NUTRIENT AND ANTINUTRIENT CONTENT OF AN UNDEREXPLOITED MALAWIAN WATER TUBER NYMPHAEA PETERSIANA (NYIKA)

By

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(ABSTRACT)

Nymphaea petersiana Klotzsch (Nyika*)* is an important wild tuber eaten in some districts of Malawi. The tubers were processed by boiling/freeze-drying(BFD) and sun-drying(USD).The tuber's nutrient and antinutrient composition was determined to produce a preliminary nutrient data base for use in sub-Saharan Africa.

There was no significant difference ($P > 0.05$) in protein content of BFDand USD samples. Sun-dried samples were significantly ($P < .05$) higher in ash than boiled samples while boiled samples were significantly higher ($P < .05$) in crude fat and total carbohydrate. The protein content of the tubers (8.0 and 8.1 %) was higher than that of the staple maize (7.9%), African millets (unspecified) (7.5%), and polished rice (7.0%), but lower than sorghum (10.7%). Protein content was higher than tubers like cassava (1.3%), potato (2%), sweet potato (1.6%), yams (1.5%) and *N. lotus* (5.2). Nyika tubers have a well balanced amino acid content, limiting only in lysine.

There were no significant ($P > 0.05$) differences in the mineral content of BFD and USD samples, except for iron, which was lower in the boiled samples. Nyika tubers have a higher calcium (1376 and 946 μg/g) and phosphorus (2250 and 2883 μg/g) content than wild and domesticated cassava, potatoes, sweet potatoes and wild and domesticated yams. Sun-dried tubers have a higher iron content (88μg/g) than maize (20μg/g). The zinc content of tuber was higher (21and 25 μg/g) than that of boiled maize flour, boiled sorghum flour, rice, cassava, and sweet potato. The predominant fatty acids

in the tubers were oleic (47%), linoleic (32%), palmitic (21%) and linolenic (7%) acids. Ascorbic acid content was very low, only 0.1 and 0.003 mg/100g.

Tannin content was lower (1 and 1.7 %) in the tubers than in *Vulgare Pers*. sorghum, DeKalb sorghum from U.S. and Kabale sorghum from Uganda. There was a significantly ($P < 0.05$) lower content of phytate in boiled (3.9 μg/g) than in sun-dried tubers (6.0 μg/g). Phytate content of the tubers was lower than that of cooked maize flour, unrefined maize flour, cassava and sweet potato. Trypsin inhibitor activity in the tubers was reduced from 463 to 55 TIU/g tuber and chymotrypsin inhibitor activity was reduced to 50 from 267 CIU/g tuber by cooking.

Nyika is a good source of iron and quality protein limiting only in lysine. Protein is comparable to staple maize and higher than root crops consumed in Malawi. It is not a good source of fat and ascorbic acid. Tannin, phytate, trypsin, and chymotrypsin inhibitor content lower than most food crops consumed in Malawi.

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CHAPTER 1
INTRODUCTION

Nymphaea petersiana (Nyika)

Nymphaea petersiana Klotzsch, a waterlily, locally known as *Nyika*, is a wild root tuber (Figs. 1.1 & 1.2) belonging to the family Nymphaeaceae. *Nyika* is an important water tuber in the southern part of Malawi especially in the Lower Shire Valley region. It grows in ponds and along the vast swamps of the Shire river. *Nyika* tubers are collected from waterways by wading into the shallow waters but mostly by using canoes (Figs. 1.3 & 1.4). These tubers are especially important as a food reserve during times of famine or poor harvest, especially in the districts of Chikwawa and Nsanje in the southern region of Malawi. Whole cooked tubers are commonly sold in markets as snack foods. *Nymphaea petersiana* tubers can be peeled, cut in small pieces, dried and ground into powder. The powder is then used for cooking into a thick porridge (locally called nsima), which can be served with different types of stews and/or vegetables.

Research on root crops in Malawi has only been conducted on the domesticated root tubers like cassava. Very little, if any research, has been done on edible wild root tubers. To date, there is, to our knowledge, no available data on the food value, (nutrient and antinutrient composition) of these tubers.

The major objective of this study, therefore, was to determine the content of selected nutrients and antinutrients in *Nymphaea petersiana* tubers. Data from this study will be used to build a nutrient data base for the tuber so that its nutrient/ antinutrient content can be compared to the domesticated root tubers and cereals currently consumed in Malawi. Such data can be utilized by the relevant governmental and non-governmental organizations concerned with the nutritional status of the Malawian population, especially the most vulnerable groups - lactating women and children and the disadvantaged poor living in the rural areas.

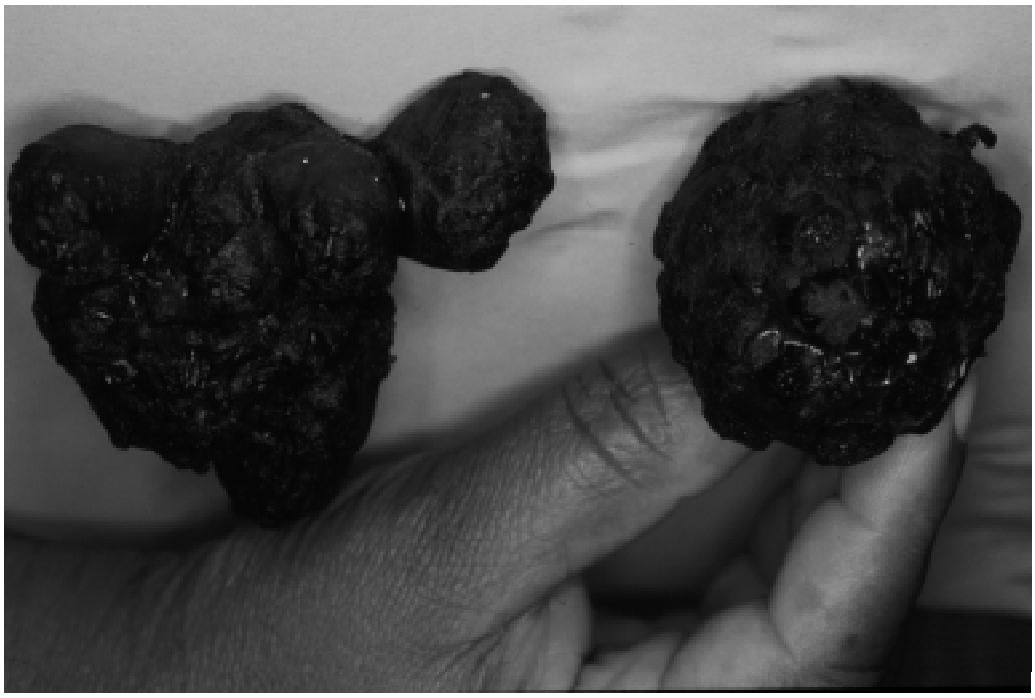


Fig.1.1 *Nymphaea petersiana* tubers

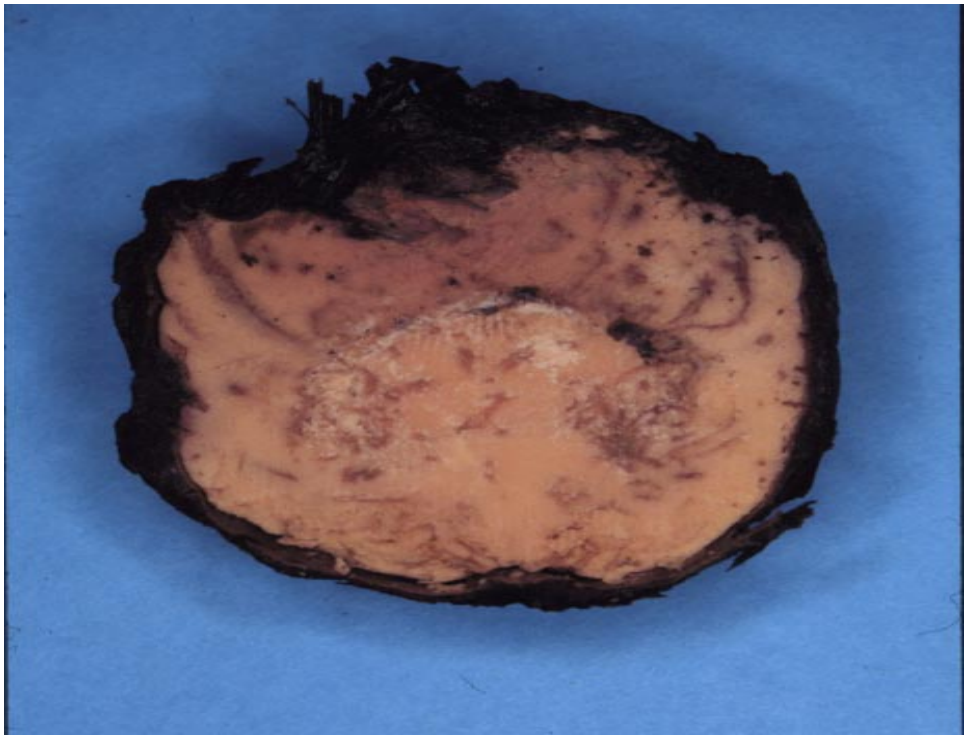


Fig. 1.2 *Nymphaea petersiana* tuber cut open

Fig. 1.3 Negotiating for canoe hire with villagers in Chikwawa District

Fig. 1.4 Local man wading out of swamp with Nyika plant in hands - Nsanje District

CHAPTER 2

PURPOSE AND OBJECTIVES

Purpose

Although *Nymphaea petersiana* tubers are widely consumed in some districts of the southern part of Malawi, there is, to our knowledge, no available data concerning the nutrient and / or antinutirent composition of these tubers. The purpose of this study was to determine the tuber's content of some selected nutrient and antinutrients, in order to establish a nutrient data base for the tuber and hopefully lay the foundation for nutrient data base for other edible wild tubers, as well as other wild foods eaten in Malawi and sub-Saharan Africa. The other purpose of this study was to determine the nutrient and antinutrient content of this tuber to help assess how the tubers might be best utilised in the Malawian diet as compared to other Malawian root tubers and cereals which are the staples in the country.

Objectives

1. To determine if *Nymphaea petersiana* tubers contain adequate quantities of selected macro and micro-nutrients to be considered a good source of each of these nutrients
2. To determine if the nutritional composition of *Nymphaea petersiana* is comparable to that of the Malawian staple - maize, and other cereals eaten in Malawi like rice, finger millet, sorghum as well as domesticated tubers like cassava, yam, potato, and sweet potato.
3. To determine if there are any antinutritional factors in *Nymphaea petersiana* tubers.

Null Hypotheses

Since the tubers are eaten either cooked or as sun-dried flour, the following null hypotheses were tested:

1. There is no significant difference in nutritional composition of sun-dried and boiled tubers of *Nymphaea petersiana.*
2. There is no significant difference in antinutritional factors found in sun-dried

and boiled tubers of *Nymphaea petersiana.*

3. There are no significant differences in the nutrient and antinutrient content of *Nymphaea petersiana* tubers and other Malawian foods such as maize, rice, sorghum, finger millet, cassava, potato and sweet potato.

CHAPTER 3

REVIEW OF LITERATURE

Malawi -Demography

Malawi is a country in South-East Africa, occupying the lower end of the Great Rift Valley of Africa. The country is 520 miles (837 km) from north to south and varies in width from 5 to 100 miles (8 to 160 km) (Fig. 3.1). The land surface area is 45,747 square miles (118,484 square kilometres), 20% of which is covered by Lake Malawi (11430 square miles; 29,604 square km). Lake Malawi is one of the largest and deepest lakes in the world, and the third largest lake in Africa (Britannica World Data, 1995). The lower end of the lake opens into the Shire River, Lake Malawi's only outlet and the country's principal river. The climate is for the most part sub-tropical without violent extremes of heat and cold, although the temperature at the lake-shore may rise to over 100°F, and in the Lower Shire Valley up to 115°F, in the hot season. The rainy season is from November to March during which the rainfall may vary from 30 to 70 inches according to the area (Berry and Petty, 1992).

The population of Malawi (1997) is about 10 million and only about 10% of this population lives in urban areas, although a rural-to-urban trend in internal migration continues. Malawi has a population growth rate of 3.1% per annum and according to the World Bank, this means that the population will double in the next twenty years. In fact, Malawi's fertility rate of 6.7% is one of the highest in the world (Ministry of Economic Planning and Development (MPED) et al., 1996). Malawi's land mass is small and as a result Malawi has one of the highest population densities in Africa of 264.0 persons per square mile (101.9/ sq km) in 1997 (Britannica World Data, 1998). This creates tremendous pressure on the land, with over one-half of rural households farming less than one hectare, while one-quarter cultivate less than a half-hectare (MPED et al., 1996).

Almost half of the population (46%) is under 15 years of age. The birth rate (1996) per 1,000 is 42.6 as compared to the world average of 25.0. The death rate per 1000 (1996) is 24.5 as compared to the world average of 9.3. The life expectancy at birth (1996) is 35.9 years for males and 36.5 years for females (Britannica World Data, 1998). A 1992 demographic and

health survey (DHS) indicated a very high infant mortality rate of 134 per 1000 live births, while the under-five mortality rate currently stands at 234 deaths per 1000 live births (National Statistical Office (NSO), 1994). This means that nearly one in seven children dies before his/her first birthday, and nearly one in four children does not reach his/her fifth birthday. The gross national product (GNP) per capita of U.S. $170 which is among the lowest in the world. The daily per capita caloric intake is 2038, 88 % of FAO recommended minimum requirement (Britannica World Data, 1998). Twenty seven per cent (27%) of the children are underweight and close to half (49%) are stunted (World Bank Data,1990) (Fig. 3.2).

According to the Human Development Index (HDI) that ranks countries based on life expectancy, education and real per capita income, Malawi is 48% below the poverty line, and the third from the bottom in the Southern African Development Community (SADC) region (Table 3.1), the last two being Mozambique and Angola (Human Development Report, 1995).

Table 3.1 Human Development Index For SADC Countries

Country	Population in millions	Per-capita income in US$	HDI
Angola	13	410	0.150
Botswana	2	2,800	0.524
Lesotho	3	400	0.432
Malawi	11	160	0.179
Mozambique	20	100	0.155
Namibia	2	2,030	0.440
South Africa	46	3,010	0.766
Swaziland	1	810	0.462
Zambia	11	350	0.351
Zimbabwe	13	590	0.413

Source: Human Development Report, 1995

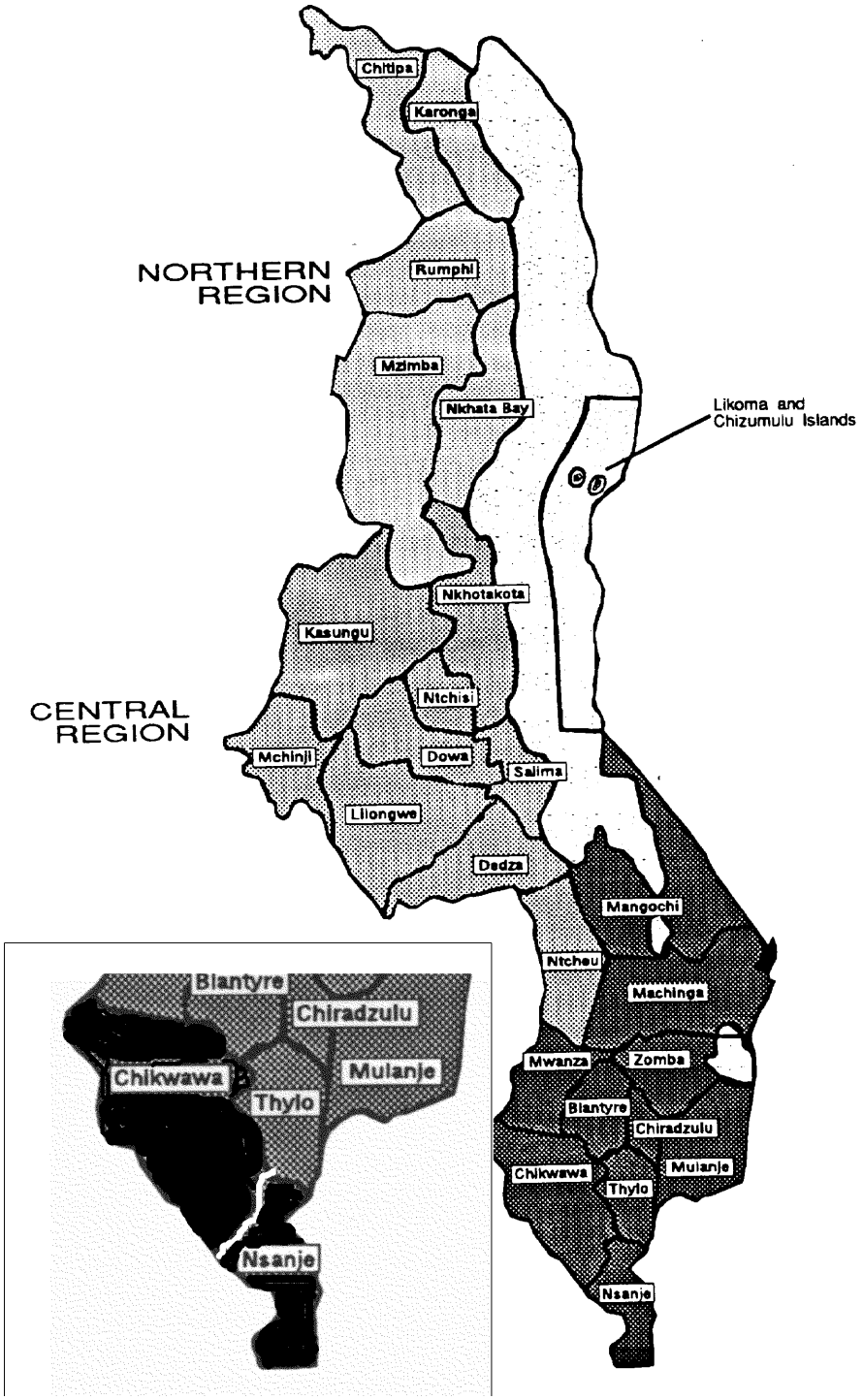


Fig. 3.1 Map of Malawi with inset showing Chikwawa and Nsanje districts - Site of study

