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Nutritional quality and organoleptic acceptability of akara prepared from germinated tepary beans

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The University of Arizona, 1987

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NUTRITIONAL QUALITY AND ORGANOLEPTIC ACCEPTABILITY OF AKARA PREPARED FROM GERMINATED TEPARY BEANS

by

Ahmed Idouraine

A Thesis Submitted to the Faculty of the

DEPARTMENT OF NUTRITION AND FOOD SCIENCE

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE
WITH A MAJOR IN FOOD SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

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This thesis has been approved on the date shown below:

Ann M. Tinsley
ANN M. TINSLEY
Associate Professor of
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July 8, 1987
Date

DEDICATION

To my mother with all my love.

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ABSTRACT

Tepary bean (Phaseolus acutifolius G.), was germinated for 48 hours and used for akara preparation. Raw and germinated teparies and uncooked and cooked akara were analyzed for proximate and amino acid composition, vitamin A and ascorbic acid contents, trypsin inhibitor activity (TIA), and compared to that obtain using cowpeas.

Protein and amino acid composition of cowpeas and raw teparies were similar. Vitamin A and ascorbic acid contents were low. Germination increased vitamin A and ascorbic acid contents but had little effect on protein content, amino acid composition, and TIA. In cooked akara, protein content dropped from 21.03-23.77% to 12.05-14.36%, ascorbic acid from 5.76-8.88% to 1.22-1.60%, and TIA from 12.41-12.98% to 9.15-11.25%. For sensory evaluation, no significant difference in color, texture, and flavor was found but for the overall acceptability, akara prepared from raw teparies was significantly ($P \leq 0.05$) preferred to akara prepared using cowpeas and germinated teparies.

CHAPTER 1

INTRODUCTION

Legumes are an important source of nutrients in major developing countries. They are grown under a wide range of environments and their dietary and economic importance is well established (Salunkhe, 1982; Aykroyd et al., 1982). Their adaptation in arid and semi-arid regions is of particular interest as these areas are among the poorest regions of the world. It has been estimated by the World Food Bank that two-thirds of the people in these regions suffer from malnutrition. They habitually or seasonally consume less protein and calories than they need for adequate health and activity. Protein calorie malnutrition (CPM) is less dramatic than famine but more widespread. It is viewed as the major nutritional problem in most developing countries.

The nutritional situation in the Sahelian Africa region is known to be precarious. The need for improved food sources of high energy and good protein quality with adaptability to the specificity of the local conditions is imperative in order to reduce the dependency of this population from international aid.

The Sahel is a transition zone of semi-arid and arid land between the Sahara and the Savannas of west and central Africa. This zone is characterized by a short rainy season (two to four months, starting around June and ending in September) and a yearly rainfall which can vary from near 0 mm, in the Sahara border, to 600 mm in the

dry Savannas (Davi, Mattei, and Salomon, 1976). Over 40% of the land is unsuitable for agriculture and the remainder is lost through desertification (United Nations, 1977). Periodic drought followed by famine or food shortage is not something exceptional. From 1858 to 1945, in the upper valley of the river Senegal, Benefice, Chevassus-Agnes, and Barral (1984) reported five serious crises of more than eight month duration and twenty two-crises of three to eight month duration. Annegers (1973b), Gast (1968), and Lhote (1955) studying the diet of various groups of Sahelian population, found chronic and periodic deficiencies in several basic nutrients.

The basic diet of this population consists of cereals (millet and sorghum) which provide the majority of the protein required and 70 to 80% of the energy supply. The energy requirements of the Sahelian population was reported satisfied to a level of $\pm$ 10%. This level may drop to 30-50% during severe drought (International Development Research Center, 1980). The protein level of the Sahelian diet is highly regional and strongly related to environmental and economic conditions. Cereals and legumes are the main source of protein. Total animal protein consumption is generally low in most Sahelian countries. Relatively high levels of animal protein intake occur only in the coastal population and among the nomadic people of the Northern Sahel (Annegers, 1973a). Protein intake is not always satisfactory for children and settled rural population (International Development Research Center, 1980). Of the eight essential amino acids necessary to

human adults, lysine, methionine, and isoleucine were reported the most deficient (Annegers, 1974). Vitamin and mineral requirements are generally met. However, deficiency may occur from season to season (Table 1).

Table 1. Daily per Capita Intakes of Nutrients by Senegalese Nomads with WHO/FAO Recommendations by Season[1].

	Intake noted	WHO/FAO requirement	Intake noted	WHO/FAO requirement	Intake noted	WHO/FAO requirement	Intake noted	WHO/FAO requirement
Period	July 1980		Aug-Sep. 1980		Jan-Feb. 1981		June 1981	
Energy Cal	2,153	2,156	2,005	2,264	2,619	1,986	2,432	2,134
Protein g	60.4	33.4	61.8	31.2	80.5	31.9	67.0	35.5
Vit. A µg	199	625	818	623	543	630	371	623
Riboflavin mg	0.93	1.19	1.43	1.25	1.62	1.09	1.16	1.17
Vit. C mg	4	28	55	28	30	28	17	29
Folates µg	128.6	325.8	100.8	345.2	156.2	349.1	127.2	351.2
Zinc mg	9.9	14.4	9.1	8.9	11.9	9.4	10.2	13.1

[1]Adapted from Benefice et al. (1984).

The consumption of legumes is low in the Sahelian zone when compared to other countries such as Mexico (14 g per head per day), Brazil (10.8 g), and India (13.1 g) (Annegers, 1973a). The total daily per capita pulse protein consumption in Sahelian region is illustrated in Figure 1.

The major pulses consumed in this zone are cowpea (Vigna spp), groundnut (Arachis hypogaea), bambara groundnut (Voandzeia subterranea), and locust bean (Parkia spp). They are grown essentially in the Sudanic zone (2 to 5 rainy months) and have a tendency to decrease further toward the North. Tepary bean, a drought tolerant legume, previously

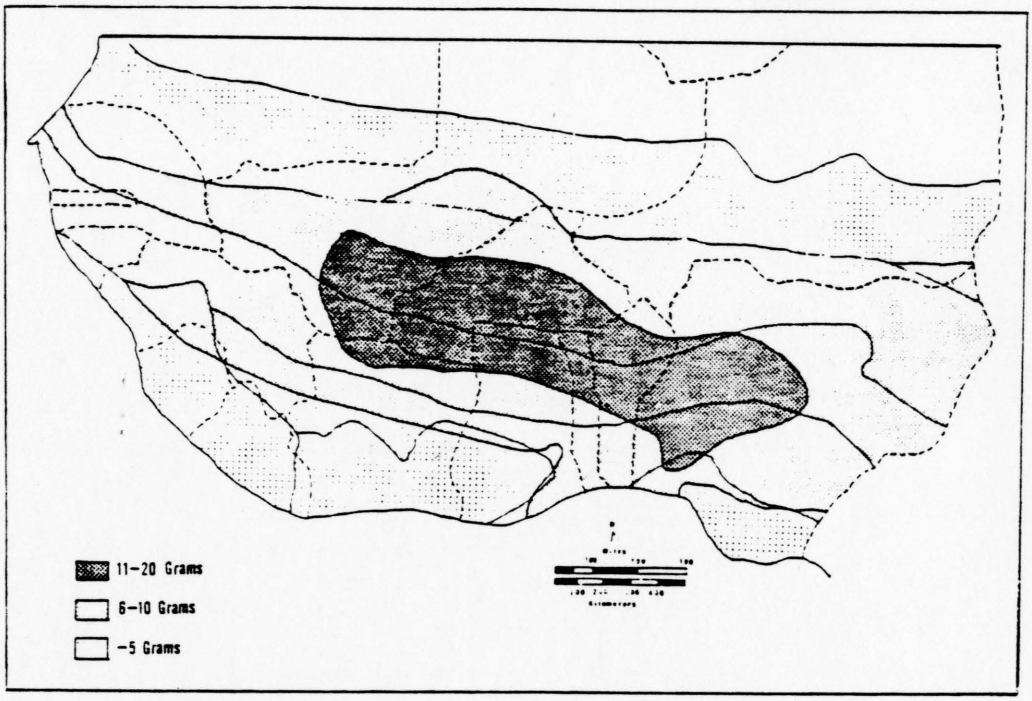

Figure 1. Daily per Capita Pulse Protein Consumption in West Africa and Sahelian Area (Annegers, 1973a).

introduced and found highly adaptable to conditions prevailing on the African continent (Leakey and Wills, 1977; Rachie et al., 1974; Stanton, 1966) may be developed in areas where the local culture cannot be successfuly grown.

In the Sahel, legumes are generally eaten fresh and prepared in many ways including cooking, roasting, frying, and baking. The traditional processing methods are mainly soaking, decortication, and conversion to a paste product before cooking (Ologhobo et al., 1986). Germinated and fermented legumes which are traditionally used in the Indian (India) diet, are not common in Africa. Very little information is available on the use of these methods on this continent. In India

the sprouted grains are eaten raw (with salt), parched, or mixed with flavorings, and fried or boiled (Aykroyd, 1982). The high level of legume consumption in India compared to that of Africa, which is low, may be due to these various processing methods and ways of preparation.

