AN ABSTRACT OF THE THESIS OF

Jean R. Kaunda for the degree of Master of Science in Nutrition and Food Management presented on January 30, 2002. Title: Determination of Vitamin B-6 and Pyridoxine-Glucoside in Selected Malawi Foods and the Effect of Preparation Techniques on Vitamin B-6 and Pyridoxine-Glucoside Content

Abstract approved: ______________________________

James E. Leklem

There were two main purposes to this study. The first was to determine the vitamin B-6 and pyridoxine β-glucoside content of selected foods commonly consumed in Malawi. The second was to examine the effect of preparation procedures of foods in Malawi on the content of vitamin B-6 and pyridoxine β-glucoside in foods. Seventeen plant foods commonly eaten in Malawi were determined for vitamin B-6 and pyridoxine β-glucoside using a microbiological assay. In addition, two commercial weaning foods, roasted maize-soy bean blend and extruded maize-soy bean blend, were also determined for vitamin B-6 and pyridoxine β-glucoside contents. Among all the foods analyzed, whole maize flour contained the highest amount of vitamin B-6 (0.66 mg/100 g), therefore, an excellent source of vitamin B-6 content in foods. Cooking decreased vitamin B-6 in pinto beans, kidney beans, sugar beans and cow peas by 34%, 45%, 14% and 48%, respectively. Roasting decreased vitamin B-6 in chick peas and soy beans by 59% and 38%, respectively. Soaking and fermentation reduced vitamin B-6 in soaked maize flour and cassava flour by 86% and 89 %, respectively. Therefore, these data suggest that some of the preparation procedures practiced in Malawi have a negative impact on the vitamin B-6 content of the processed foods. Cooked and roasted foods contained lower total amount of pyridoxine-glucoside than that of the raw food. The high pyridoxine β-glucoside content have adverse impact on

the bioavailability of vitamin B-6 content. Based on typical diets for the urban and rural populations in Malawi, the rural diet contained less vitamin B-6 compared to that of urban diet. Therefore, the rural population may be at risk of inadequate vitamin B-6 intake compared to the urban population.

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Determination of Vitamin B-6 and Pyridoxine-Glucoside in Selected Malawi Foods and the Effect of Preparation Techniques on Vitamin B-6 and Pyridoxine-glucoside content

by

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APPROVED:

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Chair of Department of Nutrition and Food Management

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Jean R Kaunda, Author

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1. INTRODUCTION

Optimum health demands an adequate intake of all macro and micronutrients from food. Since utilization of one nutrient is often dependent on the adequate supply of some other nutrients, deficiency of any one of the nutrients may affect not only that nutrient, but also the entire metabolic machinery (Latham, 1997).

Internationally, the Food and Agriculture (FAO) has a long history in developing and disseminating food composition tables and related information. The FAO started its activities of developing food composition tables in 1949. Between 1960 and 1970, the FAO prepared food composition tables for many regions including east Africa, which are still in use today. The December 1992 International Conference on Nutrition (ICN) reemphasized the need to reexamine the nutrient content of food typically consumed by specific populations (FNSP, 1992). This may stem from what has been meager data on the nutrient contents of foods and lack of inclusion of many micronutrients in older nutrient composition tables.

Recently, micronutrient studies in developing countries have focused on vitamin A, iron, iodine and zinc. Few studies have been done on B-complex vitamins.

This could be because of the obvious clinical problems manifested in the vitamin A, iron, iodine and zinc compared to B- complex, for instance, vitamin B-6 (Latham, 1997). Several studies conducted in developing countries show that a large percentage of women, men and children suffer from vitamin B-6 deficiency, as judged by erythrocyte aspartate aminotransferase activation coefficient (Walker, 1996; Korede,. 1990). Deficiencies of vitamin B-6 and other B-vitamins are seen more often during pregnancy (Schuster et al, 1981). Unfortunately very little information is available on the vitamin B-6 content of foods in developing countries. Vitamin B-6 performs a host of important functions in the body, which are critical for the good health. Vitamin B-6 is important in the metabolism of amino acids in the body (Leklem, 1991). The active form of vitamin B-6 is required for the many important protein metabolic processes including transamination, and decarboxylation reactions (Hudson et al, 1989; Leklem, 1991). Vitamin B-6 as an essential nutrient is necessary for the optimal growth and development of normal immune response (Chandra and Shakuntla, 1985; Meydani, 1991). Recently, deficiency of vitamin B-6 has been noted in persons infected with the immunodeficiency virus (Baum et al, 1991b). Therefore, it is clear that a compromised immune system brought about by a vitamin B-6 deficiency may have serious consequences for the health of an individual. Another important function of vitamin B-6 is its role in nervous system function.

In several studies, lack of the active form of vitamin B-6 has been linked to abnormal behaviors of infants and rats (McCough et al, 1990).

Vitamin B-6 deficiency could be a problem in Malawi because of the accelerated health problems. Nutritional deficiency is the most common cause of secondary immunodeficiency, for instance, infection (Chandra and Shakuntla, 1985). Chronic malnutrition is a widespread problem in Malawi and is one of the major factors responsible for the high mortality of infants under five. Malawi ranks number five among countries in sub-Saharan Africa with the highest levels of malnutrition. Fifty percent of the children in Malawi are chronically malnourished (Panpanich et al, 1999). HIV/AIDS is also a big health problem in Malawi. One million Malawians out of the total population of 10.5 million are affected with HIV (Galavotti et al, 2001). Malnutrition and vitamin deficiencies are risk factors for AIDS. The nutritional status of an individual is known to play a role in accelerating the progression of HIV/AIDS, increasing the prevalence and severity of the infectious complications of HIV/AIDS. Poor nutrition increases the risk and progression of disease. In turn, disease exacerbates malnutrition.

Reliable data on the vitamin B-6 content of foods will help in the assessment of vitamin B-6 nutritional status of the population groups. Further, there are several reports in literature suggesting that considerable loss of vitamin B-6 occurs during food preparation and processing (Ekanayake, 1990; Augustin, 1978; Gregory and Ink, 1978).

A study was therefore undertaken to provide information on the vitamin B-6 and pyridoxine-glucoside content of some of the foods that constitute the diet of Malawians and to investigate the effect of preparation procedures on the content of vitamin B-6 and pyridoxine-glucoside of nineteen foods commonly consumed in Malawi.

2. LITERATURE REVIEW

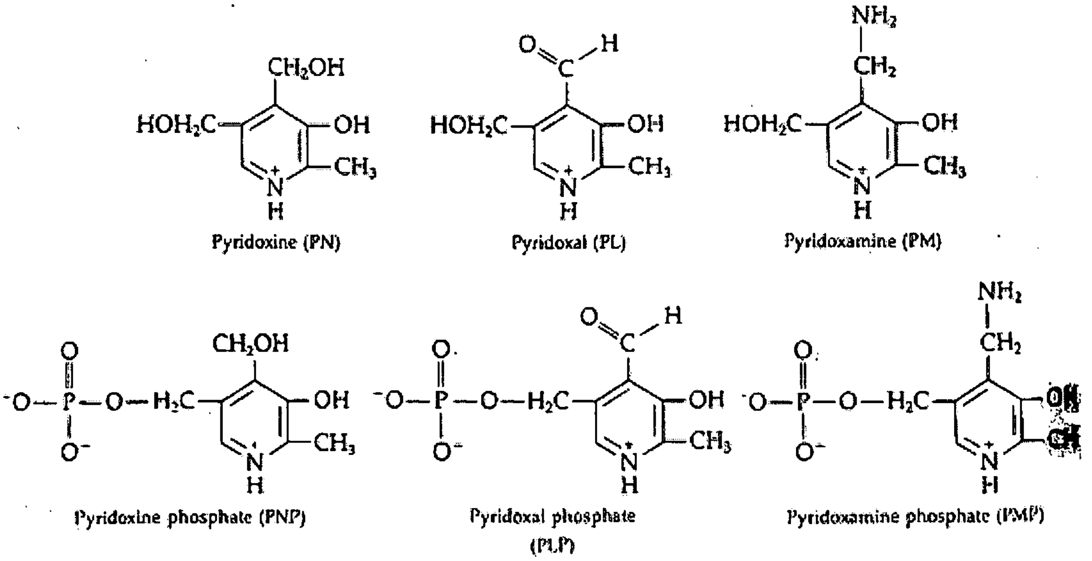
2.1 Vitamin B-6 History/Chemistry

The importance of vitamin B-6 in metabolism has been recognized since 1934 when Gyorgy established the essentially of this nutrient. Gyorgy called it vitamin B-6 to describe a rat pellagra dermatitis preventative factor distinct from other B- vitamins (Gyorgy, 1971). Vitamin B-6 is a generic term for a group of all 3-hydroxyl 5-hydroxymethyl-2 methyl pyridine derivatives, which affect the biological activity of pyridoxine in rats (American Institute of Nutrition, 1987).

Vitamin B-6 in food exists as 6 related compounds. These include the three forms, pyridoxine (PN), pyridoxal (PL) and pyridoxamine (PM), and their corresponding 5'-phosphate esters; pyridoxine 5'-phosphate (PNP), pyridoxal 5'-phosphate (PLP) and pyridoxamine 5'-phosphate (PMP). These forms are illustrated in Figure 1. Pyridoxal is the predominant form in animal sources, with the majority of the pyridoxal in the phosphorylated form. Plant foods contain vitamin B-6 mostly as pyridoxine. Animal derived foods contain high vitamin concentration compared to the plant sources.

The liver, which is the center of vitamin B-6 metabolism, is particularly a concentrated source of the vitamin B-6 (Leklem, 1991).

Excellent sources of vitamin B-6 are tuna and chicken breast. Plant foods contain vitamin B-6 mostly as pyridoxine.



Pyridoxine (PN) Pyridoxal (PL) Pyridoxamine (PM)

Pyridoxine phosphate (PNP) Pyridoxal phosphate (PLP) Pyridoxamine phosphate (PMP)

Figure2.1 Vitamin B-6 structures

A good food source of vitamin B-6 contains a substantial amount of vitamin B-6 in relation to its calorie content and contributes at least 10 percent of the U.S. Recommended Daily Allowance (U. S RDA) for vitamin B-6 in a serving. Plant foods, considered as good sources of vitamin B-6 include: vegetables such as potatoes, spinach, broccoli and cauliflower; fruits such as avocados, bananas and raisins. For cereal grains the following are considered to be good sources: whole wheat bread, barley, brown rice and corn meal. The vitamin B-6 present in grains is concentrated mainly in the bran (McDowell, 2000). Legumes such as peanuts, soybeans and lentils are also good sources. In plants, pyridoxine exists in significant amounts as glycosylated vitamin B-6 also called pyridoxine- glucoside (PN- glucoside) (Kabir et al, 1983).

Vitamin B-6 is thought to be fairly stable. In solution vitamin B-6 forms are sensitive to light (Ang, 1979) and this sensitivity is thought to be pH-dependent. Pyridoxine has been found to be more stable than the other two forms: pyridoxal and pyridoxamine. This was evident in a study where a series of diets with differing levels of pyridoxamine, pyridoxal and pyridoxine were prepared without any precautionary measures to prevent exposure to light. After a period of 5 weeks, analysis of these diets showed that pyridoxamine and pyridoxal levels of all the diets were reduced drastically while the pyridoxine levels remained unaltered (Ekanayake, et al, 1986).

2.2 Food Processing and Storage on Vitamin B-6

Food processing, preparation and storage involve a great number of different procedures some of which alter nutritional value, although this effect is not always deleterious. Losses, when they do occur vary enormously with the conditions and length of time of processing. The instability of certain of the vitamin B-6 forms during food processing, preparation and storage is a factor that may contribute to losses of the nutritional quality of foods with respect to vitamin B-6. As a result, the adequacy of food products as sources of dietary vitamin B-6 could be decreased due to losses of vitamin B-6 availability during processing, preparation and storage (Gregory et al, 1978).