Source: Ministry of Economic Planning and Development (MEPD) et al., (1996)

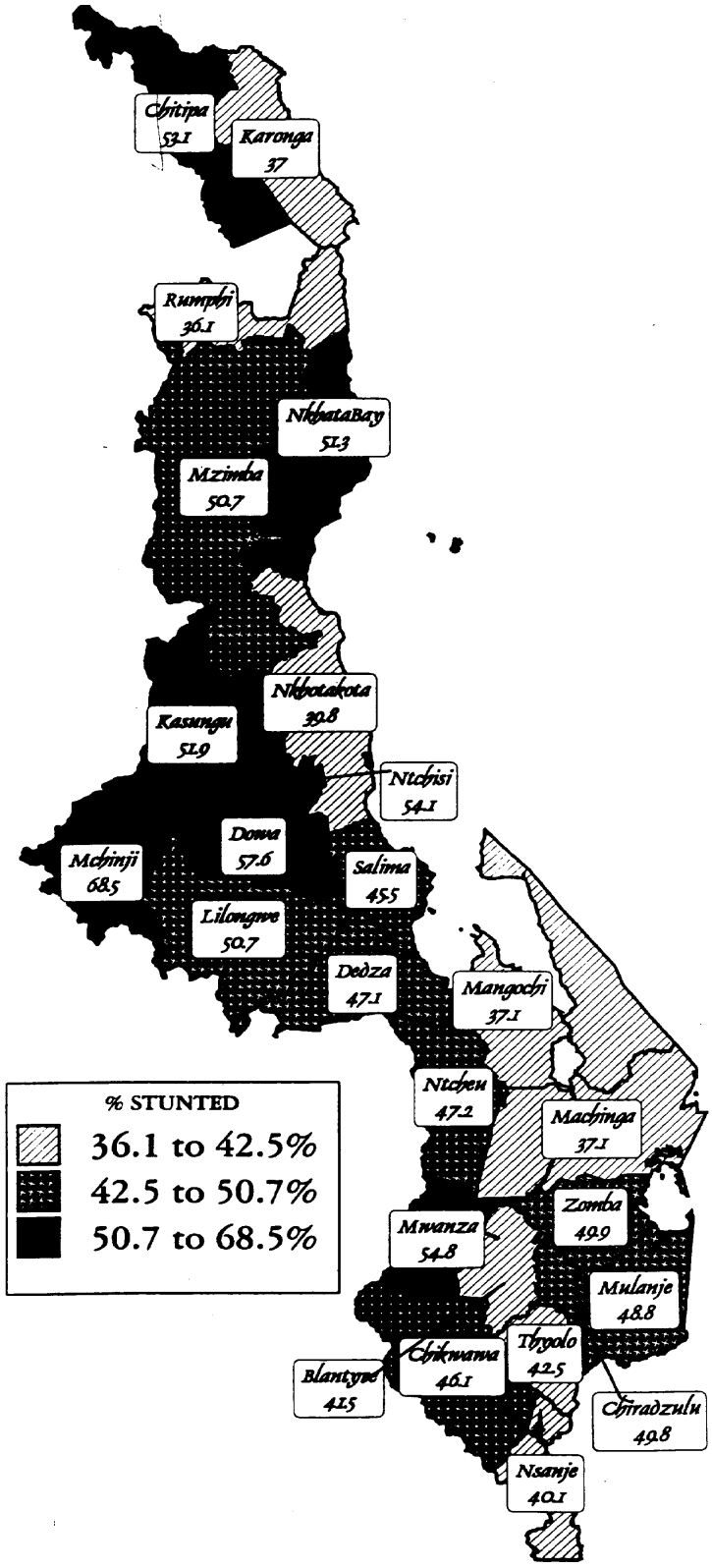


Fig. 3.2 Map of Malawi showing prevalence of stunting in children Source: (MEPD et al., 1996)

Nutritional Status Of the Malawian Population

The first comprehensive national nutritional survey (The Nyasaland survey) was carried out in the then colonial Nyasaland (now independent Malawi) between 1938-1943 and the survey results were not published until 1992. The work was carried out by a multi-disciplinary team. Included in the study were household dietary studies, a clinical survey, chemical analyses of foods and agricultural and anthropological surveys. It was not until 1980/81 that the Malawi government undertook a National Sample Survey of Agriculture (NSSA) which, for the first time, included a nutritional module. The results of the survey, which were released in 1984, showed that undernutrition was a widespread problem among the Malawian under-five population with stunting among 56% of the children (Fig. 3.2). The same study also showed that 12 % of the lactating mothers had chronic malnutition and that 42 % of rural mothers were more likely to have chronic malnutrition than urban mothers. Prior to the release of the NSSA data, the Centre for Social Research undertook a nutrition survey in six communities in Malawi, and the Ministry of Health also carried out a clinic-based survey to ascertain the nutritional status of under-fives. All of these surveys showed that malnutrition was a chronic and growing problem in Malawi (Berry and Petty, 1992).

Due to the low level of farm technology, inadequate irrigation, and a shortage of cash and credit to buy farm inputs such as seed and fertilizer, farms with between one-half and one hectare can produce only 40-70% of their staple food requirements, and by June (only a few months after harvest) most rural people are reduced to eating two meals a day (MPED et al., 1996). In some districts 70-80% of households run out of food by December (8 months after harvest). As a result, in 1992 and still in 1995, over a quarter of Malawi's under-5's were underweight, and more than one-half were stunted by long-term malnutrition. In 1990 the World Bank estimated that over one-half of the population lived below the poverty line, estimated at an annual income equivalent of US $40/adult (MPED et al., 1996).

Nutrient Intake of Malawian Children

Adequate intake of energy and essential nutrients are necessary for optimal growth, development, and health in children. In rural Malawi, children often have low intakes of energy, protein, vitamin A, thiamin, riboflavin, iodine, iron and zinc, some of which are attributed to

high intakes of cereals and/or starchy staples, and low intake of animal products (Ferguson et al., 1993a). Plant-based diets generally have a protein content which is inferior in terms of density, digestibility, and amino acid pattern compared to that of animal products. They also have high levels of dietary fibre and phytate which inhibit the absorption of certain minerals.

Results from a study done by Ferguson et al (1993b , 1995), conducted at two seasons of the year (during harvest and preharvest season), on rural children in southern Malawi showed that cereals were the major source of all the nutrients investigated, except for calcium which was provided primarily by animal products. Legumes were an important secondary source of protein, iron and zinc. Fruits and vegetables were also important secondary sources of iron and calcium. From this study it was found that rural children in the south of Malawi were at risk for suboptimal zinc and iron nutriture because of the greater consumption of unrefined cereals high in phytic acid. The bioavailability of zinc in the diets is compromised by high levels of phytic acid relative to zinc intake (Ferguson et al., 1993b).

Ferguson and colleagues (1993b), recommended nutrition intervention strategies for combating micronutrient deficiencies and strongly recommended that one of these strategies be the incorporation of indigenous foods into the diet. This would be the best strategy and the least costly. In general, Malawian cereals (boiled green maize, maize flour, sorghum flour, and rice) are low in calcium, zinc and manganese. The high calcium content of the accompanying side dishes (i.e. fish and/or leaves), compensated however, in part, for the low levels in the dietary staples. To date, there are limited comprehensive data on the dietary patterns of rural Malawian children.

Distribution of kwashiorkor in the southern region of Malawi

Kwashiorkor, means ‘disease of the displaced child. Some of the factors suggested as contributing to the disease are: dietary imbalance, infections and the psychological trauma associated with abrupt cessation of breast-feeding. A study conducted in the southern region of Malawi (Courtright and Canner, 1995), indicated an overall district-adjusted prevalence of kwashiorkor of 18/1000, which was equally divided between boys (1.03%) and girls (1.17%). Prevalence peaked at 18-23 months. Some of the causes of kwashiorkor cited in the study were: abrupt cessation of breast-feeding during weaning and substitution with a most common weaning food of a thin maize flour porridge (phala) whose protein composition was only 0.9/100g. Early

supplementation with thin *phala* is very common in Malawi and few children are exclusively breastfed. Abrupt cessation of breast-feeding with the next pregnancy is also common in the region (Courtright and Canner, 1995). Prevention of kwashiorkor in young children is currently based on recommendations to improve diets in pregnacy and early childhood. One way to achieve this would be to replace the thin maize porridge with a food that is high in protein. The exploitation of indigenous wild plants could help alleviate some of this high prevalence of kwashiorkor.

Nutrition And Poverty In Malawi

Unlike other Southern African countries, Malawi has no mineral wealth and its economy is based solely on agriculture, which accounts for about one-fourth of the gross domestic product (GDP) (Britannica World Data, 1997). The World Bank identifies two categories of poverty in Malawi - the poor and the 'core poor'. Since the poverty line is based on minimum nutrition requirements from food - estimated at 200 kg/year of the staple maize for an adult, costing roughly $40 per capita per annum - 55 per cent of the Malawi population can then be categorized as poor, including the 'core poor' who total 20 per cent. In the absence of income data about smallholder farmers, poverty is estimated on the basis of the size of their agricultural plots. The 'core' poor farm less than 0.5 hectares, while other poor households defined as those with 0.5 - 1 hectare. Thus according to the nutrition-based poverty lines, about 51 percent of all households and about 55 percent of the population are poor, while about 20 percent form the 'core poor', implying that they attain at least one-third less than the minimum recommended nutritional intake. Equally significant, female-headed households are over-represented among the poor and the 'core poor' (World Bank Data,1990).

Corn (maize) is the chief staple and is typically grown with peanuts (groundnuts), beans, and peas. Cassava is the most widely grown root crop and it is eaten as a staple food at the lake shore areas. Rice is included amongst the staples although almost everywhere it is regarded as a luxury food. Sweet potatoes are also consumed as a subsidiary food. They are eaten more often at one season (harvest sason) in the rural areas, whereas owing to better provision for storage, they are consumed all the year round in the urban areas. Potatoes and yams are also eaten frequently in both the rural and urban areas. Tobacco, tea, sugar-cane and cotton are the principal cash crops (Berry and Petty, 1992).

The population of Malawi, had for a very long time been supported by the plentiful fish from Lake Malawi with its large supply of protein and by a rich soil that resulted in high agricultural yields of maize (the staple food). Lately, however, over-population, deforestation, crumbling economies due to the devaluation of the currency and the structural adjustment reforms brought on by the World Bank and the International Monetary Fund (IMF), negligence of the more adaptable indigenous food crops, and changing weather patterns, have resulted in food shortages with devastating nutritional consequences. The onset of the AIDS epidemic and the resultant high rates of orphans in the country has confounded the problem even further and drammatically increased the number of children suffering from malnutrition, particularly in the poor rural areas (World Bank Report, 1990). Greater exploitation of wild indigenous foods and promotion of their use would help alleviate some of the food shortage and malnutrition currently being experienced in many parts of the country.

Food And Diet

The foundation of Malawian diet is white maize. In the rural areas the maize is pounded into a flour called '*ufa woyera'*. The main steps in the process of preparing *ufa woyera* is as follows: The grain is made wet and is pounded with a pole in a wooden mortar. This removes the skin (pericarp), the germ and also some soft endosperm, and these soft tissues are winnowed off as bran or *madeya* from the remaining endosperm which is known as *mphale*. The *mphale* is then soaked for several days and then it is pounded (or ground in a maize-mill) into a fine white flour called *ufa woyera*. *Ufa woyera* when boiled with water makes the stiff porridge called *nsima* which is the staple food of the country. Where there are alternative staple foods grown, such as cassava, finger millet or sorghum, it is common practice to use a mixture of flours for *nsima.* For example, in districts where adequate amounts of cassava are grown, cassava flour is mixed with maize flour in proportions of about one in three. In other areas finger millet or sorghum flour are mixed with maize flour. In the Lower Shire Valley where *Nyika* is eaten, the *Nyika* flour can be mixed with the maize flour.

The *nsima* is always eaten with a side dish called *ndiwo*, made from vegetables, legumes, fish or meat. While emphasis is put on the importance of *nsima* as the main dish, the importance of the side dish is shown by the fact that the word for hunger (*njala*) could also be applied not only when there is no maize flour to cook the main dish, but also when there are no side dishes

obtainable to eat with the *nsima* (Betty and Perry, 1992). Most of the time the side dishes are composed of legumes and vegetables. The vegetable side -dishes prepared from green leafy vegetables can be made from the leaves of sweet potato, haricot bean, cowpea, garden pea, and cassava leaves, or from numerous varieties of wild edible leaves collectively termed *masamba*. Such green leafy vegetables are usually boiled for about 20 minutes, and tomatoes and pounded groundnuts (if available) are added to the *ndiwo*. Legumes include beans, cowpeas, peas and groundnuts.

One or two meals are eaten in the course of the day. *Nsima* is eaten at each of those times, while some side dishes such as leafy vegetables and legumes maybe eaten at each of the two meals. Some side dishes such as fish or meat are only eaten at one of the meals because of their scarcity. Meat, especially beef is a prestige food in the rural areas and is often served during ceremonies or festivals. The meat is cooked by either boiling or roasting. Meat or animal products therefore, contribute only a very small percentage to the protein intake in the rural areas. One of the observations made by Berry and Petty (1992) in their research was that the urban areas had a greater variety of foods and they are eaten on a greater number of days than in the rural areas, and this is due to the fact that most people in the urban areas have money to spend on food and have storage facilities for such commodities as fresh fish and meat than those in the rural areas. By contrast food when it becomes available in the rural areas is eaten in quantity for a season only.

Numerous insects, such as some types of caterpillars and termites, are enjoyed seasonally and contibute to the protein intake of the population. If abundant, specimens may be dried for later use. Grasshoppers and locusts are also enjoyed seasonally. Snack foods such as green maize, sugarcane, roasted cassava, roasted sweet potatoes and roasted groundnuts are eaten in between meals.

Mineral Nutriture Derived From Commonly Consumed Malawian Foods

In view of the paucity of reliable food composition data on the mineral content of locally grown and prepared Malawian foods, a study was done by Ferguson and others in 1989 in the southern region of Malawi. These data were collected as part of a larger investigation of the nutritional status of rural Malawian children. Ferguson et al., (1989) analysed 44 raw and cooked Malawian foods for zinc, copper, manganese, calcium, phosphorus, magnesium, sodium, and

potassium content expressed in milligrams per 100 g edible portion. The results indicated that the more refined dietary staple (65 % extraction maize flour) was generally low in most of the minerals analyzed, whereas the less refined Malawian cereals had relatively high levels of zinc and phosphorus.

Zinc and Iron

Zinc is an essential trace element in the diets of humans for optimal health and growth. Signs and symptoms of mild zinc deficiency in young children include impaired linear growth, poor weight gain, reduced deposition of lean body tissue, anorexia, hypogeusia and impaired immunocompetence (Ferguson et al.,1993a).