The introduction of tepary beans and germination as a means of processing in tne diet of nomads and transhumants would not only improve their calorie-protein intake but might also help to eliminate some of the chronic vitamin and mineral deficiencies.

In a previous study in which interviews were conducted with inhabitants of eight different Sahelian countries, teparies were found to be "very acceptable" when used in recipes of primary foods (Tinsley, et al., 1985). Among the various African recipes using legumes, akara, a fried food prepared from cowpeas, was selected for the introduction of teparies in the African diet. Akara is one of the most popular food products and requires a short cooking time (Dovlo, Williams, and Zoaka, 1976; McWatters and Chninnan, 1985). The adaptation of raw and germinated tepary beans to the formulation used to prepare akara may enhance the tepary bean acceptability by improving palatability and sensory properties of the food as well as improving nutritional quality.

The purpose of this study was to explore the possibility of using dry and germinated tepary beans in akara preparation, to evaluate the nutritional quality and organoleptic acceptability, and to determine the antinutritional factors of the final products.

CHAPTER 2

LITERATURE REVIEW

Tepary Bean

Tepary bean (Phaseolus acutifolius G.) is a drought-adapted legume from Southwestern North America where it has been used in the native American diet for more than 5 millennia (Nabhan and Felger, 1978; Nabhan, Weber, and Berry, 1979). Teparies are generally planted with the early rainfall of July and harvested 60 to 75 days later. They require one-half to three-fourths the water of conventional crops to reach maturation (Taggart, Storey, and Bower, 1983). The cultivating techniques used by the native Americans have been described by Nabhan and Teiwes (1983) and Bouscaren, Waines, and Boykin-Bouscaren (1983). Dry seed yield may vary from 250-900 kg/ha in dry land to 900-2000 kg/ha in irrigated area (Nabhan et al., 1980).

The grain weight and protein content may be influenced by cultivars and environmental conditions (Waines, 1978; Nabhan et al., 1980). Scheerens et al. (1983) compiled information on proximate analysis (Table 2), essential amino acid patterns, minerals, and vitamins of wild and domesticated teparies. As for legumes in general, sulfur amino acid and tryptophan were reported to be the most deficient. Teparies are rich in minerals; their concentration may depend on soil composition and agricultural techniques (Calloway, Giaugue, and Costa, 1974). The vitamin content reported per 100 g of

sample were as follows: niacin (2.80 mg), thiamine (0.33 mg), and riboflavin (0.12 mg). Like most legumes, teparies contain antinutritional factors and are hard to cook (Kabbara et al., 1987; Thorn et al., 1981). These factors, which could limit the utilization of teparies, may be reduced by combining various processing methods.

Table 2. Proximate Composition of Tepary Beans[1].

Component	Wild Teparies			Domesticated Teparies		
	Sample	Mean Size	Range	Sample	Mean Size	Range
Moisture (%)	4	6.7	4.8-8.4	39	6.1	3.0-9.9
Crude Protein (%)	6	24.5	23.3-25.4	39	23.2	13.0-28.8
Crude Fat (%)	4	1.4	1.0-2.4	39	0.8	0.4-1.9
Carbohydrates						
Total[2]					59.0	
Fiber (%)					6.5	5.1-9.9
Ash (%)				5	4.4	
Calories/100g[3]				1	320.0	

[1]Values compiled by Scheerens et al. (1983).
[2]Calculated by difference.
[3]Estimated from proximate analysis.

Effect of Germination

Germination, as a means of processing, is widely used in Asia. It has also been reported as a custom in the Powamu Bean Dance of native Americans (Nabhan, Weber, and Berry, 1985). It is known to increase the nutritional quality and organoleptic acceptability of legumes. Finney (1983) reviewed extensively the effect of germination on nutritional values of cereals and legumes. He found that sprouting is a way of

greatly improving nutritional quality and alleviating the problem of malnutrition. Vanderstoep (1981) showed that germination increased protein and vitamin content and reduced the antinutritional factors of legumes.

King and Puwastien (1987) germinated mung beans from 24 to 120 hours and found no dramatic changes in protein and amino acid composition. Their results were similar to that reported by Youcef et al. (1987) in faba beans, Akapanuman and Achinewhu (1985) in cowpeas, Hsu et al. (1980) in dry peas, lentils, and faba beans, and by Finney, Morad, and Hubbard (1980) in faba beans. Sathe et al. (1983) reported a reduction in total protein and a substantial increase in essential amino acids of great northern beans germinated from 2 to 5 days, while Whu and Fenton (1953) noticed a decrease in lysine content of soybeans germinated for 6 to 7 days. Sprouting has also been reported to improve protein digestibility and iron bioavailability of legumes (Boralkar and Reddy, 1985; Subbulakshmi, Ganeshkumar, and Venkataraman, 1976; Reddy, 1985).

Akara

Akara, a deep fat-fried legume product, is widely consumed in Southwestern and Sahelian Africa. Its protein content may vary from 11.5% to 19.3% (McWatters, 1983; McWatters and Brantley, 1982). The product recipe may vary from housewife to housewife depending on the available products at the moment of preparation. Dovlo et al. (1976) described a recipe using cowpeas. McWatters and Chhinnan (1985) studied the effects of water level and hydration time on apparent viscosity of

cowpea paste and physical-sensory attributes of akara. They found that 60% water level produced an akara with better characteristics. McWatters (1983) compared the quality characteristics of akara prepared from commercial cowpea flour with traditional akara made from soaked, dehulled blackeye-peas. He noticed that small size particles of flour have a tendency to produce an akara which was less appreciated than the traditional one. Reber et al. (1983) evaluated akara supplemented with cottonseed, peanut, and soybean flours. They found the final products highly acceptable and nutritionally better than the traditional foods. McWatters and Brantley (1982) studied the characteristics of akara prepared from Dixiecream cowpeas , a cowpea cultivar with a white seed coat and little pigmentation in the eye region. They found the product acceptable even when not dehulled. McWatters and Flora (1980) reported that akara prepared from southern peas was suitable for home or institutional use in developed countries. Collins and Sanders (1973) described a tasty deep-fried snack food made from soybeans and onions. The protein content of this product varied from 10.4% to 12.1% and the lipid content from 22.9% to 27.3%.

Vitamin A

It has been recognized that vitamin A has an essential role in several human and animal physiological functions. Recently, several reviews dealing with different vitamin A aspects were reported. Elsa and McCormick (1986) and Madani (1985) reviewed the historical and general aspects of vitamin A. Hank (1985) revised the biochemical and physiological role of vitamin A and Chytil (1985) studied its function

in the respiratory tract. Biesalski et al. (1985), Goodman (1984), and Ganguly et al., (1980) reviewed the mode of action of this vitamin and its role in health and diseases. Ong and Chytil (1983) studied the relationship between vitamin A and cancer and found that a deficiency in vitamin A caused some alterations which may lead to cancer.

Vitamin A is known to be necessary for the development and health of humans and higher animals. It is required for vision, growth, reproduction and maintenance of differentiated epithelia and mucus secretion. Chen, Buccini, and Chen (1985) found that vitamin A increased resistance to cold. Zamaria et al. (1985) reported that hypovitaminemia can be responsible for malignant process, while Hennekens et al. (1982) mentioned the possible effect of retinol or beta carotene in decreasing the risk of cancer in man. Sommer, Katz, and Tarwotjo (1984) showed that mortality among Indonesian children was more closely associated with vitamin A status than with their general nutritional state. Arroyane (1969) reported that vitamin A deficiency reduces the protein utilization.

Hypovitaminosis A affects many people in developing countries. In the Sahelian Africa, Villard and Bates (1986) reported severe vitamin A deficiency in the Northern region near the Sahara desert but less in the Southern sectors of the Sahel. The recommended daily dietary allowance of vitamin A in the US has been set at 800 and 1000 retinol equivalents for the adult woman and man, respectively, the major natural sources of vitamin A for humans and animals are from plants. Retinol (vitamin A) is found only in animals, whereas, the carotenoids

(provitamin A) is the form found in plants. The carotenoids are converted to retinol in the intestinal mucosa by enzymes.

The vitamin A content of seed legumes may vary from 0 IU to more than 100 IU/100 g of sample. Germination was reported to considerably increase the carotenoid content of legumes. Fordham, Wells, and Chen (1975) studying the effect of germination on vitamin content of several peas and beans, found a significant increase. These results were similar to that of Chattopadhyay (1951).

Ascorbic Acid

Ascorbic acid or vitamin C is one of the most investigated vitamins. Several reviews dealing with different aspects of this vitamin have been published. Levine and Morita (1985) examined the historical, chemical, and biological aspects of ascorbic acid and Ashoor and Monte (1984) reviewed the retention of vitamin C in processed food. Horning (1981) studied its metabolism and requirements in man while Sato and Udenfriend (1978) its biosynthesis in mammals. In addition to its well known connection with the treatment of scurvy, ascorbic acid has several other important roles in health. Hallberg, Brune, and Rossander (1986) suggested that it promotes iron absorption in the diet while Anderson (1984) reported its role as an an anti-allergic and anti-inflammatory agent. Yoshioka, Matsushita, and Chuman (1984) noted its possible role against hypertension.