Loss of nutrients from vegetables during cooking is caused mostly by leaching into cooking water rather than by nutrient destruction (Raab et al, 1973; Miller et al, 1973; Ekanayake and Nelson, 1990). Within the range of heat treatments employed, Raab et al (1973) demonstrated that heat processing after blanching of lima beans did not result in decreased vitamin B-6 content. Raab et al (1973) suggested that the loss of vitamin B-6 in lima beans was due to leaching of the vitamin during blanching. Preparation units in lima beans processing were evaluated as to their effect on the availability of vitamin B-6 (Ekanayake and Nelson, 1990). Blanching of rehydrated lima beans resulted in the loss of about 20% of the pyridoxine. In another study (Daoud et al, 1977) the effect of blanching treatment on color and vitamin B-6 retention in canned garbanzo beans was investigated. The water blanched samples were lower in vitamin B-6 than the unblanched samples. Water blanching resulted in approximately a 10-15% loss of total vitamin B-6 while steam blanching resulted in a 5-8% loss.

Tadera et al (1986a) examined the stability of pyridoxine in products and observed the formation of 6-hydroxypyridoxine during heat incubation with various plant homogenates at 30° C for 24 hr and 90° to 120° C for 15 minutes. The 6-hydroxypridoxine is not usable in the body.

Roasting of dry foods also affects the availability of vitamin B-6. In a study by Gregory and Ink (1988) a dehydrated model food system was fortified with various forms of vitamin B-6 and subjected to roasting to determine the effect on the B-6 vitamers. Roasting at 180° C for 25 minutes resulted in a loss of 50-70% of the total vitamin B-6. Yen et al (1976) reported that roasting of shelled corn in the production of animal feed induced large losses of vitamin B-6 activity.

Milling of grains such as wheat, corn and rice has a detrimental effect on the vitamin content of the resultant flour. The bran, germ and endosperm contain significant concentrations of vitamins, which are lost as a result of removal of these components during the milling process (Eitenmiller and Landen, 1999). In a review of literature reported losses of 51.1 to 93.8 % of vitamin B-6 as a result of processing and refining grains.

Several groups have reported that vitamin B-6 increases in some foods during storage. Page and Hanning (1963) noted a continuous increase in vitamin B-6 in varieties of potatoes studied from the time of harvest until the end of the sixth month storage. Augustin et al. (1978) used U. S. potato varieties grown in various locations to analyze the content of water-soluble vitamins at harvest time and during subsequent storage. After 2 months, Augustin et al, noted a significant increase in vitamin B-6. Addo and Augustin (1988) also noted an increase in vitamin B-6 during 9 months storage of potatoes from 16 to 46%. Richardson et al (1961) reported an increase in some frozen and irradiated sweet potatoes stored for 9 months. All groups suggested that the liberation of some vitamin B-6 from a bound form during storage might have accounted for the increase in vitamin B-6 activity. Gregory et al (1978) examined the retention of vitamin B-6 using dehydrated foods fortified with various B-6 vitamers during storage for 128 days at 37° C. The retention was found to decrease with dehydration. However, Gregory et al (1978) identified the binding of pyridoxal 5-phosphate to protein to form pyridoxyllsine as the main cause of the loss in vitamin.

Drying has also been associated with vitamin B-6 retention in foods. Holmes et al. (1979) investigated vitamin B-6 retention during home drying and found that 60 to 96% of the vitamin was retained during drying.

TABLE 2.1 Vitamin B-6 Content of Foods

Food	Vitamin B-6 content mg/100 g)
Vegetables	
Potatoes, raw	0.25
Potatoes, cooked	0.39
Sweet potatoes, raw	0.25
Sweet potatoes, canned	0.07
Corn, raw	0.26
Corn, frozen	0.09
Yam, raw	0.16
Beans/Legumes	
Kidney beans, raw	0.46
Kidney beans, cooked	0.32
Soybeans, raw	0.38
Soybeans, cooked	0.63
Beans, navy, cooked	0.38
Cow peas, raw	0.56
Cow peas, frozen	0.11
Peas, green, raw	0.16
Lima beans, frozen	0.11
Lentils	0.29
Garbanzo, beans	0.65
Chick peas	0.53
Nuts/seeds	
Peanuts, raw	0.25
Peanuts, roasted	0.04
Cereal/grains	
Cornmeal	0.20
Rice polished	0.17
Rice unpolished	0.55
Rice cooked	0.14
Whole wheat flour	0.27

Sources: Leklem, 1991; Kabir et al, 1983; Schroedel, 1971; Geralds and Combs, 1992.

2.3 Bioavailability of Vitamin B-6

In human nutrition, the term bioavailability is generally defined as the amount of a nutrient absorbed and utilized by an animal as compared to the total amount of the nutrient chemically determined in the food (Tsuge et al, 1996). It is important to know not only how much of a nutrient is present in a food, but also to what extent it is available to the body. With respect to vitamin B-6, nutritional status of an individual is influenced by the amount of the vitamin ingested, the extent of the absorption and the metabolic utilization of the vitamin B-6 vitamers present in the diet, and the specific metabolic requirements of the individual (Leklem, 1991).

In a study by Leklem et al (1980), bioavailability of vitamin B-6 in whole wheat bread, white bread and white bread enriched with vitamin B-6 was studied in nine men. After adjustment of six days, subjects were randomly fed each of the three breads for one week. Whole wheat bread, white bread and white bread enriched with vitamin B-6 supplied 1.20, 0.35 and 1.18 mg of vitamin B-6 daily, respectively. The subject's constant diet supplied 0.38 mg of vitamin B-6. When white bread was fed, the subjects also received an oral dose of pyridoxine to maintain a daily intake of 1.5 mg of vitamin B-6. A higher fecal vitamin B-6 excretion and a lower urinary 4-PA excretion were observed when whole bread was fed compared to when either white bread enriched with vitamin B-6 or white bread plus an oral dose of vitamin B-6 was fed. The mean plasma pyridoxal 5'-phosphate concentration was significantly lower when whole wheat bread was fed than when white bread enriched with vitamin B-6 was fed. The results of this study suggest that vitamin B-6 is 5-10 times less available from whole bread than from white bread with an oral dose of vitamin B-6.

Several studies found lower bioavailability of vitamin B-6 in plant origin as compared to animal sources (Leklem, et al, 1980; Gregory JF, 1985). In a study comparing the bioavailability of vitamin B-6 in beef with that in soy beans, Leklem et al (1983a) demonstrated that the vitamin B-6 in soy beans was 6-7% less bioavailable than the vitamin B-6 found in beef. There are several factors that might contribute to this variation in bioavailability of vitamin B-6 including dietary fiber, pyridoxine-glucoside, and processing and storage of foods. These factors will be discussed below

2.3.1 Dietary Fiber

Fiber influences the bioavailability of vitamin B-6 (Leklem et al 1980ab); Gregory, 1980). Lindberg et al (1983) showed that wheat bran added to the diet decreased the biovailability of vitamin B-6. The effect of cooked wheat bran on the bioavailability of vitamin B-6 was determined in ten men, aged 20 to 35 years. The subjects consumed a constant diet with and without the addition of 15 g wheat bran during the three successive 18-day periods in a switchback design. The subjects were groups of five each. One group received the additional bran during periods one and three; the other group consumed the bran during period two. The bran and no bran diets supplied 1.6 mg and 1.66 mg of vitamin B-6 daily, respectively. Based on plasma total B-6 and pyridoxal-5' phosphate (PLP) concentration, urinary 4-pyridoxal acid (4-PA) excretion and fecal vitamin B-6 excretion, Lindberg et al, (1983) observed a significant increase in fecal vitamin B-6 and decrease in urinary 4-PA excretion during the bran periods. In addition, bran significantly depressed plasma vitamin B-6 and pyridoxal 5'-phosphate concentration. Therefore, this suggests that dietary fiber decreases the absorption of vitamin B-6. Another study by Nguyen et al (1983) suggested that the dietary fiber in the form of raw carrots decreased vitamin B-6 absorption in the lumen.

2.3.2 Pyridoxine- Glucoside

As mentioned earlier, pyridoxine glucoside, the glycosylated form of vitamin B-6 exists in plant foods. This compound was first identified as a constituent of rice bran by Yasumoto et al (1977) as 5-O-(D-glucopyranosl) pyridoxine. Various researchers including Kabir et al (1983) and Gregory et al (1987) have confirmed the existence of pyridoxine-glucoside in many plant-derived foods. Pyridoxine-glucoside accounts for 8-50% of the total B-6. Table 2.2 lists the foods with varying amounts of pyridoxine-glucoside. The first in depth descriptive study on pyridoxine-glucoside in common plant food was provided by Kabir et al (1983), who showed that the level of pyridoxine-glucoside is variable in most plant foods and is not detectable in animal products. Kabir et al (1983a) reported that 47% of the vitamin B-6 present in orange juice is in a form of pyridoxine-glucoside. Owing to the high presence of pyridoxine-glucoside in plant foods, bioavailability of pyridoxine-glucoside in humans has recently received much attention.

Bioavailability of pyridoxine-glucoside is considerably lower than the other B-6 forms (Nelson et al, 1976; Lekelm et al, 1980b). In vivo evaluating the bioavailability of pyridoxine-glucoside has reported inefficient utilization (Ink et al, 1986; Trumbo et al, 1988a; Gregory et al 1989). Kabir et al (1983b) initiated the first human study to examine the impact of pyridoxine-glucoside on the bioavailability of vitamin B-6 from plant foods. In a 52 -day study, Kabir et al (1983) compared the vitamin B-6 bioavailability from tuna, whole wheat bread and peanut butter. Dietary intake of pyridoxine-glucoside, its subsequent excretion into urine, and various indices of vitamin B-6 status were measured. Compared to the vitamin B-6 in tuna, vitamin B-6 in whole wheat bread and peanut butter was 25% and 37% less available. The level of pyridoxine-glucoside in these foods was inversely correlated with vitamin B-6 bioavailability based on urinary vitamin B-6 and 4-pyridoxic acid.

It is also interesting to note that the work of Gregory et al (1991) highlighted the fact that pyridoxine is poorly hydrolyzed to pyridoxine, though it seems to be well absorbed. The primary site of limited conversion to pyridoxine is the intestinal mucosa, where glucoside activity toward pyridoxine-glucoside has been demonstrated (Gregory et al, 1991).

Also a review of the literature (Gilbert and Gregory, 1992); Zhang et al, 1993; Nakano and Gregory, 1995; Hansen et al, 1996; Nakano et al, 1997) relating to the pyridoxine-glucoside utilization suggests that quantitatively pyridoxine-glucoside alters the metabolism of pyridoxine in vivo, hence, the metabolic utilization of co-ingested non-glycosylated forms of vitamin B-6 is partially impaired. Therefore, in plant food-based diets, the content of pyridoxine-glucoside in whole grain cereals, legumes, vegetables and fruits could be an important factor for the vitamin B-6 bioavailability as it is a potent inhibitor of pyridoxine metabolism.

