Nutritional Evaluation of Marama Bean (*Tylosema esculentum*, Fabaceae): Analysis of the Seed[1]

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*Marama bean (*Tylosema esculentum, *Fabaceae), a potential arid-land legume crop from the Kalahari Desert of Botswana, was analyzed for protein, amino acids, oil, fatty acids, fiber, caloric value, trypsin inhibitor, and mineral content. Results indicate that the bean is adequate in these nutrients for a human diet, but the trypsin inhibitor activity should be destroyed prior to consumption.*

Berechnungen betreffend des Ernährungswertes der Marama Bohne (*Tylosema esculentum,* Fabaceae): Die Analyse der Samen. *Die Marama Bohne ist potentiell ein Legumen aus der Kalahari Wüste für Ernten in öden Ländern. Es wurde nach Proteinen, Aminosäuren, Öl, Fettsäuren, Fasern, Heizwert, Trypsin Hemmungstoffen, und anorganischem Gehalt analysiert. Die Ergebnisse zeigen, dass die Bohne adäquat ist für menschliche Ernährung in diesen Nährstoffen, aber das die Trypsin hemmende Aktivität sollte vor dem Verzehrt zerstört.*

The marama bean, *Tylosema esculentum* (Burchell) Schreiber (Fabaceae)—also known as morama, mangetti, moramma nut, tsin, braaiboontjie, and maramma—is a perennial legume native to the arid and semi-arid grasslands of southern Africa (Bousquet 1981–1982; Keith and Renew 1975). The stems are prostrate and trailing, up to 3 m in length; the bi-lobed leaves are leathery and glaucous-green at maturity. The yellow flowers, borne in racemes, produce an oval-oblong pod 5–6 cm long, usually bearing two (but up to six) round seeds about 2 cm in diameter. Mature beans, brownish black and very hard, are often called "nuts" by peoples of the Kalahari (Coetzer and Ross 1976; Keith and Renew 1975); when roasted, the beans are considered a delicacy. The sweet-tasting, tuberous root is the size of a sugar beet (Vietmeyer 1986). For many Kalahari Desert foragers the marama bean has been the preferred staple for centuries (Bousquet 1981–1982; Keegan and von Staden 1981).

The National Research Council in 1979 noted that marama bean is a neglected legume of great potential (National Academy of Sciences 1979). At an annual meeting of the AAAS, Bousquet (1983) summarized the work done prior to 1983, noting that the plant is being studied for possible cultivation in the United States at research centers in Texas and elsewhere (Bousquet 1981–1982; Miller 1981). The results of some of these efforts have recently appeared in this journal (Powell 1987). Marama has also been suggested as a plant worthy of cultivation in arid and semi-arid regions of Africa (Keegan and von Staden 1981). Here we report on some selected nutritional qualities of the marama bean.

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TABLE 1. SELECTED NUTRIENTS OF MARAMA SEEDS.[a]

	Our study	Literature[b,c,d]
Protein (g) (N × 6.25)	31.8 ± 1.1	31.6, 34.3, 29.5
Oil (g)	42.2 ± 1.6	36.1, 34.8, 42.8
Fatty acids	34.0 ± 1.6	N.D.[e]
Waxes	8.2 ± 1.9	N.D.[e]
Carbohydrate (g)[f]	18.9 ± 2.2	23.0, 23.1, 24.3
Ash (g)	3.2 ± 0.1	2.9, 3.2, 3.2
Moisture (g)	3.9 ± 1.0	5.2, 5.6, N.D.[e]
Energy (MJ)	2.66 ± 0.08	2.27, 2.68, 2.34

[a] Values are based on an as-received (5–7 yr post harvest) weight of 100 g of mature, viable, de-shelled seeds. The average weight of a seed before de-shelling was 1.8 g, with the shell comprising 50% of this weight.
[b] Ripperger-Suhler and Longenecker (1982).
[c] Wehmeyer et al. (1969).
[d] National Academy of Sciences (1979).
[e] No data.
[f] Obtained by difference.