Ascorbic acid is present in most all vegetables and fruits, but it is easily destroyed during processing and storage of foods. Ascorbic acid content of seeds has been reported by several authors to increase

considerably during germination. Prudente and Mabesa (1981) reported a significant increase in mung bean with the vitamin C content increasing from about 0 mg to 111 and 145 mg/100g of sample after 48 and 60 hours of germination, respectively. Hsu et al. (1980) reported an increase from 2.2 to 64 mg/100 g in yellow peas and 0.9 to 77.5 mg/100 g in faba beans after 4 days of germination. Fordham et al. (1975) studied the effect of germination on vitamin C content of several types of peas and beans. The ascorbic acid content in dried seeds varied from 2.2 to 9.0 mg/100 g, while in sprouts it ranged from 18.8 to 50.0 mg/100 g. More recently, El-Shimi and Damir (1984) studied the nutrient changes in fenugreek seed during germination. They found that ascorbic acid increased from 4.23 to 8.87 mg/100 g after 4 days of germination but also reported that soaking caused a 5.67% loss in ascorbic acid content.

Trypsin Inhibitor

Legumes are known to contain several antinutritional and toxic factors which have been demonstrated to be responsible for the low protein digestibility. Rackis and Gumbman (1981) and Liener and Kakade (1980) reviewed trypsin inhibitor and, more recently, Mounts and Rackis (1985) edited a special issue on this subject.

The pancreas is known to be responsible for the production of most enzymes necessary to digestive process. As a consequence, any substance which affects pancreatic function may lead to some changes in nutrient availability. Rackis, Gumbman, and Liener (1985) reported that trypsin inhibitors affect pancreatic function, although the mechanism is not well understood. Green and Lyman

(1972) and Green and Miyasaka (1983) have suggested that it involves negative feedback controlled by the level of free trypsin in the small intestine. According to this mechanism when the free trypsin drops below a certain level, the pancreas is induced to secrete and produce more enzymes.

Temler (1980) found that trypsin inhibitor activity (TIA) and high dietary protein (soya flour) are responsible for pancreatic hypertrophy and hyperplasia. Rackis et al. (1985) reported that dietary peptides interact directly with duodenal endocrine tissue to release cholecystokinin (CKK) hormone which then interacts between pancreas and trypsin level, whereas soybean TI forms a stable complex with trypsin followed by the release of CKK which causes hypersecretion of pancreatic enzyme. Liener et al. (1985) showed that increased secretory activity, hypertrophy, and hyperplasma of the pancreas were associated with higher levels of TIA and the growth rates were inversely related to the TI content of the diets. Pancreatic hypertrophy, growth inhibition, reduction in protein digestibility, and increase in sulfur amino acid requirements and stimulation of gallbladder activity were also reported.

Doell, Ebden, and Smith (1981) found that the daily intake of TIA from the average British diet was 330 mg per person (about 4.1 mg TIA/g of protein). He showed that in most traditional Oriental food such as tofu, soyu, chine bean curd, the TIA has been removed or inactivated by processing and cooking. Trypsin inhibitors are widely distributed in nature. Weder (1985) gave information on the structure, reactive sites and inhibitory activity of trypsin inhibitors found in some Leguminosae.

In order to improve the nutritional quality of legumes, removal of the antinutritional and toxic factors is essential. This is often done by combining several processing methods such as soaking, cooking, germination, fermentation, roasting, and frying. Several papers concerning the effect of these processing methods have been published.

Desphande and Cheryan (1983) and Kakade and Evans (1966) found a TIA decrease of 13 and 28%, after soaking navy beans and dry beans for 18 hrs and 4 days, respectively. Cooking generally inactivates trypsin inhibitors, however this is not always true, particularly when using dry heat. Kadam et al. (1986) found only 10 and 18% TIA reduction after incubating mothbeans at 80°C and 100°C, respectively for 60 min. Tan and Wong (1982) reported that TIA in winged bean meal were extremely resistant to dry heat treatment (less than 5% of the TIA was inactivated after 2 hrs at 100°C). However, in another publication, Tan, Wong, and Lumen (1984) found that dry heat (200°C for 30 min) destroyed all winged bean TIA. Ochoa (1987), using cowpeas, reported a 55% destruction after 55 min of dry roasting at 150°C.

Fermentation as a way of processing is known to reduce raffinose, stachyose and verbascose content of legumes. Little has been published on the effect of this treatment of TIA. However, Padhy and Salunkhe (1978) reported an increase in TIA in soy idli, while Tallent (1979) and Zamora and Fields (1979) found a decrease in the same product.

The literature relating to the effect of germination on TIA seems to favor a reduction even though some articles reported an increase. King and Puwastien (1987) found no significant change on TIA of winged beans after 48 and 120 hrs of germination. Chitra and Sadasivam (1986) reported a TIA reduction of 88 and 58% on black gram beans germinated for 48 and 72 hrs respectively. The authors observed a decrease up to 48 hrs and then a sharp increase. However, the overall effect was a decrease. Kadam et al. (1986) did not detect any activity on moth beans germinated for 48 hrs. Khokhar and Chauhan (1986) showed that germination of moth beans for 60 hrs lowered TIA considerably. These results were similar to those found by Abdullah, Baldwin, and Minor (1982) for chickpeas, horse beans, lentils, and mung beans. Ologhobo and Fetuga (1983), studying the effect of TIA on 18 varieties of lima beans, found that germination for six days produced marked losses in TIA after the 4th day. The losses were between 39.9 and 63.84% in the different varieties.

Subbulakshmi et al. (1976) reported a slight decrease of TIA in horse gram and moth beans germinated for 72 hrs. Kakade et al. (1966) noticed a slight reduction in TIA (7.5%) then an increase (5.4%) in navy beans germinated for respectively 2 and 4 days. They suggested the reduction may have been produced by the mobilization and degradation of protein, including trypsin inhibitors. The increased activity has not been explained.

CHAPTER 3

MATERIALS AND METHODS

Sample Preparation

Raw Samples

White tepary beans from the 1986 harvest were obtained from Mexico. Teparies were cleaned manually and placed in jars for storage at 40% relative humidity and 3-4°C. Cowpeas were obtained from a local store.

Germination

White tepary beans were washed four times and soaked in tap water in a glass jar (tepary beans to water ratio 1:3 w/v) for 14 hrs at room temperature. After soaking, tepary beans were washed twice and divided in two equal portions. The first portion, used to prepare akara from ungerminated teparies, was dehulled manually, washed, drained, and frozen for future use. The second portion was spread on trays covered with a flannel cloth previously sprinkled with 200 ml of tap water. The trays were covered with transparent plastic film. The following morning, the seeds were sprinkled with an additional 50 ml of tap water. After 48 hrs of germination, sprouted seeds (0.6 to 1.5 inches long) were collected, washed, manually dehulled, and immediately frozen for future analysis and akara preparation.

Cowpeas were soaked and dehulled in the same manner and stored under the same conditions. Dry samples were ground, without removing testa, in a Wiley mill and passed through a 30 mesh screen. Germinated tepary beans used for analysis were dehulled and freeze-dried, then ground in a Wiley mill and pressed through a 30 mesh screen.

Akara Preparation

A 200 g sample of defrosted cowpeas, and both ungerminated and germinated tepary beans were separately blended with chopped onions, black pepper, garlic powder, curry, and water (see Table 3 for formula) for three minutes in a blender to a smooth paste consistency. This paste (raw akara) was then whipped for two additional minutes to incorporate air. Tablespoon portions of the mixture were dropped in peanut hot oil (380°) and fried for 3-5 min in an automatic fryer. The fried product (akara) was drained on absorbent paper and kept warm for sensory evaluation. A portion of the raw and the cooked materials from each separate sample was removed and freeze-dried for future analysis.

Table 3. Ingredients and Formula Used for Akara Preparation.

Products	Beans (g)	Water (ml)	Onions (g)	Salt (g)	Garlic (g)	Pepper (g)	Curry (g)
Cowpea Akara	200	100	30	8	5	2	2
Tepary Bean Akara	200	100	30	8	5	2	2
Germinated Tepary Akara	200	70	30	8	5	2	2

Proximate Composition

Proximate composition of cowpeas, raw and germinated tepary beans, uncooked and cooked akara were determined following the procedures recommended by the Association of Official Agricultural Chemists (AOAC, 1980). Moisture content was determined by weight-differences of samples dried in a vacuum oven overnight at 60°C. Protein analysis was determined by the micro-Kjeldahl method using a conversion factor N x 6.25.

Crude fat values were obtained by extracting samples with hexane for five hours in Goldfisch apparatus. The acid detergent fiber was determined by the Van Soest (1963) method and ash by weight difference of sample after combustion overnight at 600°C. Carbohydrates were calculated by difference.

Amino Acid Analysis

The amino acid profile of all samples was determined according to the Spackman, Stein, and Moore (1959) method.