Widespread suboptimal zinc nutriture constitutes a notable health risk for children in terms of growth and development. Zinc adequacy depends on both the amount and bioavailability in the diet in relation to physiological requirements. Certain dietary components, such as phytic acid, dietary fiber, calcium, and protein also affect zinc absorption. Of these, phytic acid has the greatest inhibitory effect on zinc absorption. Ultimately, the amount of zinc absorbed depends on the complex interactions among these dietary constituents, as well as on the zinc status and health of the individual. Rural African children consuming cereal-based diets high in phytic acid and low in animal products are likely to be particularly vulnerable to suboptimal zinc nutriture (Ferguson et al., 1993b).

Phytate, Zinc And Calcium

The paucity of food composition data on phytic acid contents of African foods is unfortunate, since the habitual diets in many African countries are high in cereals. Consequently, calcium, phytate and zinc contents of 30 representative raw and cooked foods consumed by 4 -6-year old children living in rural Malawi were analyzed and the corresponding Zn: phytate, Ca:phytate, and (Ca:phytate/Zn) molar ratios were calculated (Ferguson et al., 1988). The phytate content of foods in the legume and cereal groups was markedly higher than those of vegetables, roots and fruits. Phytic acid contents expressed on a freshweight basis ranged from 166- 1297 mg/100g for legumes; 211-1089 mg/100g for cereals; 4-97 mg/100g for leaves; 10-59 mg/100g for roots and 11-25 mg/100g for fruits (Ferguson et al., 1988). All food groups showed marked variability in phytic acid content. Groundnuts, fresh kidney beans, cowpeas, and maize

bran had the highest content (i.e. greater than 1000 mg/100 g dry wt). In contrast, bananas, avocados, sweet potatoes, and chinese cabbage had the lowest phytate content.

Phytate content of food varies considerably, depending upon environment, maturation and processing procedures. Results showed that cooking had little or no effect on the phytate content of maize flour (i.e. 219 mg/100g for cooked maize flour vs 234 mg/100 g for raw maize flour). This was in agreement with other investigators who had found no difference in the phytic acid content of cooked compared to raw cereals (Ferguson et al., 1993b). Conversely, the more refined maize flour (*white ufa*) analyzed in the study had lower phytate and zinc contents compared to the less refined maize flour (*mgaiwa*). This was not unexpected, as the zinc and phytate fractions of maize are concentrated in the germ, which is removed by milling. Furthermore, the processing of white *ufa*, but not *mgaiwa,* also involves the 1-to-3-day fermentation stage. It is not clear to what extent this fermentation increases the hydrolysis of phytic acid. Results also indicated that the Malawian milling and fermentation practices decrease the phytate:Zn molar ratio and hence have important implications in terms of the bioavailability of zinc in the Malawian diet. The analyzed Ca:phytate ratios of cooked Malawian cereal flours were all below the critical molar ratio of 6:1 (Ferguson, 1988). In the Malawian diet, however, a leaf, legume or fish relish is always consumed with the cooked cereal flours. Such relishes, with the exception of legumes, are high in calcium (i.e. pumpkin leaf, chinese cabbage, and amaranthus sp.). Hence, the calcium content of the relishes in these diets may be high enough to promote a phytate-induced decrease in zinc bioavailability.

Selenium

Studies of dietary selenium intakes of children in less industrialized countries are limited, in part because of the absence of data on the selenium content of locally grown foods. Children in less industrialized countries tend to consume a restricted number of locally grown food items. Consequently, if the soil is deficient in selenium, and/or if the trace element content of foods is reduced by preparation and processing techniques, the selenium nutriture of the children may be compromised. Preliminary evidence in experimental animals indicates that selenium may play a role in both cell-mediated and humoral immunity. Consequently, the existence of low selenium status in children in areas of the world where the prevalence of infectious diseases is high, may be particularly significant.

Selenium intakes of groups of children living in rural Malawi who consume diets based on cereals and starchy staples, was determined by Donovan and colleagues in (1991). Selenium intakes were calculated from dietary intake data of all the staple foods consumed. Seasonal variations in the production, processing and consumption of cetain foods in Malawi resulted in seasonal differences in the dietary intakes and major sources of selenium for the children. Cereal products were the major source of selenium in all three seasons, contributing 35 - 60 % of dietary selenium. Of the cereals, maize is the main staple, but sorghum, millet, and cassava flours can also be used for *nsima*, depending on the season and agricultural labour demands. For example, percentages of selenium from grain products increased from the harvest to the postharvest period (35% v.s. 60%) because of a rise in the consumption of sorghum flour with a higher selenium content. The overall results showed that the average dietary intakes of selenium for the Malawian children consuming plant-based diets was well below the average selenium intakes of children in some industrialized countries, such as the U.S.A. and Australia, who consume omnivorous diets (Donovan, et al., 1991).

Use Of Indigenous Food crops

Although modern agriculture and the food supply of industrial societies is based on a handful of plant species, traditional agriculturalists, pastoralists and hunters/gatherers in most African countries use a myriad of plants for food, medicine, construction, etc. Central to such practices is the exploitation of wild food resources. However, dietary utilization of non-domesticated plants has received very little attention in economic development efforts. Paralleling this omission has been the revelation that despite increased food production in some sectors, there has been a drastic narrowing of the food base in many traditional societies. By focusing on a limited number of cultivars of a few staple food crops, a vulnerable position is created, not only because diversification assures dietary balance and facilitates intake of micro-nutrients but through danger of domesticated crop destruction by drought or insect pests (Ogle and grivetti, 1985). Although indigenous crops may not be as high yielding as crops of global importance, they can provide stable production under adverse ecological conditions, such as high aridity. Alternatively, they may be harvestable during seasons when other foods are scarce (Johns, 1994). Finger millet (*Eleusine coracana)*, for example, suffers relatively few diseases and

pests, is tolerant to soil moisture stress and has excellent storage qualities (Barbeau and Hilu, 1993). It also has a growth cycle of only three months and thus can provide two harvests a year.

Although nutrient composition of most indigenous crops has not been analyzed, these species may be rich sources of vitamins, minerals or amino acids that complement other components of the diet. (FAO, 1983). The nutrient data on those species that have been analyzed, reveal that they are often comparable, superior in some instances, to most staple foods. For example, finger millet has been found to be a good nutrient source with relatively high calcium content (Barbeau and Hilu, 1993). Carr (1958), reported high calcium and phosphorus values for *Adenia gummifera, Amaranthus gummiferria, Amaranthus thunbergii, Bidens pilosa, Corchorus tridens and Gynandropsis gynandra.* He also identified fruits of *Adansonia digitata* as exceptionally high in ascorbic acid with values among the highest for any African plant, wild or domesticated. Some indigenous leafy vegetables have been found to be particularly valuable sources of provitamin A, vitamin C, folate, iron and protein. Examples include *Amaranthus caudutus, Amaranthus gracilis, Amaranthus thunbergii, Bidens pilosa, Corchorus tridens, Momordica balasania and Gynandropsis gynandra* which provide a complement to diets high in carbohydrate (Akhtar, 1987). It has also been found that an increased incidence of chronic diseases, such as diabetes, is experienced by many indigenous people as they adopt western diets and lifestyles. Plant foods in traditional diets are higher in fibre than western diets and the carbohydrates they contain are digested more slowly (Thorburn et al., 1987). Hence, dietary incorporation or maintenance of indigenous food crops could be highly advantageous to marginal populations or to specific vulnerable groups within populations.

In addition, researchers have, during the last 20 years, documented declining interest among younger people for traditional food sources. This change has led to substantial losses in knowledge about edible wild resources. Rapid economic and technological development may further contribute to a decline in the customery use of indigenous dietary resources. When this occurs, lost is both the knowledge and skills of recognition and identification of climatically adapted food resources that have previously sustained societies (Ogle and Grivetti, 1985a).

Background Information On Wild Foods

Knowledge of wild gathered foods has been passed on from previous generations. In different regions of the world, there is a great variation in species, in preparations and use of such

foods. They can be used during the whole year, but more often they are used during a time of scarcity. They are important during periods of famine. Although there seems to be an increase in the interest for, and understanding of wild gathered foods as an important resource, including as food, the potential nutritive values of most of these foods have not been analyzed systematically, and it is difficult to find literature and food composition tables which include gathered wild foods (Nordeide et al., 1994).

The first comprehensive composition tables of Australian Aboriginal foods have been reported by Miller et al. (1993). The information in Miller's book is not only used by Aborigines themselves but by a variety of professionals like dietitians, school teachers, anthropologists, epidemiologists and agricultural scientists (Nordeide et al., 1994).

Review of the literature reveals that there is some documentation on the use of wild foods in Africa. Grivetti et al. (1987) gives a systematic assessment of published literature on the dietary use of wild plants in many African societies. Others who have done similar work include Story (1958), Murdoch (1960), Clark (1960; 1962; 1968, Allan (1965, Metz et al. (1971), Lee (1973; 1979), and Darby et al. (1977). From West Africa, Diarra (1977) lists more than 40 edible plants used in different seasons near Bamako in Mali. Glew et al., (1997) in their study of 24 indigenous plants of Burkina Faso found out that these plants were good sources of protein (some three plants had as high as 20 to 37 % protein), essential amino acids, essential fatty acids such as linoleic and linolenic acids and micronutrients such as iron and zinc. A study done by Kim and colleagues (1997) in the Republic of Niger on the seeds of *Boscia senegalensis*, which is used a famine food, revealed that these seeds contain substantial quantities of arginine, tryptophan and essential fatty acids such as linoleic acid, as well as zinc and iron. Ogle and Grivetti (1985a,b) in their articles on wild gathered foods, discussed the dietary utilization of edible wild plants in the kingdom of Swaziland, southern Africa. Salih et al. (1992) compared wild grasses with local staple cereals in an area of Western Sudan. They found that wild grains of *C. biflorus* were particularly high in protein concentration and the locally grown sorghum had unusually high lysine levels. Becker (1983; 1986) studied the contribution of wild plants to human nutrition in Northern Senegal and the Sahelian Zone. Among the plants growing in Northern Senegal, 50% have valuable edible parts. They also found out that these plants were important sources of vitamins A, C and riboflavin.

Despite all the documentation on the identity, distribution and uses of edible wild foods in Africa in general, and in Malawi in particular, there is a paucity of information on their chemical composition. Most of the work on wild foods documented from Malawi has been on edible wild fruits (Jardin, 1967; Williamson, 1975; FAO, 1983; Saka et al., 1988; Campbell, 1987; Kalenga- Saka et al., 1992). Williamson (1975), in her listing of 'useful plants of Malawi' does include root tubers. *Nymphaea caerulia* is listed as being edible in Nkhota-kota district (in the central region of the country). There is, however, no mention of *Nymphaea petersiana*. In general, there is little, if any information on the wild root tuber *Nymphaea petersiana,* which is eaten widely in the Lower Shire Valley in the southern region of the country. This study therefore, was undertaken to obtain basic data on the chemical composition for this wild tuber *Nymphaea petersiana* and to begin the process of compiling data on the chemical composition of wild root tubers of Malawi.

Tropical Root Crops

Tropical Root crops are important staple foods for about a third of the world's population. Most of these people live in the less developed countries of the tropics and subtropics, where poverty, malnutrition and hunger are day-to-day problems. Three of the world's leading producers and consumers of tropical root crops are China, Nigeria, and Brazil. The annual, global per capita consumption is about 70 kg. The main domesticated tropical root crops are cassava (*Manihot esculenta*) sweet potatoes (*Ipomea batatas*), potatoes (*Solanum tuberosum*), yams *(Dioscorea spp.* and taro (*Colocasia esculenta*). At the world level potatoes have the largest cultivated area of all tropical root crops. Among the other root crops listed, the most important in terms of cultivated area is cassava (55.4 %), followed by sweet potatoes (30.3%), while yams (9.9%) and taro (4.4%) are far less important (Chandra, 1986).

Tropical root crops are renowned for high outputs of food energy or kilojoules (kJ) per unit area of land. The food energy of tropical root crops in kJ per Kg of edible portion varies from about 2550 for giant taro to 5650 for cassava. The crude protein content of tropical root crops is low compared to grain crops ranging in g per Kg from 6 for giant taro to 22 for taro. Nevertheless, the purpose of tropical root crops in the diets of peoples where they are consumed as staples is to supply the food energy or carbohydrates, not the protein. The protein is often contributed by grains, legumes, and animal-based products in the diet. On the whole the tropical

root crops have adequate amounts of minerals and vitamins. Taro and cassava leaves are particularly high in crude protein, carotene and vitamins (Chandra, 1986).

Species of Nymphaea

All species of *Nymphaea* are perennial herbs. They are rooted in what is always a rich organic substrate at the bottom of ponds or sluggish streams, with leaves and flowers elevated to the water surface on elongate petioles and peduncles. A single mature plant may occupy an area from half a meter in diameter in smaller species up to three meters or more in larger species. Flowers are floating or may be emergent as much as 40 cm on stout peduncles (Wiersema, 1987). Plants of *Nymphaea* withstand seasonal periods of cold or drought either as seeds or as tuberous rhizomes. In fact, following cessation of growth at least a brief period of drying out appears to be necessary for resprouting of tubers. Seeds of these species may germinate directly following their release or may require a period of drought (Wiersema, 1987).

The stems of some species of *Nymphaea* are elongate and horizontal. Inland species may develop a smooth and globose tuber or one that is more ovoid and densely woolly. In many species these tubers are developed on lateral shoots, either at the nodes of elongate stolons or, as in two species, within abortive tuberiferous flowers. Certain species produce stolons only at the time of germination of tubers; in others stolons are formed throughout the vegetative period and are an effective means of vegetative propagation. In species which do form additional tubers, a tuberous rhizome serves as the resting state. Several sprouts may develop from this dormant structure at the next growing season (Wiersema, 1987).

The genus *Nymphaea* is the largest genus of the waterlily family and occurs world-wide with more than 50 species in tropical and temperate climates (Cronquist, 1988). There are seven species of the genus *Nymphaea* in the Flora Zambesiaca (FZ) region. The FZ region encompasses Mozambique, Zimbabwe, Zambia, Botswana and Malawi. The seven species in the FZ region include *Nymphaea sulphurea, Nymphaea divaricata, Nymphaea maculata, Nymphaea caerulea, Nymphaea capensis., Nymphaea lotus,* and *Nymphaea petersiana*. The genus *Nymphaea* is represented by four species in Malawi, namely, *N. caerulia, N. capensis, N. lotus* and *N. petersiana* (Exell and Wild, 1960).

A diagonistic description of the genus *Nymphaea* and its seven species is well documented in the Flora Zambesiaca region. The four species occuring in Malawi can be

recognized by the following diagnostic features: the first diagnostic characteristics are based on the colors (white flowers vs blue, pink or yellow) of the flowers and correlated flower parts. This isolates *N. lotus* (which has white flowers) from the six species. These six species whose flower colors are blue, pink or yellow (rarely white) are segregated into two groups, based on whether the flowers are blue or pink against flowers that are yellow and have floating leaves. The latter describes the species *N. sulphurea*. This leaves 5 species which have pink or blue flowers.These are divided into two groups based on whether they have floating and deeply incised-cordate, orbicular to elliptic leaves or leaves that are submerged etc. *N. divaricata* has submerged leaves. The remaining 4 species are separated first on whether leaves are sinuate-dentate or sinuate-lobulate margins - the former describes *N. petersiana* and the latter *N. capensis*. The other alternative of the key is whether the leaves have entire margin, and if they are large to medium, 6 -30 cm in diameter then it is *N. caerulia*, if smaller and less than 5 cm in diameter, then it is *N. maculata* (Exell and Wild, 1960).

Use Of Nymphaea spp. As Food By Humans

Different parts of *Nymphaea* are used for food in various parts of the world. In some parts of the world, it is the leaves that are used, other parts of the world use the seeds, stems or the rhizomes. As early as 1909, Stuhlman pointed out that rhizomes of *N. caerulea* were used as a famine food in Egypt. Stuhlman (1909) went on to caution that the rhizomes of *N. caerulea* were "poisonous" unless boiled (Stuhlman, 1909). The implication is that there is an unidentified toxin in the rhizome that is destroyed or leached into the cooking water during the boiling stage (Emboden, 1981). The rhizomes of this blue water lily, when cooked are regarded as "famine food" to be used only in dire circumstances.

In Nigeria, West Africa, seeds from *N. lotus* are harvested, dried in the sun and used as a grain. Preparation is usually done by boiling in somewhat salty water for several hours. The food prepared from the seeds is called *Bado* (Oestroem et al., 1980). The *Bado* seeds increase considerably in volume, by uptake of water, during the preparation. The dish resembles raw or unpolished rice and it is eaten together with a stew or vegetable stew or other saucy dishes. In Mali, West Africa, the roots of *Nymphaea lotus* are used as a food by the nomadic tribes in the Malian Gourma (Nordeide et al., 1994). Nutritional analysis and calculations of the chemical scores showed the roots of the *N. lotus*, to have a balanced amino acid composition.

