CHARACTERIZATION OF THE PROTEINS AND FLOUR

OF *DISTICHLIS PALMERI* (VASEY) GRAIN

AND *DISTICHLIS SPP.* FIBER

by

Susana Bojórquez Yensen

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Susana Bojorquez Yensen

entitled Characterization of the Proteins and Flour of Distichlis palmeri (Vasey) Grain and Distichlis spp. Fiber

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy

Charles W. Weber — Date 9/8/95

Edward Sheehan — Date 9/8/95

Ralph L. Price — Date 9/8/95

Date

Scottie Misner — Date 9/8/95

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

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Charles W. Weber

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ABSTRACT

Proteins from a halophyte cereal *Distichlis palmeri*, whole-grain flour were fractionated on the basis of their solubility. Five fractions were obtained and each analyzed for fifteen amino acids. The amino acid pattern of the whole-grain was similar to that of a cereal. The percent of amino acids for the ethanol extraction ranged from a high of 35% glutamine to a low of 0.18% lysine. The fractions were rich in asparagine and glutamines. The lysine content of this grain is higher than that of wheat and the highest percentage (6.3%) was found in the water soluble fraction. Protein recovery of the different fractions ranged from 78% to 87%.

SDS polyacrylamide gel electrophoresis showed that the water soluble fraction had a wide range of molecular weight proteins; the NaCl fraction had circa 10 proteins in high concentrations; the prolamine fraction had a band at about 22,000 daltons with two weak bands at 45,000 and 66,000 daltons; the 2-mercaptoethanol fraction had few weak bands, and the SDS fraction had circa 6 bands present in high concentrations.

The foaming capacity and stability of the albumin fraction of *Distichlis palmeri* was excellent. The whole-grain flour of *Distichlis palmeri* had a better water and oil holding capacity as g/g protein. The emulsion capacity of the whole-grain flour was also better than in its protein fractions.

When evaluated with a Hedonic scale for taste preference there was no significant difference in flavor acceptance between the "exclusive" eaters and "frequent" eaters of whole-wheat products. Overall, the "exclusive" eaters of whole wheat rated *Distichlis*

palmeri muffins significantly higher than either the "occasional" eaters or the "frequent" eaters. The 50/50 mix received the highest rating.

The grain of *Distichlis palmeri* has 5.15% soluble fiber and 12.7% of insoluble fiber. No trypsin inhibitor activity was detected in the flour. The two forages (*Distichlis spp.)* analyzed had 6.10% and 15.20% of protein respectively. Acid detergent fiber was 33% and 20%. Low lignin was found in both grasses (6.8% and 5.10%). Mineral content for both forages was: Ca (0.3% and 0.5%); Mg (0.09% and 0.2%); P (0.12% and 0.21%); Zn (6.2 ppm and 20 ppm), and Cu (5.5 ppm and 6.7 ppm).

CHAPTER 1

INTRODUCTION

Agriculture, throughout the world, has been facing a growing problem: salinization of the lands. This is not a new problem. It is well known that in ancient Mesopotamia, as the soils became more salinized the yields for wheat came down. Barley, which is more tolerant to salt, was then used more and more, up to the point where even barley's yields were also too low. In this way, one of the major agricultural civilizations collapsed.

There are several reasons why land becomes salinized. The two major factors for the salinization of the soils are the irrigation methods currently used, and the intrusion of salty water into the fresh water supplies, i.e. agricultural regions close to the ocean.

One such place is in the Costa de Hermosillo, Sonora, Mexico, where the water table is currently being contaminated with the intruding sea water form the Gulf of California. As the wells became saltier the lands lost their use for agricultural purposes and the people abandoned the lands. These barren lands are now slowly eroding.

Currently, on a worldwide basis, of the cultivated lands, about 0.34 x 10^9 ha. (23% of the total cultivated lands) are saline and another 0.56 x 10^9 (37%) are sodic (Massoud, 1981). Most of these areas are in arid and semi-arid regions. According to the U. S. Department of Agriculture, the United States has a moderate to severe potential for a salt problem, especially in the West. A prime example of which is the San Joaquin Valley that was used to hold water from drains instead of dumping these waters to the San Francisco

Basin. As the water evaporated the soils became too salinized to be used for agriculture. This area now represents a pollution problem due to the erosion of its top soil that creates dust storms.

If we can utilize the salty water from the wells and/or utilize the abandoned salinized lands and grow a crop, for animal or human consumption, there will no longer be a waste of this resource. Countries throughout the world are taking measures to deal with this increasing problem. In Australia, for example, a National Programme for Productive Use of Saline Land was formed to help with the vast areas of salinized soils and waterlogging (Malcolm, 1993). This Commitee was formed to make productive use of the one million hectares that would be salt-affected land throughout Australia by the year 2000.

The salts in the water decreases the ability of plants to take up soil water, and thereby reducing the germination rate, plant growth, and subsequent yields. This problem has been puzzling agriculturalists for years. It used to be that whenever a piece of land was salinized the farmer would just move away from that area to solve his problem. As a result, there is essentially little new land for cultivation. Furthermore the problem continues to grow day after day.

Researchers have been trying to find a solution to land salinization, but with limited success. One possibility is to develop a strain of plants which isgenetically predisposed to salt tolerance.

Such genetic studies are, at best, a solution that typically does not provide immediate results. For instance Soybeans are a case in point as are some of the grasses, and peppers (Blum, A., 1988; Staples and Toenniessen, 1984).

However, there are plants that are already adapted to salty soils and waters. Plants were gathered by our ancestors for several uses. These plants are called Halophytes. Halophytes have evolved to withstand the harsh saline habitats created either due to man or climatic conditions.

Halophytic plants have adapted to salty environments in several ways. One way is the exclusion of the salts at the root level. Others, have salt glands that excrete the salts from the leaves as some tolerant grasses do. Others, like the succulents, accumulate water in the leaves, and in this way dilute the salt in the tissues.

This study deals with one halophytic grass: *Distichlis palmeri* (Vasey). This plant, from the family Poaceae, produces an edible seed similar to wheat and rice (Yensen, 1984). The nutritional analysis of the grain had been published already (Yensen and Weber, 1986, 1987). The purpose of this dissertation is to characterize the proteins and fiber of the grain and to evaluate its functional properties. In doing so we will get a better idea as to what food systems this new grain might be useful. By analyzing it's leaves, its potential as a forage ca be determined.

CHAPTER 2

LITERATURE REVIEW

Definition of salinization.

Salinity is defined as the concentration of dissolved mineral salts present in waters and soils on a unit volume or weight basis. The major solutes are the cations Na^+, Ca^{++}, Mg^+, and K^+, and the anions Cl^-, $SO_4^=$, HCO_3^-, CO_3^-, and NO_3^- (Tanji, 1990). Salinity in the water and soils is measured in various ways depending on the concentrations of the minerals, i.e conductivity, refractive index etc.

Salt tolerance of conventional crops.

The following two tables are adapted from E.V. Mass, from the U.S. Salinity Lab (Mass, 1990). Table 1 shows some conventional vegetables and their salt tolerance. From this table zucchini squash is more tolerant of salt, followed by asparagus and red beets. Table 2 shows some cereal crops and their salt tolerance. In Table 2 rye, with 11.4 dS/m, tolerates the most salt, followed by guar, 8.8 dS/m, and barley with 8.0 dS/m.

Table 1. Salt Tolerance of Vegetable and Fruit Crops[a].

Crop		Electrical conductivity of saturated-soil extract
Common name	**Botanical name**	**Threshold dS/m**[b]
Asparagus	*Asparagus officinalis*	4.1
Bean	*Phaseolus vulgaris*	1.0
Beet, red	*Beta vulgaris*	4.0
Broccoli	*Brassica oleracea*	2.8
Carrot	*Daucus carota*	1.0
Celery	*Apium graveolens*	1.8
Corn, sweet	*Zea mays*	1.7
Lettuce	*Lactuca sativa*	1.3
Onion	*Allium cepa*	1.2
Potato	*Solanum tuberosum*	1.7
Pumpkin	*Cucurbita pepo*	1.2
Squash, zuchini	*C. pepo melopepo*	4.7
Strawberry	*Fragaria sp*	1.0
Tomato	*Lycopersicon esculentum*	2.5

[a]These data serve only as a guideline to relative tolerances among crops. Absolute tolerances vary, depending on climate, soil conditions, and cultural practices.
[b]In gypsiferous soils, plants will tolerate EC_e about 2 dS/m higher than indicated.

Table 2. Salt Tolerance of Herbaceous Crops[a]-Grasses and Forage Crops[a].

Crop		Electrical conductivity of saturated-soil extract: threshold dS/m[b]
Common name	Botanical name	
Alfalfa	*Medicago sativa*	2.0
Barley (forage)	*Hordeum vulgare*	6.0
Bermuda grass	*Cynodon dactylon*	6.9
Cotton	*Gossypium hirsutum*	7.7
Guar	*Cyamopsis tetragonoloba*	8.8
Rice	*Oryza sativa*	3.0
Rye	*Secale cereale*	11.4
Wheat (forage)	*Triticum aestivum*	4.5

[a]These data serve only as a guideline to relative tolerances among crops. Absolute tolerances vary, depending on climate, soil conditions, and cultural practices.
[b]In gypsiferous soils, plants will tolerate EC_e about 2 dS/m higher than indicated.

Measurement of salinity.

Electrical conductivity (EC) is usually used in soil extracts and measured in decisiemens per meter (dS/m), which is equivalent to millimhos per cm. A soil with an EC > 4 dS/m in the soil saturation extract is considered a saline soil (Richards, 1954).

Throughout the world there is the problem of measuring salt content. This is because there are a number of different units of measurement. To help with this problem, a scale, called the NyPa Scale by Dr. Nicholas P. Yensen (1993), and printed here by permission, contains virtually all the units used to date (ppm, ppt, dS/m, %, etc). Appendix A shows this scale. This scale is specially helpful at international salinity conferences where many presenters use different units to express the salinity of waters or soils.

Uses of halophytes.

In the Sahara Desert there are already plants that have adapted to the harsh conditions of salt and high temperatures, and that have been used for centuries. Such is the case of the oasis and salt marshes widely distributed in the Sahara and along the Arabian Gulf.

Artesian wells, with water as salty as seawater, can also be found in the Sahara Desert, where only *Tamarix, Cressa cretica*, and *Juncus sp* occur (Batanouny, 1993). Halophytes here are used as range plants, timber, fuel wood and coal, windbreaks, for tannins, and as medicines (the ash of *Typha domingensis* was used for wounds, the ash of *Zygophyllum* was used for treating scabies in camels) (Batanouny, 1986).

Other halophytes (*Seidlitzia rosmarinus*) are used for cleaning clothes and utensils, due to the saponins present (Rizk, 1985). It has been reported that some halophytes are used for their aphrodisiac powers e.g. *Avicennia sp.* and *Cynomorium coccineum* .

Some halophytes have edible fruits (*Lycium sp.*). Others have seeds that have a high oil content (*Salicornia spp.*), and contains saponins. *Atriplex spp.* are used in Argentina and Mexico as well as other countries for cattle grazing.

There are grasses used by animals as forage, such as *Distichlis sp.* This grass grows throughout the Americas in arid and humid climates. In Mexico City *Distichlis spicata* was used in the 70's to control the dust produced by the Ex-Lago Texcoco, that polluted the area close to the airport. This grass was irrigated with saline waste water from the water treatment plant at Lago Texcoco. The halophyte grew well and cattle was grazed on it.

Ethnobotany of *Distichlis palmeri*.