Acid Hydrolysis

For acid hydrolysis, 5 ml of 6 N HCl was added to a 100 mg defatted sample weighed in a "Kontes" hydrolysis vial. A deoxygenation was then accomplished by alternately applying vacuum and flooding with dry nitrogen over a period of several minutes. The vials were then sealed by heat and left in an oven for 24 hours at 105-110°C.

Sample Preparation

After sample hydrolysis, the vials were cooled gradually to room temperature. Each sample was then transferred to a round bottom flask for HCl removal by rotary evaporation. All HCl was removed by washing the residue three times with deionized water.

Sample Removal

Each sample was removed from the rotovac flask by adding 10 ml of dithioglycol buffer (pH 2.2) to suspend the residue. The hydrolysate was then filtered through Whatman # 40 filter paper. An aliquot of 1 ml filtered hydrolysate was diluted to 10 ml with citrate buffer. An aliquot of this solution was injected directly into a Beckman Model 121 Automatic Amino Acid Analyzer. Eluted amino acids were measured colometrically after exposure to ninhydrin at 570 nm and proline was measured at 440 nm.

Vitamin A Determination

The procedure described by Parrish et al. (1985) and the AOAC (1980) method was followed for vitamin A determination.

Reagent Preparation

Acetone-in-hexane solution (4%). For this reagent, 40 ml acetone were transferred to 1000 ml flask and brought to volume with hexane.

Acetone-in-hexane solution (15%). Preparation of this reagent consisted of diluting 150 ml acetone with 850 ml hexane.

Adsorption alumina. An aliquot of 5 g water was weighed in a small bottle and thoroughly mixed by shaking with 95 g of alumina. The mixture was then cooled for at least 2 hrs.

Potassium hydroxide solution. KOH (70 g) was dissolved in 70 ml of deionized distilled water. This solution was prepared fresh each time.

Antimony trichloride reagent (20%). A 100 g portion of antinomy crystals from an unopened sealed bottle was dissolved in 500 ml of pre-warmed chloroform. After cooling, 15 ml acetic acid was added. The solution was then filtered through Whatman # 2 filter paper. This reagent was stable for up to two months.

Alkaline Digestion

Raw beans, freeze-dried samples of germinated teparies, and uncooked akara were ground in a Wiley mill and passed through a 30 mesh screen. Cooked freeze-dried samples were finely ground in a mortar with a pestle. Twenty grams of each sample was weighed in a 250 ml flask, covered with aluminum foil, then mixed thoroughly with 80 g of alcohol using a stirring bar. Twenty grams of KOH was added to the mixture. The samples were refluxed at about two drops/sec for 30 min. During the refluxing period, the flasks were agitated three times to disperse the aggregates. After refluxing, 25 ml alcohol-water (3V+1V/V) solution was added to the mixture. The flasks containing the samples were then adjusted to 175 ml with the alcohol-water solution. This mixture was vigorously shaken and then allowed to settle.

Sample Extraction

A portion of the settled solution (80 ml) was transferred to a separatory funnel and completed with 32 ml of deionized water and 80 ml of hexane. After shaking, the mixture was allowed to stand until two layers appeared. The lower layer was drained to another separatory funnel and mixed again with an equal amount of hexane. The lower layer of this second separatory funnel was discarded and the remaining solution was combined with the first extract. The extract was then washed three times with 80 ml of water and transferred, after filtration through Whatman # 2 paper containing 5 g Na_2SO_4, to a 250 ml Erlenmeyer. This solution was then evaporated until a dry residue was formed.

The residue of each sample was mixed with 10 ml of hexane and transferred to a test tube. If no color was present, the carotene absorbance was determined at 440 nm against a blank set with hexane. If yellow pigments were present, as was the case with all samples, chromatography was used.

Chromatography

Chromatographic columns were packed with 6-7 cm alumina covered with 0.5-1 cm Na_2SO_4 and immediately washed with 20 ml hexane.

Elution of sample with 4% acetone-in-hexane solution. When about 0.2 ml of hexane was left in the top of the column, 10 ml of the solution to be chromatographed, was added. The sample was then eluted with 4 % acetone-in-hexane solution. The carotene band was eluted with this solution. At this time a brief inspection of the column with UV light showed a light fluorescent band, characteristic of retinol, on the

top of alumina if vitamin A was present. The evolution of retinol (Vitamin A) through the column was followed by a periodic inspection with UV light.

Elution of sample with 15% acetone-in-hexane solution. When the retinol was about two-thirds of the way down the column, the receiver (125 ml Erlenmeyer) containing carotene was removed and replaced by another flask for retinol collection. Before drying, a 15% acetone-in-hexane solution used to elute retinol was added.

Readings. Three readings were made as follows:
First reading - the carotene fraction was transferred to a test tube and read at 440 nm against a blank containing 4% acetone-in-hexane solution,
Second reading - when retinol was completely out of the column (no fluorescent band) 13 to 18 ml, depending on the collected solution, was transferred to a test tube (the carotenoid was read at 440 nm against a blank containing 15% acetone-in-hexane solution), and
Third reading - the test tube contents of the 2nd reading was then evaporated to dryness. One ml chloroform and nine ml $SbCl_3$ were added to the residue of each sample and retinol absorbance was determined at 620 nm versus a blank holding 1 ml chloroform and 9 ml $SbCl_3$.

Ascorbic Acid

The vitamin C content was determined according to the method described by Pelletier (1985) and Pelletier and Brassard (1977).

Reagent Preparation.

Metaphosphoric acid solution (17%) (Reagent 1). A 50 g of metaphosphoric acid pellets (Baker Analyzed Reagent) was dissolved in deionized distilled water and diluted to 100 ml. This solution was stable up to one week when stored in refrigerator.

Metapnosphoric acid solution (0.85%) (Reagent 2). An aliquot of 50 ml of 17% HPO_3 solution was made up to 1000 ml with water. This solution was prepared daily.

Ascorbic acid standard (Reagent 3). A 100 mg portion of ascorbic acid was dissolved in 0.85% HPO_3 solution and brought up to 100 ml. Ten ml of this solution was then transferred to a 100 ml flask and diluted to volume with 0.85% HPO_3. From this second solution, five dilutions containing 2, 4, 6, 8, and 10 ml, respectively, were prepared in 100 ml flasks and brought up to 100 ml with 0.85% HPO_3. These diluted standards contained 2, 4, 6, 8, and 10 µg/ml vitamin C. These solutions were stable up to one week at 3°C.

2,6-dichloroindophenol solution (0.1%) (Reagent 4). This solution was made by dissolving 200 mg sodium salt of 2,6-dichloroindophenol in 150 ml of hot water, cooled and diluted to 200 ml with water. The solution was filtered and kept in refrigerator. This solution was stable for two weeks at 3°C.

Boric acid (5%) (Reagent 5). An aliquot of 50 g boric acid was dissolved in water and diluted to 1 liter. This solution was stable for two weeks at room temperature.

Boric acid solution (3.33%) (Reagent 6). This solution was prepared by diluting 66.6 ml H_3PO_3 (5%) solution to 100 ml with water.

Potassium phosphate dibasic solution (45%) (Reagent 7). A 45 g portion of K_2HPO_4 was dissolved and diluted to 100 ml with water. This solution was prepared daily.

2,6-dichloroindophenol (0.1%) and boric acid (5%) solution (Reagent 8). An aliquot (13.3 ml) of 0.1% 2,6-dichloroindophenol solution and 66.6 ml of 5% H_3PO_3 was diluted to 100 ml with water. This solution was filtered before use and prepared daily.

Homocysteine and K_2HPO_4 solution (Reagent 9). DL-homocysteine (150 mg) was dissolved in 25 ml water and mixed with 42.5 ml of 45% K_2PO_4. This solution was prepared daily and filtered before use.

DNPH solution (1.5%) (reagent 10). DNPH (1.5 g) was dissolved in 9 N H_2SO_4 and brought to 100 ml volume with the same acid. This solution was prepared daily and passed through a sintered filter.

DNPH (1.2%) and thiourea (3%) solution (Reagent 11). Thiourea (1.5 g) was dissolved in 7.5 ml 9 N H_2SO_4 mixed with 40 ml 1.5% DNPH and then diluted to 50 ml with 9 N H_2SO_4. This solution was prepared daily.

Nitric and citric acid mixture (Reagent 12). A 50 g portion of citric acid was dissolved in methanol to make 100 ml, then 150 ml concentrated HNO_3 was added slowly while stirring. This solution was cooled and used 90 min after preparation.

Sample Extraction

Samples were prepared following the same procedure used for vitamin A extraction. One gram of each sample was extracted for three minutes with 10 ml 0.85% HPO_3. This solution was filtered through Whatman # 2 filter.