In North-West Himalayas, India, leaves of *Nymphaea alba* L. are consumed as a vegetable and are documented as a good source of minerals such as calcium, and phosphorus (Katiyar et al., 1985). In Pakistan, roots of *Nymphaea lotus* L., locally known as *Bhes* or *Barsanda*, are used as a cooked starchy vegetable in large areas of the country. *Bhes* are cylindrical roots 20- 50cm long and 2-3 cm diameter. Freshly harvested light brown roots turn darker on storage and become dark brown on cooking. The roots are either sliced into 0.5 cm thick rings or cut into pieces of 2.5 cm length before they are cooked or fried in ghee. Meat-like flavor of the cooked *Bhes* is very much relished by the common populace of Pakistan (Shah et al., 1976). Fresh *Bhes* contain 6.5 mg/100g (dry weight basis) of ascorbic acid, which upon dehydration decreases to 2.3 mg/100g.

In Australia, *N. violacea*, is a major aquatic macrophyte in the waters of the Alligator Rivers Region, Northern Territory. It is used as a traditional Aboriginal diet item and it is called 'bush' food in the region (Petterson et al., 1993). Diaz (1977) records the use of *Nymphaea ampla* in Chiapas where the rhizome is ingested.

In the Lower Shire Valley of the Southern Region of Malawi, *Nymphaea petersiana* locally known as *Nyika*, is an established widely eaten root tuber. Its significance surfaced in the late 1940's when there was famine in the country and many people in this part of the country survived by eating this wild tuber. The tuber is cooked in its coat, and the coat is peeled off and the inside of the tuber is eaten with any type of vegetable and/or stew. It can also be peeled, sun-dried and ground to powder, and the flour can be cooked into a hard porridge (*nsima*), which again can be eaten with any vegetable and /or stew.

Use of Nymphaea spp. As Food By Other Animals

Besides humans, other mammals, rodents, ducks, geese and different types of insects use different parts of *Nymphaea spp*. for food. For example, in Chikwawa and Nsanje districts, where the root tubers of *N. petersiana* are eaten as a food, the leaves are also voraciously eaten by pigs. In the Kafue Plains of Zambia, in Central Africa, seven commonly occuring species of filter-feeding ducks feed on *N. capensis* between May and August (Douthwaite, 1977). Doucet and Fryxell, (1993), investigated forage preferences of beavers (*Castor canadensis*). They

discovered that among the five forage species that the beavers fed on, the white water lily, *N. odorata* was the second best preferred food for the beavers. Studies of muscovy ducks' (C. moschata) food habits was done in Mexico (Woodyard and Bolen, 1984). In wetland settings, 66% of the diet of muscovy ducks consisted of seeds from common aquatic plants - *Nymphaea spp*. and Avicennia. In the Ivory Coast, West Africa, the large mammals, called *Manatees* live in the lagoon systems extending over 300 km and totalling about 1200 km^2 of brackish waters, and also between the Sassandra and Cavally rivers. These mammals (who are now an endangered species) feed on leaves of *Nymphaea* spp. (Roth and Waitkuwait, 1986). The blue-winged teal (*Anas discors*) found in the wetlands of Cienaga Grande de Santa Marta, Colombia, mostly feed on seeds of *Nymphaea spp.* (Botero and Rusch, 1994). The pigmy gees (*N. auritus*), found on the Thamalakane river, in northern Botswana, Southern Africa, feed on ripe fruits of the water-lily *N. caerulea*, as their principal food, which is most abundant from late September to January (Douthwaite, 1980). In the Upper Peninsula of Michigan, U.S.A., five species of hebivorous insects were commonly found on water lilies (Nymphaeacea). One primary aquatic insect, a caddisfly larva (Trichoptera: Limniphilidae), had a generalized diet of water lilies (*Nympheae spp*.), in addition to other macrophytes and algae.

Uses of Nymphaea spp. As Medicinal Plants

Various species of *Nymphaea* have been used as a medicinal plant by people of different cultures. An analysis of the glyphs and pictorial evidence in the Dresden Codex of the Maya provides an insight into the diversity of plants employed by these ancient people. The codex reveals a host of plants of medicinal value with a disproportionately large representation of narcotic plants. The codex reveals that the white water lily of that region, *Nymphaea ampla,* is especially frequent in depictions. This inclusion is attributed to its narcotic properties (aporphine and quinolizidine). Diaz (1977) reported extracting aporphine from the bulbs and roots of *Nymphaea ampla*. Further work on apomorphine conducted by Tamminga et al., (1978) has resulted in the conclusion that when administered in relatively low doses apomorphine appears primarily to influence presynaptic dopamine receptor sites producing beneficial effects in patients suffering from schizophrenia. However, at certain doses apomorphine might exert the opposite effect (Emboden, 1981). Emboden, (1984), cited a few papers which suggest that certain water lilies (Nymphaea) have served as hallucinogens in Maya civilizations and are still in use in some

areas of Chiapas, and that the concept that water lilies might be used as ritual psychotogens was extended to ancient Egyptian civilizations. The collective evidence on water lilies in the Old World and the New World suggest a contextual use as a ritual psychotogen. This evidence has been distilled from Maya and post-Maya ceramics, codices, stone reliefs and frescos. The ancient Egyptians left similar information on tomb sculpture, ceramics, frescos and papyri (Emboden, 1979a; 1984).

CHAPTER 4

Materials and Methods

Purpose

Although *N. petersiana* tubers are widely consumed in the southern part of Malawi, there is, to our knowledge, no available data concerning the chemical composition of these tubers. The purpose of the study was to determine the tuber's proximate composition, mineral content, amino acid profiles , fatty acid composition, mono and disaccharide content, vitamin C content, and content of selected antinutritional factors; and to determine if there were any statistical differences in the nutrient and antinutrient content of tuber samples due to processing method: boiled and freeze-dried versus sun-dried samples.

Survey Site

The Nyika tubers for this study were collected from Chikwawa and Nsanje districts of the Lower Shire Valley (Fig. 3.1). The Lower Shire Valley forms the south-eastern end of the East African Great Rift Valley and the southern-most districts of Malawi. The area is low-lying (200 feet above sea level). The mean annual temperature is around 32 °C. The mean annual rainfall varies between 20" - 35", mostly falling in December to April (Pelletier and Msukwa, 1991). Most residents are involved in subsistence maize production. Other crops grown are sorghum, a millets, legumes and other vegetables. Cotton is produced as a cash crop. Because of its geographical position, this area is usually one of the worst hit areas during drought or flooding. In 1992, during the 1991-1992 Southern Africa drought, agriculture figures showed a 98% maize crop failure in the Lower Shire Valley, the highest in the country (Courtright and Canner, 1995).

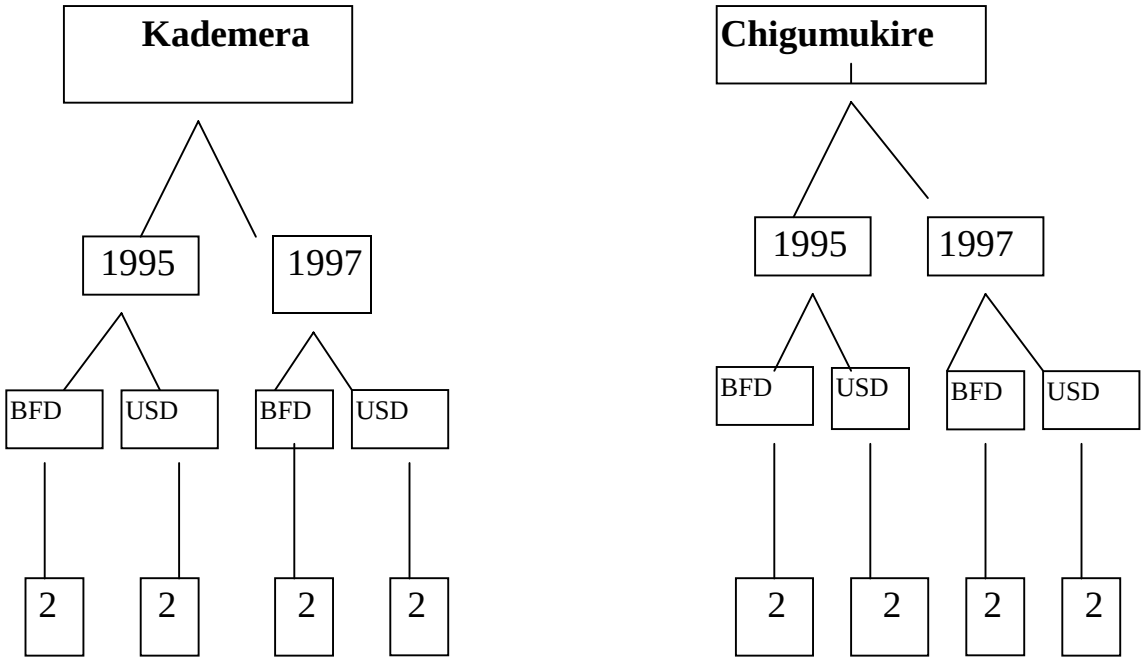
Samples for Analysis

Nymphaea petersiana tuber samples were collected from Chikwawa and Nsanje districts in the Southern Region of the country, where this tuber is habitually eaten. Permission to bring the tubers to our laboratories at Virginia Tech., Blacksburg, Virginia, was obtained through the United States Department of Agriculture (USDA). Samples were collected at two different times. One batch of samples was collected in September, 1995 and the other batch of samples was collected in September, 1997. Tubers were collected from two locations, Kademera and Chigumukire swamp areas covering a seventy-mile distance along the Malawi's Shire River

swamps. All the samples were assumed to be of a similar stage of maturity because the samples used in this study were harvested in September which is the normal harvesting season for this crop. After harvesting, the samples were put in cooler boxes with blue ice and transported to University of Malawi laboratory, Blantyre, Southern Malawi. They were frozen at - 60° C for one week. They were then shipped in the frozen state to Blacksburg, USA. The tubers were again kept frozen at - 60° C prior to analysis.

Experimental Design

The tuber samples were blocked into two categories according to the time the samples were taken (1995 and 1997). Within each time block, the samples were further blocked according to the location where they were collected from (Kademera and Chigumukire). Random numbers were used to allocate the tubers into two processing methods -uncooked/sun -dried (USD) and boiled/freeze-dried (BFD). Three-digit numbers were drawn from random number tables. Each 3-digit number was written on a piece of paper and on a tape and the tubers were tagged with the taped three-digit numbers. The three digit numbers on the papers were put in a box and drawn out randomly. The taped tubers were then assigned to each processing method based on the random numbers matching those drawn from the box. Each group of tubers drawn this way formed a composite sample. Each of the samples were analyzed in duplicate, which means that a total of 4 samples were analyzed per location. Overall, 16 samples were analyzed for each nutrient assayed. The experimental design is shown in Fig. 4.1 below:



BFD Boiled/freeze-dried samples
USD Uncooked/Sun-dried samples

Fig. 4.1 Experimental Design

Sample Treatment

The tubers in each batch formed a composite, and each composite was processed in one of two ways. One batch of tubers (composed of 10-12 tubers) was boiled in water (until they were soft to touch) for about 40 minutes, and cooled. The outer coats were removed from the boiled tubers and each tuber was sliced into smaller pieces and freeze dried for about 24 hours. The freeze dried samples were ground to powder using a cyclotec 1093 sample mill, and the powder was used for the analysis. Each of the tubers in the other batch (composed of 10-12 tubers) had the outer coat removed using a knife, then they were sliced into small pieces, and the pieces were spread out in the sun to dry (process was carried out between September and October). The tubers were considered dry when they were brittle and would be readily ground to

powder. It took from 24-48 hours for the tubers to be dry. The sun-dried tubers were ground to powder as sstated above. The powder was used for the analysis. The samples are referred to as boiled/freeze-dried (BFD) and uncooked/sun-dried (USD) throughout the text.

Statistical Analysis

A randomized complete block design model was chosen for statistical analysis of the data. of this study. Location and time factors were completely blocked and the only variable tested for was the processing method. Analysis of variance (ANOVA) was used to determine if there were any significant differences in the content of nutrients and antinutrient factors in the *Nymphaea petersiana* samples, processed by the two methods. Significant differences were tested at P = 0.05 (summary of statistical analysis shown in Appendix E). The analysis of data was done using SAS statistical package (SAS Institute Inc. Copyright©, 1989 - 1995, Cary, NC.)

Proximate Analysis

All tuber samples were analysed in duplicate. Moisture content was determined by drying in a Brabender Moisture Tester oven (Brabender Instruments Inc., NJ) at 135° C for 2 hours, according to AOAC (1984) approved method 7.007 (Appendix B). Nitrogen content was estimated by the Kjeldahl nitrogen method 2.057 (AOAC, 1984), (Appendix C). Protein content was calculated by multiplying % nitrogen by 6.25. Crude fat was estimated by the chloroform-methanol extraction method described by Phillips, et al., (1997), which is a modification of AOAC (1990) method 983.23. Dietary fiber analysis was determined using a total dietary fiber Sigma (1991) assay kit method (Appendix D). Ash content was determined by decomposition in a muffle furnace at 450° C - 620° C for 12 hours according to method 7.009 (AOAC, 1984), (Appendix A). A standard reference material, SRM 1544 - a frozen diet composite, purchased from the National Institute of Standards & Technology (NIST), was run simultaneously with the tuber samples for method validation. All analyses were performed in duplicate. Total crude carbohydrate was obtained by difference [100 - (crude protein + crude fat + fibre + ash)] (Muller and Tobin, 1980).

Mineral Analysis

Nyika flour samples were wet ashed using prior to mineral analysis using a mixture of 3 parts concentrated (16N) nitric acid and one part (70% w/v) perchloric acid. Wet ashing according to AOAC (1984) method 7.099 (b), was done to avoid mineral decomposition and

destruction (volatilization) that may occur while ashing in a muffle furnace. The ashed samples were analyzed for calcium, zinc, phosphorus, iron, and selenium. Calcium and zinc content of the Nyika samples were determined by atomic absorption spectrophotometry (AAS) (AOAC (1984) method 7.100), using a Pelkin-Elmer 2100 spectrophotometer (Norwalk, Connecticut). Phosphorus was determined colorimetrically (AOAC (1984) method 7.126) using a Milton Roy Spectronic 601 spectrophotometer. Iron and selenium content were determined by the above AAS method using a Pelkin-Elmer ICP Emission Spectrometer Plasma 400 (Norwalk, Connecticut). The mineral content of each of the samples was determined from standard curves (standards for each mineral were analyzed prior to and after each set of samples). Standard reference material, SRM 1515 (Apple Leaves)
purchased from the National Institute of Standards & Technology (NIST), were run simultaneously with the tuber samples for method validation. All analyses were performed in duplicate.

Fatty Acid Analysis

Total Lipid Extraction

A simplified gravimetric determination of total fat after chloroform-methanol extraction method (Phillips et al., 1997) was used for the extraction of the total fat from the samples. This method is a modification of the AOAC (1990) method 983.23.

Chemicals used were ACS-certified chloroform and HPLC-certified methanol (Fisher Scientific, Fair Lawn, NJ). Sodium acetate (Sigma Chemical Co., St. Louis, MO) was prepared as a 0.5M solution in distilled deionized water. Standard Reference Material (SRM 1544), a frozen diet composite, from the National Institute of Standards and Technology (NIST, Gaithersburg, MD) was used for method validation.