In the Sonoran Desert there is a halophytic grass, *Distichlis palmeri*, which was once used by the Cocopa Indians. Every May the Indians made special trips to the mouth of the Colorado river where they gathered the seeds of the *Distichlis palmeri* known to them as nypá or trigo gentil (Williams, 1987). *Distichlis palmeri* produces a seed similar to wheat and rice; and, it was used to condiment their soups and pan bread. It was their first "crop" at the end of the spring. The Indians made trips to the Gulf near the mouth of the Colorado River to gather the seeds. The Cocopa cut the seedheads, dried them by fires and then threshed them to separate the seeds from the chaff. The movement of the tides also "collected" the seeds for them and left piles of seeds along the seashore.

As a result of their encounter with the Spaniards around 1540's, and their subsequent visits to the delta around 1875, the Cocopa abandoned their traditional foods. They exchanged them for the "civilized" foods.

Thanks to Ethnographer, Anita Alvarez de Williams, who published information about the Cocopa (1975, 1987), it was possible to obtain more information about the uses of nypá or trigo gentil. She arranged interviews with the Cocopa elders where we learned that it was around 1950's when they last collected these seeds.

This plant was thought to be extinct, since it was difficult to find it. And, it wasn't until 1980 that Drs. Nicholas Yensen and Miguel Fontes rediscovered it. Dr. Yensen later confirmed the rediscovery by comparing his collected specimens to the Dr. Edward Palmer's original type specimens from 1889, currently located at the Kew Royal Botanic Gardens in London.

Distichlis palmeri composition compared to cereals.

Table 3 shows the proximal composition of the grain of *Distichlis palmeri*. It does not have gluten. Therefore, a mix of *Distichlis palmeri* flour with wheat flour has to be used to produce good quality bread. The flour, though, can be used for cookies and biscuits; it can also be cooked like rice (Yensen, 1984).

Table 3. Proximal analysis of *Distichlis palmeri*, hard red winter wheat, barley, and brown rice grain (dry weight basis).

	Distichlis palmeri	Wheat	Barley	Brown rice
Protein, %	8.7	13.7	13.0	9.5
Crude Fiber, %	8.4	2.6	6.0	1.0
Fat, %	1.8	1.9	1.9	1.9
Ash, %	1.6	1.9	3.4	1.2
Available Carbohydrate, %[1]	79.5	79.9	75.7	86.5
Starch, %[2]	69.6	-	-	-
Sugar, %[2]	3.8	-	-	-
Gross Energy, Kcal/g	3.9	4.1	4.5	4.1

[1] By calculation.

[2] Determined by the Anthrone method (Clegg, 1956).

CHAPTER 3

FRACTIONATION AND PARTIAL CHARACTERIZATION OF *DISTICHLIS PALMERI* (VASEY) PROTEINS

ABSTRACT

Proteins from a halophyte cereal *Distichlis palmeri*, whole-grain flour, were fractionated on the basis of their solubility in: 1) 0.001 M phosphate buffer at pH 7, 2) 0.5 M sodium chloride, 3) 70% ethanol, 4) 0.6% 2-mercaptoethanol at pH 10, and 5) sodium dodecyl sulfate (SDS). Total protein extraction was 72%. The SDS fraction was the predominant fraction.

Each of five fractions, residue, and whole-grain sample were analyzed for fifteen amino acids. The percent of amino acid ranged from a high of 35% glutamine for the ETOH extraction to a low of 0.18% lysine, also from an ETOH extraction. The fractions were all rich in asparagine and glutamines which are common in seed storage proteins. The lysine content of this grain is higher than that of wheat. Of the fractions the highest lysine percentage, 6.3%, was found in the water soluble fraction. Protein recovery of the different fractions ranged from 78% to 87%.

SDS polyacrylamide gel electrophoresis showed: 1) the water soluble fraction had a wide range of molecular weights, 2) the NaCl fraction had circa 10 proteins in high concentrations, 3) the prolamine fraction had a band at about 22,000 daltons with two weak bands at 45,000 and 66,000 daltons, 4) the 2-mercaptoethanol fractions had weak

bands, 5) the SDS fraction had circa 6 bands, which were also present in low concentrations. The amino acid pattern of the whole grain is similar to that of a cereal.

INTRODUCTION

The recent trend to use new unconventional crops does not always mean the crops themselves are new to us. Most were known to our ancestors who gathered plants and roots for food and medicinal purposes. The developments of modern agriculture is embodied in the high-yielding annual cereal crops: wheat, rice, corn, barley, sorghum, and millet, which provide the bulk of human food. But, concurrent with this historically unprecedented agricultural development has been the serious accumulation of salt in lands and waters throughout the agricultural world.

Like the "new" unconventional crops, salinization is not a "new" problem. For example, it is well known that in ancient Mesopotamia, as the soils became more salinized, the yields for wheat came down. Barley, which is more tolerant to salt, was then used more widely to the point where even barley's yields were also too low and one of the world's first major agricultural civilizations collapsed. The process of salinization has continued. Presently, on a worldwide basis, hundreds of thousands of acres are lost to salinization every year.

In order to utilize these salinized lands, otherwise wasted lands, many scientists have proposed the use of unconventional crops that are capable of growing in salty soils. Research surveys of the various known salt-loving plants, or halophytes, as potential crops are now widely conducted in many countries in an effort to find solutions to the salinization problem.

One potential halophyte crop is *Distichlis palmeri*, or salt grass. The genus *Distichlis* can be found from Canada to South America. It is capable of growing in sea water as well as brackish or fresh waters (Yensen et al., 1985). *Distichlis palmeri* produces a grain that resembles wheat and rice, see Figure 1. The Cocopa Indians of the Colorado River Delta area used this grain to make a pan bread and as a thickening agent for their soups. While wild grain yields are only a few pounds to the acre, the action of the tides and waves would gather the grain and deposit it in wind rows along the beach. Nearly twenty years of research, selection, and breeding has resulted in domesticated, patented varieties which can produce tons to the acre (Yensen and Yensen, 1987).

With a possible commercial value for this unconventional crop, the grain is under intensive study to fully evaluate its potential uses (Yensen and Weber, 1986, 1987; Yensen and Bedell, 1993). Proximal analysis indicate that it is similar to other cereals. Baking trials indicate that the flour behaves more like a cookie flour than a bread-making flour (Tapia, 1988). The protein content, 8%, falls in the range of other cereals. The present study deals with the characterization of the proteins obtained by fractionation with different solvents. This study furthers our knowledge of this unusual grain and its potential uses.

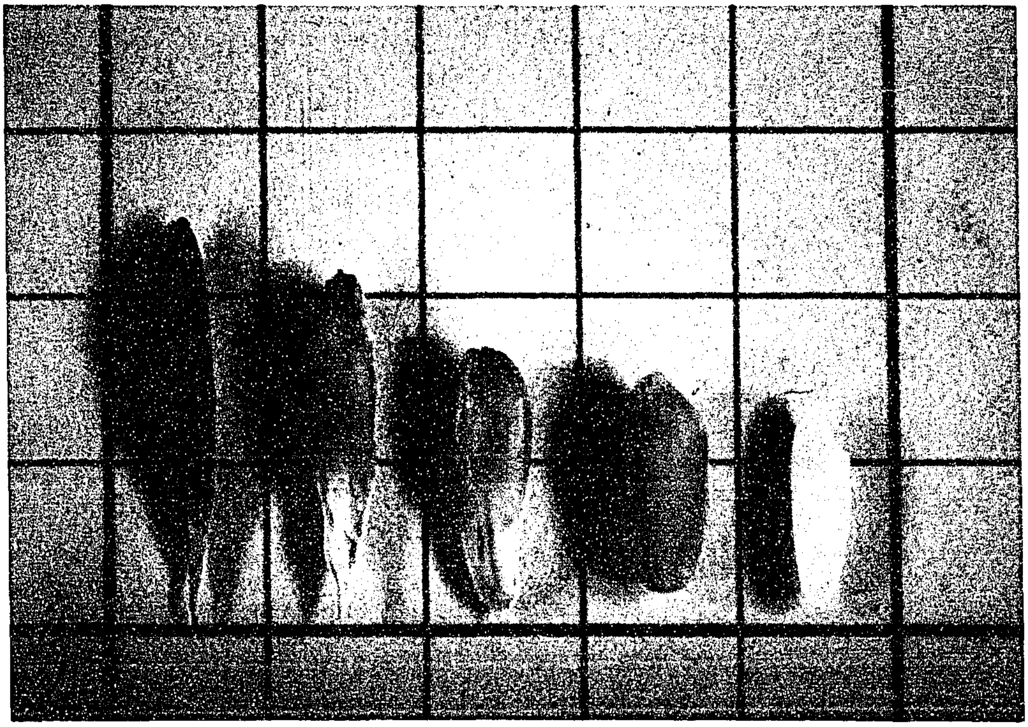

Fig. 1. A. B., and C. = *Distichlis palmeri* seeds; D = wheat, and E = rice

MATERIALS AND METHODS

Sample preparation of *Distichlis palmeri* grain.

The seeds of *Distichlis palmeri* were ground to pass through a 24 mesh screen in a Weber mill and then defatted by adding to the sample 10 times its volume in hexane and stirred for 2 hours under a hood. The supernatant was decanted, and, the sample was then rinsed 3 times with hexane until the hexane was clear. The residue was then filtered and collected on Whatman #1 filter paper. This was later lyophilized and stored in a freezer at -12°C. Note that because the grain has a relatively low fat content (Yensen and Weber, 1986), it is not absolutely necessary to defat it, but was initially done to maintain procedure consistancy and to eliminate possible errors.

Protein fractionation.

The fractionation of the proteins was on the basis of their solubility according to the Laundry and Moreaux method, modified by Robutti et al. (1974). Samples of 50 g each were sequentially extracted with 300 ml of solvent with a magnetic stirrer at 4° C., except for the sodium dodecyl sulfate (SDS) which was extracted at room temperature. Table 4 shows the solvents used as well as their sequence and times of extraction. The extraction solvent solutions were prepared either at 4°C or at room temperature, according to their use. The soluble fractions were separated by centrifugation at 19,200 x g for 25 minutes in a refrigerated (at 4°C) centrifuge (Beckman Mod. J-21). The supernatants for each solvent were combined and dialyzed against deionized distilled water for 48 hours with frequent water changes. They were then centrifuged and freeze dried. The dried samples were stored in a freezer for subsequent analysis. Protein was determined on the soluble fractions using the BCA reagent (Pierce Biochemicals) and on the insoluble protein using the Kjeldahl method (N x 6.25) (AOAC, 1985).

Amino acid determination.

For the amino acid determination, 30 mg of the samples were placed in ampoules with 5 ml of 6 N HCl. The ampules were flushed with nitrogen, to evacuate the oxygen, and sealed under vacuum; then placed in an oven at 110°C for 18 hours (Jones et al., 1981). After the digestion, the mixtures were transferred to flasks and evaporated to dryness in a rotary evaporator. Ten ml. of 0.1 N HCl was then added to the flasks to dissolve the amino acid residues and then filtered through Whatman No. 1 paper. The amino acids were determined by HPLC (Spectraphysics Mod. SP8000A). The percent protein calculations for each amino acid, was determined from integrated values and protein levels.

A computer program was used for the amino acid content calculations.

Table 4. Solvents, order, and length of extractions used for the fractionation of *Distichlis palmeri* seed proteins.

Solvents and order of Extraction	Extraction time (Hrs.)		
	1st.	2nd.	3rd.
Sodium Phosphate buffer, 0.001 M Ph 7.0	2	1	1
Sodium Chloride, 0.5 M	2	1	1
Ethanol, 70 %	2	1	1
2-Mercaptoethanol, 0.6 % in carbonate buffer, 0.1 M, Ph 10.0	2	1	1
Sodium dodecyl sulfate, 0.5 % in carbonate buffer, 0.1 M, pH 10.0	2	1	1

Sodium dodecyl sulfate polyacrylamide gel electrophoresis.