Test Tube Preparation

Two sets of test tubes (one for the total test and the other for the blank test) were prepared in duplicate. One ml of the appropriate dilution (2, 4, 6, 8, and 10 μg/ml) of ascorbic acid standard and appropriate amount of reagents were added as follows:

Sequence	Blank Test	Total Test
0	Reference: 1 ml of 0.85% HPO_3	Reference: 1 ml of 0.85% HPO_3
1	Addition of 0.5 ml reagent 9 to all blank test tubes. Time was set to proceed with sequence 4 in about 90 min.	none
2	none	Without delay, after seq. 1, 0.5 ml reagent 8 were added to all test tubes in 2 min. The sequence 3 was immediately followed.
3	none	Addition of 0.5 ml reagent 9 to all test tubes. Samples were left for about 90 min before proceeding with sequence 5.
4	Addition of 0.5 ml reagent 6 to all blank test tubes.	none
5	Starting with blank test, reagent 11 was added to all blank test tubes and total test tubes. They were shaken for a few seconds and then held in water bath at room temperature for 3 hrs.	
6	Addition of 2 ml reagent 12 to all tubes which were cooled to 15°C by adding ice and mixed gently by bubbling nitrogen.	

Within 30-90 min after completion of sequence 6, the absorbance of each solution was read using a Beckman DU Spectrophotometer, Model 2400, at 520 nm against a reference treated in the same way as the other samples.

Trypsin Inhibitor activity

The procedure of Kakade et al. (1974) was used for trypsin inhibitor activity determination.

Reagent Preparation

Tris-buffer. Tris(hydroxymethyl) aminomethane "Trizma base" (6.05 g) and $CaCl_2.2H_2O$ (2.94 g) were dissolved in 980 ml of deionized distilled water. The pH was adjusted to 8.20 $\pm$ 1 with 1 N HCl and brought to 1000 ml. This solution was stable for 1-2 months at 7°C.

Substrate Solution. An aliquot of 80 mg of benzoyl-DL-arginine-p-nitroanaline hydrochloride (BAPA) was dissolved in 2 ml of dimethyl sulfoxide and diluted to 200 ml with tris-buffer prewarmed to 37°C. This solution was kept at 37°C in water bath. A new solution was prepared for each two runs.

Trypsin Solution. A 10 mg portion of trypsin was weighed in a 50 ml beaker and transferred to 500 ml volumetric flask with 0.001 N HCl. This solution was then brought to volume with the same dilutant and stored at 5-10°C. This solution may be stored for one month.

Sample Extraction

Two grams of each defatted sample was extracted for two hours with 50 ml of 0.01 N NaOH in a 125 ml beaker with a stirring bar. The suspension was then centrifuged at 2000 RPM for 10 min. The surfactant was filtered through filter paper and then diluted so that 1 ml of the sample extract would inhibit approximately 40 to 60% of the trypsin used as a standard in the analysis. The appropriate dilutions were determined through repeated analysis.

Portions of the diluted extract were then pipetted into duplicate sets and poured into 50 ml Erlenmeyers according to the following scheme:

Flasks	1	2	3	4	5	sample blank	trypsin standard
ml extract	0.0	0.5	1.0	1.5	2.0	0.0	2.0
ml DD H_2O	2.0	1.5	1.0	0.5	0.0	2.0	0.0

Two ml of the trypsin solution was added to each flask. One ml of 30% acetic acid was immediately added to the same blank flask and to the trypsin standard flask.

Samples 1, 2, 3, 4, and 5 (in duplicate) were then placed in a gyratory water bath kept at constant temperature (37°C), while the sample blank flask and trypsin standard were left at room temperature. After five minutes of temperature equilibrium, 5 ml of the prewarmed BAPA solution was pipetted into each flask at 15 seconds intervals.

After exactly ten minutes, the reaction was terminated by blowing 1 ml of 30% acetic acid into each flask. Each flask, containing 10 ml of reaction mixture, was then filtered (Whatman # 1) into test tubes. The absorbance of each solution was then determined in a spectrophotometer.

Sensory Evaluation

A set of three samples consisting of akara made from cowpeas and dry and germinated tepary beans was evaluated at each session by 11 volunteer panelists. The coded samples were arranged in random order on white plates and served warm to the sensory panelists in individual booths. Samples were evaluated for color, texture, flavor, and overall acceptability on a hedonic scale of 9 to 1 with 9 = "I like very much" and 1 = "I dislike very much" (Appendix D). The untrained panel consisted of 8 African students and 3 non-African students.

Statistical Analysis

Statistical analysis of color, texture, flavor, and overall acceptability of cooked akara as determined by sensory evaluation was performed by analysis of variance ($P \leq 0.05$) using the statistical package program Costat (Cohort Software, 1986).

CHAPTER 4

RESULTS AND DISCUSSION

Proximate Composition

Proximate composition of cowpeas, raw and germinated tepary beans, and uncooked and cooked akara are shown in Table 4. Results are the average of duplicates expressed on a dry weight basis.

Table 4. Proximate Composition of Cowpeas, Raw and Germinated Tepary Beans, and Uncooked and Cooked Akara[1].

Products	Moisture	Crude Protein	Crude Fat	Carbohydrate		Ash
				Crude Fiber	Total Carbohydrates[2]	
	%	%	%	%	%	%
Cowpeas	7.03	24.06	1.11	6.04	64.32	4.47
Tepary Beans	7.25	22.02	1.06	5.49	66.80	4.63
Germinated Tepary Beans	58.28	24.70	0.87	2.83	67.19	4.41
Uncooked Cowpea Akara	70.22	23.77	0.65	2.97	66.11	6.50
Uncooked Tepary Akara	69.95	21.03	0.56	3.09	68.88	6.44
Uncooked Germinated T. Akara	74.32	21.35	0.62	3.82	67.65	6.55
Cooked Cowpea Akara	45.38	14.36	33.86	3.33	42.15	6.30
Cooked Tepary Akara	33.34	12.05	38.31	3.03	40.59	6.02
Cooked Germinated Tepary Akara	31.29	12.33	38.11	3.04	41.99	4.53

[1]Percentage calculated on dry weight basis.
[2]Calculated by difference, including starch and other carbohydrates.

The moisture content of cowpeas and raw teparies was similar and are in the range reported by Scheerens et al. (1983) for tepary beans. Moisture content of uncooked akara varied from 69.95 to 74.32%. In cooked products, the moisture content of akara prepared from cowpeas (45.4%) was higher than that of raw and germinated teparies

(31.3 and 33.4% respectively). Similarly, McWatters (1983) reported a moisture content varying from 40.8 to 48.8% in akara prepared from cowpea paste and Nigerian cowpea flours.

The protein content of cowpeas (24.06%) was higher than that of raw teparies but lower than that of sprouted teparies (24.70%). Protein synthesis may have been responsible for this slight increase. These results were similar to those reported by Akpanuman and Achinewhu (1985) for Nigerian cowpeas. In uncooked akara, the protein content was lower than the protein of raw beans. The decrease may have been the result of soaking and washing the teparies before adding other ingredients. Cabral (1987) found that soaking soybeans resulted in a loss of protein. The sharp protein decrease in cooked akara may have been due to protein destruction by heat and removal of fat from the samples. McWatters and Brantley (1982) reported a protein content (wet basis) ranging from 12.8 to 13.4% in akara made from cowpeas.

Fat content of raw teparies decreased by about 20% after germination. This finding is similar to that reported by King and Puwastien (1987). The reduction was probably produced by beta-oxidation with the fat being used for energy purposes. In uncooked akara, fat content was similar in the three products. In cooked products, akara from teparies seemed to retain more fat that akara made from cowpeas (38.3% vs 33.9%). McWatters (1983) found a fat content ranging from 20.8 to 31.8% in akara prepared from cowpeas, while fat content in cowpea akara in the present study was slightly higher (33.9%).

Acid detergent fiber (fiber) in cowpeas (6.04%) and in raw tepary beans (5.4%) was in the range of that reported by Scheerens et

al. (1983) for teparies. The decrease of fiber in germinated teparies was apparently due to the removal of seed coat, although Akpanuman and Achinewhu (1985) did not notice changes in fiber after germinating cowpeas for 72 hrs. In uncooked and cooked akara, fiber content was similar. No reference for fiber content for akara was found.

Carbohydrate content, calculated by difference, was similar in raw and germinated teparies. This result was comparable to that of Akpanuman and Achinewhu (1985) for germinated cowpeas. Carbohydrate decrease in cooked akara was due to fat removal and effect of heat.

Ash content was similar in raw beans and uncooked akara. The small reduction observed in cooked akara was probably due to some loss during frying. McWatters (1983) reported 5.5% ash in akara prepared from dehulled California blackeyed peas, while Youcef et al. (1987) found a slight increase of ash in faba beans germinated for 3 days.

Amino Acids

The amino acid profile of cowpeas, raw and germinated teparies and uncooked and cooked akara is shown in Table 5.

There were no appreciable differences in the amino acid content of raw cowpeas and tepary beans. The essential amino acid profile of teparies was in the range of that reported by Scheerens et al. (1983). Germination and cooking did not produce appreciable changes in the percentage composition of amino acids. Methionine and cysteine were low. These results were similar to those reported by Finney et al. (1980) in faba beans and by Hsu et al. (1980) in peas, lentils and faba beans after 4 days of germination.

Table 5. Protein and Amino Acid Profile of Cowpeas, Raw and Germinated Tepary Beans, and Uncooked and Cooked Akara[1].