TABLE 2.2

Vitamin B-6 and Pyridoxine-glucoside content of different foods

Food	Vitamin B-6 µg /100 gram	Nonconjugated Vitamin B-6 Mg /100 gram	Nonconjugate Vitamin B-6 %	Pyridoxine-Glucoside µg /100 gram
Vegetables				
Broccoli, raw	168	140	84	n.d
Broccoli, frozen	119	48	23	78
Cauliflower, raw	156	148	95	9
Cauliflower,frozen	84	20	23	69
Green beans, ra	60	51	85	6
Green beans, ca	28	16	56	8
Carrots, raw	170	75	44	87
Fruits				
Bananas	313	308	98	10
Avocados, fresh	443	221	50	15
Orange juice, fres	43	18	42	16
Orange juice, con	165	54	33	78
Peaches,canned	9	7	71	2
Nuts				
Filberts, raw	587	707	120	26
Almonds, raw	86	69	81	n.d
Grains				
Corn, frozen	88	38	44	6
Rice (white) coo	138	50	37	19
Rice bran	3515	600	17	153
Whole wheat bread	169	69	40	29
Wheat bran	903	117	13	236
Whole wheat flour	265	129	48	19
Legumes				
Navy beans,coo	381	143	37	159
Peanut butter	302	49	16	54
Soybeans, cook	627	130	21	357
Animal produc				
Beef grounded,	263	83	31	n.d
Tuna, canned	316	158	50	n.d
Chicken				
breast, cooked	684	316	46	n.d
Leg, cooked	306	150	49	n.d
Milk, skimmed	5	4	79	n.d

Reproduced from Kabir, 1983

n.d=notdetected

2.4 Absorption, Transportation and Metabolism of Vitamin B-6

All the forms of vitamin B-6, which are ingested are freely absorbed by passive diffusion in the jejunum and enter the circulation in mainly the non-phosphorylated forms (Merrill and Henderson, 1990). Absorption of the phosphorylated forms can occur (Mehansho et al, 1979; Hamm et al, 1979), but only in very limited amounts. Substantial portion of the absorbed vitamin B-6 forms are transported to the liver, where these forms are converted back to their respective phosphorylated forms (Merrill, 1984). The vitamin B-6 forms enter the hepatocytes by diffusion followed by metabolic trapping (Merrill and Henderson, 1990). The interconversion of the vitamers is depicted in Figure 2.2

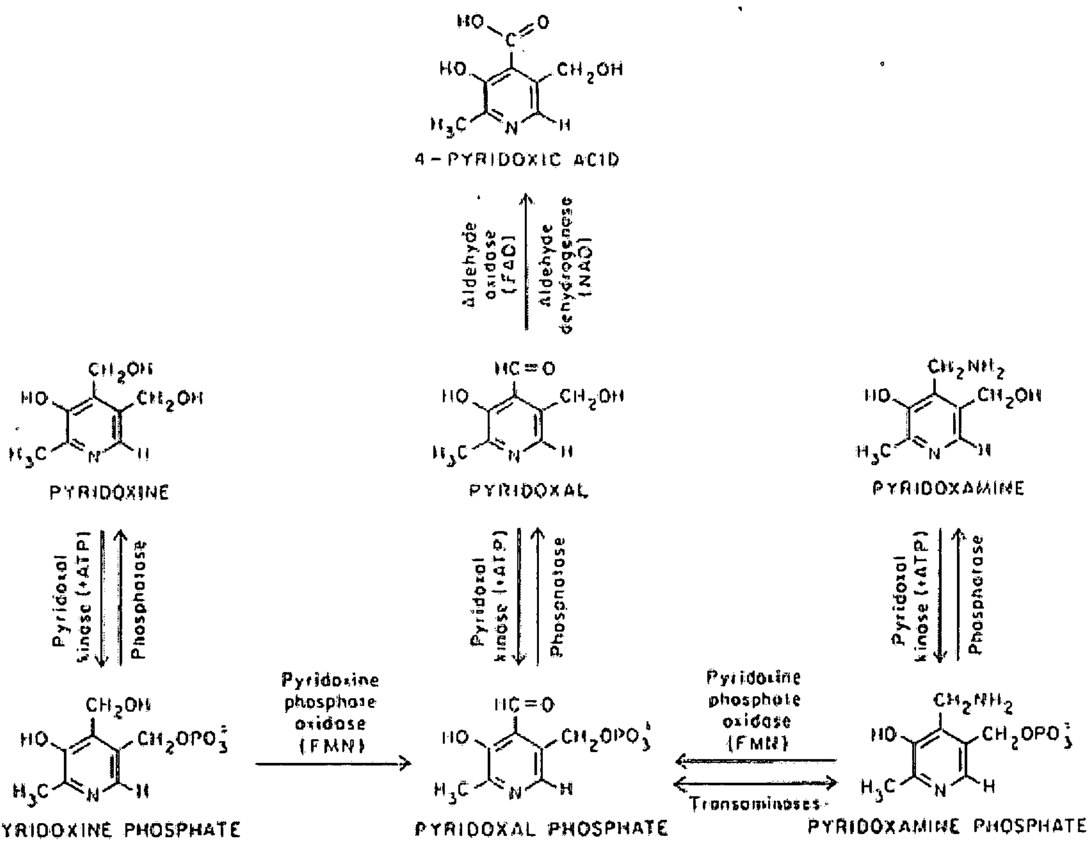


Figure 2.2 Metabolic interconversions of B-6 vitamers (Adapted from: Leklem:1991)

In the liver, pyridoxal kinase catalyzes the conversion of pyridoxine, pyridoxamine and pyridoxal to their phosphorylated forms. The phosphorylated forms of pyridoxine and pyridoxamine are then oxidized to pyridoxal 5'-phosphate by the flavin mononucleotide (FMN) oxidase. Pyridoxal 5'-phosphate formed in the liver is either used directly in the liver in a number of enzyme reactions involving amino acid, or released from the liver bound to albumin and transported to other tissues (Leklem, 1988). The binding of pyridoxal 5'- phosphate to albumin provides a means for transport of pyridoxal 5'-phosphate. The dephosphorylated pyridoxal 5'-phosphate and pyridoxal from the circulation can be converted to 4-pyridoxic acid probably by the FAD-dependent aldehyde dehydrogenase. The 4-pyridoxic acid is not biologically active and is the major excretory product of vitamin B-6 metabolism. A majority of ingested vitamin B-6 is converted to 4-pyridoxic acid and is absent in the urine of vitamin B-6 deficient individuals (Merrill et al, 1984; Leklem, 1991).

2.5 Body Pool and Stores

In the body, the majority (75-80%) of vitamin B-6 in the form of pyridoxal 5'-phosphate is located in muscle (Coburn et al, 1988). Kreb and Fisher (1964) proposed that the amount of pyridoxal 5'- phosphate is primarily associated with muscle glycogen phospharylase based on experimental data showing high content of vitamin B-6 in muscles. Black et al (1978) have shown that muscle phosphorylase acts as a reservoir for vitamin B-6 and that starvation causes depletion of muscle phosphorylase. Black et al. (1978) also noted that vitamin B-6 in the muscle was made available during caloric deficit. The second pool of pyridoxal 5'- phosphate in the body is in the liver. This pool accounts for 5-10% of the vitamin B-6.

The majority (66%) of the pyridoxal 5'-phosphate is found in the liver in the cytosolic compartment (Coburn, 1990) where the metabolic conversion of dietary precursors to pyridoxal 5'-phosphate takes place. Smaller amounts of vitamin B-6 in the form of pyridoxal 5'-phosphate are found in the kidney and brain.

2.6 Biochemical Functions of Vitamin B-6

There are many functions of vitamin B-6 in the body. Over 100 enzymes are known to require pyridoxal 5'-phosphate as an activating enzyme (Allgood, 1991). A major role played by vitamin B-6 as pyridoxal 5'-phosphate in human is in amino acid metabolism. Reactions in which pyridoxal 5'-phosphate is required as coenzyme include transaminations, decarboxylations and deaminations. Other cellular reactions that require pyridoxal 5'-phosphate are gluconeogenesis, immune functions, niacin formation via tryptophan metabolism, nervous system, erythrocyte function and hormonal modulation (Leklem, 1991).

2.6.1 Gluconeogenesis

Vitamin B-6 is involved in the formation of glucose from amino acid during a process called gluconeogenesis. The two processes include the breakdown of muscle and liver glycogen to glucose and deamination of amino acids, making the carbon skeletons available for gluconeogenesis. Gluconeogenic process is the central to maintaining optimal physical activity. During physical activity, muscle tissue becomes the main user of energy in the body. Again there is breakdown of muscle glycogen to meet the energy needs of the muscle. Glycogen phosphorylase is the enzyme responsible for the conversion of muscle glycogen to glucose-1-phosphate (Black et al, 1977). Pyridoxal 5'-phosphate serves as a coenzyme for glycogen phosphorylase

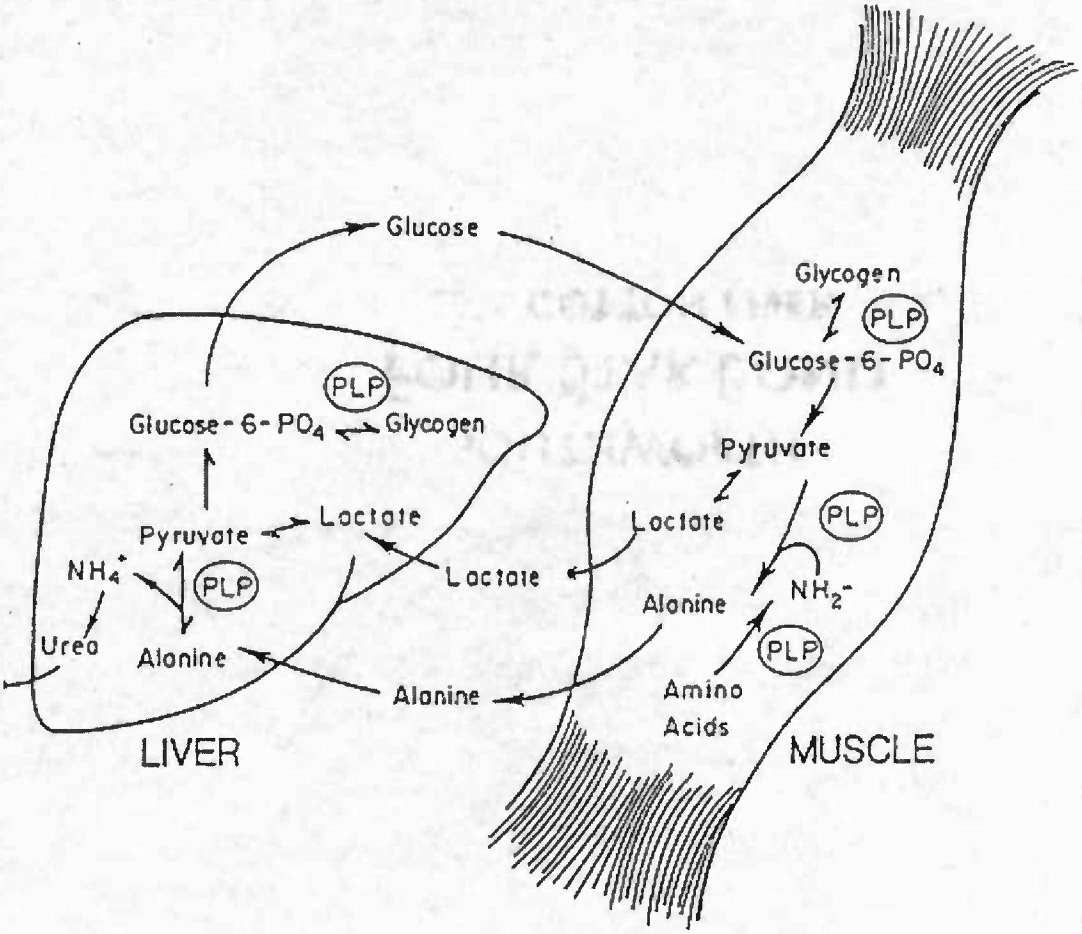


Figure 2.3 Cori-alanine cycle, the involvement of PLP in gluconeogenic metabolism (Adapted from: Leklem:1985)

2.6.2 Immune Function

Vitamin B-6 contributes to optimum functioning of the immune system (Meydani et al, 1991; Miller et al, 1987; Chandra et al, 1985). PLP serves as coenzyme for serine transhydroxymethylase, one of the key enzymes involved in 1-carbon metabolism (Leklem, 1991). Proper functioning of 1-carbon metabolism is required for normal nucleic acid synthesis. Studies in animals (Axelrod et al, 1971; Robson et al, 1975) have shown that vitamin B-6 deficiency impairs both primary and secondary antibody production and the number of antibody-producing cells. In a human study, Talbott et al, (1987) found that the immune system of 11 elderly females was impaired and treatment with 50 mg pyridoxine per day for two months improved their immune system as judged by lymphocyte response. Meydani et al (1991) found that vitamin B-6 deficiency impairs in vitro measures of cell-mediated immunity in healthy elderly adults, and that the impairment was reversed by vitamin B-6 administration. Deficiency of vitamin B-6 has also been associated with immunological changes in persons infected with HIV (Coodley, 1993). Abnormally high prevalence of low serum vitamin B-6 has been reported in populations of human infected with immunodeficiency virus type 1 (Coodley et al, 1993). Development of low vitamin B-6 status over 18 months was also associated with a significant worsening of surrogate markers of HIV disease stage (Baum, 1995). Baum (1995) also stated that AIDS patients had a low vitamin B-6 level in the blood even though the patients were consuming adequate amounts of vitamin B-6 in their diets. This suggests either a malabsorption problem, disease or that the drugs used in the treatment of AIDS may interfere with pyridoxine absorption and /or metabolism.