METHODS AND MATERIALS

Samples of marama bean [*Tylosema esculentum*; *Storey 1016*, COCO] were a gift from Joseph Bousquet, then of the Department of Sociology and Anthropology, Southwestern Texas State University, San Marcos, Texas. The samples were originally harvested from 1978 through 1980 from the Kalahari Desert, Ghantsi, Botswana. These seeds were germinated and grown to maturity in our greenhouse.

Mixed samples of the dry, feral bean were cracked with a hammer to remove the hard seed coat. After homogenization, a portion of the shelled beans was dried at 110°C overnight to obtain the percent moisture. The same portion was then ashed overnight at 550°C.

A second portion of the shelled beans was defatted with diethyl ether in a Soxhlet extractor. Extracted oil was subsequently reacted with methanol and boron trifluoride to produce methyl esters of the fatty acids (Welcher 1975) that were identified and quantified using a gas chromatograph with a mass selective detector (Hewlett-Packard model 5995 equipped with an OV-101 methyl silicone, capillary column). A Perkin Elmer 900 gas chromatograph equipped with 10% diethlyene glycol succinate and FFAP (Varian) columns and a flame ionization detector was also used. The fatty-acid-ester-to-wax ratio in the oil was determined by using the infra-red carbonyl peak calibrated with a series of oleic acid:nujol oil standards. Free acid versus ester content was estimated using the free-acid hydroxyl peak, which was essentially absent from the marama oil spectrum.

A third portion of the beans was defatted with a Soxhlet extractor and the resulting lipid-free meal was analyzed for its Si, P, S, Cl, K, Ca, Mn, Fe, and Zn content after placement in a polyethylene cell (Spex model 3529) covered with polycarbonate (Spex model 3522) using x-ray fluorescence (Rigaku model 3064). Calibration curves and quality assurance were achieved by analyzing National Bureau of Standards biological standards numbers 1566, 1567, 1571, 1573, 1575, and 1577 for the metals. Because this method is non-destructive, 0.1 g of the same sample was digested in 5 ml concentrated HNO_3 for analysis of Na by atomic emission and Mg by atomic absorption spectrophotometry (Varian model 275B).

TABLE 2. PERCENT COMPOSITION OF THE FATTY ACIDS IN MARAMA BEAN OIL.

Fatty acid	Ratio of chainlength to number of pi bonds	Our study[a]	Literature[b,c]
Myristic	(14:0)	1.3 ± 0.3	N.D.,[e] Trace
Palmitic	(16:0)	13.8 ± 5.0	16.9, 14.1
Palmitoleic	(16:1)	1.7 ± 0.3	1.8, 0.7
Stearic	(18:0)	9.7 ± 7.0	10.0, 6.5
Oleic	(18:1)	48.5 ± 8.0	34.8, 47.9
Linoleic[d]	(18:2)	19.2 ± 9.5	26.3, 24.6
Linolenic	(18:3)	2.0 ± 1.5	2.3, N.D.[e]
Arachidic	(20:0)	2.8 ± 1.3	3.4, 3.3
Arachidonic	(20:4)	N.D.[e]	2.1, N.D.[e]
Others	—	1.2 ± 1.0	N.D.,[e] 2.8

[a] Data are normalized to 100%. Total fatty acid was 34.0 g/100 g of seed. The presence of a pi bond indicates unsaturation in a hydrocarbon chain.
[b] Bousquet (1983).
[c] Engelter and Wehmeyer (1970).
[d] Essential fatty acid.
[e] No data.