For electrophoresis, a slab SDS polyacrylamide gradient gel was employed according to the Laemmly system (1970), using a Bio Rad Mini Protean II gel electrophoresis apparatus. A gradient gel (5-20%) was run starting at 70 volts until the bands were aligned and then the voltage was turned to 160 volts until the dye line was almost out of the gel. The gel slabs were fixed in a solution of 50% methanol, 10 % acetic acid and 0.25% Coomassie Brilliant Blue R-250. Then, they were destained with a solution of 0.50% methanol, 0.75% acetic acid in deionized water. The molecular weights were estimated by use of the Sigma low and high molecular weight standards (Sigma Chemical Co.). The proteins included in the standard were: myosin, 205,000 daltons; b-galactosidase, 116,000; phosphorylase, 97,400; albumin, 66,000; albumin, 45,000; glyceraldehyde-3-phosphate dehydrogenase, 36,000; carbonic anhydrase, 29,000; trypsonogen, 24,000; trypsin inhibitor, 20,100, and a-lactalbumin, 14,200.

Statistical analysis.

The data are expressed as means $\pm$ standard error of the mean (SEM), where

$$SEM = \frac{\sigma}{\sqrt{N}} .$$

RESULTS AND DISCUSSION

Isolation of *Distichlis palmeri* grain protein fractions.

The total protein extraction was 72%. The residue protein was 4.7%. The total protein extracted plus the residue protein gives a total recovered protein of 76.7%. This leaves 23.3% of the nitrogen "unaccounted for". The unaccounted for portion could be related to nonprotein nitrogen (which would have increased the original protein calculation), and/or to soluble protein lost during dialysis and/or discarded with the supernatant after centrifugation. This level of unaccounted for nitrogen is similar to other values reported in the literature for this type of fractionation (Sathe and Salunkhe, 1981; Rahman, 1988; Tjahajadi et al., 1988; Idouraine, 1993).

Table 5 shows the protein content obtained from each of the solvent extractions. The fraction obtained with SDS was the most abundant one, as was the case for rice glutelins (Wall, 1962), followed by the ethanol, phosphate buffer, sodium chloride and 2-mercaptoethanol. The lowest value obtained was from the 2-mercaptoethanol fraction, 22.80 mg. The freeze dried sample was then used for the determination of the amino acid composition. Table 6 shows the values obtained for the amino acids. Here it is shown which fractions were poor, rich or intermediate in a given amino acid. The fractions were all rich in asparagine and glutamines a condition which is common in seed storage proteins. The lysine content of this grain was higher than that of wheat (Yensen and Weber, 1986). The highest values for lysine were found in the water soluble part. Like other cereals, the prolamine fraction had the lowest value for lysine.

Table 5. *Distichlis palmeri* protein content[1].

Solvent	mg of protein[1]	% of total protein[2]
Phosphate buffer pH 7.0	583.00	14.80
Sodium Chloride, 0.5 M	362.00	9.20
Ethanol, 70 %	603.00	15.30
2-mercaptoethanol, 0.6 % in pH 10.0 buffer	22.80	0.60
SDS, 0.5 % in pH 10.0 buffer	1,283.00	33.00
TOTALS	2,853.80	72.90

[1]Calculated with the BCA method (Pierce Biochem., Rockford, IL).
[2]Total protein = 3.94 g (calculated as N x 6.25).
Total protein extraction was 72%.
These values are the average of triplicate extractions.

Gel electrophoresis protein patterns.

In Figure 2, the electrophoretic patterns of the relatively large protein molecules are shown. The water soluble fraction shows several bands with a wide range of molecular weights. The sodium chloride fraction had circa 10 proteins found in higher concentrations.

The prolamine fraction had a major band at 22,000 daltons. This was similar to that reported for rice prolamine (Juliano and Boulder, 1976) which has a single subunit of 23,000 daltons similar to the one shown in Figure 2. The prolamine fraction, however, was interesting in that it had two protein bands at relatively low concentrations: 45,000 and 66,000 daltons (not shown in Figure 2).

The 2-mercaptoethanol fraction had few bands. These bands were weak, indicating that very small quantities were present. The SDS fraction had about 6 bands, in low concentrations.

Table 6. Amino acid composition (% of protein) of *Distichlis palmeri* protein fractions.[1]

Amino Acid	EXTRACTION SOLVENT					
	Water	NaCl	ETOH	2-ME	SDS	Residue
ASP	9.12	6.73	3.53	9.89	6.55	5.80
GLU	13.90	15.42	35.00	17.96	18.80	11.19
SER	3.79	4.45	3.76	3.93	3.12	3.57
HIS	1.74	3.04	1.45	2.24	3.51	5.13
GLY	5.07	5.02	0.56	5.65	3.51	5.13
THR	3.93	2.34	2.29	3.20	2.86	2.77
ARG	5.56	14.58	0.89	6.09	5.86	6.42
ALA	5.64	5.09	6.33	6.13	5.17	5.18
TYR	2.56	2.76	4.96	2.89	3.64	2.21
MET	1.63	1.51	1.65	1.99	2.07	1.13
VAL	5.25	5.50	6.17	5.51	5.23	4.43
PHE	3.01	3.81	3.77	3.53	3.91	3.66
ILE	3.45	3.33	4.89	3.65	3.95	3.06
LEU	5.67	5.29	11.41	6.48	7.54	6.45
LYS	6.32	2.94	0.18	5.14	3.47	4.64
Recovery, %	78	82	87	84	78	-

[1]Average of triplicate extractions.

CONCLUSIONS

While these results suggest that the protein fractions of *Distichlis palmeri* are perhaps most similar to rice, they fall in the general nature and composition of other cereals such as wheat, oats, etc. The lysine content, while higher than wheat, is still relatively low compared to that of legumes, and, as in wheat, the high levels of glutamines do not optimize the potential nutritional value of the proteins. The high consumption of cereals in the world gives all cereals, and potentially *Distichlis palmeri* grain, great nutritional importance. Agriculturally speaking, *Distichlis palmeri* grain thrives in salinized lands irrigated with salty water far above the level at which conventional crops can survive! This makes the grain very unique.

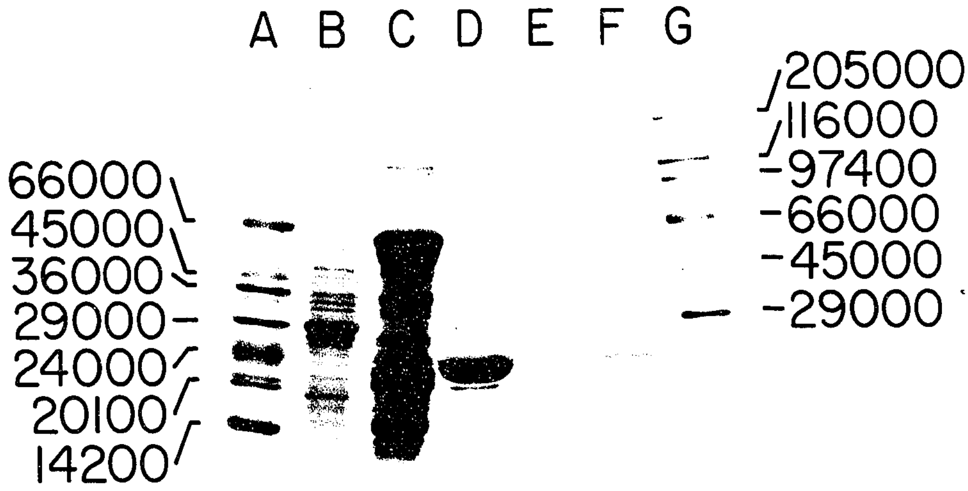

Figure 2. Sodium dodecyl sulfate polyacrylamide gradient gel electrophoresis patterns of *Distichlis palmeri* seed extracted with different solvents: A = Low molecular weight standard; B = Water soluble; C = NaCl soluble; D = Ethanol soluble; E = 2-mercaptoethanol soluble; F = SDS soluble; G = High molecular weight standard.

CHAPTER 4

FUNCTIONAL PROPERTIES OF THE GRAIN OF *DISTICHLIS PALMERI*

INTRODUCTION

Agriculture throughout the world has been facing a growing problem: salinization of the lands. This is due to several processes including the irrigation methods currently used. In other places, however, the salinization is due to the contamination of well waters with salty waters i.e. agricultural regions close to the ocean. One such place is located in the Costa de Hermosillo, in the State of Sonora, Mexico, where the water table is currently being contaminated with the intruding sea water from the ocean.

As wells became salty the lands were abandoned and no longer used for agricultural purposes. People then abandoned the lands. Now, the lands has been slowly eroding. If the people can utilize the salty water from the wells and grow a crop, for animal or human consumption, then these lands can be reclaimed.

The use of halophytes in recent years has given a promising alternative for these salinized lands. Already, these plants that are growing in countries such as Mexico, Namibia, Morocco and Australia. These countries are taking advantage of these previously unusable lands and salty wells. The plants growing in these countries belong to the true grass family, Poaceae, and are divided in three use categories: grasses, forages, and grain producing plants. The present study considers the nutritional characteristics of the grain producing plant and particularly the functional properties of the grain. The species name of the grain plant is: *Distichlis palmeri* (Vasey). *Distichlis palmeri* grows in lands with salty soils and/or water. The grain resembles wheat and can be used to make cookies and muffins (Yensen et.al., Unpublished data). Baseline nutritional analyses of this grain have been published already (Yensen and Weber, 1986, 1987).

The purpose of the current study was to evaluate the functional properties of *Distichlis palmeri* flour and its protein fractions. Knowing the functional properties will give us a better idea as to for what food systems this new grain will be useful.

MATERIALS AND METHODS

Preparation of samples.

The grain was finely ground and the flour was sequentially extracted with 0.001M sodium phosphate buffer at pH 7, 0.5 M sodium chloride, and 70% ethanol. The extractions with each solvent were repeated three times, followed each time by centrifugation at 18,000 x g. The extracts were then dialyzed against distilled water for 48 hrs. with frequent water changes. The supernatant with water soluble proteins and the precipitates for globulins and prolamines were lyophilized and stored in air-tight containers in a freezer.

Protein Solubility.

Protein solubility was determined by the method of Balmaceda et. al. (1984). Duplicate dried samples of the whole flour and the water, salt and ethanol fractions were individually prepared at 1% weight/volume (w/v) protein solutions. Each suspension was adjusted to a pH ranging from 1 to 12 by adding 0.1 N HCl or 0.1 N NaOH, stirred for 15 minutes at room temperature and centrifuged at 28,000 x g for 30 min. Protein content (N x 6.25) of the supernatant and the samples was determined by Kjeldahl by the AOAC (1990) method. Protein solubility was calculated by dividing the protein content of the supernatant by the protein content of the dry sample.

Water and oil absorption.

Water absorption capacity was determined by a modified method of Balmaceda et al. (1984). Duplicate samples of about 1 gram equivalent protein sample were individually weighed into a pre-weighed 50-ml round bottom centrifuge tube, mixed with 20 ml deionized water and adjusted to pH 7, and stirred for 15 minutes. The tubes were centrifuged at 18,000 x g for 15 min, decanted, and reweighed again. Water absorption capacity was determined with respect to both the sample weight and the protein weight by dividing the absorbed water weight (grams) by the sample weight (grams) and then the absorbed water weight (grams) by the protein weight (grams). Oil absorption capacity was done in the same way as water absorption, except that oil was used instead of water. The sample was then stirred for 30 minutes instead of 15 minutes. Oil absorption capacity was expressed as: ml oil held/grams of sample or grams of protein.

Foaming capacity and foam stability.

Foaming capacity was determined by passing nitrogen through a calibrated chromatographic column containing 50 ml of 1% protein water solution previously adjusted at pH 7 (Balmaceda et al., 1984). The column (ID = 46 mm) was equipped with a removable coarse fritted disk (50/50) connected to a T joint. The nitrogen flow was adjusted to 100 ml/minute. Foaming capacity was quantified by measuring the volume of foam after 2 minutes of bubbling. Foam stability was determined by measuring the volume of foam remaining after 15 and 30 minutes after gas flow stopped. All measurements were done in duplicate.