Five grams ($\pm$ 0.1g) of a thoroughly mixed sample of SRM 1544 were weighed into a 500 ml polypropylene centrifuge bottle. Based on the moisture content of the sample, enough 0.5M sodium acetate was added so that the total volume of water in the sample plus sodium acetate solution was 32 ml. Next, 80- ml methanol and 40- ml chloroform were added to the sample. The resulting proportion of chloroform:methanol:water was 1:2:0.8 (vol/vol/vol). The centrifuge bottles were capped and shaken on an orbital platform shaker (New Brunswick Scientific Co., Inc., Edison, NJ) for 2 hours at 325 rpm. Next, precisely 40 ml of chloroform

were added to each sample, and the mixture was shaken for 30 min at 300 rpm. The final ratio of chloroform:methanol:water in the samples was 2:2:1.8 (vol/vol/vol). The samples were centrifuged at 2300 rpm at a temperature between 4 and 22 ° C for 10 min to clarify the bottom layer. The samples were placed in a 25 °C water bath and allowed to equilibrate for 15 min. The samples then were stored undisturbed at room temperature for up to 24 hrs. The lower chloroform layer was transfered into weighed test tubes which had been heated in a drying oven at 101 ± 2 ° C. A pipetter was used to transfer accurately and precisely the chloroform (bottom) layer. The layer was evaporated to dryness in a 60 °C water bath under a stream of nitrogen. The test tubes were reweighed, and the amount of total lipid was calculated as follows:

Wt.of lipid (mg) = (Wt of test tube after chloroform removed) - (Wt of empty test tube)

% Lipid = (mg lipid/ Wt of sample) x 100

Analysis of Fatty Acids by Gas Chromatography

A Shimadzu gas chromatograph GC14A with a model AOC-14 Autoinjector and a Chromatopac C-R4AX processor (Columbia, MD) was used. It was operated by a temperature programming of 60° C to 220° C (at 10° C per min, hold for 2 min at 100° C, then 10° C per min to 220° C; Injector-220° C; Detector-230° C). Helium the carrier gas, was used at a gas flow-rate of less than 1 ml/min, with flame ionisation detectors (FID). An SP 2330 capillary column (30 meter length capillary column with 0.32 mm i.d., Supelco, Bellefonte, Penn.) was used. Heptadecanoic acid (17:0) was used as an internal standard. Short - chain standards C_4 - C_{12} and long - chain standards C_{16} - C_{20} were used to compare retention times of these standards to retention times of sample peaks eluted from the column as a means of tentatively identifying fatty acids in the tubers (Appendices P, Q, R, S, and T)

Procedure

All extracted lipids were used in the fatty acid analysis. Procedure used was a modification of AOAC (1990) method 983.23 by Phillips et al. (1997). Replicate lipid samples from the extraction process were transfered into 15 ml glass test tubes with teflon liner, and toluene (2 ml) was added to each test tube. A base reagent (0.5 N methanolic base, Supelco 3 - 3080) (2 ml) was also added to each test tube. The mixture was mixed and heated at 75 °C in a heating block for 15 minutes, and then cooled. The cooled samples were decanted into a 60 ml

separatory funnel, and the tube was rinsed with 6 ml of deionized water. Petroleum ether (6ml) was added to the funnel, mixed and allowed to separate. The bottom layer was removed and discarded. The top layer was passed through a sodium sulfate tube into a small test tube with teflon cap with liner to remove any traces of water. It was evaporated down (in an atmosphere of nitrogen) to 2.5 cm, and an aliquot was transferred to an autosampler vial and placed in the Autoinjector tray of the Shimadzu gas chromatograph GC 14A. The program was loaded, and the sample was run for 30 minutes.

Quantification of fatty acids

Quantification of the fatty acids was done using the following formula:

$$\text{Conc of Unknown Fatty acid} = \frac{\text{Peak Area of Unknown FA} \times \text{Conc of Int. Std.}}{\text{Peak Area of Internal Standard}}$$

Amino-acid Analysis

Nymphaea petersiana flour samples (BFD and USD) were subjected to amino acid analysis using the AOAC (1990) method 982.30 E (a,b,c) and F. The amino acids were separated and quantified using a Beckman 6600 automated amino acid analyzer, with a Beckman anion exchange column. Norleucine was used as an internal standard.

Sample Hydrolysis - AOAC (1990) method 982.30 E (a)

The sample (0.1g) was weighed into a hydrolysis tube and 10 ml 6N HCl was added to it and mixed. The mixture was freezed in dry ice-alcohol bath. The tube was sealed under vacuum (vacuum of $\leq 50\mu$ was drawn and held for 1 min). The flour samples were hydrolyzed with the 6N HCl for 24 hr at 110°C under a steady stream of nitrogen. The samples were cooled, and the hydrolysate was filtered through Whatman No.1 paper. Filtrate was dried at 65 ° C under vacuum. This hydroysate was used for the determination of all amino acids except methionine, cystine and/or cysteine, and tryptophan.

Perfomic Acid Oxidation and Acid Hydrolysis - AOAC (1990) method 982.30 E (b)

Nymphaea petersiana flour samples (0.1g) were placed in a hydrolysis tube, and 2 ml cold performic acid was added to each test tube and let to sit overnight at 0-5 °C. Cold HBr (3ml) + 0.04 ml 1-octanol (antifoam) was added to each test tube, and the contents were immediately mixed in ice water bath and evaporated to dryness at 40 °C under vacuum. To each test tube

was added 10 ml of 6N HCl and acid hydrolysis was performed as described above. This treatment quantitatively converted methionine to methionine sulfone and cystine and/or cysteine to cysteic acid. This hydrolysate was used to determine methionine (MET) and cystine/cysteine (CYS).

Alkaline Hydrolysis - AOAC (1990) method 982.30 E (c)

Nymphaea petersiana flour samples (0.1g) were placed into glass hydrolysis tubes having a Nalgene polypropylene centrifuge tube as an internal liner. Hydrolyzed potato starch (25 mg) was added to the sample. Fresh 0.6 ml 4.2 N NaOH + 0.04 ml 1-octanol was added to the test tube. The contents of the tube were mixed for 2 min under partial vacuum (the vacuum ≤ 50μ was drawn and held for 1 min). The tube contents were frozen in dry ice-alcohol bath. The tube was sealed while under vacuum. The contents of the tube were hydrolyzed for 22 hours at 110 ± 1 °C. The tube was cooled, and contents were transfered to 5 ml volumetric flask containing sufficient cold 6N HCl to neutralize the hydrolysate; the hydrolysate was diluted to volume using buffer appropriate for amino acid analyzer. The hydrolysate was centrifuged and filtered and it was used to determine tryptophan (TRP).

Analysis of Soluble Sugars by High-Pressure Liquid Chromatography (HPLC)

The method of Conrad and Palmer (1976) as modified by Johnson and Harris (1987) was used. The HPLC system used for this study was a Perkin-Elmer , with an 10 HPLC pump and an LC-25 Refractive Index Detector and Series. The samples were analyzed on an Alltech NH_2 (Amine) column. The column length was 250 mm with 4.6 mm inner diameter, 5μ film. A Hewlett-Packard 3390A integrator was used to calculate the peak areas (Appendices F, G, H, I, and J).

Nymphaea petersiana samples were analysed using the method as described by Johnson and Harris (1987). The samples (1.5 g) were dissolved in HPLC-grade water (20ml) and extraction was by solid phase extraction (SPE), using a B&J solid phase system column (Burdick & Jackson, Inc., Muskegon, Michigan). The column was activated by rinsing with acetonitrile (2ml), then washing it with hexane (2 ml). The sample was then applied to the column, and it was eluted from the column with acetone (2 ml). The analyte was concentrated by evaporation of the eluted solvent to 2 ml with nitrogen gas. Lactose wa used as the internal standard.

Each sample was analysed in duplicate on the LC-NH_2 column at 22 ° C with pressure adjusted to 700 psi. The mobile phase, was composed of filtered-degassed acetonitrile:water (75:25). The flow rate was 1 mL/min. Twenty (20) μL of filtered sample was injected. Equal amounts of standards (glucose, sucrose, fructose and maltose) were combined for injection. Lactose was used to test for precent recovery. The peak areas were calculated on the integrator. The saccharides were identified from their relative retention times compared to those of mono and disaccharide standards. Each component was calculated as a percent of total sugars using the formula:

$$\text{Sugar \%} = \frac{\text{Peak area of the individual component}}{\text{Total peak areas for all components}}$$

Analysis Of Ascorbic Acid

Nymphaea petersiana samples were analyzed for ascorbic acid content by high-performance liquid chromatography (HPLC), using the method of Wimalasiri and Wills (1983). The HPLC system used for this study was a Perkin-Elmer , with an 10 HPLC pump and an LC-25 Refractive Index Detector and Series. The samples were analyzed on a Phenosphere 5 micron NH_2 80A column; length x ID (250 nm x 4.6-mm). The mobile phase was composed of acetonitrile/water (70:30). The solvents used were optima HPLC solvents containing 0.01M ammonium dihydrogen phosphate.

Method used was by Wimalasiri and Wills (1983). Tuber samples (5.0 g) were weighed into a teflon tube. Twenty five (25) ml of 3% citric acid was added to the sample and homogenized using a polytron homogenizer (Kinematica, Switzerland). The contents were filtered using teflon filters and an aliquot of the filtered fluid was analysed for ascorbic acid by the HPLC method. L-ascorbic acid ((Aldrich) was used as a standard (Appendices K, L, M, N, and O). Spiking for conclusive identification of the ascorbic acid peaks was not done, therefore the results got in this study are inconclusive .

Analysis of Antinutritional Factors

The antinutritional factors - trypsin and chymotrypsin inhibitors, tannins, and phytate were analysed. The colorimetric procedure of Wheeler and Ferrel (1971) was used to estimate phytate. The colorimetric method of Kollipara and Hymowitz (1992) was used to determine the trypsin

and chymotrypsin inhibitor activity. Tannins were analysed using the method of Burns (1971).

Assay Of Trypsin And Chymotrypsin Inhibitor Activity

The Nyika flour samples were analysed for the possible presence of trypsin and chymotrypsin inhibitor activity using the colorimetric method of Kollipara and Hymowitz (1992). A Perkin-Elmer Lambda 3B UV/VIS spectrophotometer (Norwalk, Connecticut) was used for the analysis. Both trypsin and chymotrypsin inhibitor activity assays were conducted according to Hummel's (1959) method using p-toluenesulfonyl-L-arginine methyl ester (TAME) and N-benzoyl-L-tyrosine ethyl ester (BTEE), respectively as substrates. Soya beans were simultaneously analyzed with the *N. petersiana* samples for quality control.

Extraction

A buffer solution containing 0.023 M $CaCl_2$ and 0.092 M tris-HCl [tris(hydroxymethyl)aminomethane, pH adjusted with HCl], pH 8.1 buffer) was added to reach a precise final concentration of 20 mg of tuber flour/mL in a centrifuge tube. All the samples were left in the refrigerator (4°C) overnight before being clarified by centrifugation at 10,000g for 2 minutes. The supernatant from each sample was removed to a new tube and used immediately or stored at -20°C for later use.

Trypsin Analysis

A total of 2.6 ml of assay buffer (10.34 mM $CaCl_2$, 41.38 mM Tris-HCl, pH 8.1) was added to a substrate (10 mM TAME, prepared fresh the same day by dissolving 37.9 mg of TAME/mL of assay buffer) volume of 0.3 mL in a quartz cuvette (10-mm path length, 3.5 mL capacity). An aliquot of 0.1 mL of bovine trypsin (Sigma Chemical Co., catalog no. T-8253, dissolved and diluted to a final concentration of 20 µg/mL in 1 mM HCl), was added and mixed with the buffer and substrate immediately before the initiation of recording absorbance at 247 nm wavelength (A_{247}). Assay buffer (2.6 mL), substrate (0.3 mL), and 1 mM HCl without enzyne (0.1 mL) was used as a reference blank. The assays were monitored for 6 min in a UV-vis spectrophotometer (Hitachi Model U-2000) to confirm the linear progression of the reaction curve.

For inhibitor activity assays, 2.6 mL of assay buffer, 0.1 ml of the enzyme and 2-6 µL of the flour extract were mixed in a cuvette and incubated for 6 min. Substrate (0.3 mL) was added after the incubation period, and recording was immediately initiated at A_{247}. The

spectrophotometer was set to auto-zero with the assay tube against the reference blank just before the start of the recording. Inhibitor activity assays were monitored for 3 min. The trypsin inhibitor units per gram of tuber flour was calculated as follows:

TIU/g of tuber flour = [($T\Delta A_{247}$/min - $I\Delta A_{247}$/min) x 3 x 1000]/ (540 x gram flour weight)

where $T\Delta A_{247}$/min is the change in A_{247}/min in the absence of inhibitor (sample); $I\Delta A_{247}$/min is the change in ΔA_{247}/min in the presence of inhibitor, and gram flour weight is the gram equivalent of flour extract used in the assay.

Chymotrypsin Assay

Assay buffer (1.4 ml), (0.1 M $CaCl_2$, 0.1 M Tris-HCl, pH 7.8) was added to 1.5 mL of substrate solution. The substrate, 1mM BTEE, was prepared fresh on the same day by dissolving 15.7 mg of BTEE, in 50 mL of 50% (w/w) aqueous, spectral grade methanol (i.e., 3:2 methanol/H_2O by volume). To this assay solution was added 0.1 mL of bovine -alpha-chymotrypsin (Sigma, catalog no. C-4129), dissolved and diluted to a final concentration of 20 µg/mL in 1 mM HCl) just before the assay started. The progress of the reaction was monitored like that of trypsin (described above) for 6 min at wavelength 256 nm, and the linearity of the reaction was confirmed. The reference blank contained the same solution as above except 0.1 mL of 1 mM HCl was used in place of enzyme solution.

The inhibitor assay was conducted by incubating 7.5-15 µL of flour extract with 0.1 mL of enzyme solution (i.e., 2µg of µ--chymotrypsin) and 1.4 mL of the assay buffer for 6 min. At the end of the incubation period, the reaction was started by adding 1.5 mL of 1 mM BTEE solution. The initial absorbance reading was adjusted to zero (auto-zero) against the blank, and immediately the reaction was monitored for 3 min. Chymotrypsin inhibitor units (CIU) were calculated as follows:

CIU/g of tuber flour = [($C\Delta A_{256}$/min - $I\Delta A_{256}$/min x 3 x 1000)] / (964 x gram flour weight)

where $C\Delta A_{256}$/min is the change in A_{256}/min in the absence of inhibitor (sample), $I\Delta A_{256}$/min is the change in A_{256}/min in the presence of inhibitor, and gram flour weight is the gram equivalent of flour extract used in the assay

Estimation of Phytic Acid Content

Method of Wheeler and Ferrel (1971) was used. Phytate in the tuber samples was extracted with trichloroacetic acid and precipitated as the ferric salt. The iron content of the precipitate was determined colorimetrically and the phytate phosphorus content was calculated from this value assuming a constant 4 Fe: 6 P molecular ratio in the precipitate. The iron content of the samples was, therefore, calculated from a $Fe(NO_3)_3$ standard run at the same time as the samples or read from a previously prepared standard curve, and calculation of the phytate phosphorus was done from the iron results, assuming a 4:6 iron: phosphorus molecular ratio.

Procedure

The colorimetric procedure of Wheeler and Ferrel (1971) was followed to estimate the phytic acid content of *Nymphaea petersiana* tubers. Sorghum (*Vulgare Pers.)* was simultaneously analyzed with the *N. petersiana* samples for quality control. A finely ground sample estimated to contain 5 to 30 mg phytate P was weighed into a 125-ml Erlenmeyer flask. The sample was extracted with 50 ml 3% TCA for 30 min with mechanical shaking. The suspension was centrifuged and a 10 ml aliquot of the supernatant was transfered into a 40 ml conical centrifuge tube. A solution of $FeCl_3$ (4 ml) was added to the aliquot , and the contents were heated in a boiling water bath for 45 min, followed by centrifugation (10-15 min) and decanting of the clear supernatant. The precipitate was washed twice by dispersing well in 20 to 25 ml 3% TCA, heating in boiling water bath 5 to 10 min, and centrifuging. The precipitate was dispersed in a few ml of water and 1.5 N NaOH was added with mixing. The volume was brought to approximately 30 ml with water and heated in a boiling water bath for 30 min, filtered hot through a moderately retentive paper (S & S 597, Whatman No. 2). The precipitate was washed with 60 to 70 ml hot water and filtrate was discarded. The precipitate from the paper was dissolved with 40 ml hot 3.2 N HNO_3 and transferred into a 100 ml volumetric flask. The flask and its contents were cooled to room temperature and diluted to volume with water. An aliquot (5 ml) was transfered to another 100 ml volumetric flask and diluted to approximately 70 ml. Potassium thiocyanate (1.5 M KSCN; 20 ml) was added to the flask and contents were diluted to volume. The absorbance of this aliquot solution was read immediately at 480 nm. A reagent blank was run with each set of samples.

Estimation of Tannin in the Nyika Tubers

The method of Burns (1971) was used. The method involved overnight extraction of tuber flour with methanol at room temperature. An aliquot of the extract was added to a solution of vanillin and hydrochloric acid and the resultant color was read on a spectrophotometer (Milton Roy Spectronic 401 spectrophotometer) at 500 nm wavelength. Sorghum (*Vulgare Pers*.) was analyzed simultaneously with the samples for quality control.

Procedure - (Burns, 1971)

The reagents were prepared by combining equal volumes of 8% concentrated hydrochloric acid (HCl) in methanol and 4% vanillin in methanol (prepared daily and mixed just before use).