The defatted meal was also analyzed for protein content and composition by a modification of the procedure by Storey et al. (1982). The albumins were obtained as the water soluble fraction after repeated dialysis with water at 4°C. The remaining proteins (Table 5) were separated by successive homogenization with a polytron (Brinkman) and centrifugation at 12,000 rpm (each for 10 min) using 1 N NaCl buffered with 50 mM sodium phosphate (pH 7) for the globulins. The extractant was replaced with 50% (v/v) n-propanol for the prolamines, 50 mM borate buffer (pH 10) for the alkali soluble glutelins, and 1% (v/v) acetic acid for the acid soluble glutelins. In each extraction, 12 ml of solution were used per gram of seed. The completeness of each extraction was monitored by measuring the absorbance of the extraction solution at 280 nm. Typically, five or six repetitions were needed for extraction of 95% of the protein from the meal, giving a total of 60–72 ml of extraction solution for each of the five fractions. Protein from each extraction step was immediately quantified using the biuret method (Welcher 1975). The purity of the various protein fractions was further examined using sodium dodecyl sulfate (SDS) one-dimensional disk and plate gel-electrophoresis (O'Farrell 1975) with bovine albumen, egg albumen, pepsin, carbonic anhydrase, and lysozyme (Sigma Chemical Co.) as molecular weight standards. The extracts were tested for trypsin inhibitor activity before and after boiling using N-a benzoyl-L-arginine p-nitroanilide as the substrate (Kassell 1970). A portion of defatted meal was also dry baked for 20 min at 140°C and extracted to determine how much of the trypsin inhibitor in the various fractions would be destroyed under typical roasting conditions.

Total protein was measured in the defatted material by the Kjeldahl method, and the caloric content of the meal and oil was measured with a Parr oxygen bomb calorimeter. Amino acids were determined with an amino acid analyzer (Carlo Erba 3A29) after acid hydrolysis under nitrogen using norleucine as an internal standard, except for tryptophan, which was determined spectrophotometrically (Hewlett-Packard 8450A) using a slight modification of the procedure by Bencze and Schmid (1957) after a base hydrolysis. Acid hydrolysis can also

TABLE 3. PROTEIN, FIBER, STARCH, ENERGY, AND MINERAL CONTENT OF DEFATTED MARAMA BEAN MEAL.[a]

	Marama meal	Tepary bean[b]	Jojoba[c]
Protein (g) (N × 6.25)	550 ± 5	224	270
Energy (MJ)	1.94 ± 0.6	N.D.[d]	1.4
Fiber (g)	16 ± 2	25	120
Ash (g)	66 ± 3	39	39
Sodium (g)	0.24 ± 0.02	0.45	0.27
Magnesium (g)	5.8 ± 0.1	1.5	2.8
Silicon (mg)	40 ± 20	N.D.[d]	N.D.[d]
Phosphorus (g)	6.7 ± 0.4	3.0	3.5
Sulfur (g)	3.7 ± 0.2	2.0	3.0
Chlorine (g)	0.3 ± 0.1	1.8	3.0
Potassium (g)	16.0 ± 1.3	0.14	13.2
Calcium (g)	4.2 ± 0.2	2.1	0.74
Manganese (mg)	50 ± 20	13	42
Iron (mg)	40 ± 20	45	92
Zinc (mg)	100 ± 20	35	32
Available starch (g)	130 ± 13	N.D.[d]	N.D.[d]

[a] Amount per kg of defatted, dry matter feed.
[b] From Taggert et al. (1983).
[c] From Utz et al. (1982).
[d] No data.

destroy cystine, so low results were expected for this sulfur-containing amino acid. However, since the total sulfur was known, an upper limit could be determined for the cystine content. Crude fiber and the availability of starch were measured according to standard methods (Horowitz 1975; Porter et al. 1973). The precision (%RSD) of most of the analyses was 10% or better.

RESULTS AND DISCUSSION

The mean values (and one standard deviation, based on triplicate analyses) of our analysis of selected nutritional components of the whole bean (Table 1) are in general agreement with previous studies, although the total oil reported is slightly greater, and the carbohydrate slightly lower, than given in earlier reports. The protein and fat in marama bean are higher than in several types of bean (Kanamori et al. 1982) and many other potential crops (Vietmeyer 1986). Table 1 gives a breakdown of the marama bean oil into fatty acid esters and waxes. Less than 5% of the fatty acids is present as the free acids; the balance is esterified in the oil. Compared to soybeans, which contain about 17% lipid (Street and Opik 1975), the amount of oil in marama is quite high and is present largely as mono-saturated or unsaturated fatty acid esters (Table 2). Unsaturated fats are usually recommended over saturated fats for the human diet, but this is controversial (Marshall 1986).