Emulsion capacity.

Emulsion capacity was measured using electrical resistance as described by Balmaceda et al. (1984). Duplicate samples 50 ml of 1% protein suspensions of whole flour, globulin and prolamine fractions were adjusted to pH 7 according to Balmaceda (1994). Each sample was separately transferred to a high speed blender (Waring Comm. Blender model 31 BL 91), equipped with two electrodes connected to a volt-ohm-milliammeter (VOM) set at R x 1000. After 30 seconds of blending at high speed, gradual titration with corn oil was initiated and continued until the VOM read infinite resistance. This meant that an inversion from oil-in-water to a water-in-oil emulsion had taken place. Emulsion capacity, expressed as ml of oil/100 mg protein, was defined as the amount of oil added to the sample up to the inversion point (infinite resistance).

RESULTS AND DISCUSSION

The protein solubility profile (Figure 3) shows the prolamine fraction soluble only at pH 9 and higher, whereas the albumin was soluble at all pH's but more so at a higher pH. The globulin fraction exhibited a solubility typical of the proteins with an isoelectric point at pH 4.5. The whole flour of *Distichlis palmeri* was also soluble at all pH's but more so at a pH higher than 8. At this pH the quantity of tannins and other colored compounds were higher, too. This makes it difficult to prepare a protein concentrate with a light color.

Table 7 shows the water holding capacity and oil holding capacity of *Distichlis palmeri* flour and albumin, globulin, and prolamin fractions. The whole flour of *Distichlis palmeri* had a better water and oil holding capacity when expressed as g/g protein than when given as g/g sample. Even though the other macro nutrients present in the flour, i.e. starch, were also contributing to the absorption of both water and oil.

Table 8 shows the foaming capacity and foaming stability for *Distichlis palmeri* flour; albumin, globulin, and prolamin fractions. Foaming capacity for the albumin fraction was excellent producing a foam volume equal to 400% of the pre-foamed volume. This is higher than soy bean protein isolate. The foam produced very fine bubbles and retained a foam volume of 100% after 30 minutes. The whole *Distichlis palmeri* flour did make some foam but it was very coarse and unstable.

Figure 4, shows the emulsion capacity of *Distichlis palmeri* flour (WF) and its globulin (GLO) and prolamine (PRO) fractions expressed as ml of oil emulsified/100 mg of protein. The emulsion capacity was better in the whole flour than in the other fractions, globulins or prolamines. This could be due to the protein interaction as well as the starch present in the whole grain.

The functional properties are affected by food systems making it difficult to control for other parameters present, i.e. starch, sodium chloride concentrations, fat, etc. The purpose of obtaining the functional properties in a given protein is to find a suitable food system for its use; but, the final test is always to taste test a given food. *Distichlis palmeri* does not have gluten, yet it makes a good quality bread at 50% wheat flour substitution, with flavor and color and overall acceptability not being affected (see Chapter 5). The flour behaves more like a cookie flour; however, muffins and cookies can be made with an excellent acceptability.

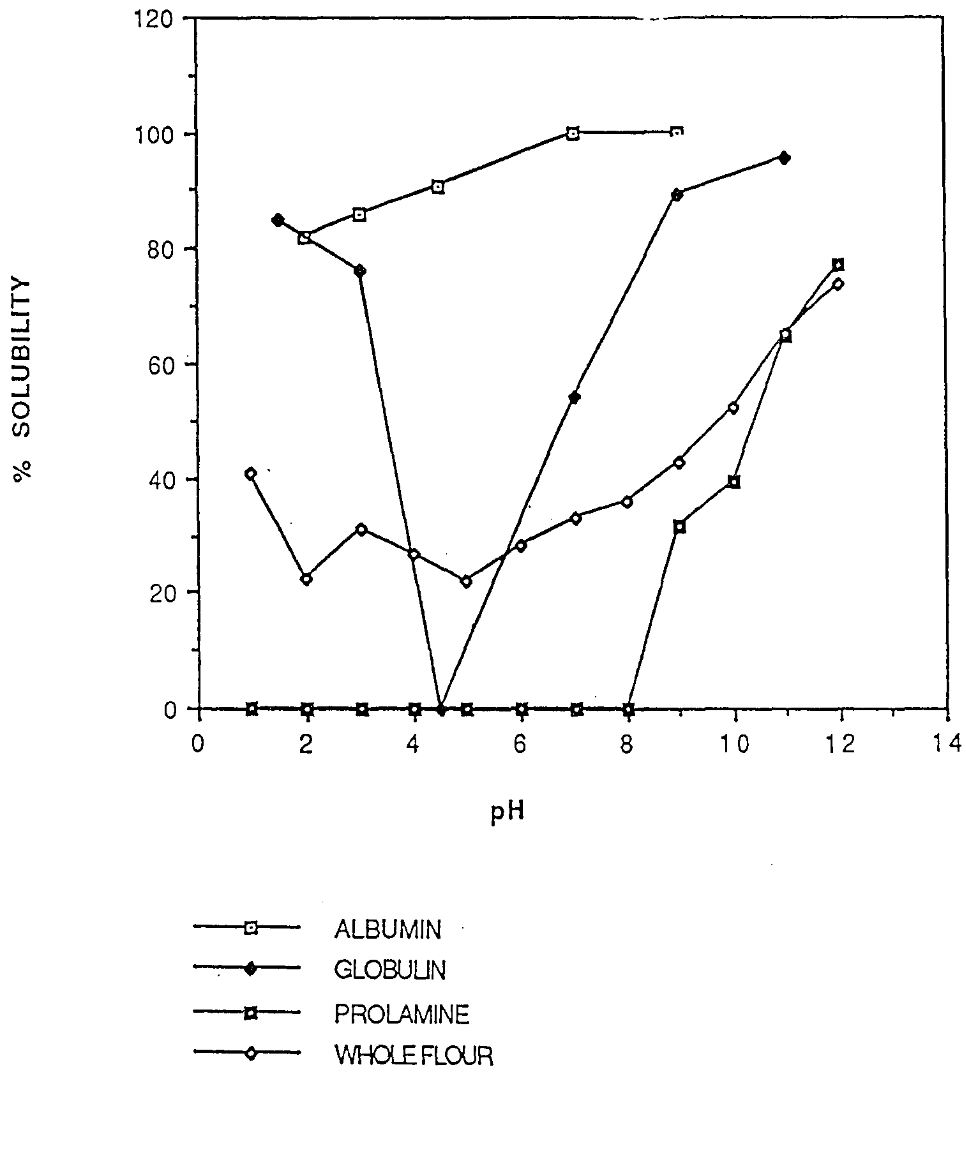

Figure 3. Protein solubility of *Distichlis palmeri* flour, albumin, globulin and prolamine fractions.

Table 7. Water holding capacity and oil holding capacity for *Distichlis palmeri* flour, and albumin, globulin, and prolamine fractions.

Fraction	Water Holding Capacity		Oil Holding Capacity	
	g/g sample	g/g protein	g/g sample	g/g protein
Whole Flour	0.99 ± 0.007	11.14 ± 0.09	0.80 ± 0.001	8.96 ± 0.007
Albumins	ND	ND	1.43 ± 0.005	7.38 ± 0.005
Globulins	ND	ND	2.96 ± 0.25	4.09 ± 0.35
Prolamins	1.99 ± 0.04	2.99 ± 0.07	3.37 ± 0.09	4.78 ± 0.26

Table 8. Foaming capacity and foaming stability for *Distichlis palmeri* flour, albumin, globulin and prolamin fractions.

Samples	Foaming capacity (%)	Foam stability (%)	
		15 min	30 min
Whole flour	55.16	0	0
Albumins	400.0	100	100
Globulins	27.6	0	0
Prolamines	0.0	0	0

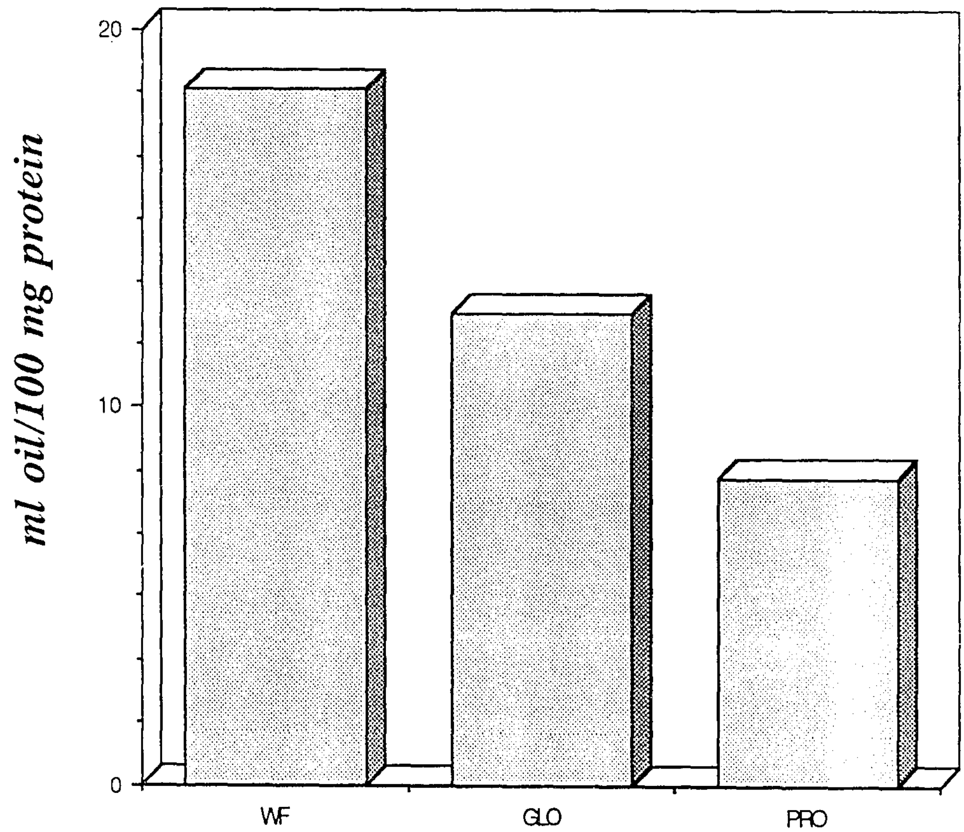

Figure 4. Emulsion capacity of *Distichlis palmeri* flour (WF) and its globulin (GLO) and prolamine (PRO) fractions expressed as ml of oil emulsified/100 mg of protein.

CHAPTER 5

SENSORY EVALUATION OF *DISTICHLIS PALMERI* MUFFINS

ABSTRACT

Whole wheat and *Distichlis palmeri* muffins were evaluated using a Hedonic scale. Muffins were prepared using 0%, 25%, 50%, 75%, and 100% substitution of the whole wheat flour. The evaluations were segregated into "occasional", "frequent", or "exclusive" eaters of whole wheat. The occasional eaters of whole-wheat breads preferred the lighter color of the 100% whole-wheat muffins. There was no significant difference in flavor acceptance between the "exclusive" eaters and "frequent eaters" of whole wheat. Overall, the "exclusive eaters" of whole-wheat bread rated *Distichlis palmeri* muffins significantly higher than either the "occasional" eaters or the "frequent eaters". The "exclusive eaters", however, did not significantly prefer the whole wheat over any of the mixtures. The 50/50 mix received the highest rating.