				Uncooked			Cooked			
	Cowpeas %	Teparies %	Germinated Teparies %	Cowpea Akara %	Tepary Akara %	Germinated Akara %	Cowpea Akara %	Tepary Akara %	Germinated Akara %	FAO/WHO Requirement[2]
Protein	24.06	22.02	24.70	23.77	21.03	21.35	14.36	12.05	12.33	-
Amino Acid										
Lysine	6.36	6.12	6.16	5.77	4.73	5.75	5.90	5.77	5.05	12
Histidine	3.17	2.43	2.60	3.06	2.22	2.53	2.98	2.50	2.34	-
Ammonia	1.70	1.38	1.39	1.56	1.39	1.49	1.68	1.51	1.54	-
Arginine	6.40	5.26	5.30	6.00	4.87	5.42	6.18	5.46	5.23	-
Aspartic Acid	10.50	10.56	10.10	9.47	9.60	10.10	10.75	10.49	10.43	-
Threonine	4.02	4.25	4.24	3.55	3.88	4.16	4.07	4.34	4.17	7
Serine	5.08	5.49	5.70	4.76	5.48	5.45	5.46	5.87	5.84	-
Glutamic Acid	14.83	14.44	14.38	13.91	14.03	14.29	15.91	15.00	15.17	-
Proline	2.31	T	T	2.23	T	0.61	2.62	T	T	-
Glycine	3.99	4.00	3.80	3.34	3.61	3.88	3.84	4.07	3.91	-
Alanine	4.38	4.24	4.27	3.85	4.04	4.22	4.37	4.31	4.38	-
Cysteine	1.12	1.14	1.21	1.30	1.61	1.55	1.57	1.52	1.69	-
Valine	4.57	4.71	4.62	3.83	4.09	4.89	4.56	4.54	4.27	10
Methionine	1.46	1.37	1.34	1.42	1.34	1.35	1.51	1.41	1.47	-
Isoleucine	3.89	3.62	3.70	3.21	3.08	3.89	3.70	3.52	3.23	10
Leucine	7.36	7.03	7.12	6.51	6.41	7.14	7.36	7.18	6.86	14
Tyrosine	3.19	3.62	3.88	3.15	3.73	3.80	3.09	4.26	4.08	-
Phenylalanine	5.24	5.06	5.45	4.89	4.95	5.38	5.07	5.57	5.36	-
Met & Cys	2.58	2.51	2.55	2.72	2.95	2.90	3.08	2.93	3.16	13
Phe & Tyr	8.43	8.68	8.33	8.04	8.68	9.18	8.16	9.83	9.44	14

[1]Percentage calculated on dry weight basis.
[2]mg per kg of adult body weight per day.
T = trace.

Vitamin A

Vitamin A content of cowpeas, raw and germinated tepary beans, and uncooked and cooked akara are reported in Table 6. The different steps followed for vitamin A determination are shown in Appendix A.

Table 6. Vitamin A Content of Cowpeas, Raw and Germinated Tepary Beans, and Uncooked and Cooked Akara[1].

Products	Vitamin A (IU/100g)
Cowpeas	23
Tepary Beans	16
Germinated Tepary Beans	25
Uncooked Cowpea Akara	26
Uncooked Tepary Akara	25
Uncooked Germinated Tepary Akara	27
Cooked Cowpea Akara	25
Cooked Tepary Akara	24
Cooked Germinated Tepary Akara	25

[1]Calculated on dry weight basis.

The vitamin A content of cowpeas, dry and germinated teparies was successively 23, 16, and 25 IU per 100 g of samples. The vitamin A content of cowpeas was higher that that of dry tepary seeds but lower than the germinated teparies. These values were lower than those reported by Watt and Merrill (1974) for cowpeas (30 IU/100 g of samples), mung beans (80 IU), and dry peas (120 IU), but much higher than that of common beans (0 IU) and lima beans (trace). Forty eight hours of germination increased the vitamin A content of tepary beans by approximately 56%. This increase conforms to that found by Fordham et al. (1975) and Chattopadhyay and Banerjee (1951) for peas and beans.

Vitamin A content of tepary beans and akara has not been previously reported.

In uncooked akara, the vitamin A content varied from 25 to 27 IU/100 g of sample. There was no dramatic increase of vitamin A even though onions and garlic powder were added. Frying akara for 3-5 min did not produce noticeable change in vitamin A content.

Ascorbic Acid

The ascorbic acid content of dry cowpea seeds, dry and germinated tepary beans, and uncooked and cooked akara is reported in Table 7. Calculation methods are indicated in Appendix B.

Table 7. Vitamin C Content of Cowpeas, Raw and Germinated Tepary Beans, and Uncooked and Cooked Akara[1].

Products	Vitamin C (mg/100g)
Cowpeas	1.03
Tepary Beans	0.27
Germinated Tepary Beans	6.23
Uncooked Cowpea Akara	5.76
Uncooked Tepary Akara	5.57
Uncooked Germinated Tepary Akara	8.88
Cooked Cowpea Akara	1.41
Cooked Tepary Akara	1.22
Cooked Germinated Tepary Akara	1.60

[1]Calculated on dry weight basis.

The vitamin C content of cowpeas (1.03 mg/100 g) was in the range (1.00 to 2.00 mg/100 g) reported by Souci, Fackman, and Tarwotjo (1986). That of tepary beans was in the range of the values reported

for french beans, white dry beans, and lentils by the same autnors. Germination for 48 hours considerably increased the vitamin C content. These results are similar to those found by El-Shimi and Damir (1980) in fenugreek and in mung beans by Tsou and Hsu (1978).

In uncooked akara, the vitamin C content varied from 5.57 to 8.88 mg/100 g of samples. Onion and garlic were apparently responsible for this increase. In cooked akara, ascorbic acid content varied from 1.22 to 1.60 mg/100 g of sample. This sharp decrease was the result of heat. Vitamin C content of tepary beans and akara has not been reported previously.

Trypsin Inhibitor Activity

Trypsin inhibitor activity of the raw cowpeas, raw and germinated tepary beans, and uncooked and cooked akara is shown in Table 8. Calculations for germinated teparies are shown in Appendix C.

Table 8. Trypsin Inhibitor Activities of Cowpeas, Raw and Germinated Tepary Beans, and Uncooked and Cooked Akara[1].

Samples	TIU/mg	Reduction in TIA (%)
Raw Cowpeas	13.72	
Tepary Beans	14.80	
Germinated Tepary Beans	14.37	2.9
Uncooked Cowpea Akara	12.41	9.5
Uncooked Tepary Akara	12.98	10.3
Uncooked Germinated Tepary Akara	12.08	9.7
Cooked Cowpea Akara	10.85	13.0
Cooked Tepary Akara	11.25	15.3
Cooked Germinated Tepary Akara	9.15	24.3

[1]Calculated on dry weight basis.

The trypsin inhibitor activity of cowpeas (13.72 TIU/mg of sample) was lower that that of raw tepary beans (14.80 TIU/mg) but not of germinated teparies (13.37 TIU/mg). The trypsin inhibitor activity of raw tepary beans was similar to that obtained in the same laboratory by Thorn (1981) for raw white tepary beans (15.4 and 17.4 TIU/mg), but lower than that found by Tinsley et al. (27.4 TIU/mg) (1985). Forty eight hours of germination did not produce an appreciable effect on trypsin innibitor activity. The reduction in TIA was only 2.9%. This result was in accord with that reported by King and Puwastien (1987) for winged beans, by Ologhobo and Fetuga (1983) for lima beans (10% loss after 48 hrs germination), and by Chitra and Sadasivam (1986) for black gram beans.

In uncooked products, the TIA was 12.41 TIU/mg for akara prepared from cowpeas, and 12.98 and 12.08 TIU/mg for that from raw and germinated tepary beans, respectively. This is equivalent to a reduction of about 9.5% TIA. This slight decrease in TIA was probably due to the concomitant effect of soaking and washing bean samples before the addition of ingredients. Kadam et al. (1986) reported a 20% decrease in motn bean TIA after 8 hours of soaking.

Deep-fat frying of tepary meal for 3-4 min and cowpea meal for 4-5 min was not very effective in reducing TIA. The reduction, when compared to uncooked products, was only 13.0% for cowpea akara, 15.3% and 24.3, respectively, for tepary and germinated akara. This was probably due to the short cooking time, the bean variety, or to the heat resistance of some trypsin inhibitors. No report relating to the effect

of the frying process on TIA was found; however, these results are similar to those reported by some authors when using dry heat. Kadam et al. (1986) found a TIA destruction of only 10 and 18% for moth beans incubated for 60 min at 80°C and 100°C respectively. Elias, Hernandez, and Bressani (1976) reported a TIA reduction of 22.3% in cowpeas after 30 min toasting at 240°C while Ochoa (1987) found a reduction of 55.1% after 55 min of dry roasting at 150°C.

It is obvious that the best reduction in TIA was obtained with akara prepared from germinated teparies (24.3%) when compared to the uncooked products. No data on the antinutritional factors of fried legumes was available for comparison.