The protein, fiber, available starch, total energy, and mineral content of defatted seed meal of marama bean is given in Table 3. These values are compared to two other potential crops that are now in limited cultivation—tepary beans and jojoba (Taggart et al. 1983; Utz et al. 1982). Jojoba meal is very high in fiber compared to marama and tepary, while the samples of marama are quite low in sodium and

TABLE 4. AMINO ACID COMPOSITION OF MARAMA BEAN PROTEIN, IN PERCENT.

Amino acid	Marama bean		Soybean literature[a]
	Our study	Literature[a]	
Essential amino acids[a]			
Arginine	5.96	9.58	7.93
Cystine	0.78[b]	2.01	1.78
Histidine	2.25	3.57	2.62
Isoleucine	3.75	5.66	5.90
Leucine	5.58	7.70	8.46
Lysine	5.22	6.51	6.93
Methionine	0.76–1.32[b]	1.55	1.34
Phenylalanine	4.58	5.81	5.42
Threonine	2.89	4.97	4.32
Tyrosine	11.13	14.54	3.49
Tryptophan	1.55	1.29	1.51
Valine	4.18	6.12	5.75
Nonessential amino acids		N.D.[c]	N.D.[c]
Alanine	2.98		
Aspartic acid	10.31		
Glutamic acid	14.89		
Glycine	5.36		
Proline	6.60		
Serine	5.09		
Ammonia	1.18		
Total	95.04%		

[a] Bousquet (1983).
[b] Unprotected. Upper limit determined by difference.
[c] Not determined.

chlorine. Other values are relatively close, except that protein content in marama bean is much higher than tepary and jojoba meal. In fact, marama, with 32% protein, is apparently higher in total protein than most other legumes, including lupine (31%), lens (24%), pea (23%), broadbean (23%), and phaseolus (22%), though lower than some soybean varieties, which can have 38–40% protein (Boulter 1977).

Because of the relatively high total protein content of defatted marama bean meal (55%), we analyzed it for some of the essential and non-essential amino acids (Table 4). From literature data and the data obtained in our study, marama bean protein appears to be comparable to soybean in essential amino acid content, with methionine as the limiting amino acid. Both methionine and cystine appear

TABLE 5. PROTEIN COMPOSITION OF MARAMA BEAN MEAL, IN PERCENT.[a]

	Marama	Soybean[b]	Jojoba[c]	*Phaseolus*[b]
Albumins	23.3 ± 3.0	10	36–45	15
Globulins	53.0 ± 2.5	90	11–13	75
Prolamines	15.5 ± 0.7	0	0	0
Alkali-soluble glutelins	7.7 ± 0.2	0	36–44	10
Acid-soluble glutelins	0.5 ± 0.1	0	0	0

[a] About 10% of the marama bean protein was lost during the extensive fractionation procedure.
[b] From Boulter (1977).
[c] From Storey et al. (1982).

to have concentrations about half of those reported by Ripperger-Suhler and Longenecker (1982), but our values are corroborated by the total sulfur in the meal (Table 3). In spite of the low values obtained for cystine and methionine, marama protein quality is generally superior to most common legume crops, such as garden bean and pea (Boulter 1977; Kanamori et al. 1982).

As mentioned before, Table 5 shows the seed protein of marama broken down into the constituent proteins. This distribution is different from other legumes and jojoba. Surprisingly, in light of the relatively low content of cystine and methionine, marama bean albumin content is higher, and globulin is lower, than soybean. Like phaseolus, marama contains glutelins. The presence of alcohol soluble protein (prolamine) in a legume seed is also unusual (Boulter 1977). SDS gel electrophoresis indicates that the albumin fraction contains proteins with molecular weights of 14,000, 22,000, 82,000, and 280,000 daltons; the globulin fraction, 19,000, 21,000, 37,000, 73,000, 143,000, and 286,000 daltons; the prolamine fraction, 15,500, 19,000, 66,000, and 220,000 daltons; and the alkali-soluble glutelin fraction, 14,500 and 100,000 daltons. The acid-soluble glutelin fraction was in such low concentration in the initial extract that no data on molecular weights were obtained.