A standard curve was prepared by adding 100 mg catechin to 50 ml methanol. This was used at various dilutions from full strength to 1:10; I ml of each dilution was pipetted into each of two separate tubes. After the 10 dilutions (20 tubes) had been prepared, 5 ml of vanillin-HCl reagent was quickly added to each tube. Absorbance readings were taken on a spectrophotometer at 500nm, after a uniform incubation time (20 minutes). The vanillin-HCl was used for the 100% transmittance blank.

Tannin Determination - (Burns, 1971)

Nymphaea petersiana flour samples (1g) were put in a 125-ml flask. Methanol (50 ml) was added to each flask, and stoppered. The mixture was mixed occasionally by swirling. After 20 to 28 hours swirl it was allowed to settle. Supernatant (1 ml) was pippeted into each of two tubes and procedure proceeded as with standards above. All the samples were extracted at one time.

A plot of absorbance versus catechin gave a typical logarithmic curve. By using 1g of tuber in 50 ml methanol the readings were easily converted to percent catechin. The values for tannin obtained from this test were based on catechin equivalents.

CHAPTER 5

RESULTS AND DISCUSSION

Proximate Composition

The proximate composition of *Nymphaea petersiana* is presented in Tables 5.1, 5.2, 5.3 and 5.4. The processing method did not have a significant ($P > 0.05$) effect on the crude protein and total dietary fiber content of the tubers. There was, however, a significant difference ($P< 0.05$) in the ash content, crude fat content and the total carbohydrate content between the two processing methods (Table 5.2). There could have been some leaching of minerals during the boilimg of the tubers, hence the significantly higher content of ash in the USD samples as compared to the BFD samples. The higher amounts of the crude fat and total carbohydrates in the boiled samples as compared to the uncooked samples might have been due to the lower moisture content of the boiled sample (about 20 times less as compared to the uncooked samples), consequently the content of these nutrients were more concentrated in the cooked samples as compared to the uncooked samples. The total carbohydrates content was higher in the boiled samples as compared to the uncooked samples. This might have been due to the fact that since calculation of carbohydrates was by difference, the boiled samples had a higher moisture content (Table 5. 1) than the uncooked samples, therefore upon subtraction, the amount of carbohydrates is higher in the boiled samples which had a smaller amount of moisture.

A comparison of the proximate composition of *Nymphaea petersiana* with some cereals eaten in Africa indicated that except for sorghum (10.7%), the protein content of the tubers (8.0 and 8.1% for BFD and USD respectively) was comparable to that of the other cereals including that of the Malawian staple, maize (7.9%). The protein content was slightly higher than the protein content of African millets (unspecified) (7.5%), and that of milled and polished rice (7.0%). Since this tuber is used as a famine food, the presence of this high amount of protein means it can be substituted for the Malawian staple maize without compromising the protein content of the diet. The tuber, however, had a higher fiber content (14 % for both USD and BFD samples, respectively) than all the cereals indicated in Table 5.3. The presence of this high amount of dietary fiber in the tuber could reduce the bioavailability of some minerals such as zinc, and the biological availability of other nutrients such as proteins and carbohydrates, because

a high fiber content could trap and protect a good proportion of nutrients, such as proteins and carbohydrates from hydrolytic breakdown, thus reducing digestibility and utilization of end products of digestion. On the other hand, dietary fiber has also been associated with a number of therapeutic effects. For example, dietary fiber from plant foods is said to be rich in associated polyphenol compounds which act as antioxidants (Saura-Calixto, 1998). Increased consumption of dietary fiber is related to a reduction in the intestinal absorption of bile acids and dietary lipids by enhancing the fecal excretion of these. Reduced concentrations of total bile acids indicates improvement in risk for colon cancer (Haak et al., 1998).

Table 5.1 **Proximate Composition of Nymphaea petersiana (Nyika) Tubers**[a]

Location		**Chigumukire**		**Kademera**		
Year	1995	1997	1995	1997	SRM[d]	NIST Value[e]
		Processing Method 1[b]				
components[g] **(%)**						
Moisture	0.5 ± 0.13	0.48 ± 0.021	0.68 ± .035	1.1 ± .15	73.6 ±.22	73.1 ±.79
Crude protein	7.9 ± .06	8.8 ± .14	7.95 ± .021	7.1 ± .07	5.1 ± 0.0	5.28 ±.30
Ash	1.86 ± .029	1.93 ± .023	1.78 ± .013	1.90 ± .008	1.01 ± .011	0.97 ±.22
Total dietary fiber	12.3 ± 0.023	14.95 ± 0.82	12.97± 0.14	15.56± 0.17	ND[f]	ND
Crude fat	1.06± 0.001	1.09 ± 0.015	0.97± 0.087	1.0 ± 0.01	3.92 ±.127	3.68 ± .48
Total carbohydrates	76.28	72.75	75.65	73.34	-	-
		Processing Method 2[c]				
Components (%)						
Moisture	10 ± 0.6	11.76 ± .035	10.3 ± .49	11 ± .7	73.6 ± .22	73.1 ± .79
Crude protein	8.2 ± .31	8.1 ± 0	8.0 ± .68	8.1 ± .20	5.1 ± 0.0	5.28 ±.30
Ash	2.59 ± .050	2.29± .026	2.49± .028	2.37 ± 0.034	1.01 ± .011	0.97 ± .22
Total dietary fiber	11.9 ± 0.93	12.3 ± .02	14.8 ± 1.67	17.0 ± 0.67	ND	ND
Crude fat	0.87 ± 0.139	0.88 ± 0.002	0.86± 0.005	1.07 ± 0.002	3.92 ± .127	3.68 ± .48
Total carbohydrates	66.44	64.67	63.55	59.76	-	-

[a]All values are means and standard deviations of duplicate assays of single pooled samples
[b]Processing method 1 - boiled/freeze-dried (BFD)
[c]Processing method 2 - uncooked/sun-dried (USD)
[d]SRM - Standard Reference Material 1544 used for validation of method
[e]NIST - National Institute of Standards & Technology certified values for the SRM
[f]ND - not determined (LOD 1 μg/g)
[g]All values are on a dry weight basis

Table 5.2 **Comparison of Proximate Composition Of Nymphaea petersiana (Nyika) Tubers By Two Processing Methods**[a]

Components[b] (%)	Boiled and freeze-dried (BFD)	Uncooked and sun-dried (USD)
Moisture*	0.70 ± .285	10.77 ± .785
Crude protein	8.0± 0.70	8.1 ± 0.08
Ash*	1.9 ± 0.065	2.4± 0.13
Total dietary fiber	14.0 ± 1.56	14.0 ± 2.4
Crude fat*	1.03 ± 0.055	0.9 ± 0.10
Total carbohydrates*	74.37	63.83

*Significant ($P < 0.05$)
[a]All values are an average of two locations in two different years (1995 and 1997)
[b]All values are on a dry weight basis

Table 5.3 **Comparison Of The Proximate Composition Of Nyika Tubers With Some African Cereals**

Crop	Protein (%)	Ash (%)	Total CHO (%)	Fat (%)	Fiber (%)
Nyika[b] (BFD)	8.0	1.9	74.37	1.03	14.0
Nyika[b] (USD)	8.1	2.4	63.83	0.9	14.0
Maize, soaked, sun-dried[a]	7.9	0.4	73.1	1.5	0.6
African millets red[a]	7.5	3.3	73.1	1.47	3.4
Rice, milled, polished[a]	7.0	0.6	79.5	0.5	0.4
Sorghum[a]	10.7	1.9	71.7	3.2	2.4

[a]Source: Food Composition Table For Use In Africa (Leung, 1968)
[b]All Nyika values are on a dry weight basis

A comparison of the proximate composition of this tuber and other tubers eaten in Africa (Table 5.4) indicates that this tuber has a higher protein content than cassava (1.3% both domesticated and wild), potato (2.0%), sweet potato (1.6%), yams (3.2 and 1.5 % wild and domesticated, respectively) and the Egyptian *Nymphaea lotus* (5.2%). Staple foods with protein contents below 3% (cassava and potato) do not meet the protein requirements of humans even when ingested in amounts supplying more than the caloric requirements. On the contrary, a diet of cereals (with an 8-10 % protein content) meets the protein requirements of adults, provided enough is eaten to supply the caloric requirements (Cheftel et al., 1985). This probably explains why, in developing countries, where root tubers are the staple food, protein-calorie malnutrition (marasmus) is much more common than malnutrition attributable solely to protein deficiency (Cheftel et al., 1985).

Nymphaea petersiana tubers have a much higher protein content (Table 5.4) than all the root tubers eaten on the continent and in Malawi, and its protein content is comparable to the staple maize, which means that when eaten in sufficient quantities it can meet the caloric requirements of an adult. It would also be a better substitute and/or complement to the weaning food often used in the rural areas, which is made from refined maize, which is cooked into a thin porridge called *phala*, whose protein composition was found to be only 0.9/100g (Courtright and Canner, 1995). The crude fat content (1.03 and 0.9 %) and ash content (1.9 and 2.4 %) of the tubers were also higher than those of the other root tubers listed in Table 5.4, which was an indication that this tuber could successfully substitute and/or complement the domesticated tubers eaten in Malawi and on the African continent.

Table 5.4 **Comparison Of the Proximate Composition Of Nyika[b] With Other African Root Tubers**

Crop	Protein (%)	Fat (%)	Total CHO (%)	Fiber (%)	Ash (%)
Nyika, (BFD)	8.0	1.03	74.37	14.0	1.9
Nyika (USD)	8.1	0.9	63.83	14.0	2.4
Cassava wild[a]	1.3	0.0	27.6	-	1.1
Cassava sweet[a]	1.3	0.5	84.8	1.8	2.9
Potato[a]	2.0	0.1	19.0	0.4	1.0
Sweet potato[a]	1.6	0.2	27.5	1.0	0.9
Yam, wild tuber[a]	3.2	0.1	19.0	0.8	1.1
yam (African), tuber[a]	1.5	0.1	27.5	0.9	0.9
Nymphaea lotus, root (Egyptian)[a]	5.2	0.2	25.6	1.0	1.1

[a]Source: Food Composition Tables For Use In Africa (Leung, 1968)
[b]All Nyika values are on a dry weight basis

Composition Of Some Selected Minerals In Nymphaea petersiana Tubers

The content of some selected minerals of *Nymphaea petersiana* are presented in Tables 5.5, 5.6, 5.7 and 5.8. Statistical analysis of the data revealed that there were no significant differences ($P < 0.05$) in the mineral content of BFD and USD samples except for iron. A large percentage of the iron might have leached into the water during the cooking process. Determination of iron in the water used to boil the tubers would have shed some light on this high loss of iron in the boiled tubers. This means that the two processing methods used (in Malawi) for preparing these tubers, do not cause much difference (except for iron) in the content of these selected minerals.

Comparison of the mineral content of the tuber to that of the staple maize (250 μg/g for Ca; 20 μg/g for Fe) showed that it had a higher calcium (1376 μg/g and 946 μg/g (BFD and USD respectively) and iron 88μg/g (UDS) content, and had comparable mineral content to the African cereals that are also eaten in Malawi (Table 5.7). A comparison of the mineral content of this tuber to other African root tubers (Table 5.8) indicated the tuber had a higher calcium content (1376 μg/g for BFD) than cassava (480 μg/g wild and 1210 μg/g domesticated), potato (110 μg/g), sweet potato (330 μg/g) and yams (520 μg/g wild and 690 μg/g domesticated), all of which are also eaten in Malawi. Phosphorus (2200μg/g and 2600μg/g) content was also higher than all the tubers listed in Table 5.8. The USD tuber had a higher iron content (88.0 μg/g) than values given for cassava (78.0 μg/g), potato (7.0 μg/g) and sweet potato (20 μg/g).

Table 5.5 **Composition Of Some Selected Minerals In Nymphaea petersiana (Nyika) Tubers**[a]

Location	**Chigumukire**		**Kademera**			
Year	1995	1997	1995	1997	SRM Values[f]	NIST Values[g]
		Processing Method 1[b]				
Mineral (μg/g)						
Calcium	932 ± 15	1500 ± 238	1251± 113	1820 ± 18	15260 ± 150	15360 ± 220
Phosphorus	2521± 432	2250± 47	2350± 6.0	1874± 162	1580 ± 64	1590 ±10
Zinc	19.2 ± 0.48	25.0 ± 0.90	19.3 ± 0.19	19.7± 1.5	12.5 ±0.13	12.5 ±0.3
Iron	15.4 ± 0.23	16.8 ± 0.71	14.9 ± 0.32	33 ± 1.5	86 ±2.1	83 ± 5
Selenium	ND (LOD 1 μg/g)	ND (LOD 1 μg/g)	ND (LOD 1 μg/g)	ND (LOD 1 μg/g)	-	-
		Processing Method 2[c]				
Mineral (μg/g)						
Calcium	948± 46	1099 ± 102	779 ± 39	1191 ± 20	15260 ± 150	15360 ± 220
Phosphorus	3244 ± 435	2500 ± 0	2768 ± 156	3020 ± 419	1580 ± 64	1590 ± 10
Zinc	22.0 ± 0.75	29.7 ± 0.65	22.3 ± 0.46	26.0 ± 0.68	12.5 ± 0.13	12.5 ± 0.3
Iron	71.5 ± 2.2	88 ± 5.3	70.0 ± 6.3	170 ± 0.63	86 ± 2.1	83 ± 5
Selenium	ND (LOD 1 μg/g)	ND (LOD 1 μg/g)	ND (LOD 1μg/g)	ND (LOD 1 μg/g)	-	-

[a]All values are means and standard deviations of duplicate assays of single pooled samples
[b]Processing method 1 - boiled and freeze-dried (BFD) - values on a dry weight basis
[c]Processing method 2 -uncooked, sun-dried (USD) - values on a dry weight basis
[d]ND - not detected
[e]LOD - limit of detection
[f]SRM 1515 - Apple Leaves used for method validation
[g] NIST Values - National Institute of Standards & Technology certified values of the SRM

Table 5.6 **Comparison Of Mineral Composition Of Nymphaea petersiana (Nyika) Tubers By Two Processing Methods**[a]

	Boiled and freeze-dried (BFD)	Uncooked and sun-dried (USD)
Mineral[b] (μg/g)		
Calcium	1376.75 ± 376	946.75 ± 233
Phosphorus	2250 ± 275	2883 ± 321
Mineral (μg/g)		
Zinc	21.0 ± 2.8	25.0 ± 3.6
Iron*	20.0 ± 9.0	100.0 ± 45.5

*Significant ($P < 0.05$)
[a]All values are an average of two locations in two different years (1995 and 1997)
[b]All values are on a dry weight basis

Table 5.7 **Comparison Of The Mineral Composition Of Nyika[b] With Some Cereals Eaten In Malawi**

Crop	Calcium (μg/g)	Phosphorus (μg/g)	Iron (μg/g)
Nyika (BFD)	1376	2250	20
Nyika (USD)	946	2883	100
Maize,soaked, sun-dried	250	-	20
African millets, (unspecified)[a]	3970	2440	171
Rice, milled, polished[a]	90	1270	17
Sorghum[a]	1380	3200	553

[a]Source: Food Composition Table For Use In Africa (Leung, 1968)
[b]All Nyika values are on a dry weight basis

Table 5.8 **Comparison Of The Mineral Composition Of Nyika[b] with Other African Root Tubers**

Crop	Calcium (μg/g)	Phosphorus (μg/g)	Iron (μg/g)
Nyika, (BFD)	1376	2250	20
Nyika, (USD)	946	2883	100
Cassava, wild[a]	480	-	78
Cassava,sweet[a]	1210	1180	-
Potato[a]	110	380	7
Sweet Potato[a]	330	560	20
Yam, Wild, tuber[a]	520	450	-
Yam (African), tuber[a]	690	290	-
Nymphaea lotus, root (Egyptian)[a]	930	1510	-

[a]Source: Food Composition Table For Use In Africa (Leung, 1968)
[b]All Nyika values are on a dry weight weight basis

There were no values for the mineral zinc for tubers llisted in the Africa composition tables; consequently, zinc values have not been discussed together with the other minerals.

Research on the zinc content of Malawian foods was done by Ferguson, et al., (1989) and results are listed along with the zinc content of *Nymphaea petersiana* tubers, in Table 5.9. The zinc content of the tubers is higher (21μg/g and 25μg/g for BFD and USD respectively) than boiled maize flour (8μg/g) (65% extraction); boiled sorghum flour (12%); rice (16 μg/g); boiled cassava (6 μg/g) and sweet potato (2 μg/g). Generally it was found that cereals and root crops in Malawi have a low zinc bioavailability, which was attributable to the high dietary fiber and phytate content of plant foods eaten as staples (Ferguson, et al., 1989).