Sensory Evaluation

Sensory mean scores and significant differences among akara prepared using cowpeas and akara prepared from raw and germinated teparies are indicated in Table 9. Sensory scores of akara products are

Table 9. Sensory Mean Scores and Significant Differences Among Akara Prepared from Cowpeas and Akara from Raw and Germinated Teparies.

	Sensory Characteristics[1]			
	Color	Texture	Flavor	Average Acceptability
Cowpea Akara	$2.64^{a} \pm 0.67$	$2.45^{a} \pm 0.52$	$2.55^{a} \pm 0.69$	$2.55^{ab2} \pm 0.69$
Tepary Akara	$3.00^{a} \pm 0.00$	$2.91^{a} \pm 0.30$	$2.73^{a} \pm 0.65$	$3.00^{b} \pm 0.00$
Germinated Tepary Akara	$2.55^{a} \pm 0.69$	$2.45^{a} \pm 0.69$	$2.00^{a} \pm 0.89$	$2.09^{a} \pm 0.83$

[1]Where 3 = good, 2 = medium, and 1 = poor.
[2]Difference in Letters (a,b) in the Same Column Indicates Significant Differences ($P \leq 0.05$).

illustrated in Figure 2. The evaluation sheet used by panelists is shown in Appendices D.

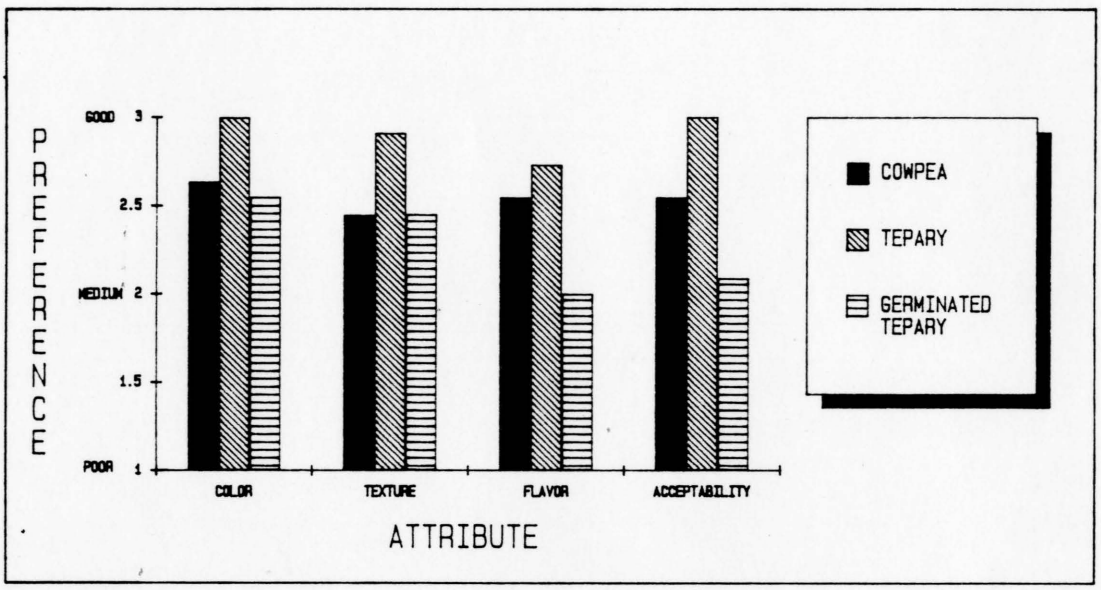

Figure 2. Sensory Scores of Akara Products.

For better analysis, the hedonic scale was divided into three equal parts corresponding to three categories. For each category, a score related to the degree of preference was attributed. Score 3 = good was assigned to akara highly preferred (category one), score 2 = medium to akara in between (category two), and score 1 = poor to akara disliked (category three). Sensory evaluation data related to these scores are indicated in Appendix E.

No significant difference ($P \leq 0.05$) in color, texture, and flavor was found among the akara products. For the overall acceptability, a significant difference ($P \leq 0.05$) was found between akara prepared from

raw teparies and that prepared using germinated teparies. There was no significant differences between akara made from cowpeas and that made using germinated teparies. However, panelists ranked akara prepared from raw teparies the best followed by that made from cowpeas and germinated teparies. Akara prepared from cowpeas was described as chewy with good flavor while that made from raw teparies, as crispy, and crunchy. Akara from germinated teparies was qualified as crispy with a bitter after taste.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

Legumes are an important source of calories, proteins, vitamins, and minerals in most developing countries. Their introduction in arid and semi-arid regions is of particular interest as these areas are among tne poorest regions of the world.

In tne Sanel, dietary intake is closely related to the amount and duration of rainfall. Any substantial changes in the rainy season may affect the nutritional status of the agricultural population. Seasonal food shortages leading to under-nutrition are not exceptional. The need for improved sources of high energy and high protein quality crops that are adaptable to the local conditions is apparent.

Tepary beans are a potential food crop for this area. Their introduction into the Sahelian diet should pose no problem. Studies of primary foods conducted with African students native to this zone, nave demonstrated that teparies were found "very acceptable" when used in local recipes.

The antinutritional factors and the lengthy cooking time phenomenon reported for tepary beans, and for legumes in general, could be moderated by combining two or more processing methods. Germination has been reported to increase the nutritional value of legumes. Introducing germinated legumes into the Sahelian diet might improve the

nutritional quality and reduce the seasonal vitamin and mineral deficiencies of the nomadic and transhumant peoples.

Teparies did not require much water for germination. Less than one liter of tap water was used to soak and germinate 200 g of samples. The sprout length varied from 0.5 to 1.5 inches.

Tepary seeds were found to be a good source of protein but a poor source of vitamin A and vitamin C. Forty eight hours of germination did not cause appreciable changes in protein and amino acid composition. The vitamin A and vitamin C contents were, however, considerably increased. Trypsin inhibitor activity was reduced by approximately 3%.

In cooked akara, protein content decreased by approximately 50% when compared to raw beans. Frying did not produce noticeable changes in amino acid composition or vitamin A content. Vitamin C was, however, dramatically reduced. Trypsin inhibitor was not extensively reduced. Cooking-time was not a problem in akara preparation. Akara prepared from both germinated and ungerminated teparies cooked in less time than that prepared from cowpeas. No significance difference ($P \leq 0.05$) in color, texture, and flavor was found among akara prepared from cowpeas and raw and germinated teparies. For the overall acceptability, a significant difference ($P \leq 0.05$) was noticed between akara made from raw and germinated teparies. Akara prepared from raw teparies was preferred to akara made from cowpeas and germinated teparies, respectively.

Nutritionally, teparies are an excellent potential food crop. Several studies related to seed composition of this plant have been publisned but information on the vitamin content of these seeds is lacking. More research on methods to improve the nutritional values of these beans is also needed.

The few studies reported on akara have stressed only the physical characteristics and organoleptic properties with little being reported on the nutritional quality; more research related to the nutritional aspect is needed. Literature on the effect of frying on antinutritional factors of legumes appears to be non-existent. More information is also needed on this.

Despite their apparently successful introduction in Africa, teparies have not yet been reportedly used in the Sahel area. This may have been due to their lack of popularization among the transhumant people and local farmers. Regular availability of these seeds in local markets with acceptable prices would be primary steps for their possible introduction. More information on tepary adaptability to the local conditions is also necessary before considering their introduction on a larger scale.

APPENDIX A

DETERMINATION OF VITAMIN A CONTENT

Standard Curve and Sample Absorbances

Vitamin A Standard. A stock solution containing 28.31 IU vitamin A/ml hexane was prepared from an unopened capsule of UPS Vitamin A reference standard. The table below showed the dilutions prepared and their absorbance at 620 nm.

Dilution (ml/100)	IU Content	Absorbance
0.18	0.509	0.010
0.80	2.266	0.040
1.80	5.099	0.095
3.70	10.48	0.205
5.30	15.015	0.315
7.10	20.114	0.405
9.00	25.497	0.520

The correlation (c), slope (s), and interception (i) below were then calculated from the above absorbance.

c = 0.999

s = 0.020

i = 0.0002

Carotene Standard. The following dilutions were prepared from a stock solution holding 50 mg carotene/ml hexane and their absorbance were read at 440 nm against a blank containing hexane.

Dilution (ml/100)	IU Content	Absorbance
0.5	0.25	0.040
1.0	0.50	0.240
2.0	1.00	0.460
3.0	1.50	0.550
5.0	2.50	0.650

The correlation, slope, and interception were respectively:

c = 0.998

s = 0.088

i = 0.0097

The correcting factor for carotenoid was calculated from absorbance (A) of 6 series of carotene dilutions read at 440 and 620 nm.

Dilution (ml/100)	A_{440}	A_{620}
0.5	0.05	0.022
1.0	0.10	0.024
2.0	0.20	0.028
3.0	0.30	0.030
5.0	0.50	0.041
7.0	0.70	0.051

The correcting factor was f = 0.043.