The water-soluble and saline-soluble protein fractions also contained a potent trypsin inhibitor activity, comprising about 40% of the protein in the aqueous-soluble fraction, and 20% of the protein in the saline-soluble fraction. Thus, trypsin inhibitor was about 20% of the total seed protein in this batch of seeds. It is unusual to find trypsin inhibitors in the saline-soluble protein fraction of seeds, as they are more typically albumins (McNab 1977; Ryan 1981; Samac and Storey 1981; Storey et al. 1982). However, the presence of trypsin inhibitor is common in legumes, typically comprising 5–10% of the total protein (Ryan 1981). Other plants such as barley and potato tuber may also contain as much as 10% of the inhibitor.

Trypsin inhibitor activity in marama can be destroyed by heat. Baking the defatted seed meal at 140°C for 30 min decreased the activity in the aqueous protein-extracts by 80% and the saline protein-extracts by 50%, giving a decrease of 70% in total trypsin inhibitor activity of the meal. Boiling fresh extracts from uncooked seed meal for 2 min in a microwave oven reduced the aqueous extracts' activity by 90%, and the saline extracts' activity by 75%. This amounted to an 80% reduction in the total activity. These findings are in agreement with those of Ripperger-Suhler and Longenecker (1982), who found the marama bean trypsin inhibitor to be six times more potent than that found in soybeans when measured by the method of Kakade et al. (1969). They found no toxic hemagglutinins using the method of Jaffe and Gomez (1975). They also found that autoclaving at 15 psi and 120°C for 20 min destroyed 99% of the inhibitor, but that baking at 150°C for 20 min did not adequately denature the protein. Our work on measuring the inhibitor in the various fractions indicates that the saline-soluble fraction is twice as difficult to denature as the water soluble fraction. We intend to explore the properties of this protein further in our nutritional analysis laboratory.

CONCLUSION

Marama bean has been an important food source for the peoples of the Kalahari Desert for centuries. Our study indicates more clearly why this is true, and we

suggest that it would be valuable to further pursue its cultivation as a food for humans or animals or both. Proper cooking appears to render the bean more palatable, and it significantly reduces the trypsin inhibitor activity. The level of the dietary nutrients tested was indicative of a high quality food. In fact, marama cultivation could prove more valuable than some of the established crops as a source of complete protein, minerals, carbohydrate, and lipid in the diet of humans and animals that inhabit arid or semi-arid regions of the world.

ACKNOWLEDGMENTS

We wish to thank Joseph Bousquet for providing us with numerous reprints and preprints on the marama bean, and for the seeds used in this analysis.

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Book Review

Non-Traditional Oilseeds and Oils of India. N.V. Bringi (ed.). Oxford & IBH Publishing Co., 66 Janpath, New Delhi 110 001. 1987. 254 pp. approx. $25.00.

Designed primarily for the Indian audience, this book is written as a reference source for scientists, technologists, industry managers, entrepreneurs, and planners that are interested in exploring the full potential of non-traditional oilseeds and oils of India. Reviews presented in ten chapters provide information on the characteristics of each of the oils and fats, the different processes developed for isolation and upgradation, and the technologies relevant for application in industry. Included are data on the chemical nature of materials other than seeds, as well as lists of components with their potential applications; free-hand drawings of some of the species accompany the introductions to a number of chapters.

The first four chapters deal with speciality confectionery fats, e.g., sal (*Shorea robusta*), mowrah (*Madhuca indica*), mango (*Mangifera indica*), kokum (*Garcinia indica*), dhupa (*Vateria indica*), and phulwara (*Bassia butyracea*), which may serve as substitute sources for cocoa butter. Three additional chapters review studies that explore the value of karanja (*Pongamia glabra*) and neem (*Azadirachta indica*) in agriculture and soap-making, and kusum (*Pongamia glabra*) for its cyano-lipids. Two additional chapters summarize recent advances using pilu and khakan (*Salvadora oleoides* and *S. persica*), and pisa (*Actinodaphne hookeri*), as sources of scarce lauric and myristic acids. The final chapter deals with 11 selected oilseeds offering good commercial scope for development.

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