2.6.3 Niacin Formation

Vitamin B-6 plays an important role in the production of niacin from tryptophan. The enzyme kynureninase catalyzes the conversion of 3-hydroxykynurenine to 3-hydroxyanthranilic and is pyridoxal 5'-phosphate dependent. Vitamin B-6 deficiency impairs kynureninase activity. This impairment results in the urinary excretion of abnormal amounts of tryptophan metabolites, particularly of xanthurenic acid. Leklem et al (1975) examined the effect of vitamin B-6 deficiency (0.19 mg PN/day) on the conversion of tryptophan to niacin by measuring the excretion of two urinary metabolites of niacin, N-methylnicotinamide and N-methyl-2-pyridone-5-carboxamide. After four weeks of consuming a low vitamin B-6 diet, the total excretion of these metabolites decreased following a tryptophan load test. In a study by Hansen et al, (1996) nine women were studied to measure the effect of varying levels of dietary protein on vitamin B-6 status with a constant vitamin B-6 intake. Following a 2-g L trytophan load test, Hansen et al (1996) observed a significant increase in the urinary excretion of the tryptophan metabolites xanthurenic and kynurenic acid by the nine women. This suggests that there is abnormal tryptophan degradation following a diet low in vitamin B-6.

2.6.4 Erythrocyte

Vitamin B-6 is also necessary in the synthesis of heme. In the mitochondria, pyridoxal 5'-phosphate is required for delta-aminolevulinic acid synthetase, the enzyme which catalyzes the condensation of lysine with succinyl CoA to form aminolevulinic (ALA) (Kikushi et al., 1958). ALA is the initial precursor in heme synthesis and deficiency of vitamin B-6 in animals can lead to hypochromic, microcytic anemia (Leklem, 1991).

2.6.5 Nervous System

There are many neurotransmitters, for example, dopamine, norepinephrine, serotonin, histamine and gamma amino butyric acid (GABA) whose synthesis depends on the action of pyridoxal 5'-phosphate dependent enzymes. Vitamin B-6 deficiency has detrimental effects on neural development and function (Loo, 1980; Groziak et al, 1990). The observation that there are neurological abnormalities in animals and human infants, and the involvements of pyridoxal 5'-phosphate in neurotransmitter formation support the role of vitamin B-6 in nervous system function. A study conducted in pregnant rats fed vitamin B-6 restricted diets during gestation and in their pups through 196 days postnatal showed that rats had generalized hypoactivity before weaning. McCullough et al, (1990) found that

infants of Egyptian mothers with marginal vitamin B-6 status have alterations in behavior compared to infants of mothers with adequate vitamin B-6 status. McCullough et al. described several infants who appeared to have altered neurological development as result of low vitamin B-6 intake. An infant was documented to have had a seizure during the first month of life. At 1 month, the mother's milk of this infant contained only 38 g vitamin B-6 per liter. Another infant received human milk that contained less than 84 g vitamin B-6 per liter for 5 months. At the age of 9 months, the infant was assessed to have poor neurological tone and could not sit alone. In this study (McCullough et al, 1990) no other assessments of nutritional status or intake of any other nutrient besides vitamin B-6 content human milk were reported. In this study, it does not mention that the study that the infant abnormal behaviors were reserved with vitamin B-6.

2.6.6 Steroid Hormone Action

Vitamin B-6 as pyridoxal 5'-phosphate has been shown to play a regulatory role in steroid hormone action. The steroid hormones, glucocorticoid, estrogen, progesterone, and androgen are involved in the regulation of growth, development, reproduction and metabolism in many tissues throughout the body. Through direct interaction, each hormone exerts its effect in target tissues with specific intracellular receptor proteins (Allgood et al 1991). The physiological effects of steroid hormones are achieved through the binding of receptors to specific sites in DNA (Carson-Jurica et al, 1990). Pyridoxal 5'-phosphate has been shown to react with lysine residues in steroid hormone receptor proteins to prevent or interfere with hormone binding (Leklem, 1991). These receptor proteins mediate nuclear uptake of the steroid hormone and the interaction of the nucleoproteins with the DNA (Allgood et al, 1991).

Several studies have shown depressed steroid action in the presence of pyridoxal 5'-phosphate (Muldoom and Cidlowski, 1980; Nishigori et al, 1978; Disorbo et al, 1980).

2.7 Vitamin B-6 Requirements

There are numerous factors that may contribute to the requirement for vitamin B-6. Some of these factors include physiological function, dietary intake and intestinal flora. Some other factors previously reviewed (Leklem, 1990) do not have sufficient data to judge the quantitative effect. Factors known to have the most significant effect are vitamin B-6 and protein intake. An increase in vitamin B-6 has been shown to increase pyridoxal 5'-phosphate concentration and an increase in protein intake decreases plasma pyridoxal 5'-phosphate concentrations (Miller et al. 1981; Leklem, 1990). Thus, protein intake influences both plasma pyridoxal 5'-phosphate concentration and urinary 4-PA excretion in an inverse relationship (Miller et al. 1985). Amino acids resulting from excess protein intake for body's growth and maintenance are metabolized, and this process requires increased transaminase levels (Leklem, 1991). Because of this increased metabolism, there is a greater need for pyridoxal 5'-phosphate in tissues. This results in retention of pyridoxal 5'-phosphate in tissues for the catabolism of excess amino acids (Miller et al. 1985).

In study by Miller et al. (1985), the effect of dietary protein on the metabolism of vitamin B-6 in humans was examined. Eight men, aged 21-31 years were fed semi-purifies diets containing 0.5 (low), 1.0 (medium) and 2.0 g (high) protein per kg body weight. Vitamin B-6 intake was kept constant at 1.6 mg per day. Each level of protein was fed for 15 days. Every third day, urinary vitamin B-6, urinary 4-pyridoxic acid, plasma total vitamin B-6 and plasma pyridoxal 5-phosphate were measured. The results showed a negative correlation between protein intake and the concentrations of urinary and plasma vitamin B-6 compounds measured. Therefore, Miller et al, (1985) suggested that with increased intake of dietary protein, vitamin B-6 is retained in the body for increased catabolism of amino acids. There is some evidence that women may have a higher requirement for vitamin B-6 than men. In a study by Shultz and Leklem (1981), plasma pyridoxal 5'-phosphate levels of women consuming self-chosen diets were lower than those of men having a similar vitamin B-6 to protein intake ratio.

Considering the relationship between protein intake and vitamin B-6, the current Recommended Dietary Allowance for vitamin B-6 is based on a ratio of 0.06 mg/ 1 g of protein. The U.S.A RDA for men aged 31-50 is at 1.3 mg and those of 51 and above is at 1.7 mg. The RDA for women is at 1.3 mg per day for ages 19 to 50 and at 1.5 mg per day from 50 years and above (National Research Council, 1999).

3. MALAWI'S GEOGRAPHY AND POPULATION

Micronutrient deficiency remains one of the common forms of nutritional disorder in developing countries. Poor growth of children is the clearest manifestation of nutrition problems in such countries, including Malawi. One of the many nutrition problems stems from the low intake of micronutrients. Hence, vitamin B-6 deficiency as a consequence of suboptimal intakes may be prevalent in such populations.

Malawi is a land-locked country situated in south-central Africa. It is bordered by Tanzania to the north and north-east, Mozambique to the east, south and south-east, and Zambia to the west. Malawi is about 850 km long and 80 to 160 km wide of which 55 percent is arable. Lake Malawi, which covers about one fifth of the country, lies about 460 meters above sea-level and extends 640 km along the border. Lake Malawi is the third largest lake in Africa. Administratively, the country is divided into three regions and 24 districts.

Based on the national census of 2000 the population of Malawi was estimated to be 10.5 million people (and rising). Malawi is one of the poorest countries in the world and one of the most densely populated in Africa, with a population density of 85 persons per sq km. Malawi's socio-economic indicators are amongst the worst in the world.

Malawi's economy is agriculture based. Agriculture usually accounts for about one-third of GDP and over 90 percent of export earnings, predominantly tobacco (90 percent), but also tea, sugar, coffee and cotton. About 90 percent of the population depends on agriculture for their livelihood. Maize is the main staple food for about 93% of the population hence food security is measured in terms of maize availability and accessibility. Maize is the major source of carbohydrates and protein for large segment of the population. It is consumed three or four times a day, when it is plentiful in the household. Maize can be prepared in several ways. Dried maize flour is prepared by pounding using a wooden pestle and hollowed out of a tree truck. Soaked maize flour is prepared by soaking the pounded maize grains in water for 2-3 days. After that, the maize is dried for about 30 minutes and then taken to the miller for milling. This soaked maize flour is commonly called ufa woyera. Ufa woyera takes about 5 days to prepare and there is a 65% extraction rate of the maize grains (Latham, 1997. Whole maize flour is unpounded maize taken to a miller directly without soaking and is commonly called Mgaiwa in Malawi. Although Ufa woyera takes longer to prepare than mgaiwa, most people prefer Ufa woyera. It is worth mentioning that Ufa woyera is less nutritious than mgaiwa because the best parts of the maize grain are discarded during processing (Nutrition Facts for Malawian Families, 1990).

A variety of dishes are prepared from green or dried maize and samp, which is coarsely, ground maize. Nsima the main dish is prepared by adding maize flour to warm water while the pot is on the stove until the mixture is thick. The nsima is always served with relishes. The relishes accompanying nsima always consists of vegetables, beans and occasionally meat or fish. Everything is boiled in water and in most circumstances onions and tomatoes are added to the relishes. In addition, other ingredients to enrich the relish sauce may include groundnut flour both dry raw and roasted; small dried fish and cooking oil.

Cassava is also cultivated as a staple food in some parts of Malawi. It is the co-dominant staple with maize. It is mostly eaten as a staple food in large parts of northern Malawi and a few areas in the south. Cassava leaves are eaten as a vegetable too.

There are two methods of processing cassava flour. The first method involves peeling the cassava tubers and slicing them into small pieces after which the peeled cassava tubers are washed. The pieces are soaked in water for two to three days until they are soft. Then the soaked cassava tubers are removed from water, cleaned, squeezed and spread out in the sun to dry (Lorri et al, 1995). The dried products are ground into flour. The resultant flour is called Kondowole. This process is common in the northern part of Malawi. For the second method cassava tubers are peeled (thick tubers being sliced in half) and put out in the sun until they are dry (Trech et al, 1983). This method is common in the southern part of Malawi and sweet cassava is usually used. The resultant unfermented dried cassava is called Makaka. The flour made from cassava is very fine compared to the maize flour. According to Trech et al (1983) cassava flour can also be prepared into nsima, a thick porridge and that is eaten with leafy vegetables, beans and animal relishes. Thin porridge can also be prepared from cassava flour and is frequently given to infants in areas where cassava is consumed widely.