Sample Absorbances. The table below shows the carotene (CeA_{440}), carotenoid (CdA_{440}), and retinol (ReA_{620}) absorbance of each sample, respectively. A correction factor (C) for carotenoid calculated as illustrated below, is also reported.

$$C = CdA_{440} \times f \qquad \text{where} \qquad f = 0.043$$

Samples	CeA_{440}	CdA_{440}	ReA_{620}	$Cx10^{-3}$
Raw Cowpeas	0	0.020	0.010	0.860
Tepary Beans	0	0.020	0.015	0.860
Germinated Tepary Beans	0	0.049	0.030	2.100
Uncooked Cowpea Akara	0	0.031	0.020	1.330
Uncooked Tepary Akara	0	0.015	0.022	0.600
Uncooked Germinated Tepary Akara	0	0.030	0.025	1.290
Cooked Cowpea Akara	0	0.017	0.023	0.731
Cooked Tepary Akara	0	0.090	0.025	3.870
Cooked Germinated Tepary Akara	0	0.010	0.022	0.430

Calculation of Vitamin A Content

The vitamin A content of each sample was determined as follow:

$$\text{IU Vitamin A/g} = (A_c \times V_i \times V_c \times V_f)/(S \times W \times V_t \times V_u)$$

where:

$A_c = A_{620} - (A_{440} \times f)$

V_i = initial volume used

V_c = total volume of 15% acetone-in-hexane collected during retinol elution

V_f = final dilution used for reading absorbance at 620 nm

S = slope vitamin A at 620 nm

W = weight of sample

V_t = total volume obtained after extraction

V_u = amount of V_c effectively used to read absorbance at 440 nm

$$\text{IU Vitamin A/100 g} = \text{IU Vitamin} \times 100$$

For illustration, the vitamin A content in IU/g of sample (germinated tepary beans) was:

$$(0.028 \times 80 \times 18.80 \times 10)/(0.020 \times 20 \times 175 \times 18.80) = 0.32 \text{ IU/g}$$

or 32 IU/100 g of sample

APPENDIX B

DETERMINATION OF ASCORBIC ACID CONTENT

Standard Curve and Sample Absorbances

The net average absorbance of the vitamin C solution standard were the following:

Dilution (ml/100)	Vitamin C (µg/ml)	Absorbance
1	1	0.013
2	2	0.023
4	4	0.045
6	6	0.073
8	8	0.093
10	10	0.118
16	16	0.188

From this table, the following correlation, slope, and interception were then calculated:

c = 0.999

s = 0.011

i = 0.0001

Two series of test tubes (one for the blank test and the other for total test) were prepared in duplicate from the sample duplicating the process used with vitamin C standard. The net average absorbance at

520 nm and the vitamin C concentration expressed in mg/100 g of sample for each sample were the following:

Samples	Absorbance	Vitamin C (mg/100g)
Raw Cowpeas	0.013	1.03
Tepary Beans	0.003	0.27
Germinated Tepary Beans	0.066	6.23
Uncooked Cowpea Akara	0.061	5.76
Uncooked Tepary Akara	0.059	5.57
Uncooked Germinated Tepary Akara	0.094	8.88
Cooked Cowpea Akara	0.015	1.41
Cooked Tepary Akara	0.013	1.22
Cooked Germinated Tepary Akara	0.017	1.60

Calculation of Vitamin C Content

The vitamin C content (X) in mg/100 g of sample was calculated as follow:

$$X = F(A-i) \times D \times 100 \times 10^{-3}/(S \times W)$$

where:

A = net absorbance of the sample (total test minus blank)

i = intercept of the standard

D = dilution used

S = slope of the standard

W = weight of the sample

F = correction factor for the possible change during analysis calculated as follow: $F = 2As/(AB+Aa)$; or

$$F = (2 \times 0.073)(0.70 + 0.70) = 1.04$$

where:

As = absorbance of the fourth higher level in the standard curve

AB and As = absorbance of the same standard placed before and after each group of samples

For illustration, the vitamin C content of germinated teparies in mg/100g was:

$$X = 1.04\ (0.066 - 0.0001) \times (10 \times 100 \times 10^{-3}) / 0.011 = 6.23$$

APPENDIX C

DETERMINATION OF TRYPSIN INHIBITORY ACTIVITY

Sample Absorbance

The absorbance of each solution was read in the spectrophotometer (Coleman Junior II Spectrophotometer Model 6/20) at 410 nm against the sample blank and corrected for the trypsin standard (TS).

The calculation of the trypsin inhibitor activity of germinated tepary bean is illustrated below:

Test Tube	ml extract	absorbance	mean	corrected absorbance
trypsin standard	2.0	0.015		
1A 1B	0.0	0.635 0.640	0.637	0.637
2A 2B	0.5	0.473 0.512	0.493	0.489
3A 3B	1.0	0.292 0.294	0.293	0.285
4A 4B	1.5	0.134 0.148	0.141	0.130
5A 5B	2.0	0.129 0.131	0.130	0.115

Absorbance (Abs) were determined against the blank tube. The sample weight was 2.0 g and the dilution of extract 16.66x (4 ml extract diluted to 100 ml with DD H_2O).

Corrected Absorbance

Corrected absorbances (CA) were calculated as follows:

CA = Abs - (ml sample extract x Abs TS)/ (TS)

and results are shown below:

Test Tubes	Corrected Absorbance
1	0.637 - (0.0ml x 0.015)/2ml = 0.637
2	0.493 - (0.5ml x 0.015)/2ml = 0.489
3	0.293 - (1.0ml x 0.015)/2ml = 0.285
4	0.141 - (1.5ml x 0.015)/2ml = 0.130
5	0.130 - (2.0ml x 0.015)/2ml = 0.115

Expression of Activity

One trypsin unit (TU) is defined as an increase of 0.01 absorbance units at 4.0 nanometers per 10 ml of the reaction mixture. Trypsin inhibitory activity is expressed in terms of trypsin units inhibited or trypsin inhibitor unit (TIU). TIU was obtained by substracting each TU sample from test tube with zero ml extract. Each TIU was divided by aliquot size to yield TIU/ml.

Test Tube	ml extract	CA	TU	TIU	TIU/(ml extract)
1	0.0	0.637	63.7		
2	0.5	0.489	48.9	14.8	29.6
3	1.0	0.285	28.5	35.2	35.2
4	1.5	0.130	13.0	50.7	33.8
5	2.0	0.115	11.5	52.2	26.1

A graph of TIU/ml extract versus ml extract was plotted and a linear regression of the line was determined. The linear regression gives the value of 34.51 TIU/ml as the vertical intercept. Since the sample was extracted with 50 ml base, and the extract was diluted 16.66x, the trypsin inhibitor activity of the germinated tepary beans, expressed as TIU/mg of sample, is:

$$34.51 \times 50 \text{ ml} \times 16.66 \times 10^{-3}/2 \text{ g} = 14.37 \text{ TIU/mg sample}$$

APPENDIX D

SENSORY EVALUATION SHEET

Name: _____ Sex: _____ Age: _____
Country: _____ Date: _____ Phone: _____

AKARA EVALUATION

1. You are being presented with 3 coded samples of Akara prepared from cowpeas, dry tepary beans, and germinated teparies. Please evaluate each product according to the following ratings on each criteria: 9 = I like very much to 1 = I dislike very much. Place a vertical slash (/) on the horizontal line at the point where you rate the product on each characteristic.

Product 202

	like very much		dislike very much
Color	9	_____	1
Texture	9	_____	1
Flavor	9	_____	1
Overall Acceptability	9	_____	1

Product 404

	like very much		dislike very much
Color	9	_____	1
Texture	9	_____	1
Flavor	9	_____	1
Overall Acceptability	9	_____	1

Product 606

	like very much		dislike very much
Color	9	_____	1
Texture	9	_____	1
Flavor	9	_____	1
Overall Acceptability	9	_____	1

2. Please rank the products in order of your degree of preference (1 = like the best to 3 = like the least).
   202 _____ 404 _____ 606 _____

   Please describe how you felt about the product:
   202 _____

   404 _____

   606 _____

3. Do you consume akara (fried ground beans) in your country? Yes No

Comments:

APPENDIX E

SENSORY EVALUATION DATA

Panel #	Cowpea Akara				Tepary Akara				Germinated Tepary Akara			
	Col	Tex	Fla	Acc	Col	Tex	Fla	Acc	Col	Tex	Fla	Acc
1	2	2	1	1	3	2	2	3	2	2	3	3
2	3	2	3	3	3	3	3	3	3	3	2	2
3	3	2	3	3	3	3	3	3	3	3	1	2
4	3	3	3	3	3	3	1	3	3	3	1	1
5	3	3	3	3	3	3	3	3	3	3	3	3
6	3	2	2	2	3	3	3	3	3	3	3	3
7	3	3	3	3	3	3	3	3	3	3	3	3
8	3	3	3	3	3	3	3	3	1	1	1	1
9	2	2	2	2	3	3	3	3	2	2	1	1
10	1	2	2	2	3	3	3	3	2	2	2	2
11	3	3	3	3	3	3	3	3	3	2	2	2

Col = Color
Tex = Texture
Fla = Flavor
Acc = Overall Acceptability

3 = Good
2 = Borderline
1 = Poor

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