Of the five minerals assayed, selenium was not detected because the limit of detection of the assay was 1μg/g (1ppm). Literature values of the selenium content of some foods eaten in Malawi indicated that the cereal products analyzed had selenium values ranging from 2.0 μg/100g to 12.9 μg/100g (Table 5.10), (Donovan, et al., 1991), which is below the limit of detection of the assay used in this study. Moreover, selenium is associated mainly with proteins, therefore, animal products and nuts/seeds (and not root tubers) are rich sources of selenium. In the foods analyzed for selenium in Malawi (Donovan et al., 1991), fish had the highest selenium concentration (10.8 to 73.2 μg/100g), followed by duck eggs (19.5 μg/100g) and grasshoppers (8.9 μg/100g). Vegetables and fruits tended to have low selenium concentrations (less than 1 μg/100g). There was no analysis of selenium content in the tubers eaten in Malawi. Since some selenium compounds in foods occur as unstable heat-sensitive and volatile forms of the element, refining or cooking of cereal grains can result in food products with a selenium content less than that of the parent material (Donovan et al., 1991). The researchers in this study, however, found that cooking and refining (95% and 65% extraction) of the maize flour had little effect on its selenium concentration (Table 5.9) (Donovan et al., 1991).

Table 5.9 **Zinc Content Of Selected Raw And Cooked Malawian Cereals And Root Crops As Compared to Nymphaea petersiana Tubers**

Food	Zinc Content (μg/g)
Maize flour	
65 % extraction, boiled	8
95 % extraction, boiled	22
Sorghum flour, boiled	12
Rice	16
Cassava, boiled	6
Sweet potato, boiled	2
Nymphaea petersiana	
Boiled and freeze-dried (BFD)[a]	21
Uncooked and sun-dried (USD)[a]	25

Ferguson et al., (1989)

[a]All values are on a dry weight basis

Table 5.10 **Selenium Content Of Selected Raw And Cooked Malawian Cereals**

Food	Selenium (μg/100g)
Maize flour (95% extraction)	2.5 ±0.8
Maize flour (65 % extraction)	
Uncooked	2.6 ± 1.0
Cooked	2.0 ± 2.2
Millet (unspecified) Flour	8.0 ± 0.6
Rice	2.4 ± 1.0
Sorghum	12.9 ± 3.9
Sorghum Flour	9.3 ± 1.6
Nymphaea petersiana	ND[a] (LOD 1 μg/g)

(Donovan et al., 1991)
[a]ND - not detected

Amino Acid Composition Of Nymphaea petersiana Tubers

The amino acid content in *Nymphaea petersiana* tubers is shown in Tables 5.11a and 5.11b. There were significant ($P < 0.05$) differences in amino acid content for a few non-essential amino acids - hydroxyproline, aspartic acid and arginine due to the two processing methods

used. There was no effect on the amino acid content of the rest of the non-essential amino acids due to the type of processing method used. There were also significant ($P < 0.05$) differences in amino acid content of two of the essential amino acids - leucine and lysine, due to the two processing methods used (Table 5.13). For the rest of the essential amino acids, the type of processing method used had no effect on the content of the amino acids. This might have been due to the fact that the affected amino acids might have been heat labile and were therefore affected during the boiling of the tubers; or some of these amino acids might have been water soluble and might have been leached during the boiling process.

The nutritional quality, of a food protein depends on the kinds and amounts of amino acids it contains, and represents a measure of the efficiency with which the body can utilize the protein (Cheftel, et al., 1985). A balanced or high quality protein contains essential amino acids in ratios commensurate with human needs. This can be determined by comparing the amino acid contents of various proteins with the FAO reference pattern. The FAO reference pattern based on the essential amino acid requirements of young children is considered the preferred reference protein (Cheftel, et al., 1985). The essential amino acid profile of *Nymphaea petersiana, (* BFD and USD) are compared with the FAO reference pattern (1985) presented in Tables 5.12 and 5.13. The tuber is well balanced in essential amino acids. The only limiting amino acid is lysine (Table 5.12).

Table 5.11a **Amino Acid Composition Of Nymphaea petersiana Tubers**[a]

Location	**Chigumukire**		**Kademera**	
Year	1995	1997	1995	1997
		Processing Method 1[b]		
Amino Acid (mg/g crude protein)				
Taurine	15.0 ± 0.7	14.6 ± 0.07	15.3 ± 0.42	16.8 ± 0.05
Hydroxyproline	1.16 ± 0.05	1.1 ± 0.01	1.2 ± 0.03	1.3 ± ND[c]
Aspartic Acid	155.5 ± 0.05	160 ± 0.8	158 ± 3.5	154.9 ± 0.42
Threonine	39 ± 0.6	39 ± 0.9	40 ± 2.8	39 ± 1.0
Serine	52 ± 1.4	53 ± 1.8	60 ± 7.0	53 ± 2.0
Glutamic Acid	75 ± 2.2	89.3 ± 0.37	91 ± 1.7	90.4 ± 0.25
Proline	33.5 ± 0.17	33.7 ± 0.16	34.6 ± 0.12	34.9 ± 0.10
Glycine	47.4 ± 0.23	48.3 ± 0.23	48.1 ± 0.34	49.1 ± 0.13
Alanine	58.9 ± 0.29	57 ± 0.5	59 ± 0.80	60.7 ± 0.17
Cysteine	24 ± 0.8	23 ± 0.9	23 ± 0.5	24.5 ± 0.07
Valine	53 ± 1.1	52 ± 0.7	50 ± 6.0	54 ± 0.8
Methionine	21 ± 0.7	21.4 ± 0.10	22 ± 0.6	21.9 ± 0.06
Isoleucine	47 ± 1.1	46 ± 0.9	42 ± 5.5	47 ± 0.8
Leucine	86 ± 1.2	85.9 ± 0.38	86 ± 1.0	87 ± 1.4
Tyrosine	41 ± 0.6	41.6 ± 0.20	40 ± 3.0	42 ± 0.8
Phenylalanine	60 ± 0.5	59.6 ± 0.28	59.9 ± 0.02	60 ± 0.7
Histidine	28.9 ± 0.14	29.2 ± 0.14	29 ± 0.8	29.7 ± 0.08
Ornithine	0.58 ± 0.82	1.12 ± 0.01	ND[c]	2 ± 0.9
Lysine	49.7 ± 0.24	48 ± 1.4	47 ± 2.0	50.4 ± 0.14
Arginine	68.8 ± 0.48	68.5 ± 0.33	68 ± 2.1	65 ± 0.2
Tryptophan	28.9 ± 0.14	28.7 ± 0.66	28.8 ± 0.04	16.8 ± 0.05

[a]All values are means and standard deviations of duplicate determinations on a dry weight basis
[b]Processing Method 1 - boiled and freeze-dried (BFD)
[c]ND - not detected (LOD 50 μg/g)

Table 5.11b **Amino Acid Composition Of Nymphaea Petersiana (Nyika) Tubers**[a]

Location	**Chigumukire**		**Kademera**	
Year	1995	1997	1995	1997
		Processing Method 2[b]		
Amino Acid[d] **(mg/g crude protein)**				
Taurine	15.8 ± 0.5	15.2 ± 0.05	15.8 ± 0.10	16.6 ± 0.03
Hydroxyproline	1.32 ± 0.012	1.26 ± 0.005	1.32 ± 0.09	1.39± 0.003
Aspartic Acid	151.7± 0.45	153 ± 0.5	151.9± 0.06	151 ± 1.7
threonine	39 ± 1.3	37.9 ± 0.14	39 ± 0.7	38.8 ± 0.08
Serine	53 ± 2.4	52 ± 1.1	52 ± 2.5	52.6 ± 0.10
Glutamic Acid	94.3 ± 0.05	89.6 ± 0.32	94.0 ± 0.32	91.4 ± 0.18
Proline	35 ± 0.6	34 ± 1.2	35 ± 1.2	34.6 ± 0.07
Glycine	47.5 ± 0.44	46.7 ± 0.17	47 ± 0.6	48 ± 1.1
Alanine	58 ± 0.5	56.8 ± 2.0	57 ± 1.5	57 ± 1.1
Cysteine	23 ± 0.7	23 ± 1.7	23 ± 0.1	22 ± 2.0
Valine	52 ± 1.9	51 ± 0.7	51.3 ± 0 33	52.6 ± 0.10
Methionine	21.1 ± 0.20	21 ± 0.8	21.0 ± 0.14	20 ± 0.9
Isoleucine	45 ± 1.4	45 ± 0.7	45 ± 1.2	45.7 ± 0.09
Leucine	84 ± 1.1	83.3 ± 0.30	85 ± 0.6	84.5 ± 0.17
Tyrosine	40.9 ± 0.38	41.9 ± 0.44	41 ± 1.2	42 ± 1.1
phenylalanine	58 ± 0.5	57 ± 0.5	57.9 ± 0.38	57 ± 0.9
Histidine	29.0 ± 0.03	29.0 ± 0.10	28.9 ± 0.19	29.1 ± 0.06
Ornithine	ND[c]	1.26 ± 0.005	0.7 ± 0.93	0.7 ± 0.98
Lysine	52.1 ± 0.45	53.0 ± 0.19	52.6 ± 0.34	53 ± 1.1
Arginine	71 ± 0.7	78.3 ± 0.28	71.7 ± 0.46	73 ± 0.8
Tryptophan	27.7 ± 0.26	30 ± 1.0	28.9 ± 0.19	27.7 ± 0.05

[a]All values are means and standard deviations of duplicate determinations
[b]Processing method 2 - uncooked, sun-dried (USD)
[c]ND - not detected (LOD 50 μg/g)
[d]All values are on a dry weight basis

Table 5.12 **Essential Amino Acid Composition Of Nymphaea petersiana Tubers**[c]

Location	**Chigumukire**		**Kademera**		
Year	1995	1997	1995	1997	
		Processing method 1[d]			
Amino Acid (mg/g crude protein)					FAO/WHO[a] (1990) Reference Pattern[b]
Threonine	39	39	40	39	34
Cystine + methionine	45	44	45	45	25
Valine	53	52	50	54	35
Leucine	86	86	86	87	66
Isoleucine	47	46	42	47	28
Phenylalanine + Tyrosine	101	101	99	102	63
Lysine*	50	48	47	50	58
Tryptophan	29	29	29	17	11
		Processing Method 2[e]			
Amino Acid (mg/g crude protein)					
Threonine	39	38	39	39	34
Cystine + Methionine	44	44	44	42	25
Valine	52	51	51	53	35
Leucine	84	83	85	85	66
Isoleucine	45	45	45	46	28
Phenylalanine + Tyrosine	99	98	99	99	63
Lysine*	52	53	53	53	58
Tryptophan	28	30	29	28	11

[a]Based on preschool child data from FAO/WHO/UNU (1985)
[b]Source: FAO/WHO/UNU (1985) which includes all references to the original data
[c]All values are means and standard deviations of duplicate determinations on a dry-wt basis
[d]Processing Method 1 - boiled and freeze-dried (BFD) - all values are on a dry weight basis
[e]Processing method 2 - uncooked, sun-dried (USD) - all values are on a dry weight basis

*Limiting amino acid

Table 5.13 **Comparison Of Essential Amino Acid Composition Of Nymphaea petersiana (Nyika) Tubers[d] By Two Processing Methods To Reference Pattern**

	Boiled and freeze-dried (BFD)	Uncooked and sun-dried (USD)	FAO/WHO[a] (1990) Reference pattern[b]
Amino Acid[e] (mg/g crude protein)			
Threonine	39.3 ± 0.50	39 ± 0.5	34
Cys + Met	45 ± 0.5	46 ± 1.0	25
Valine	52 ± 1.7	52 ± 1.0	35
*Leucine	86 ± 0.5	84 ± 1.0	66
Isoleucine	46 ± 2.4	45 ± 0.5	28
Phe + Tyr	101 ± 1.3	99 ± 0.5	63
*lysine[c]	49 ± 1.5	53 ± 0.5	58
Tryptophan	26 ± 6.0	29 ± 1.0	11

*Significant ($P < 0.05$)
[a]Based on preschool child data from FAO/WHO/UNU (1985)
Source: FAO/WHO/UNU (1985)
[c]Limiting amino acid
[d]All values are an average of two locations in two different years (1995 and 1997)
[e]All values are on a dry weight basis

In most chemical methods, a protein's nutritional value is assessed on the basis of its content of essential amino acids as compared with the human requirements for these amino acids (Cheftel et al., 1985). Tables 5.14 and 5.15 show the chemical scores of the amino acids in *Nymphaea petersiana* tubers. Although lysine is a limiting amino acid, the chemical scores for lysine of 84% and 91 % (for the boiled and uncooked tubers respectively), are reasonably high. The other essential amino acids have very high amino acid scores of greater than 100. Knowledge of the chemical score helps in planning for calculation of the complementary value of different proteins in a food mixture. In this case, the chemical score values would help in deciding what legumes would complement the tuber if it had to be used as a weaning food, or what side dishes should be eaten with the tuber to complement the limiting amino acid lysine. The chemical scores, however, do not account for differences in the digestibility of proteins or in the biological availability of specific amino acids. The chemical score values are improved when used in correlation with bioassays and the scores are corrected based on overall protein digestibility. Unfortunately it was not possible to do animal tests in this particular study, due to lack of sufficient tuber samples.

Table 5.14 **Amino Acid Scores of the Essential Amino Acids Of Nymphaea petersiana (Nyika) Tubers**[a]

Location	**Chigumukire**			**Kademera**	
Year	1995	1997		1995	1997
			Processing Method 1[b]		
Amino Acid[d] **Chemical Score (%)**					
Threonine	115	115		118	115
Cysteine + Methionine	180	178		180	180
Valine	151	149		143	154
Leucine	130	130		130	132
Isoleucine	168	164		150	168
Phenylalanine + Tyrosine	160	161		157	162
Lysine*	86	83		81	87
Tryptophan	263	261		262	153
			Processing Method 2[c]		
Amino Acid[d] **Chemical Score (%)**					
Threonine	115	111		115	114
Cystein + Methionine	176	176		176	168
Valine	149	146		147	150
Leucine	127	126		129	128
Isoleucine	161	161		161	163
Phenylalanine + Tyrosine	157	156		157	157
Lysine*	90	91		91	91
Tryptophan	252	273		263	252

*Limiting amino acid
[a]All values are means and standard deviations of duplicate determinations
[b]Processing method 1 - boiled and freeze-dried (BFD)
[c]Processing method 2 - uncooked and sun-dried (USD)
[d]All values are on a dry weight basis

Table 5.15 **Comparison Of Amino Acid Scores In Nymphaea petersiana (Nyika) Tubers[a] By Two Processing Methods**

	Boiled and freeze-dried (BFD)	Uncooked and sun-dried (USD)
Chemical Score[b] (%)		
Threonine	116 ± 1.5	114 ± 1.9
Cys + Met	180 ± 1.0	174 ± 4.0
Valine	149 ± 4.6	148 ± 1.8
Leucine	131 ± 1.0	128 ± 1.3
Isoleucine	163 ± 8.5	162 ± 1.0
Phe + Tyr	160 ± 2.2	157 ± 0.5
Lysine*	84 ± 2.8	91 ± 0.5
Tryptophan	234 ± 55	260 ± 10

*Limiting amino acid
[a]All values are an average of two locations over a two-year period (1995 and 1997)
[b]All values are on a dry weight basis

Literature values (Table 5.16) indicate corn (maize) to be low in lysine and tryptophan; rice to be low in lysine; millet, sorghum and potato to be low in lysine and threonine, cassava to be low in methionine and yam to be low in methionine and lysine. Compared to the cereals and root tubers listed in Table 5.16, however, *Nymphaea petersiana*'s only limiting amino acid is lysine. This means that *N. petersiana* tubers have a better balanced amino acid profile than the staple maize, and most root tubers eaten in Malawi. The tuber is habitually eaten with animal products such as meat or fish, which are rich in lysine, and would therefore make up for this deficiency. If used as a weaning food, the tuber flour could be cooked as a thin porridge (*phala)* to which some groundnuts (rich in lysine) could be added, to supply the limiting amino acid.