Leafy vegetables are eaten almost everyday in most households in Malawi. Usually these are cooked vegetables since consumption of raw vegetables is very rare in a Malawi. Dried vegetables leaves (Mfutso) are consumed in season, in particular, after harvest.

Sweet potatoes are consumed nationwide. Sweet potato is a common food for breakfast in most homes including urban and rural areas. It is often eaten as a snack for schoolchildren during recess. Peanuts, which are locally grown are well liked and eaten often. Peanut flour, both raw and roasted, is usually added to both fresh and dried vegetables to thicken the vegetables sauce. It can also be added to children's porridge to enrich it.

Dried beans play an important role in the diet of Malawians. Dried beans are readily available and a popular method of preparation is boiling. In most homes onions and tomatoes are added to cooked beans to add flavor and taste. Numerous dishes are prepared from beans including stewed whole maize grains with cooked beans. According to Ruth Ayoade, (Aykroyd et al, 1982) the Food and Nutrition Officer in Malawi, soy beans have never been considered a food for human consumption in Malawi. Nevertheless, recently use of soy beans has increased particularly among urban poor and rural population. A soy bean is a major source of nutrients used for fortification of less nutritious cereals, for example, maize and sorghum (Aykroyd et al, 1982). Soy beans are about 40% protein and contain more protein than any of the common vegetables or animal food sources found in Africa. In Malawi, soy beans are commonly used in the preparation of weaning foods locally known as Likuni Phala. Rab processors Ltd process the sunshine likuni phala, which is a blend of extruded maize and soy beans. This product is fortified with vitamins like vitamin A, iodine and vitamin E. Skimmed milk is also added. The World Food Program produces the roasted maize -soy beans blend.

Most people drink thobwa, a homemade beverage that is thin fermented millet porridge. This beverage is taken intermittently throughout the day, becoming very common in dry season. According to Lorri (1995) a long the border of Malawi and Tanzania, it is believed that fermented millet gruel helps in the treatment of children suffering from measles. The beneficial effect might be the replacement of fluid lost as a result of the disease (Lorri, 1995). Thus, it is used as local oral dehydration therapy.

3.1 Seasonality of the Diet

In Malawi, there are seasonal variations in the quantity of the foods that make up the diet. Basically, there are two seasons in Malawi. The first is the rainy season (preharvest) from November to April. This is a period of relatively low food availability. The second is the dry season (post harvest) extending from May to October, a period of sufficient food availability (FNSP, 1992). The variety of foods available in the dry season is less than in the period after the harvest. The characteristics and consequences of the hunger have been well documented elsewhere (FNSP, 1992). Hunger in Malawi like in other countries, is referred to as maize shortage in the household since this is the main staple food. Severity of the shortage varies from year to year and people usually ration their supplies during the last few months before harvest. Granaries are carefully monitored usually by men, and sometimes a decision has to be made whether to eat the grain stored for the next year's seed. At times in most rural homes grains must be purchased in the market to carry the household over. If a household is particularly short of cash, cassava flour may be eaten or mixed with maize flour during nsima preparation. The mixture helps to extend the maize flour.

During the hungry season, most activities shift to finding alternative sources of food. Boys hunt for grasshoppers and small animals like mice. Children may borrow their father's hoe and go to the fields to see if they can unearthern a few groundnuts or sweet potatoes overlooked in the last year's harvest. This is also the time when young men look for temporary labor jobs commonly called Ganyu in order to find food or cash for the family. A trend has also been observed towards the movement of young girls and boys to urban areas during the hungry season. Usually these youths are sent by their parents to look for jobs, mainly household jobs so that they help feed their families at home (Nurse, 1975).

High incidence rates of malnutrition are more cute during the planting season when food is in short supply. Such seasonal variations in food could be considered as contributing to transitory food insecurity of poor households, which over time, escalate into chronic food insecurity and nutritional deterioration.

3.2 Vitamin B-6 Status in Africa

Suboptimal vitamin B-6 nutriture may be more prevalent in Africa population than generally believed. The Food and Nutrition Board reports that although vitamin B-6 is widely distributed in plant foods, there are significant portions of the populations with intakes less than the Recommended Dietary Allowance (RDA) (FNB, 1989). The few studies that have been conducted in Africa suggest that intake of vitamin B-6 is also low, particularly in pregnant women and lactating and children. Although few studies have been conducted on vitamin B-6 status in Africa, two studies conducted during the 1990's have suggested that in Africa a large percentage of women of child bearing adolescents and men have vitamin B-6 intakes significantly lower than the Recommended Dietary Allowance (Walker, 1996; Korede, 1990).

Ajayi (1990) indicated a high incidence of vitamin B-6 deficiency among young adults in Africa. A recent report on the nutritional status of Nigeria adolescents indicated that 34% of the adolescents in Nigeria were vitamin B-6 deficient. In a study conducted by Korede (1990) in which the vitamin B-6 status of 120 healthy adolescent (83 males and 37 females) was assessed using dietary vitamin B-6 intake, erythrocyte alanine aminotransferase activity, and its in stimulation by pyridoxal 5'-phosphate revealed that 34% of these adolescents had poor vitamin B-6 status. The similarity in the incidence of vitamin B-6 deficiency among the males and female adolescents showed that both sexes are equally at risk to vitamin B-6 deficiency (Korede, 1990).

In another study in Nigeria, Ajayi et al, (1989) noted vitamin B-6 deficiency among university students. The subjects consisted of 14 healthy males and 20 healthy females. The 20 female students were non-pregnant and not taking oral contraceptives agents. The mean average height and weight of the subjects were 23.3±3.3 years and 58.9±7.1 kg respectively. Following the same dietary regimen throughout the study, each subject received 12.5 mg pyridoxine hydrochloride (PN-HCL) twice daily for two days. On the third day, 12.5 mg PN-HCL was administered together with a 5 g L-tryptophan load dose after an overnight fast. The tryptophan load tests were administered before pyridoxine (pre-pyridoxine) and after pyridoxine (post-pyridoxine) supplementation. Urinary xanthurenic acid (XA) was measured in urine collected on three occasions from each subject and these were pre-tryptophan (basal), post-tryptophan (pre- pyridoxine) and post-tryptophan (post pyridoxine). After tryptophan load (pre-pyridoxine), urinary XA excretion increased significantly (266.4±209.6 µmoles/ g creatinine). However, after pyridoxine supplementation (post pyridoxine) urinary XA excretion decreased to 36.7 ± 13.4 µmoles/g creatinine. The mean XA excretion after pyridoxine supplementation was similar to basal (pre-tryptophan) values in both females and men. According to the researchers, the reduction of XA excretion after pyridoxine supplementation was an indication of vitamin B-6 deficiency.

In South Africa, macro and micronutrients were measured in the diet of Venda African, (20 men and 41 women). In comparison with the National Research Council (1990) (RDA), the mean intake of vitamin B-6 was low in both men and women. The mean intakes were 0.53 and 0.76 mg in men and women respectively (Walker, 1996). In a study conducted in Egypt, suboptimal vitamin B-6 status was observed in one-third of breastfeeding women, based on low breast milk concentration of vitamin B-6 (McCullough et al, 1990). In addition, these researchers documented behavioral abnormalities in 3-6 months old infants whose mothers had vitamin B-6 levels in their milk below 0.085 mg/L.

Other risk factors may exacerbate vitamin B-6 deficiency in African populations. Alcohol and cigarette smoking have been linked to poor metabolic functions of vitamin B-6 (Li and Lumeng, 1985). Vitamin B-6 deficiency is viewed as an important nutritional complication in alcoholism. In addition, it has been established that alcoholics absorb less vitamin B-6 than do control subjects. Bonjour (1980) reported that alcoholics have a decreased ability to liberate vitamin B-6 from its bound form. Several studies suggest that alcohol interferes with conversion of pyridoxine to pyridoxal 5'phosphate. Additionally, smoking has been associated with low plasma pyridoxal 5'-phosphate concentrations (Serfontein et al, 1986). In a study comparing smoker, nonsmoker and exsmokers, the investigators found that plasma pyridoxal 5'-phosphate and pyridoxal concentrations were significantly lower in smokers than in the nonsmokers and exsmokers (Vermaak et al, 1990).

To the best of my knowledge, there have been no data published of the vitamin B-6 content of the typical Malawi foods. Therefore, there were two principal objectives of the present study. Firstly, to determine the vitamin B-6 and pyridoxine-glucoside vitamin B-6 content of foods commonly consumed in Malawi. Secondly, to examine the effect of preparation procedures of foods in Malawi on the content of vitamin B-6 and pyridoxine-glucoside vitamin B-6 in the diet.

4. MATERIALS AND METHODS

4.1 Food Sample Collection

Eighteen samples were selected to represent the common foods commonly consumed in Malawi. The foods were purchased from local markets in the central and southern parts of Malawi. The samples were fermented and unfermented cassava flour (*Manihot esculenta)*; whole and soaked maize flours (*Zea mays)*, and millet flour (*Eleusine corana).* The legumes selected were soy beans (*Glycine max)*, sugar beans (*Phaseolus vulgaris*), kidney beans, pinto beans (*Phaseolus vulgaris)*, cow peas (*Vigna sinensis,)* dried green peas (*Pisum sativum)*, chick peas (*Cicer arientinum)* and peanuts (*Archis hypogaea).* The vegetables selected were dried cow pea leaves (*Vigna sinensis)*, dried pumpkin leaves (*Cucurbita maxima*) and pumpkin (*Cucurbita maxima*). Sweet potato (*Ipomoea batatas*) was also purchased from local markets

Two commercial weaning formulas for children were also selected. These were extruded maize-soy bean blend commonly called Sunshine likuni phala processed by Rab Processors and a World Food Program product, which is a blend of roasted maize and soy beans in the ratio of 80:20.

Most samples were purchased within three to four months after harvest. All samples were shipped to Oregon State University, Corvallis, OR U.S.A. Upon arrival, sweet potato and pumpkin were frozen at -20°C and the rest were stored for a week in a cool dry place until prepared and /or analyzed.

4.2 Preparation of the Food Samples

Whole and unsoaked maize flour, fermented and unfermented cassava flour, and germinated millet had been processed into usable forms; therefore no further preparation was performed. Prior to processing in a blender, a representative sample of raw legumes were frozen with liquid nitrogen and then homogenized with a blender until a fine powder was obtained: Kidney beans, sugar beans, pinto beans, cow peas, chick peas and roasted soy beans and peanuts were processed using the same procedure. The powdered sample was then transferred into tightly sealed plastic container and stored in a freezer until assayed. Raw samples of soy beans and chick peas were roasted on baking sheet. The baking sheets were placed in a preheated gas oven (350° F). The roasting time was determined during pretrial experiments with soy beans from the United States. The roasting time determined was 15 minutes. Both soy beans and chick peas were roasted for 15 minutes, and then cooled for approximately 15 to 20 minutes. The samples were then treated with liquid nitrogen before being processed in a blender into a powder form. The roasted sample were then transferred into a tightly sealed plastic container and stored in the freezer at -20°C until further analysis for vitamin B-6.

Approximately 100 grams of the dried kidney beans, sugar beans, cow peas, and pinto beans were soaked over night at room temperature in 1000 ml of tap water. After soaking, the beans plus the water were boiled and simmered to complete cooking. Cooking was begun each time at 160° F, but the temperature during the cooking period ranged from 170-190° F. The amount of water varied with the kind of legume and ranged from 1200 ml for cow peas to 1350 ml for sugar beans. Based on preliminary trials, the time for cooking ranged from 67 minutes for the cow peas to 75 minutes for sugar beans. After cooling, the cooked beans were mashed without draining the water in a blender to uniform consistency. The cooked mashed beans were then transferred into a plastic container and frozen at 34° C for analysis. Moisture content was measured in both raw and cooked beans by drying weighed portions in an oven at 70° C for six hours (AOAC, 1970).