INTRODUCTION

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 salinity caused by extensive irrigation. *Distichlis palmeri*, a halophyte capable of growing in seawater, can also grow in these salinized lands. Therefore, waste land can ideally be brought back into production. This plant was thought to be extinct until recently; hence, little has been published about it. It has a grain similar in composition to wheat or barley (Yensen, 1984). The proximate analysis of the grain is given in Table 3. The Cocopah Indians used the flour to make bread as well as atole. The objective of this sensory evaluation was to evaluate the acceptability of muffins prepared with the *Distichlis palmeri* flour when compared to muffins prepared with whole wheat.

MATERIALS AND METHODS

Sample preparation.

Both grains, *Distichlis palmeri* and wheat, were ground in a Weber mill (mesh 024). *Distichlis palmeri* flour was substituted for whole-wheat flour in a honey-wheat muffin recipe (see Appendix B) at 0, 25, 50, 75, and 100% levels. All muffins were prepared the day before the sensory evaluation and stored refrigerated in plastic bags. The samples were warmed by means of a microwave oven just prior to the testing.

Sensory evaluation.

The affective test of ranking and rating via the hedonic rating scale were used. There were 30 untrained panel members participating in the test. The panelists were asked how often they consumed whole-wheat products. The questionnaire included the color, appearance, texture, mouthfeel, flavor, and overall acceptance of the products. The hedonic scale used ranged from 1 to 9 (1 = don't like it at all, and 9 = like it very much). The panelists were also asked to rank the 5 products in order of preference with 1 being the best and 4 the worst. The 5 samples were presented at the same time. Water was provided for use between samples. Appendix C shows a copy of the questionnaires.

Statistical analysis.

The data was analyzed by using the Dice-Leraas graphs, as well as the chi-square test (Kendall, 1975).

RESULTS AND DISCUSSION

Figure 5 shows the appearance of the muffins made with different concentrations of *Distichlis palmeri* flour. Figure 6 shows a color closer view of a muffin made with 100% *Distichlis palmeri* flour. The "occasional eaters" and the "frequent eaters" of whole-wheat bread were strongly influenced by color and appearance as opposed to the "exclusive eaters" of whole-wheat bread. For both color and appearance the "occasional and frequent eaters" gave a monotonically declining response with increasing *Distichlis palmeri* concentrations ($P<0.05$)(Figures 7 and 8). Surprisingly, color explained the largest portion of the variance followed by appearance.

The characteristics, texture, and mouth feel (Figures 9 and 10), explained the least portion of the variance. Indeed there was no significant difference between the "exclusive", "frequent", and "occasional" eater of whole-wheat bread for these characteristics. This held true at all concentrations of *Distichlis palmeri.*

With respect to flavor (Figure 11), the "exclusive" and "frequent" eaters of whole-wheat bread showed no significant change over the range of *Distichlis palmeri* concentrations. The "exclusive" eaters of whole-wheat bread, however, rated the flavor of the *Distichlis palmeri* significantly higher than the "frequent" eaters of whole-wheat bread. On the other hand the "occasional" eaters of whole-wheat bread had a gradual, but not necessary significant, decrease in preference with increased *Distichlis palmeri* concentrations.

Overall (Figures 12 and 13), the "exclusive" eaters of whole-wheat bread, showed no significant preference for muffins with 0, 25, 50, and 75% concentrations of *Distichlis palmeri*. The 100% *Distichlis palmeri* muffins, however, were less preferred by this group. Oddly, the main characteristics responsible for this reduced preference were the ones with the low variance, texture and mouth feel. It was observed that the 100% *Distichlis palmeri* muffins had a slightly "gritty" taste. This may be more likely due to a small pieces of pumice that were inadvertantly ground into the mixture and/or to the high fiber content.

It was interesting to note that the "frequent" eaters of whole-wheat bread consistently gave all concentrations of *Distichlis palmeri* a higher rating than the "occasional" eaters. Furthermore, the "occasional" eaters' ratings were generally significantly lower.

Overall, the "exclusive" eaters of whole-wheat bread rated the *Distichlis palmeri* muffins significantly higher than either the "occasional" or the "frequent" eaters of whole-wheat bread. More importantly, the "exclusive" eaters of whole-wheat bread did not significantly prefer the whole-wheat muffins over any of the mixtures with *Distichlis palmeri*. Indeed, the 50/50 mix received the highest rating.

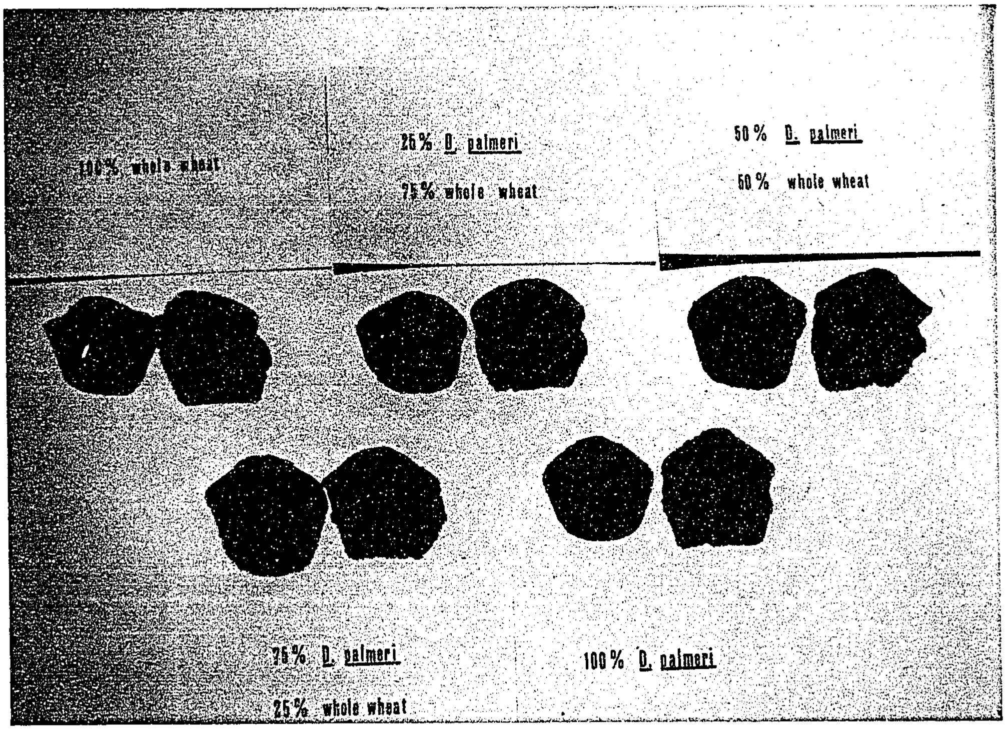

Figure 5. Muffins made with different concentrations of *Distichlis palmeri* flour.

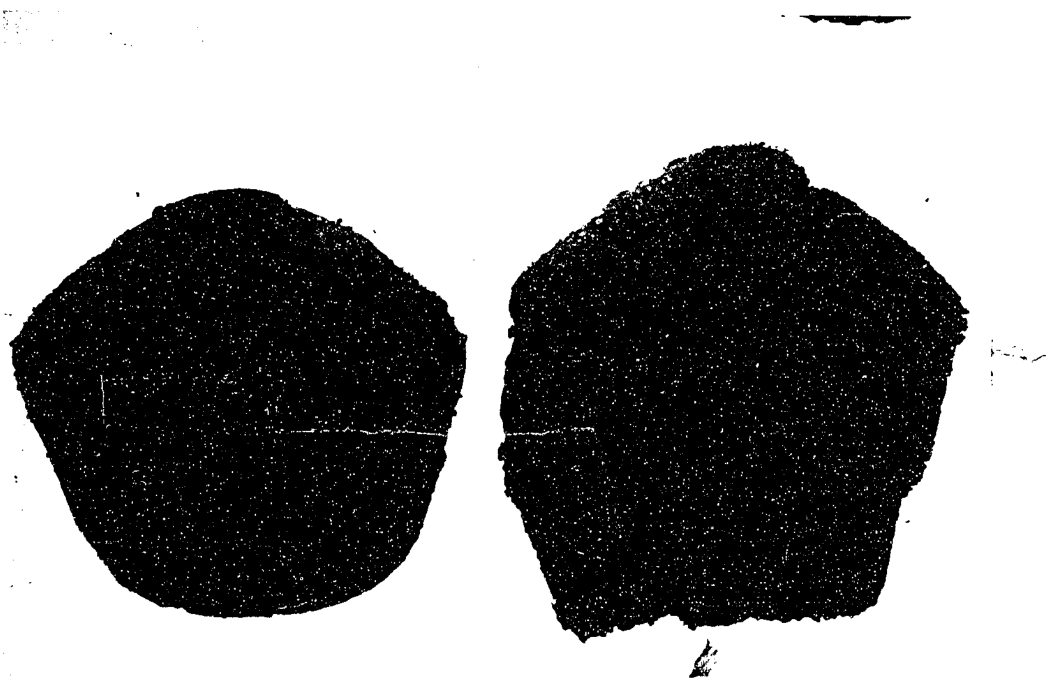

Figure 6. Cross section of a muffin made with 100% *Distichlis palmeri* flour.

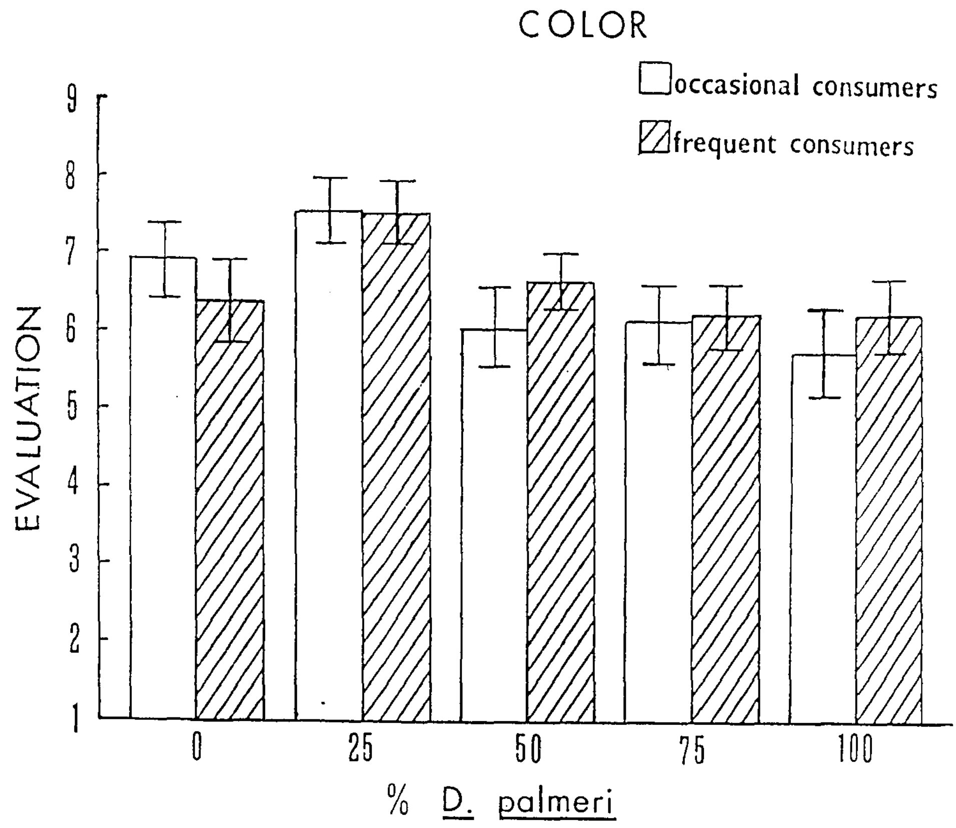

Figure 7. Dice-Leraas graph of "occasional" and "frequent" eaters color evaluation of whole-wheat bread at 0, 25, 50, 75 and 100% concentrations of *Distichlis palmeri* flour substituted for whole-wheat flour, showing the standard error of the means (note: non-overlapping standard errors of the mean are significant).

