolysis in the first method was not strong enough to hydrolyze the phosphates from the inositol compound. Whereas in the second method the TCA did hydrolyze the compound more effectively. Although the values for the percent phosphorus in methods II and III differ, they were closer than those obtained with method I. This

Table 9. The determination of % P and % phytate in *D. palmeri* grain with three different methods.

Method	% phosphorus	% phytate
I	0.02	0.11
II	0.16	0.46
III	0.20	ND

ND= not determined.

suggests that the phytate content of D. palmeri grain is near 0.46%.

Table 10 shows the phytate content of different grains compared to D. palmeri grain. In this table wheat bran (4.59-5.52%), and sesame seed (4.71%), show the highest values for phytates, followed by wild rice (2.20%), and wheat grain (0.62-1.35%). Still, D. palmeri grain showed the lowest percent of phytates (0.46%). This low phytate value may be advantageous according to Rackis and Anderson (1977), eventhough the phosphorus content in D. palmeri (0.2%), is also lower than in wheat (0.43%). Still, more studies are needed to better evaluate the phytate content of this peculiar grain.

Table 10. Phytate content of wheat, wild rice, soybean, sesame seed and *D. palmeri*.

	% phytate
Wheat grain	0.62-1.35
Wheat bran	4.59-5.52
Soybean	1.00-1.47
Sesame seed	4.71
Wild rice	2.20
D. palmeri	0.46

Reference: Reddy, 1982.

CONCLUSIONS

In recent years, the search for new salt and drought resistant crops has begun. It is important for underdeveloped, as well as developed countries, to find new crops that can stand the salinities caused by extensive irrigation. Halophytes are now being studied around the world to solve in part this problem. Distichlis palmeri a salt grass that produces a wheat-like grain could be part of the solution for the salination problem. This plant was used in the past by the Cucapa Indians from the Colorado River. They used to make bread and mush (atole) from the flour. The flour was also added to their soups. Unfortunately today D. palmeri seeds are very rare due in part to river channeling.

Although this plant had been sought for some time, three years ago the search for this particular plant begun in ernest. The variety used by the Cucapa Indians was rediscovered (Yensen and Yensen, 1984), and sufficient seed was obtained to conduct nutritional analyses. It should be mentioned that the seeds came from a wild population with a high variability in size and color. This high variability, however, opens a new field for breeding. It is speculated that through breeding this plant can be improved considerably in protein content.

Distichlis palmeri seeds were found to have a rather interesting composition for a plant that grows in sea-water. Its fiber content (8.4%) makes it suitable for the American diet which is generally low in

fiber. Its high carbohydrate content makes this seed a good source of starch with the whiteness of the starch perhaps being a good indicator of its purity. The percentage of fat was found to be surprisingly very low (1.8%) when compared to wheat, rice and barley. The percentage of protein was relatively low (8.7%) but is in the range found for sorghum, milet and wheat.

Gluten was not found using an old conventional method Further analysis are necessary to establish if this halophytic grain has gluten. However, when the dough was made, it was found to be very elastic and have a consistency in between that of wheat and corn flour. It has been proposed that the pentosans present in the seed may give it its plasticity. Still, additional studies will be necessary to elucidate the rheological properties of the grain.

Another interesting finding was the seed's low value for ash (1.6%). For a plant that grows in sea-water, a higher mineral content would be expected. D. palmeri has the capability to excret the salts through its leaves, therefore the grain does not accumulate the salts. Moreover, it should be mentioned that this versatile plant can grow in fresh water as well as saline water up to 40 ppt. For example, D. palmeri grows well in water with 5 ppt of salts (NaCl), which is near the salt limit for most conventional crops. Distichlis palmeri also prodeuces seed under fresh water irrigation in either sandy or clay soils.

An amino acid profile was also done to determine the limiting amino acids in the seed. Methionine, lysine and threonine were found to

be the first three limiting amino acids in the protein. Two experiments were conducted with mice to evaluate for this protein. In the first experiment, whole egg and lactalbumin were used as the control protein. D. palmeri was fed as the sole source of protein, fiber and carbohydrate. With D palmeri, growth was supported but with low weight gains (6.4 g in three weeks). The corrected PER was 1.31. The second experiment was designed to test the protein when the first 3 limiting amino acids were supplemented. A significant improvement in the PER (1.90) and the weight gains (12.3 g) was found by amino acid supplementation. Still, these values were lower when compared to the control protein , whole egg. Therefore, another study using the first 4 limiting amino acids would be necessary to determine if the PER and weight gains can be improved up to the whole egg control. The protein absorption was found to be similar to other cereals (79%). The animals looked healthy throughout the experiment which could be an indication of the abscence of anti-nutritional factors in the seeds.

Phytate content (0.46%) was found to be lower than that for wheat, wild rice, sesame seed and soybean. The variability in the results suggested that more studies should be done to verify the real phytate content of D. palmeri seeds.

The nutritional study of Distichlis palmeri grain has just began. More studies are needed to complete the information about its nutritional value. While it is already known that these seeds were used for human consumption, one might speculate that this grain of the past may become the grain of the future.

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