Fifty grams of the unpeeled sweet potato was boiled in 250 ml of water for 25 minutes. The boiled potato was then cooled to a room temperature and the thin outer skin was removed. The boiled peeled potato was mashed without draining the water and frozen until assayed. Moisture content was measured in both raw and cooked potato samples by drying weighed portions in an oven at 70°C for six hours (AOAC, 1970). In a similar manner, 100 grams of unpeeled pumpkin was boiled in a 350 ml of water until the pumpkin was soft (15 minutes). The cooking temperature was between 180° to 190° F. After draining, the pumpkin was cooled to room temperature and then mashed before assayed. Likewise, the moisture content was measured in cooked pumpkin by drying a weighed sample in an oven at 70°C for six hours (AOAC, 1970)

4.3 Assay Procedures

4.3.1 Total Vitamin B-6

Total vitamin B-6 of each sample was determined using a microbiological method with *Saccharomyces uvarum* (*S. uvarum*) as the assay organism (Miller and Edwards, 1981). Briefly, 2 grams of duplicate food samples were mixed with 200 ml of 0.044 N HCL and autoclaved at 121° C for 2 hrs. The individual samples were then adjusted to pH 4.5, filtered through Whatman No.1 filter paper, diluted to an approximate concentration of 1 to 2 ng/ ml pyridoxine equivalents and assayed for vitamin B-6 content by measuring the growth of *S. uvarum* (Haskell and Snell, 1970).

4.3.2 Pyridoxine-Glucoside

The method of Kabir et al (1983) was used for the determination of pyridoxine-glucoside. One gram of food and 100 ml of 0.1 M phosphate buffer (pH 6.8) were combined and stirred for 2 hrs in the dark at room temperature. This stirring was carried out in order to further break down the food material and obtain a homogenous mixture for the enzyme treatment. The pH was then adjusted to 5.0 then 6.0 units of -D-glucosidase (β-D-glucoside glucohydrolase from almonds, Sigma Chemical Company, St. Louis, MO) was added to one of the samples in duplicates. The samples were then incubated for 2 hours in shaking water bath at 37° C. Ten ml of 1 N HCL was then added and the mixture was steamed for 5 minutes to stop enzyme action. The pH was adjusted to 4.5 using KOH, the volume diluted to 250 ml, and the mixture filtered through Whatman No. 1 filter paper. The vitamin B-6 activity was measured by microbiological assay using *S. varum.* To measure nonconjugated vitamin B-6, 1 gm of food was subjected to the previous treatment with the exception that -D-glucosidase was not added prior to the 2-hour incubation at 37° C. The percent pyridoxine-glucoside was calculated as the difference between the value for the β-D-glucosidase treated and no enzyme treated sample divided by the total hydrolyzed vitamin B-6 value times 100.

4.3.3 Control

Orange juice concentrate was used as a control for each of the pyridoxine-glucoside assay. Preliminary studies (Kabir, 1982) found orange juice to contain a substantial amount of pyridoxine-glucoside. The mean value for the duplicate orange juice samples was 0.83 mg and the standard deviation was 0.01 mg.

4.4 Results

Values for the total vitamin B-6 and glycosylated vitamin B-6 content of the seventeen foods are given in Table 4.1. The foods were classified into food groups; cereals and grains, legumes, nuts, vegetables, fruits and roots. In addition, the values for the two formulated weaning food products are also given.

Among the cereal group, whole maize flour contained the highest amount of vitamin B-6 compared to soaked maize flour and millet flour. Whole maize flour was approximately 7 times higher in vitamin B-6 content than soaked maize flour and 2 times higher than millet flour. The total vitamin B-6 values for raw dry legumes ranged from 0.15 mg/ 100 grams for the green dry peas to 0.65 for the cow peas per dry weight basis. Dry raw peanut flour also contained appreciable amount of vitamin B-6 (0.48 mg/ 100 gram dry weight). Based on the dry weight basis, the dry cooked pumpkin leaves contained 23 percent more vitamin B-6 than the cow pea leaves. The cooked unpeeled pumpkin contained an appreciable amount of vitamin B-6 (Table 3). In the case of the root crops, sweet potatoes contained approximately 5 % more vitamin B-6 than unfermented cassava flour. The two formulated weaning food products, roasted maize-soybean blend (80:20 % of maize to soybeans) and extruded maize-soybean blend (80:20 % of maize to soy beans) contained an average of 0.48 mg/100 grams wet weight basis.

TABLE 4.1

Vitamin B-6 and Pyridoxine-glucoside content of Malawi foods

Food	Total vitamin B-6[a] wet/wt.	Total vitamin B-6[a] dry/wt.	% vitamin B-6 from raw To processed	Pyridoxine-glucoside[b] wet/wt.	Pyridoxine-glucoside[b] dry/wt.	%Pyridoxine-glucoside[c]
	Mg/100 grams			Mg/100 gram		
CEREAL/GRAINS						
Whole maize flour	0.66			0.01		1
Soaked maize flour	0.09		86[e]	nd[d]		nd[d]
Millet flour	0.34			0.05		
LEGUMES						
Pinto beans, raw		0.61			0.15	25
Pinto beans,cook		0.40	34		0.06	15
Kidney beans, raw		0.56			0.12	15
Kidneybeans,cook		0.31	45		0.11	35
Sugar beans, raw		0.50			0.09	18
Sugar beans,cook		0.43	14		0.07	16
Cow peas, raw		0.65			0.16	20
Cow peas, cooked		0.34	48		0.09	26
Chick peas, raw		0.61			0.28	15
Chick peas, roasted		0.21	59		0.14	56
Soy beans, raw		0.34			0.19	25
Soy beans, roasted		0.21	38		0.14	49
Green peas, raw		0.15			0.001	1.5
NUTS						
Peanuts, raw		0.48			0.17	15
VEGETABLES						
Cow pea leaves, Cooked		0.10			0.01	10
Pumpkinleaves,coo		0.13			0.03	30
Pumpkin, cooked		0.60			0.11	18
ROOTS						
Sweet potato, raw		0.20			0.01	
Sweet potato, cook		0.20	0		0.01	5
Cassava flour, unfermented	0.19			0.05		5 26
Cassava flour,ferm	0.02		89[e]	0		
WEANING FOODs						13
Roasted maize-soy	0.37			0.12		32
Extruded maize-soy	0.60			0.03		5

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a.Total vitamin B-6 refers to the amount of vitamin B-6 microbiologically after acid hydrolysis

b. Pyridoxine-glucoside refers to the difference between the enzyme treated value and the free vitamin B-6 values

c. Pyridoxine-glucoside as a percentage of the total vitamin B-6

d. n.d= not detected by the enzyme treatment

e. As commercially available.

Table 4.1 also lists the pyridoxine-glucoside content of the foods studied. According to Kabir et al (1983) pyridoxine-glucoside refers to the difference in vitamin B-6 content between the enzyme treated sample and the nonconjugated vitamin B-6. Among the cereals, soaked maize flour contained no pyridoxine-glucoside while whole maize flour contained 1% pyridoxine-glucoside. The pyridoxine-glucoside percentage ranged from 1.5%for the whole maize flour to 55% for the soy beans. Pyridoxine-glucoside has been reported to occur widely in plant foods ranging from 0 to 80 percent (Kabir et al, 1983; Gregory and Ink, 1987)

Figure 4.1 shows the vitamin B-6 content of the raw and processed foods. Nine foods were analyzed after the preparation treatments. According to Figure 4.1, whole maize flour was 86 percent higher in total vitamin B-6 than soaked maize flour. Cooking reduced the vitamin B-6 content of beans by 34%, 45%, 14% and 48 % from the same lots of pinto beans, kidney beans, sugar beans and cow peas, respectively. The total vitamin B-6 content was reduced by 59% in chick peas and 38% in soy beans, by the roasting treatment employed. The fermented cassava flour was 89% lower in vitamin B-6 than the unfermented cassava flour. There was no difference in vitamin B-6 of the raw and cooked sweet potato (Figure 4.1).

Table 4.1 gives the pyridoxine-glucoside content of the nine foods in both the raw and processed forms. When raw values were compared to processed, the percent of the pyridoxine-glucoside was found to increase with processing in four foods, kidney bean, cow peas, chick peas and soybeans. The raw pinto beans, kidney beans and cowpeas contained 11 to 18 percent pyridoxine-glucoside and after cooking, the content of pyridoxine-glucoside increased to 25 to 35 percent of the total. In contrast, in sugar beans, the pyridoxine-glucoside was reduced by 11 percent after cooking (Table 3). The roasting treatment of chick peas and soy beans increased pyridoxine-glucoside by 18 and 20 percent, respectively.

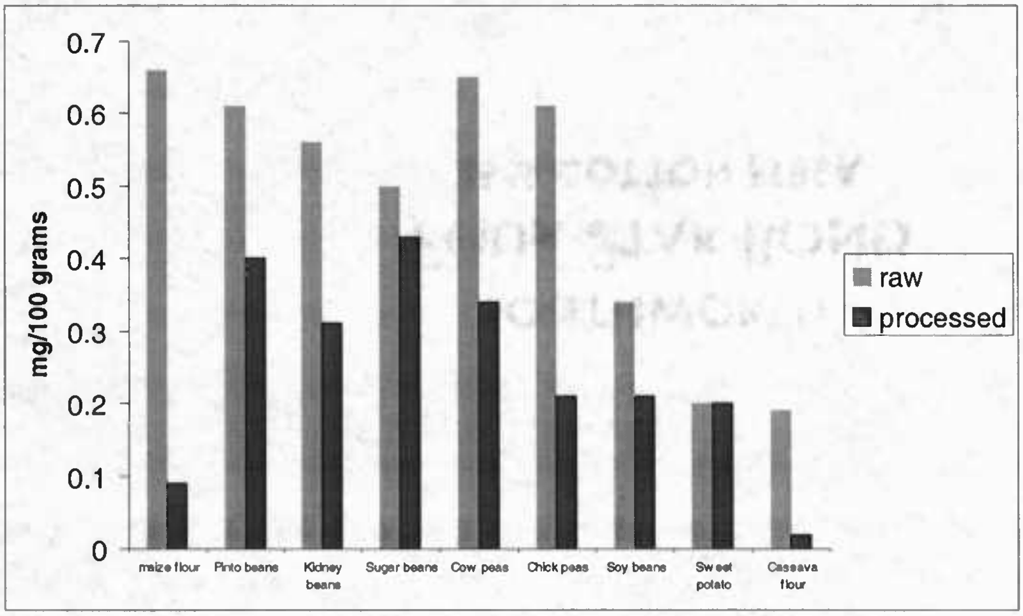

Figure 4.1 Vitamin B-6 content of raw and processed foods

No pyridoxine-glucoside was detected in soaked maize flour (Ufa woyera) compared to the whole maize flour. However, the amount of pyridoxine-glucoside detected in whole maize flour was small (1%). The percentage of pyridoxine-glucoside of the raw and cooked sweet potato was the same (5%). Based on a wet weight basis, the pyridoxine-glucoside content determined from unfermented cassava flour was 13% higher than that of the fermented cassava flour (Figure 5). In general, based on percentage, roasted chick peas and soy beans contained the highest amount of pyridoxine-glucoside than that in the rest of the food samples analyzed.

4.5 Discussion

Table 4.2 provides a comparison of the total vitamin B-6 content of some of the foods analyzed in this study with those reported in the literature. The values of the food samples analyzed in this study agreed well with those of the literature (Table 4.2). Whole maize flour was 85% higher in total vitamin B-6 than the soaked maize flour.