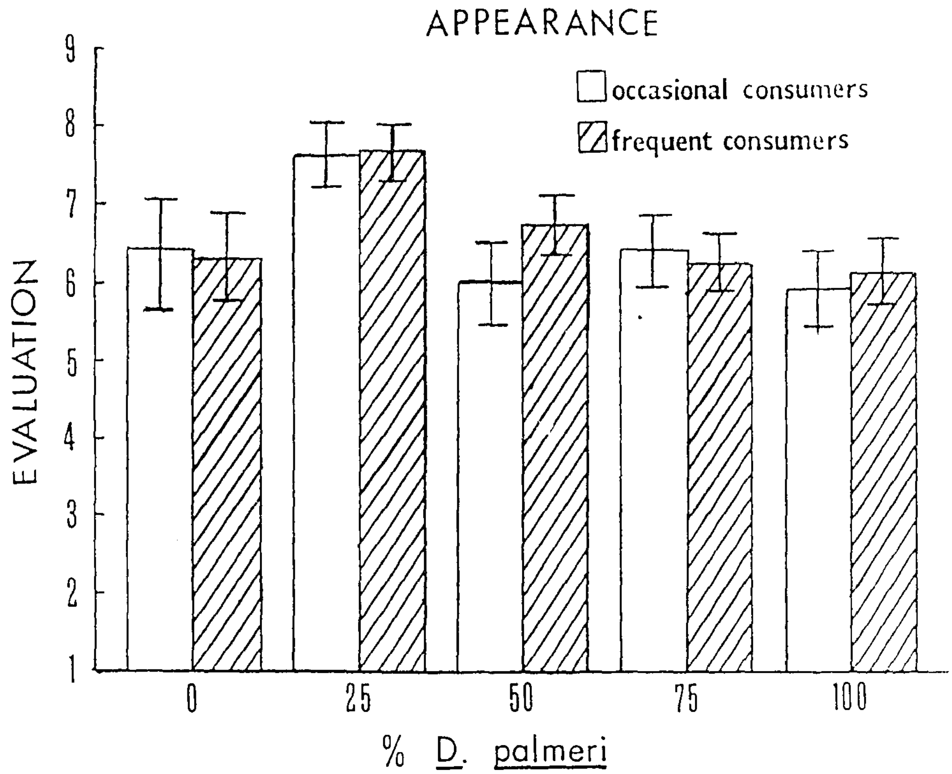

Figure 8. Dice-Leraas graph of "occasional" and "frequent" eaters appearance evaluation of whole-wheat bread at 0, 25, 50, 75 and 100% concentrations of *Distichlis palmeri* flour substituted for whole-wheat flour, showing the standard error of the means (note: non-overlapping standard errors of the mean are significant).

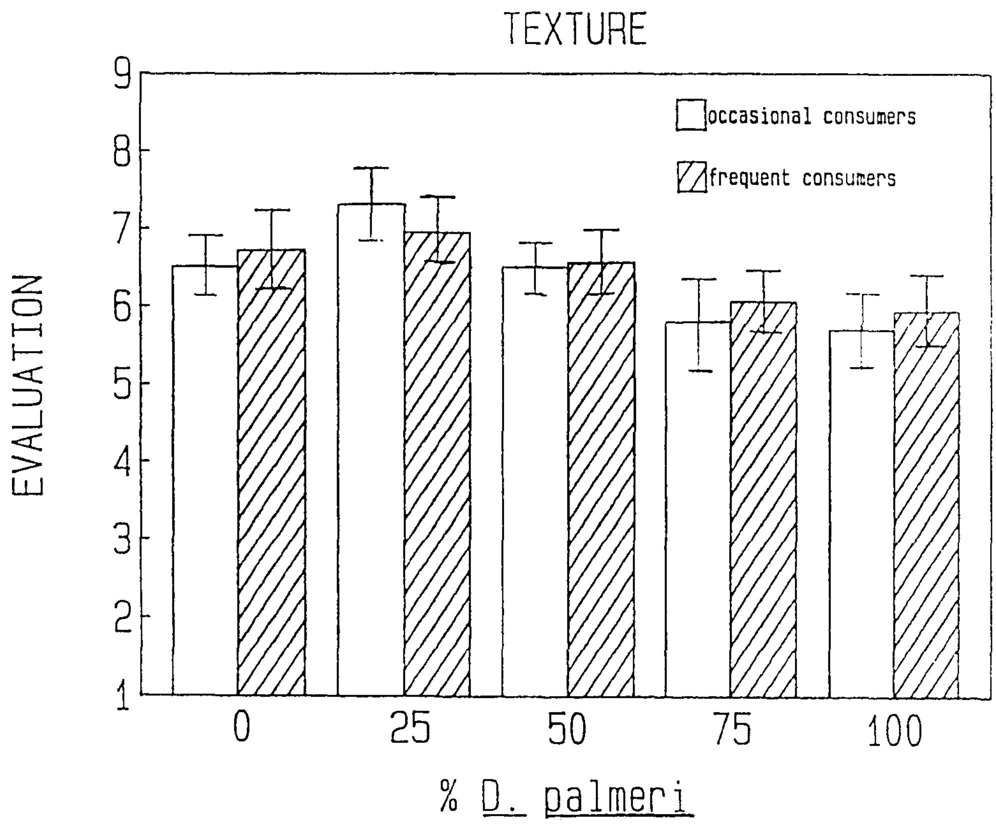

Figure 9. Dice-Leraas graph of "occasional" and "frequent" eaters texture evaluation of whole-wheat bread at 0, 25, 50, 75 and 100% concentrations of *Distichlis palmeri* flour substituted for whole-wheat flour, showing the standard error of the means (note: non-overlapping standard errors of the mean are significant).

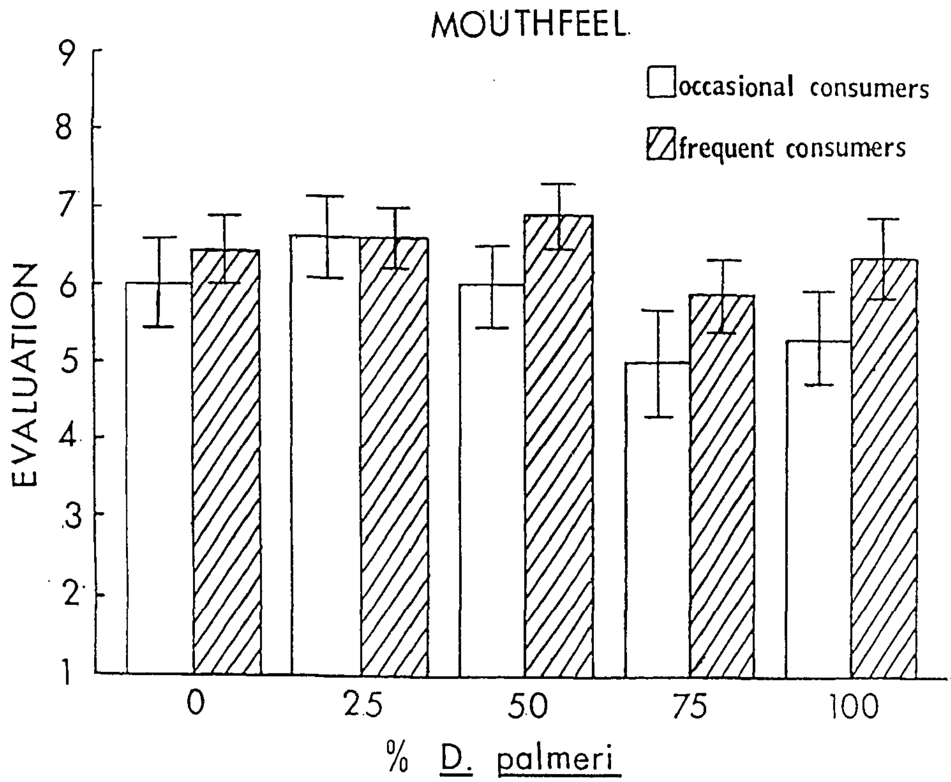

Figure 10. Dice-Leraas graph of "occasional" and "frequent" eaters mouthfeel evaluation of whole-wheat bread at 0, 25, 50, 75 and 100% concentrations of *Distichlis palmeri* flour substituted for whole-wheat flour, showing the standard error of the means (note: non-overlapping standard errors of the mean are significant).

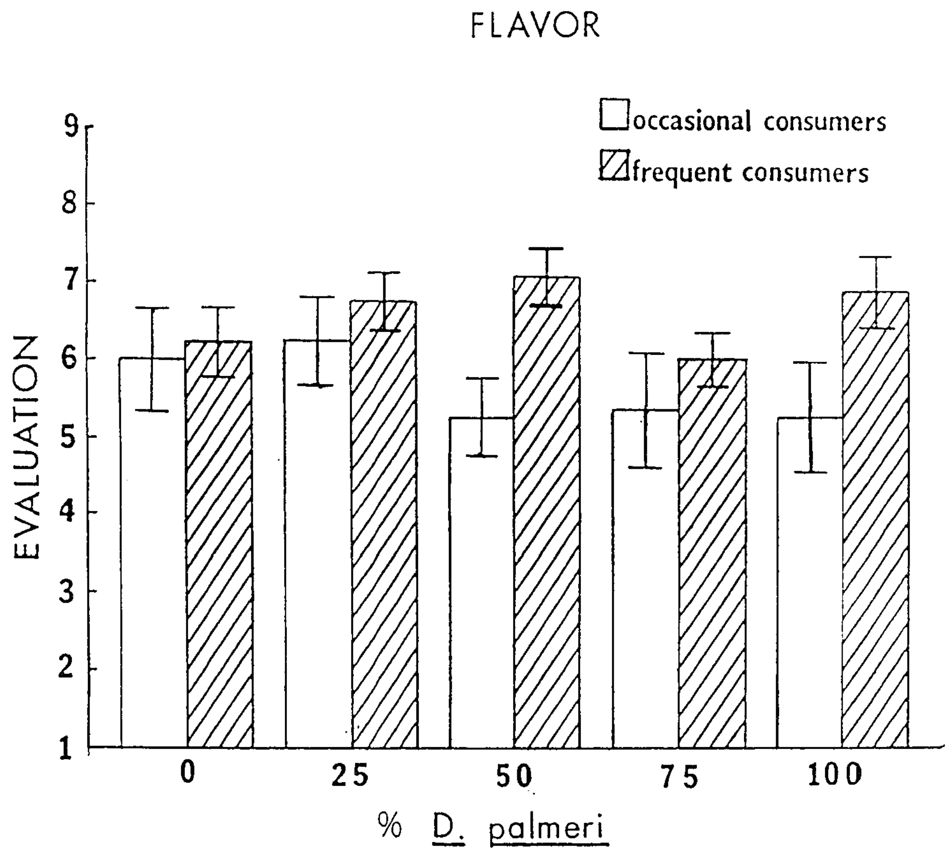

Figure 11. Dice-Leraas graph of "occasional" and "frequent" eaters flavor evaluation of whole-wheat bread at 0, 25, 50, 75 and 100% concentrations of *Distichlis palmeri* flour substituted for whole-wheat flour, showing the standard error of the means (note: non-overlapping standard errors of the mean are significant).

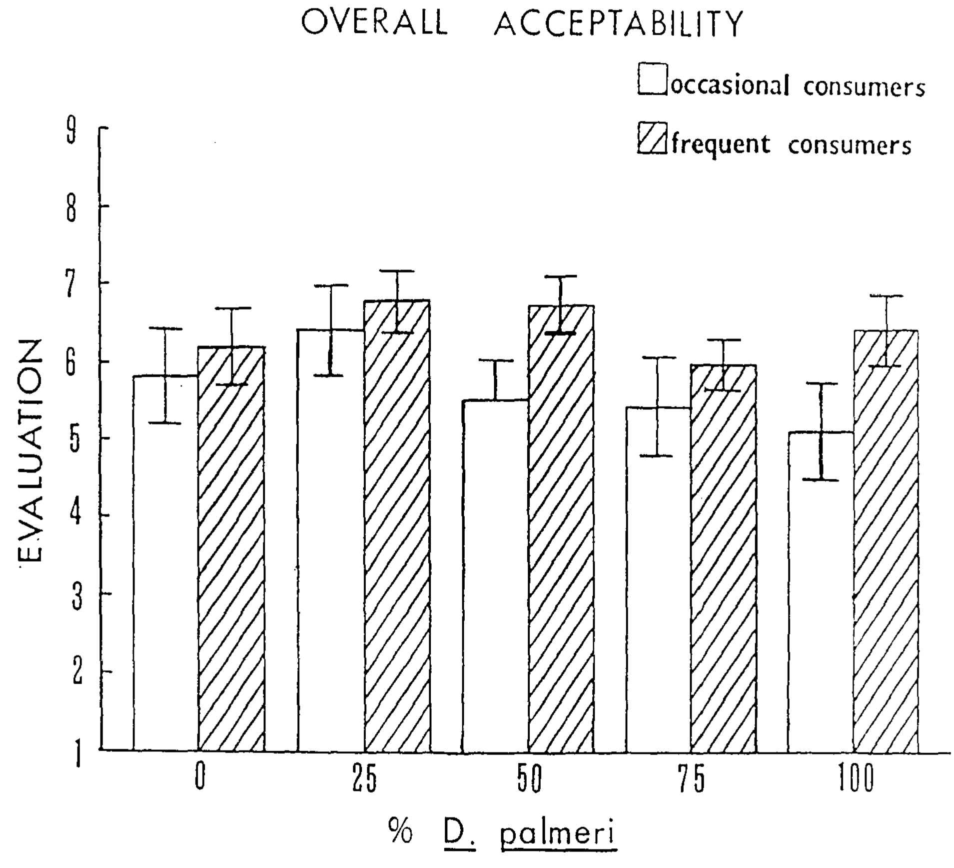

Figure 12. Dice-Leraas graph of "occasional" and "frequent" eaters overall acceptability of whole-wheat bread at 0, 25, 50, 75 and 100% concentrations of *Distichlis palmeri* flour substituted for whole-wheat flour, showing the standard error of the means (note: non-overlapping standard errors of the mean are significant).

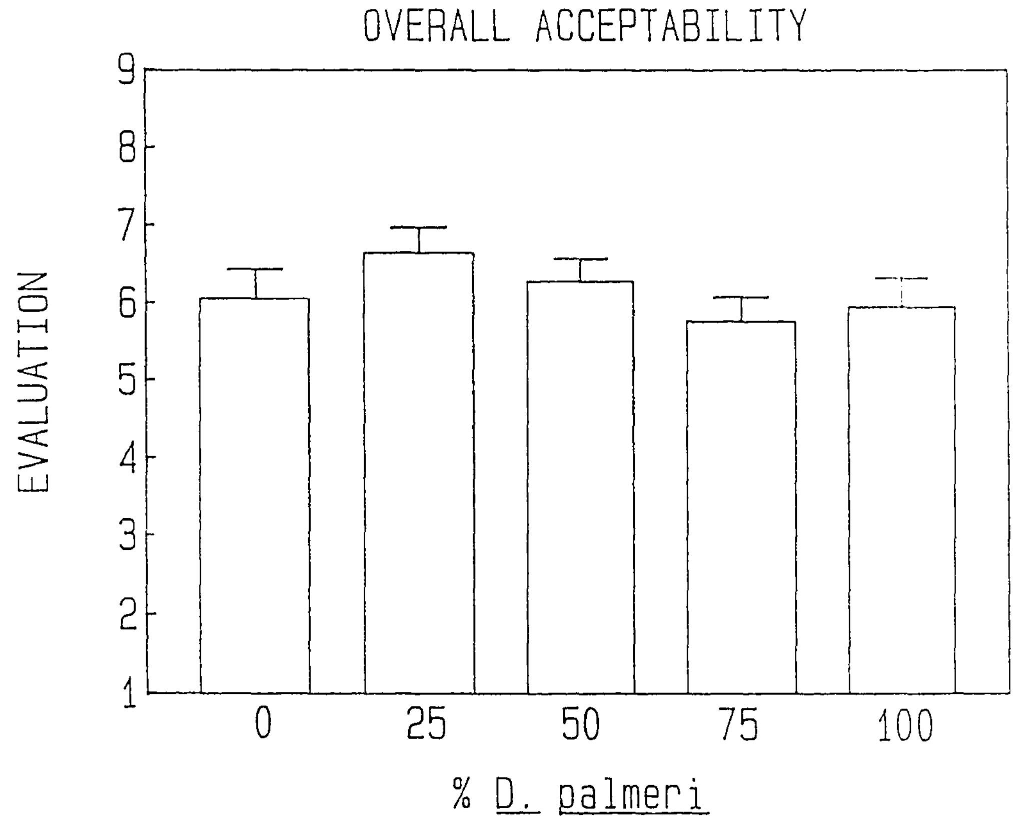

Figure 13. Dice-Leraas graph of overall acceptability of whole-wheat bread at 0, 25, 50, 75 and 100% concentrations of *Distichlis palmeri* flour substituted for whole-wheat flour, showing the standard error of the means (note: non-overlapping standard errors of the mean are significant).

CONCLUSIONS

Acceptance of a new product always presents a problem when first introduced. Part of this is due to the new flavor. In the case of *Distichlis palmeri* grain products, this was not a problem.

The nutritional value of *Distichlis palmeri* grain might be of importance for developing and developed countries. For instance, its high fiber content is important for the American diet which is generally low in fiber. The crop is also of agronomic importance because *Distichlis palmeri* can grow better in salt water than in fresh water. Therefore, abandoned salinized fields could be brought back into production.

CHAPTER 6

CHEMICAL COMPOSITION AND FIBER CONTENT OF GRAIN AND FORAGE OF *DISTICHLIS PALMERI*

ABSTRACT

The grain of *Distichlis palmeri* (Vasey) was analyzed and found to have 5.15 ± 1.2% soluble fiber and 12.7 ± 1.5% insoluble fiber. No trypsin inhibitor activity was observed in the flour. The two varieties of forage analyzed had good contents of protein (6.10% and 15.20%) and acid detergent fiber (33.0% and 20.20%) as well as low lignin (6.80% and 5.10%). Mineral content for both forages was: Ca (0.3% and 0.5%); Mg (0.09% and 0.2%); P (0.12% and 0.21%); Zn (6.20 ppm and 20.0 ppm), and Cu (5.50 ppm and 6.7 ppm).

INTRODUCTION

While the consumption of wheat is increasing worldwide, the land available for planting is decreasing due to several factors. One of those factors is the salinization of the lands and/or the irrigation water. The varieties of *Distichlis spp.* have shown a good potential for cultivation in such abandoned lands (Yensen et al, 1988). Previous work with the grain of *Distichlis palmeri* shows a good nutritional composition comparable to other cereal grains, including its digestibility and uses (Yensen and Weber, 1986, 1987; Tapia, 1988). NyPa Inc. has developed varieties of the genus *Distichlis,* some of which nutritionally resembles alfalfa, except for the fact that they grow in saline water. One patented forage variety is currently being grown in The United States, Mexico, Namibia, Australia, Saudi Arabia, and Morocco (Yensen, 1990). While more research needs to be done to continue evaluating the full potential of these plants in the food and forage industry, these new crops are already being grown commercially to feed people and animals.

The present study gives more data on two varieties. The objective of the study was to evaluate the content of soluble, insoluble and total dietary fiber, phytates and trypsin inhibitor activity in the grain of *Distichlis palmeri*. In addition the purpose was to determine the chemical composition of two varieties of *Distichlis*, a tall variety that could be used as a forage and a short variety that could be used as a turf.

MATERIALS AND METHODS

Sample preparation.

The grain of *Distichlis palmeri* was ground in a Weber mill (024 mesh). The leaves of the *Distichlis spicata* varieties, grown at the University of Arizona's Maricopa Agricultural Center farm, were freeze dried and ground in a hammer mill (40 mesh).

Proximal analysis in the grass.

Analysis of moisture, fat, ash, acid detergent fiber and lignin were done according to the AOAC methods (1984).

Soluble and insoluble fiber determination in the grain.

Analysis of soluble and insoluble dietary fibers was carried out according to Prosky et al. (1985). Briefly, the method consisted of taking quadruplicate 1 g samples and blanks and treating them with a heat stable amylase to break down the starch. This was followed by a protease and an amyloglucosidase treatment. This was done to remove the protein and any residual starch. For the soluble and insoluble fiber determinations the samples were treated with the same enzymes used in the total dietary fiber determination with the exception that the insoluble fiber was separated from the soluble fiber by filtration. Once separated the soluble fiber was then precipitated with ethanol (4 volumes). Both soluble and insoluble fiber residues were analyzed for ash and protein. Three replicates were done to obtain mean values.

Tripsin inhibitor.

The trypsin inhibitor activity was also determined in the grain by the AOAC method (1984). Basically, the sample was extracted with 0.01 N NaOH and then treated with the benzoyl-DL-arginine-p-nitroanalide reagent (BAPNA). Trypsin inhibitor activity is defined as an increase of 0.01 absorbance units/10 ml of mixture used at 410 nm.

Mineral analysis.

Mineral content was done in triplicate by a wet ash digestion method using nitric and perchloric acid plus heat. The sample was diluted and it was then analyzed with an atomic absorption spectrophotometer Hitashi Mod. 180-70 (Thompson and Weber, 1979).

Phosphorus analysis was run in triplicate with a nitric and perchloric acid digestion and made up to 100 ml volume. An aliquot was then taken from the 100 ml solution. This reacted with the molybdate solution in the presence of ferrous sulfate. A blue color was developed and read at 660 nm. A standard curve and a blank were also run at the same time (Koenig and Johnson, 1942).

RESULTS AND DISCUSSION

More attention has been given to fiber in the last decade than ever before. Scientists have now recognized the importance of fiber not only because of its benefits in the digestive tract but because of its linkage to the prevention of some diseases. Fiber has been shiown to lower the absorption rate of glucose and decrease the absorption of cholesterol. In some instances, soluble fiber is responsible for these benefits and in others the insoluble fiber.

In this study the soluble and insoluble dietary fiber of *Distichlis palmeri* was measured and compared with some of the most common staples (i.e wheat and rice).

Table 9 shows the levels of the soluble fiber content of the grain of *Distichlis palmeri* at 5.15 ± 1.2 % and the insoluble fiber at 12.7 ± 1.5 %. Likewise, soluble and insoluble levels of fiber are shown for wheat and rice.

Trypsin inhibitor is one of the factors responsible for lowering the digestibility of proteins. It is found mainly in legumes; but, it is also found in cereals (Sosulski, et al., 1988). Attempts were made to determine the trypsin inhibitor content in *Distichlis palmeri*. Several dilutions of the flour sample were tried with negative results for detection of trypsin inhibitor activity. Therefore, we concluded that there is no or an insignificant amount of this antinutritional factor in the flour of *D. palmeri*.

Table 10 shows the proximate composition of the forage and the turf varieties. The forage as well as the turf have a good acid detergent fiber content. They also have low fat and lignin content which makes them suitable for animal consumption.

The values of the turf variety for Ca, Mg, P, Zn, and Cu were all similar to the range found for alfalfa. However, the values for Ca, and P in the forage sample were lower than the range given for alfalfa.