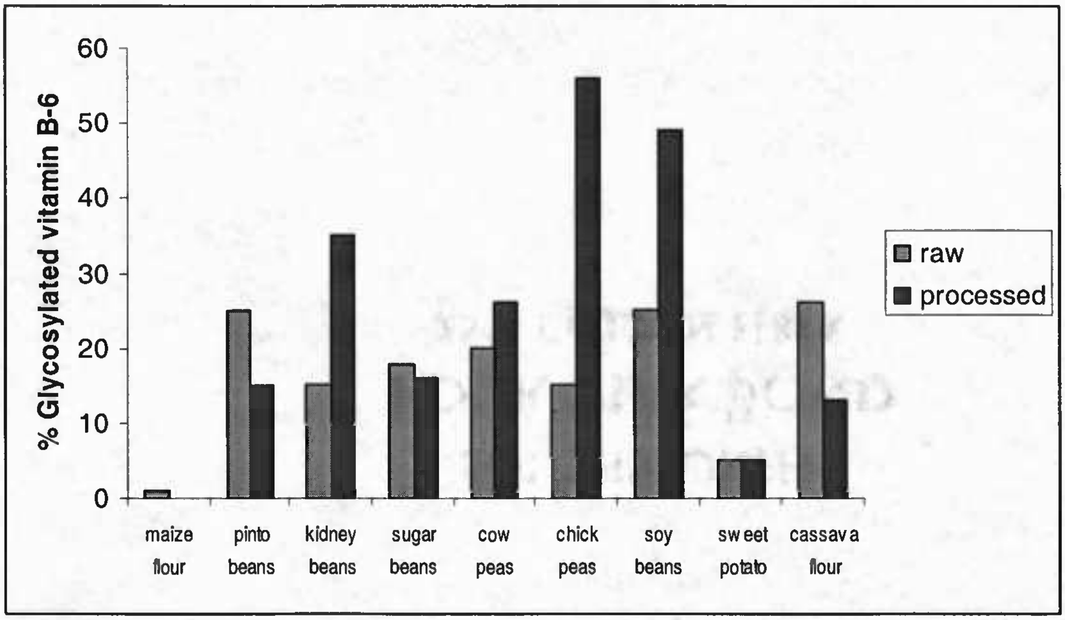

Figure 4.2 Percent Pyridoxine-glucoside content of raw and processed foods

There are possibilities that could contribute to the difference in vitamin B-6 content between the two maize flour samples. First, the removal of the bran during pounding or processing of the soaked maize flour could lead to the low vitamin B-6 content. In cereals, vitamin B-6 is concentrated in the germ and bran. Large amounts of the vitamin B-6 can be lost during processing and milling of cereals and grains (McDowell, 2000; Gregory and Ink, 1978). There is approximately a 95 percent extraction rate in whole maize flour and 65 percent extraction rate in soaked maize flour (Latham, 1997). It is important to point out that no previous study has been done on the vitamin B-6 content of whole maize flour. One study measured vitamin B-6 in corn meal in which presumably the germ and bran have been removed during processing. For example, Schroeder (1995) reported a 0.20 mg/100 grams for the corn meal which is 3 times lower in vitamin B-6 than whole maize flour. In corn meal, the germ is removed from the kernels in order to improve the keeping qualities of the meal. Therefore, it is not possible to make reliable comparisons for these two maize products. Though no study has been done on maize bran, whole maize flour could be one of the best sources of vitamin B-6 in Malawi. Second, in the case of soaked maize flour, the pounded grains with a 65% extraction rate (Latham, 1997) are soaked in water for 3 to 4 days. After soaking, the grains are washed with water, drained and dried before milling. There could be a significant loss of vitamin B-6 due to leaching during soaking and physical washing.

Table 4.2

Comparison of values for the Vitamin B-6 content of foods in current study and those reported in the literature

Food	Current study Mg/100 grams	Literature values Mg/100 grams
Pinto beans		
Raw	0.61	0.51 dry wt. (Polansky, 1969) 0.61 dry wt.(Miller, 1973) 0.50 dry wt. (Augustin, 1981)
Cooked	0.4	0.40 dry wt.(Miller, 1973) 0.40 dry wt. (Augustin, 1981)
Kidney beans		
Raw	0.56	0.56 wet wt.(Scot and Bishop, 1975) 0.44 dry wt. (Polansky, 1969)
Cooked	0.31	0.32 dry wt. (Augustin, 1981)
Cow peas		
Raw	0.65	0.56 dry wt (Polansky, 1969)
Chick peas		
Raw	0.61	0.53 wet wt.(Scot and Bishop, 1975)
Soy beans		
Raw	0.61	0.81 dry wt.(Orr, 1969)
Green peas		
Raw	0.15	0.16 dry wt. (Orr, 1969)
Peanuts		
Raw	0.2	0.25 wet wt.(Scot and Bishop, 1975)

The loss of vitamin B-6 in dry cooked pinto beans in this study is similar to (Miller, 1973) or slightly higher than those reported in other studies (Augustin, 1981) (Table 4.2). The vitamin B-6 values for the raw dry kidney beans 28% greater than those reported in the literature. In cooked kidney beans, the values for vitamin B-6 are similar with those reported by Augustin (1981). Cooked sugar beans and cow peas contained appreciable amount of vitamin B-6 with an average of 0.38 mg/100 grams dry weight basis. However, suitable literature values are not available for comparison with the values presented in this study for sugar beans and cowpeas. In case of the raw chick peas, the vitamin B-6 values found in this study were 13% higher than published values (Scot and Bishop, 1975). There are no data available on the roasted chick peas for comparison. Orr (1969) reported a 58 percent higher in vitamin B-6 for the soy beans than in this study. No suitable values were found in the literature for the roasted soy beans for comparison. It is not surprising to note some variations in vitamin B-6 concentration in dry beans reported in literature and in this study because vitamin B-6 content varies according to the type, growing environment, preparation procedures and storage conditions as well as the method used for determining vitamin B-6 content (Gregory and Kirk, 1981). Augustin et al (1981) studied the vitamin B-6 of the raw and commercial phaseolus vulgaris, bean classes grown in different areas. The vitamin B-6 content was 0.40 and 0.32 mg per 100 grams for kidney beans from California and Michigan, respectively. Another possible reason for the discrepancy between the vitamin B-6 content obtained in this study and those reported in the literature could be the difference in the sampling time following harvesting. Data reported in literature do not specify such time, and it is possible that researchers included samples stored up to one year or even longer following harvest. As pointed out earlier, in this study, samples were collected and prepared for analysis within 3 to 4 months following harvest. Although no investigation has been reported on the possible changes in vitamins during storage of dry beans, the possibility of such changes though remote, has to be considered (Augustin et al, 1981).

The data obtained in this study and those reported in the literature indicate that cooking decreases the vitamin B-6 content of the beans. The loss in total vitamin B-6 ranged from 14 percent for sugar beans to 62 percent for kidney beans when comparing the raw to the cooked dry beans. Thermal processing has been found to decrease the content of vitamin B-6 in foods (Gregory and Kirk, 1978a). Thermal losses of 0-10 % have been reported for vitamin B-6 in bean products and tomato juice (Miller et al, 1973; Raab et al, 1973). Augustin et al (1981) reported a 30 percent decrease in vitamin B-6 when comparing raw and cooked pinto beans. Although pyridoxine is the major form of vitamin B-6 in plant foods, Orr (1969) reported the presence of the other two free forms, pyridoxal and pyridoxamine in most plant-derived foods. For example, raw dry soybeans contained 44 percent as pyridoxine, 44 percent as pyridoxal and 12 percent as pyridoxamine. In the Polansky study (1981), cow peas contained 26% pyridoxal and 12% pyridoxamine. Likewise in pinto beans, 35% of the vitamin B-6 came from pyridoxal and pyridoxamine. The stability of vitamin B-6 during heat treatment depends on the form of the vitamin. Pyridoxine, the predominant form of vitamin B-6 in plants (Orr, 1969) is stable to heat, while pyridoxal and pyridoxamine are much unstable to heat. Therefore, the loss of total vitamin B-6 during cooking and heat processing could be due to the loss of these two less stable forms. The three forms of vitamin B-6 were not separately analyzed in this study.

As indicated in Table 4.1, roasted chick peas and soy beans contained appreciable amounts of vitamin B-6. Kabir et al (1981) found the vitamin B-6 content of cooked soybeans to be 0.62 mg/100 grams. This cannot validly be compared to the 0.21 mg/ 100 grams that was found in roasted soybeans because there was no information available on the roasting processing of chick peas and soy beans. In Table 4.1, green raw peas were found to contain an amount of vitamin B-6 similar to that reported in literature (Schroeder, 1971) or slightly higher than that reported in literature (Orr, 1969). The dry raw peanut flour contained an appreciable amount of vitamin B-6 (0.58 mg/100 grams). Scot and Bishop (1975) reported a slightly higher vitamin B-6 content for dry raw peanuts (Table 4.2).

Among the root crops (Table 4.1), cooked sweet potato contained more vitamin B-6 compared to the cassava flour. No data was available for the sweet potato and cassava flour for comparisons. While fermentation increases the content of other nutrients in foods, for example niacin (Briggs et al, 1997), in the present study, it was found to significantly decrease the vitamin B-6 content of the fermented cassava. The vitamin B-6 content was probably leached out during the physical washing and sun drying of the fermented cassava.

Dry cooked pumpkin leaves contained a higher amount of vitamin B-6 than cow pea leaves. Although the dry cooked vegetables contained high amounts of vitamin B-6, the content of vitamin B-6 in the fresh leafy vegetables could be much higher than that determined in dry cooked vegetables analyzed in the present study. As stated earlier, the fresh vegetables are exposed to long preparation procedures before being consumed. First, the fresh leafy vegetables are blanched in hot water for 20 minutes or longer. Second, the water is drained and the blanched vegetables are dried in the sun, which often takes a week to dry (Nutrition Facts for Malawian Families, 1990). In addition to these procedures, the dried vegetables are boiled again in water before being consumed. Since vitamin B-6 is water soluble, all these procedures could expose the vitamin to leaching through blanching and boiling. There is no data available on cooked dry cow pea and pumpkin leaves for comparison.

Cooked unpeeled pumpkin also contained an appreciable amount of vitamin B-6; however, leaching of the vitamin B-6 into water cannot be overlooked in this situation. There is no information available on the vitamin B-6 content of cooked pumpkin for comparison.

Pyridoxine-glucoside comprises of 5-80% of the total vitamin B-6 in many fruits and vegetables (Gregory and Ink, 1987); Kabir et al, 1983). Table 4.1 data clearly show that the level of pyridoxine-glucoside is quite variable. In roasted chick peas and soy beans greater than 50% of the vitamin B-6 was in the pyridoxine-glucoside form. When the nine raw food samples were compared to processed foods, based on percentage, the pyridoxine-glucoside seemed to increase with processing (Figure 4.2). In roasted chick peas and soy beans greater than 50% of the vitamin B-6 was in the pyridoxine-glucoside form. In pinto beans and sugar beans, when the pyridoxine-glucoside was expressed as percentage, there was a loss of pyridoxine-glucoside form during cooking. One reason for this difference could be that pyridoxine-glucoside may be more prone to loss in such types of beans or converted to pyridoxine. In addition to this, the pyridoxine-glucoside form may not be fully released from its bound form in foods during processing or analysis. On the other hand, on percent basis, there was an increase in pyridoxine-glucoside for the cooked kidney beans and cow peas. In the study by Kabir et al (1983), pyridoxine-glucoside was identified in cauliflower, broccoli, green beans and soy beans, and based on percent basis, the pyridoxine-glucoside increased during freezing, canning and cooking, respectively. Kabir et al (1983) attributed this increase to the denaturation of the glucosidase activities present in raw foods during processing.