Pre-feeding trials conducted in Morocco have shown that several types of animals (cows, sheep, goats, rabbits, and horses) like the taste of these forages. Figure 14 shows part of the study in which cows were used. The unpublished results of an initial feeding trial with yearling Pinot Noir dairy cattle in Morocco showed similar weight gains to their standard formulated ration (N.P. Yensen, per. comm.) Still, a feeding trial for these animals will give us a better idea about the value of these forages.

Although the samples for the forage and turf varieties analyzed were taken during spring, sampling throughout the year, and at different heights when cut, would give a better understanding of the proximal composition of these varieties.

Table 9. Comparison of soluble and insoluble dietary fiber in *Distichlis palmeri* grain, wheat and rice.

GRAIN TYPE	Soluble Dietary Fiber	Insoluble Dietary Fiber
Distichlis palmeri	5.15 ± 1.2 %	12.7 ± 1.5 %
Wheat/rye[1]	2.5 - 3.3 %	8.5 - 9.0 %
Oats[2]	4.2 %	5.7 %
White wheat flour[2]	1.2 %	1.6 %
Potatoes[2]	2.1 %	4.9 %
Rice (enriched long grain)[2]	0.2 %	0.8 %

[1](Asp et al 1983)
[2](Prosky et al 1988)

Table 10. Proximal analysis of the leaves of NyPa *Distichlis spicata* forage and turf varieties (dry weight basis).

	Forage	Turf	Alfalfa[1] /mean
Protein, %	6.1	15.2	13.0 -25.0 /19
Acid detergent fiber, %	33.0	20.2	14.0 -39.0[2] /27
Fat, %	1.7	2.8	1.4 - 3.2 / 2.3
Ash, %	6.8	10.8	5.0 -15.0 /10
Lignin, %	6.8	5.1	5.5 -10.6 / 8
Ca, %	0.3	0.5	0.76- 1.76 / 1.26
Mg, %	0.09	0.2	0.06- 1.7 / 0.88
P, %	0.12	0.21	0.21- 0.61 / 0.41
Cu, ppm	5.5	6.7	1.5 -20.2 /10.9
Zn, %	6.2	20.0	5.7 -35.1 /20.4

Values shown are averages of duplicate determinations.
[1] Ranges. From: National Acad. Sci., 1971.
[2] Crude fiber.

Figure 14. A cow eating the leaves of *Distichlis spp* as a part of a study conducted in Morocco.

CONCLUSION

It appears that in the *Distichlis palmeri* grain there is either no trypsin inhibitor or it is present in insignificant quantities. As such, it would not have antinutritional effects on the digestibility of proteins.

The forage, as well as the turf, have: 1) a good acid detergent fiber content, 2) low fat and low lignin content, and 3) Ca, Mg, P, Zn and Cu contents similar to alfalfa; all of which are desirable characteristics of an animal fodder. The palatability is good for dairy and meat cattle, sheep, goats, rabbits, chickens and horses. Initial feeding trials show similar weight gains compared to a standard formulated ration.

CHAPTER 7

CONCLUSIONS

While these results suggest that the protein fractions of *Distichlis palmeri* are perhaps most similar to rice, they fall in the general nature and composition to other cereals. The lysine content, while higher than wheat, is still relatively low compared to that of legumes; and, as in wheat, the high levels of glutamines do not optimize the potential nutritional value of the proteins. Total protein extraction was 72%.

The fraction obtained with SDS was the most abundant one, as was the case for rice glutelins, followed by the ethanol, phosphate buffer, sodium chloride and 2-mercaptoethanol. The lowest value obtained was from the 2-mercaptoethanol fraction, 22.80 mg. The fractions were all rich in asparagine and glutamines, a condition which is common in seed storage proteins. The lysine content of this grain was higher than that of wheat. The highest values for lysine were found in the water soluble part. Like other cereals, the prolamine fraction had the lowest value for lysine.

The protein solubility profile showed the prolamine fraction soluble only at pH 9 and higher, whereas the albumin was soluble at all pH's but more so at higher pH. The globulin fraction exhibited a solubility typical of the proteins with an isoelectric point at pH 4.5.

The whole flour of *Distichlis palmeri* had a superior water and oil holding capacity when expressed as g/g protein than when given as g/g sample. This was so even though the other macro nutrients present in the flour, i.e. starch, contributed to the absorption of water and oil.

Foaming capacity for the albumin fraction was excellent. It produced a foam volume equal to 400% of the pre-foamed volume. This was higher than soy bean protein isolate. The foam produced was of very fine bubbles and retained a foam volume of 100% after 30 minutes. The whole flour did make some foam; but, it was very coarse and unstable.

The emulsion capacity was better in the whole flour than in the other fractions, globulins and prolamines. This could be due to the protein interaction as well as the starch present in the whole grain.

The "occasional eaters" and the "frequent eaters" of whole-wheat bread were strongly influenced by color and appearance as opposed to the "exclusive eaters" of whole-wheat bread. For both color and appearance the "occasional and frequent eaters" gave a monotonically declining response with increasing *Distichlis palmeri* concentrations ($P < 0.05$). Surprisingly, color explained the largest portion of the variance followed by appearance.

The characteristics, texture, and mouth feel explained the least portion of the variance. Indeed there was no significant difference between the "exclusive", "frequent", and "occasional" eater of whole-wheat bread for these characteristics. This held true at all concentrations of *Distichlis palmeri*.

With respect to flavor, the "exclusive" and "frequent" eaters of whole-wheat bread showed no significant change over the range of *Distichlis palmeri* concentrations. The "exclusive" eaters of whole-wheat bread, however, rated the flavor of the *Distichlis palmeri* significantly higher than the "frequent" eaters of whole-wheat bread. On the other hand the "occasional" eaters of whole-wheat bread had a gradual, but still significant, decrease in preference with increased *Distichlis palmeri* concentrations.

Overall the "exclusive" eaters of whole-wheat bread, showed no significant preference for muffins with 0, 25, 50, and 75% concentrations of *Distichlis palmeri*. The 100% *Distichlis palmeri* muffins, however, were less preferred by this group. Oddly, the main characteristics responsible for this reduced preference were the ones with the low variance, texture and mouth feel.

It was interesting to note that the "occasional" eaters of whole-wheat bread consistently gave all concentrations of *Distichlis palmeri* a higher rating than the "frequent" eaters. Furthermore, the "occasional" eaters' ratings were generally significantly higher.

Overall, the "exclusive" eaters of whole-wheat bread rated the *Distichlis palmeri* muffins significantly higher than either the "occasional" or the "frequent" eaters of whole-wheat bread. More importantly, the "exclusive" eaters of whole-wheat bread did not significantly prefer the whole-wheat muffins over any of the mixtures with *Distichlis palmeri*. Indeed, the 50/50 mix received the highest rating.

The soluble fiber content of the grain of *Distichlis palmeri* was 5.15 ± 1.2 % and the insoluble fiber, 12.7 ± 1.5 % . Trypsin inhibitor was not found in the flour of *Distichlis palmeri*.

The forage as well as the turf varieties have a good acid detergent fiber content, as well as low fat and lignin. This makes them suitable for animal consumption.

The values of the turf variety for Ca, Mg, P, Zn, and Cu were all within a similar range found for alfalfa. The values for Ca, and P in the forage sample were lower than the range given for alfalfa.

Although the samples for the forage and turf varieties analyzed were taken during spring, sampling throughout the year should be done. This would give a better understanding of the proximal composition of these varieties.

This plant has shown potential for becoming the first halophytic grain to be used for land reclamation. The development of this crop will certainly not compete with any other grain. I would merely make use of something that has become a great problem for agriculture: salt. This and other halophytic plants could well be an answer for developing countries where fresh water is precious for human survival.

APPENDICES

APPENDIX A

1A. THE NyPa® SCALE (for comparing different salinity measurements)

THE NyPa® SCALE*

CROP TYPE:	Drinking water	SENSITIVE	RESISTANT	SALT-LOVING (HALOPHILIC) CROPS			SEA WATER

WEIGHT (Total Dissolved Solids = TDS)•

ppm (= mg/L = g/m³ = µg/ml)		10,000	20,000	30,000	35,000
ppt (= g/L)		10	20	30	35
%	0	1	2	3	3.5

Tons/acre-ft.	0	10	20	30	40

Grains/gallon	0	500	1,000	1,500	2,000
Chlorinity (ppt)	0	5	10	15	19.4

ELECTRICAL CONDUCTIVITY (EC) •

µS/cm		10,000	20,000	30,000	40,000	46,000
mS/m		1,000	2,000	3,000	4,000	4,600
dS/m = mS/cm = K_e		10	20	30	40	46
S/m (= mho/m = 0.01 S/cm)	0	1	2	3	4	4.6

PRESSURE ★•

Pascals = Newtons/m²		1,000,000	2,000,000	2,700,000
- Bars [+2% = Atm.]		10	20	27
MPa [X10⁷ = dynes/cm²]	0	1	2	2.7

MOLECULES

mmoles/L = moles/m³ = meq		100	200	300	400	500	600
moles/L (-2%~ moles/Kg)	0	0.1	0.2	0.3	0.4	0.5	0.6

Scale												
Osmolality /kg H_2O	0	1	2	3	4	5	6	7	8	9	10	11

DENSITY •

Specific Gravity ρ	1.000	1.005	1.010	1.015	1.020	1.025

Refraction (R) [Refractive Index = R x 10⁻⁴+1.333]	0	9	18	26	35	44	53	61

MEASURING INSTRUMENTS:
- • Refractometer/Hydrometer
- • Conductivity Meter
- ★ Pressure Bomb

* Based on NaCl as in sea water. Other salts may skew electrical measurements, but estimates are possible if the deviation is known. Also note that descrepancies may occur with electrical instruments because conductivity and salt concentration increase at different rates.
Note: S = *Siemens* = *mho*; MPa = *Megapascals*; Atm = *Atmospheres*; meq = *milli equivalents/liter*.

(Produced in cooperation with NyPa Inc. and Richland Laboratories, SA, Australia)

APPENDIX B

B. Honey-Wheat Muffins: recipe used during the testing program.

3/4 cup	All-purpose white flour
3/4 cup	Whole-wheat flour
2 tsp	Baking powder
½ tsp	Salt
1	Egg
½ cup	Milk
½ cup	Honey
½ cup	Margarine, melted

In a large bowl mix flours, baking powder and salt. In a small bowl beazt egg with fork; beat in milk, honey and butter. Add to flour mixture; stir just until blended. Fill greased pans 2/3 full. Bake in preheated 400°F oven until pick inserted in center comes out clean. For 36 miniature muffins this will atake 15 minutes.

APPENDIX C

C. Sample questionnaire

The purpose of this sensory evaluation is to determine consumer preference among four different samples being presented. For purposes of analysis, please complete the following three personal data questions.

1) SEX Male______ Female ______

2) AGE GROUP 18 - 20 years ______

21 - 30 years ______

31 - 40 years ______

41 - 50 years ______

51 or over ______

3) HOW OFTEN DO YOU CONSUME WHOLE WHEAT PRODUCTS?

Never ______

Occasionally ______

Frequently ______

Almost exclusively ______

APPENDIX D

D. Sample test

Please circle the number on the line where you feel each product characteristic being evaluated belongs. The scale ranges from one to nine with 1 being the most negative (don't like it at all) to 9 being the most positive (like it very much). This scale will apply to all four samples.

Please evaluate this sample on the following criteria:

Sample code									
	1	2	3	4	5	6	7	8	9
Color									
Appearance									
Texture									
Mouthfeel									
Flavor									
Overall acceptability									

The sample codes were: a circle, a triangle, a square, a pentagon, and a star.

Sample test (cont...)

Please rank the samples in the order of preference wiht 1 being the best and 4 being the worst.

Sample code ______ 1

Sample code ______ 2

Sample code ______ 3

Sample code ______ 4

Sample code ______ 5

Thank you for participating in this sensory evaluation. Your time and effort has been appreciated.

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