Pyridoxine-glucoside has been noted to be only partially utilized in humans and to quantitatively alter the metabolism in vivo retention of other vitamin B-6 forms, particularly pyridoxine (Gilbert et al, 1992). In the present study, whole maize flour was found to contain the highest amount of vitamin B-6 (0.66 mg /100 grams). At the same time, the percentage of pyridoxine-glucoside was very low (1.5%). This could indicate that whole maize flour, the main staple food of Malawi, is an excellent source of vitamin B-6. However, as already said previously, most Malawians prefer soaked maize flour to whole maize flour.

Using vitamin B-6 values for the foods analyzed in this study, four typical diet meals (two for rural areas and two for urban areas) were formulated and vitamin B-6 calculated for the day to estimate the relative vitamin B-6 intake in Malawi. Suggested combinations of food items commonly consumed in rural and urban Malawi in the morning, afternoon and evening are presented in Table 4.2. Based on the estimated meals presented in Table 4.2, the urban area population may be able to get more than 100% of their recommended vitamin B-6 intake per day. About 95 percent of the vitamin B-6 intake per day could be from nsima made from whole maize flour, which is rich in vitamin B-6 (0.66 mg/ 100 g). Whole maize flour is more popular is urban areas than in rural areas.

On the other hand, rural population may not be able to reach the recommended daily intake for vitamin B-6. According to Table 4.2, rural groups may get only 40-60 percent of the vitamin B-6 required per day. This could be due, in part, to the frequent use of soaked maize flour and fermented cassava in most rural areas. However, in those areas where bananas (0.31 mg/100 g) and avocado (0.44 mg/100 g) are consumed when in season they may be able to receive additional vitamin B-6 from such fruits.

Table 4.3 Malawi's typical diets for the urban and rural areas and the relative vitamin B-6 content

URBAN

	Food	**Amount**	**Vitamin B-6 content (mg)**	**Source of value**
DAY 1	Breakfast			
	White bread	50 g	0.02	Leklem, 1991
	Tea	355 ml		
	Lunch			
	Nsima(whole maize flour)	250 g	1.65	Current study
	Cooked kidney beans	90 g	0.27	Current study
	Dinner			
	Nsima(whole maize flour)	250 g	1.65	Current study
	Cooked kidney beans	90 g	0.27	Current study
IB-6/day			3.86	
DAY 2	Breakfast			
	White bread	50 g	0.02	Leklem, 1991
	Tea	355 ml		
	Lunch			
	Nsima(whole maize flour)	250 g	1.65	Current study
	cooked fresh white fish	28 g		
	Cooked fresh vegetables	33 g	0.05	
	Dinner			
	Nsima(maize flour)	100 g	0.66	CurrentStudy
	cooked fresh vegetables	36 g	0.05	
B-6/day			2.37	
RURAL				
DAY 1	Lunch			
	Nsima(soaked maize flour)	300 g	0.27	Current study
	Cooked pinto beans	90 g	0.13	Current study
	Dinner			
	Nsima(soaked maize flour	300 g	0.27	Current tudy
	cooked fresh			
		36 g	0.05	

Table4.3(Continued)

	Vegetables			
	Peanut flour	12 g	0.15	Current study
B-6/day			0.87	
DAY 2	Lunch			
	Nsima(fermented cassava flour)	500 g	0.1	Current study
	cooked pinto beans	90 g	0.27	Current study
	Dinner			
	Nsima(fermented cassava flour)	500 g	0.1	Current study
	cooked pinto beans	90 g	0.27	Current study
	Dried cooked pumpkin leaves	36 g	0.05	Current study
B-6/day			0.79	

5. SUMMARY AND CONCLUSION

Vitamin B-6 and pyridoxine-glucoside content of seventeen foods eaten in Malawi were determined using a microbiologic assay. In this study, it was found that whole maize flour contained the highest amount of vitamin B-6 per 100 g. When the raw foods were compared to the cooked foods, cooking decreased the vitamin B-6 content in some foods. Roasting also decreased vitamin B-6 content in chick peas and soy beans. Likewise, soaking decreased the vitamin B-6 content in soaked maize flour and fermented cassava flour compared to the whole maize and unfermented cassava flours, respectively. These observations suggest that some food preparation practices, for example, boiling, water blanching, soaking and roasting of foods have an adverse impact on the micronutrient content of foods, in this case vitamin B-6. Preparation losses of vitamin B-6 in plant foods can be large due to physical loss from leaching.

Based on the percentages of pyridoxine-glucoside, cooked and roasted foods contained high pyridoxine-glucoside levels. The high pyridoxine-glucoside in such foods may add an adverse impact on the bioavailability of vitamin B-6 to the body. Bill et al (1978) states that the percentage pyridoxine-glucoside in each food is a strong predictor of the bioavailability of vitamin B-6 from that food. Thus, the foods with highest pyridoxine-glucoside will have the lowest vitamin B-6 bioavailability.

In summary, most plant foods analyzed in this study from Malawi contained appreciable levels of vitamin B-6, therefore are good sources of vitamin B-6. This study also suggests that rural population may be at risk of inadequate vitamin B-6 intake compared to urban population. Due to high prices of meat and fish, rural people with low incomes are unable to consume them in sufficient quantities. Therefore leafy vegetables constitute the principal source of nutrients, including vitamin B-6 for the Malawian rural population. While consumed as relish by both rich and poor alike, vegetables are essential to the diet of rural subsistence households throughout the country. In addition, locally preparation procedures of foods such as soaking, fermentation and prolonged cooking, which are commonly practiced in rural areas, may have adverse impact on vitamin B-6 quantitatively. Based on these results, it is desirable that vitamin B-6 status of the population groups in Malawi be assessed and that effective measures are suggested in meeting the physiological needs.

This present study had some limitations. Many people in Malawi cook over a three-stone open fire and on traditional stoves using firewood. In this type of cooking it is hard to maintain to maintain a consistent temperature and to determine the time of cooking. In daily life, women in Malawi do not always have time to stay with the fire and do not add firewood in time. As a consequence, the heat supply would not be continuous so the food would cool down and have to be warmed again, probably increasing actual cooking time. In contrast, in this present study cooking of the foods was done over a gas stove. Different cooking methods will generate different amount of heat, which will also affect the length of cooking of that particular food. In light of this, there may be some discrepancies in terms of cooking time and heat supply between the two preparation techniques. With respect to roasting grains, traditionally, roasting in Malawi is accomplished by shaking the grains in a heated pan over an open fire. But, in the present study, roasting was done in the oven. All these differences in preparation techniques may have some nutritional consequences. The food may lose some of its nutrients and sometimes the nutrients may be enhanced after cooking and roasting of the foods. Based on literature (Raab et al, 1973; Miller et al, 1973; Ekanayake et al, 1990), in the usual cooking processes of the plant foods, there is less risk of losing vitamin B-6.

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APPENDIX

Table a.1 Total vitamin B-6 data

Food sample	Duplicates	Moisture %	Hydrolyzed	Total vitamin B-6 mg/100 g
Cereal/grains				
Whole maize flour	A		0.661	
	B		0.65	0.66
Soaked maize flour	A		0.088	
	B		0.084	0.086
Millet flour	A		0.329	
	B		0.343	0.34
Legumes				
Pinto beans, raw	A	6	0.273	
	B		0.277	0.275
Pinto beans, cooked	A	73	0.079	
	B		0.08	0.0795
Kidney beans, raw	A	25	0.364	
	B		0.392	0.378
Kidney beans, cooked	A	73	0.118	
	B		0.101	0.11
Sugar beans, raw	A	8	0.284	
	B		0.283	0.284
Sugar beans, cooked	A	69	0.66	
	B		0.66	0.66
Cow peas, raw	A	6	0.361	
	B		0.361	0.361
Cow peas, cooked	A	75	0.095	
	B		0.093	0.093
Chick peas, raw	A		0.6	
	B		0.616	0.608
Chick peas, roasted	A		0.249	
	B		0.253	0.251
Soy beans, raw	A		0.34	
	B		0.34	0.34
Soy beans, roasted	A		0.215	
	B		0.209	0.212
Green peas, raw	A		0.067	
	B		0.068	0.068
Nuts				
Peanuts, raw	A		0.479	
	B		0.477	0.478
Vegetables				
Cow pea leaves, cook	A	84	0.021	
	B		0.022	0.0215
Pumpkin leaves, cook	A	85	0.049	
	B		0.05	0.049
Pumpkin, cooked	A	82	0.193	
	B		0.194	0.193

Table a.1 (Continued)

Roots				
Sweet potato, raw	A	72	0.097	
	B	71	0.105	0.101
Sweet potato, cooked	A		0.104	
	B		0.099	0.102
Unfermented cassava	A		0.195	
	B		0.192	0.194
Fermented cassava	A		0.024	
	B		0.023	0.024
Weaning foods				
Roasted maize-soy	A		0.386	
	B		0.359	0.373
Extruded maize-soy	A		0.613	
	B		0.63	0.622
	B		0.359	0.373
Extruded maize-soy	A		0.613	
	B		0.63	0.622

Table A.2. Glycosylated Vitamin B-6 data

Food sample	Duplicates	Moisture %	with Enzyme	No Enzyme	Pyridoxine-glucoside	% Pyridoxir glucoside
Cerealgrains						
Whole maize flour	A		0.214	0.207		
	B		0.215	0.205	0.01	1
Soaked maize flour	A		0.069	0.081		
	B		0.072	0.078	n.d	n.d
Millet flour	A		0.19	0.136		
	B		0.189	0.144	0.05	15
Legumes						
Pinto beans, raw	A	6	0.218	0.191		
	B		0.229	0.197	0.15	25
Pinto beans, cooked	A	73	0.058	0.031		
	B		0.057	0.032	0.06	15
Kidney beans, raw	A	25	0.343	0.268		
	B		0.306	0.276	0.12	15
Kidney beans, cooke	A	73	0.067	0.026		
	B		0.07	0.026	0.11	35
Sugar beans, raw	A	8	0.252	0.201		
	B		0.243	0.207	0.09	18
Sugar beans, cooke	A	69	0.252	0.201		
	B		0.243	0.207	0.07	16
Cow peas, raw	A	6	0.606	0.431		
	B		0.61	0.408	0.16	20
Cow peas, cooked	A	75	0.088	0.046		
	B		0.086	0.048	0.09	26
Chick peas, raw	A		0.434	0.355		
	B		0.451	0.355	0.28	15
Chick peas, roasted	A		0.164	0.028		
	B		0.168	0.027	0.14	56
Soy beans, raw	A		0.242	0.185		
	B		0.293	0.182	0.19	25
Soy beans, roasted	A		0.153	0.042		
	B		0.142	0.045	0.14	49
Green peas, raw	A		0.031	0.031		
	B		0.034	0.032	0.001	1.5
Nuts						
Peanuts	A		0.2	0.129		
	B		0.199	0.126	0.17	15
Vegetables						
Cow pea leaves	A	84	0.0074	0.0034		
cooked	B		0.0066	0.0038	0.01	10
Pumpkin leaves	A	85	0.064	0.036		

Table a.2 (Continued)

cooked	B		0.066	0.034	0.03	30
Pumpkin , cooked	A	82	0.128	0.073		
	B		0.126	0.071	0.11	18
Roots						
Sweet potato, raw	A	72	0.072	0.055		
	B		0.071	0.054	0.01	5
Sweet potato, cooke	A	71	0.085	0.059		
	B		0.082	0.059	0.01	5
Unfermented cassav	A		0.132	0.082		
	B		0.137	0.084	0.05	26
Fermented cassava	A		0.017	0.014		
	B		0.017	0.014	0	13
Weaning foods						
Roasted maize-soy	A		0.187	0.142		
	B		0.203	0.147	0.12	32
Extruded maize-soy	A		0.476	0.444		
	B		0.528	0.438	0.03	5