Table 5.16 **Limiting Amino Acids Of Some Cereals and Root Tubers Compared to Nymphaea petersiana Tuber**

Crop	**Limiting amino acid**
Wheat (grain)	lysine
Corn (maize)	lysine, tryptophan
Rice	lysine
Millet	lysine, threonine
Sorghum	lysine, threonine
Potato (tuber)	lysine, threonine
Cassava	methionine
Yam	methionine, lysine
Nymphaea petersiana (tuber)	lysine

(Cheftel et al., 1985)

Fatty Acid Composition Of Nymphaea petersiana Tubers

The fatty acid profiles of total lipids of the tuber show that despite its low crude fat content, analysis of the fatty acid composition showed it has a good proportion of essential fatty acids (Tables 5.17 and 5.18). Oleic acid (C18:1) is the predominant fatty acid (47%), followed by linoleic acid (C18:2) (33%), palmitic acid (C16:0) (21%) and linolenic acid (C18:3 %) (7%). Presence of high levels of unsaturated fatty acids, with about 79% of the total lipids is nutritionally desirable, because one of the criteria for the determination of fat quality is the content of essential fatty acids like linoleic, linolenic, and arachnodic acids. Many animals, including humans, require some of the essential fatty acids to prevent an essential fatty acid deficiency, which is manifested by skin lesions, poor hair growth, and low growth rates (Eteshola and Oraedu, 1996). The essential fatty acid, linolenic acid is converted in the body to arachdonic, which is the precursor of a group of hormones known as prostaglandins (Nawar, 1985).The prostaglandins, along with thromboxanes, exhibit a wide range of physiologic actions, including the lowering of blood pressure, diuresis, blood platelet aggregation, effects on the immune and nervous systems as well as gastric secretions, and the stimulation of smooth-muscle contraction, to name a few (Hunt and Groff, 1990).

Unsaturated fatty acids are much more susceptible to oxidation than their saturated analogs. At high temperatures their oxidative decomposition proceeds very rapidly ((Nawar, 1985). In this study, *Nyika* tubers have 78% unsaturated fatty acids, an indication that the food would be very susceptible to rancidity. Therefore there would be need to keep the processed food in places which would minimize exposure to oxygen, light and high temperatures in order to minimize rancidity and loss of the essential fatty acids.

Table 5.17 **Fatty Acid Composition of Nymphaea petersiana (Nyika) Tubers[a]**

Location	**Chigumukire**		**Kademera**	
Year	1995	1997	1995	1997
		Processing Method 1[b]		
Fatty Acid[d] (g/100g)				
Palmitic Acid (C16:0)	20.32± 0.295	20.47± 0.058	21.14± 0.058	21.80± 0.50
Stearic Acid (C18:0)	1.71± .066	1.76 ±.027	2.19 ±.61	2.21± .015
Oleic Acid (C18:1)	42.43± .278	40.41± .21	42.37± .47	42.71± .152
Linoleic Acid (C18:2)	30.26± .064	31.88± .127	29.33± .18	29.6± .17
Linolenic Acid (C18:3)	5.82± .024	5.96± .030	5.65± .097	4.80 ± .008
		Processing Method 2[c]		
Fatty Acid[d] (g/100g)				
Palmitic Acid (C16:0)	21.13 ± .197	21.49 ± .052	19.78± 1.35	23.4 ±.18
Stearic Acid (C18:0)	2.20 ± .03	2.22 ±.138	3.0 ± 1.22	2.4 ± .003
Oleic Acid (C18:1)	44.64 ± 0.015	51.02 ± .132	46.30 ± 3.1	47.7± .68
Linoleic Acid (C18:2)	36.42 ± 0.54	30.75 ± .06	32.6 ± 2.58	31.5 ± .12
Linolenic Acid (C18:3)	6.77 ± .04	7.84 ± .079	6.81± 0.8	7.4 ± .03

[a]All values are means and standard deviations of duplicate assays of single pooled samples
[b]Processing method 1 - boiled and freeze-dried (BFD)
[c]Processing method 2 - uncooked and sun-dried (USD)
[d]All values are on a dry weight basis

Table 5.18 **Comparison Of Fatty Acid Composition of Nymphaea petersiana By Two Processing Methods**[a]

	Boiled and freeze-dried	Uncooked and sun-dried
Fatty Acid (g/100g)[b]		
Palmitic acid (C16:0)*	21.0 ± 0.68	21.46 ± 1.51
Stearic acid (C18:0)	2.0 ± 0.3	2.47 ± 0.38
Oleic Acid (C18:1)	42.0 ± 1.1	47.4 ± 2.71
Linoleic acid (C18:2)	30.0 ± 1.1	32.8 ± 2.51
Linolenic acid (C18:3)	6.0 ± 0.52	7.2 ± 0.51

*Significant ($P < 0.05$)
[a]All values are an average of two locations in two different years (1995 and 1997)
[b]All values are on a dry weight basis

Sugar Content And Vitamin Content Of Nymphaea petersiana Tubers

Although the proximate composition data (Table 5.2) showed a high carbohydrate composition (75% and 66 % for the cooked and uncooked tubers, respectively), there were only small amounts of soluble sugars present in the tuber samples as shown in Tables 5.19 and 5.21. Most of the soluble sugars may have been converted to the storage form - starch, while some of it may be present as dietary fiber components of the cell wall of the plants or as phytate. Results also show that of the three soluble sugars present in the tuber, sucrose is present in the smallest quantity. This could be due to the fact that since sucrose is the normal transport sugar in plants, most of the sucrose in the tuber may have been transported and converted into the storage form, starch. There was also a significant difference ($P< 0.05$) in the content of the glucose and fructose sugars between the two processing methods, with the uncooked tubers having about twice as much of the two sugars when compared to the cooked tubers (Table 5.21). This might have been due to the heat converting the soluble sugars into insoluble gels or due to leaching losses.

The chromatograms of the soluble sugars (Appendices G to J) show unidentified peaks, which might have been mono, di and oligosaccharides resulting from hydrolysis of starch or soluble and insoluble fibre components during the boiling of the tubers since the peaks seem to be more prominent in the boiled tubers (Appendices G and H) compared to the sun-dried samples (Appendices I and J). Some of these peaks might even be glycerol resulting from the hydrolysis of some of the fatty acids during the boiling of the tubers. Spiking with the pure sample would have helped to identify these peaks.

From a nutritional point of view, L-ascorbic acid (AA) and dehydroascorbic acid (DAA) are the two biologically active forms of vitamin C, therefore, it is necessary to estimate both AA and DHA. However, in this particular assay, the levels of dehydroascorbic acid (DHA) were below the detection of this assay so no values were determined. Results presented in Tables 5.20 and 5.21 show the ascorbic acid content of the tuber samples. The amount of the vitamin in the samples was very small (0.1 and 0.003 mg/100g for BFD and USD respectively). The amount of the vitamin in the samples was very small (0.1 and 0.003 mg/100g for the BFD and USD respectively). Since no spiking was done to confirm the presence of the ascorbic acid, the values for the content of ascorbic acid in the tuber are tentative results.

In general, the largest losses of vitamin C in non-citrus food occur during heating. Leaching loss during heating far exceeds losses during other processes (Steven, et al.,1985). In this particular study, some ascorbic acid might have have been lost during the boiling process since vitamin C is heat labile. Some vitamin C might have leached out into the water during boiling. Unfortunately iron determination of the water which was used for boiling the tubers was not done and therefore there is no conclusive evidence as to how much of the iron may have been lost due to leaching. Some vitamin C might have been lost during freeze-drying of the samples, leading to significantly ($P < .05$) low values of BDF tubers compared to the USD tubers. Maybe a shorter boiling time might have resulted in lower losses. Generally, fruits and vegetables are better sources of ascorbic acid than root tubers, like *N. petersiana.*

Research done by Bradbury and Singh (1986), on tropical root crops showed that there were losses in AA + DAA varying between 3 and 30% when the crops were dried before analysis. Because of these losses in drying the samples, they recommended that fresh rather than dried samples shoud be used for analysis to obtain correct AA and DHA contents. Although *N. petersiana* tubers are not eaten raw, analysis of fresh samples would give an indication of how much of the vitamin is lost during processing. All the samples used in our assay were either freeze-dried or sun dried, so the low values obtained might have been due to loss of the vitamin during processing prior to analysis.

Although the stability of ascorbic acid generally increases as the temperature is lowered, some investigators (Steven et al., 1985) have indicated that there might be an accelerated loss upon freezing or in frozen storage. Storage temperatures above -18°C ultimately lead to significant losses of this vitamin (Steven et al., 1985). Although this tuber was stored at a frozen temperature of -60°C, there might have been significant losses on transporting the tuber from Malawi to Blacksburg, Virginia, therefore these values might not be a true representation of the actual content of ascorbic acid in the *Nymphaea petersiana* tubers. Spiking was not done to conclusively identify the peaks, therefore these results may be considered tentative.

Table 5.19 **Sugar Content Of Nymphaea petersiana (Nyika) Tubers**[a]

	Location			
	Chigumukire		**Kademera**	
Year	1995	1997	1995	1997
Sugar (mg/100g)				
		Processing Method 1[b]		
Fructose	3.38 ± 0.60	2.2 ± 0.60	2.6 ± 0.30	2.0 ± 0.43
Glucose	4.2 ± 0.9	3.0 ± 0.90	3.0 ±0.50	2.3 ± 0.54
Sucrose	ND[d]	0.2 ± 0.35	ND[d]	ND[d]
		Processing Method 2[c]		
Fructose	5.2 ± 1.75	6.50 ± 4.14	4.23 ± 0.25	4.8 ± 0.69
Glucose	6.9 ± 3.1	9.2 ± 0.59	5.64 ± 1.2	5.3 ± 0.85
Sucrose	0.47 ± 0.16	0.59 ± 0.28	0.4 ± 0.50	0.24 ± 0.08

[a]All values are means and standard deviations of duplicate determinations on a dry weight basis
[b]Processing method 1 - boiled and freeze-dried (BFD)
[c]Processing method 2 - uncooked and sun-dried (USD)
[d]ND - Not detected (LOD 0.05 mg/100g)

Table 5.20 Ascorbic Acid Content Of Nymphaea petersiana (Nyika) Tubers[a]

Location		**Chigumukire**		**Kademera**	
Year	1995	1997		1995	1997
		Processing Method 1[b]			
Ascorbic Acid[d] (mg/100g)	0.007 ± 0.009	ND[e]		0.006 ± 0	ND[e]
		Processing Method 2[c]			
Ascorbic Acid[d] (mg/100g)	0.10 ± 0.007	0.14 ± 0.05		0.09± 0.009	0.12 ± 0.020

[a]All values are means and standard deviations of duplicate determinations on a dry weight basis
[b]Processing method 1 - boiled and freeze-dried (BFD)
[c]Processing method 2 - uncooked and sun-dried (USD)
[d]All values are on a dry weight basis
eND - Not detected (LOD .0001mg/100g)

Table 5.21 **Comparison Of Sugar And Vitamin Content Of Nymphaea petersiana Tubers[b] By Two Processing Methods[a]**

	Boiled and freeze-dried (BFD)	Uncooked and sun-dried (USD)
Sugar (mg/100g)		
Fructose*	2.5 ± 0.61	5.2 ± 0.96
Glucose*	3.1 ± 0.80	6.8 ± 1.8
Sucrose	0.05 ± 0.1	0.49 ± 0.096
Ascorbic acid[b] (mg/100g)*	0.003 ± 0.0028	0.11 ± 0.022

*Significant ($p < 0.05$)
[a]All values are an average of two locations in two different years (1995 and 1997)
[b]All values are on a dry weight basis

Antinutrient Content Of Nymphaea petersiana Tubers

When the nutritional content of wild foods is being investigated, the possible presence of antinutritional factors must also be considered, because one of the major obstacles to the wider use of lesser-known wild foods is the presence of toxic substances. Common antinutritional factors found in legumes, grains, seed crops and even root crops include typsin and chymotrypsin inhibitors, cyanogenic glucosides, and phenolic compounds such as tannins, and phytates. In this study, some of these antinutritional factors (phytate, tannin, trypsin and chymotrypsin inhibitors) were determined.

Tannin Content Of Nymphaea petersiana Tubers

Tannins are known to be present mostly in food legumes, leaves, coffee, tea, wines, and beers. When present in a food, they are known to inhibit the activities of trypsin, chymotrypsin, amylase and lipase (Rao and Deosthale, 1982). Tannins have also been found to cause growth depression in rats and poultry. This effect may be due to reduction in the digestibility of dietary protein and to a lesser extent that of available carbohydrate and lipid. Tannins also interfere with dietary iron absorption (Rao and Deosthale, 1982). Tannins of high molecular weight can be both bitter and astringent (Rao and Deosthale, 1982). Although tannins are usually present in high quantities in certain cereals like sorghum and than in root tubers, the slightly bitter taste found in cooked *Nymphaea petersiana* tubers made it necessary to determine the content of tannins in this tuber.

To date there are no data (to the our knowledge) on the tannin content of legumes, cereals and other foods eaten in Malawi, consequently, data from this study were compared with literature values of Sorghum (Vulgare Pers.) grown in the green-house of Virginia Tech., local sorghum cultivar from Kabale/ Uganda, Africa and DeKalb BR 64 (high tannin) sorghum from the United States.

Tables 5.22, 5.23 and 5.26 present the tannin content of *Nymphaea petersiana*. Although there was some difference between the BFD and USD samples, these differences were not

statistically significant (P > 0.05) (Table 5.26). The tannin content of *N. petersiana* were 1% and 1.5 % (BFD and USD samples respectively) which was lower than the Kabale sorghum from Uganda (6.88%), and for DeKalb (high tannin) sorghum (7.35 %), from the United States, and for Vulgare Pers. sorghum from Virginia Tech.'s green house (USA) (3.74 %).

Studies done by Rao and Deosthale (1982) showed that the amount of tannin in a food can be appreciably decreased by decortication, overnight soaking in water, germination and cooking. This means that depending on the method used for processing, the amount of tannin can be greatly reduced. As for *Nymphaea petersiana* tubers, the amount of tannin determined here is very low compared to that found in sorghum, which may mean that this amount may not be high enough to be of nutritional concern, but if it is, other methods of processing could be devised to reduce the amount of tannin in the tuber before it is eaten.

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Table 5.22 **Tannin Content Of Nymphaea petersiana (Nyika) Tubers**

Location		**Chigumukire**		**Kademera**		
Year	1995	1997		1995	1997	
			Processing Method 1			
Tannin (%)						
N. petersiana	0.68 ± 0.002	1.06 ± 0.009		1.14 ± 0.029	1.12 ± 0.049	
			Processing Method 2			
N. petersiana	2.41 ± 0.036	1.61± 0.023		1.58 ± 0.005	1.04 ± 0.02	
Sorghum *Vulgare Pers.*						3.74 ± 0.015

[a]All values are means and standard deviations of duplicate determinations on a dry weight basis
[b]Processing method 1 - boiled and freeze-dried (BFD)
[c]Processing method 2 - uncooked, sun-dried (USD)
[d]Sorghum (*Vulgare Pers*.) - From Virginia Tech biology department green house

Table 5.23 **Comparison Of Tannin Content of Ugandan and U.S. Sorghum Grains and Nymphaea petersiana Tubers**

Sorghum Cultivar	**Tannin (%)**
Kabale local (Ugandan) cultivar	6.88
DeKalb BR 64 (U.S.)	7.36
Vulgare Pers. (Virginia Tech.)	3.74
Nymphaea petersian (BFD)[a]	1.0
Nymphaea petersiana (USD)[a]	1.7

(Mukuru et al., 1992)

[a]*N. petersiana* values are on a dry weight basis

Phytate Content Of Nymphaea petersiana Tubers

Suboptimal mineral nutrition may be prevalent in Malawi due to high intakes of phytic acid and dietary fiber in the plant-based staple foods eaten (Ferguson et al. 1989). Phytic acid decreases the bioavailability of certain minerals and may interfere with the utilization of proteins due to the formation of phytate-protein and phytate-mineral-protein complexes and also inhibits digestive enzymes (Ferguson et al, 1989). More recently, suboptimal zinc status has been noted among rural preschool children in Malawi consuming maize-based diets high in phytic acid (Ferguson et al., 1989).

The effect of phytate depends upon the relative amounts of phytate, calcium, and zinc, as well as the amount and type of dietary protein (Ferguson, et al., 1993b). Results from the study referred to above, showed that legumes and cereals, especially unfermented and less refined cereals had the highest phytate content of all Malawian plant foods analyzed.

Tables 5.24, 5.26 and 5.27 show the phytate content of *Nymphaea petersiana* tubers to be 3.9 μg/g and 5.4 μg/g (BFD and USD samples respectively). These values are very low compared to those found by Ferguson et al. (1988), for the Malawi staple white maize flour (in mg/100g) (211); cooked maize flour (55); unrefined maize flour (792); cassava (59) and sweet potato (12). This indicates that the phytate content in *Nymphaea petersiana* tuber might be low enough to be of little nutritional concern when compared to the phytate content of the other foods eaten in Malawi.

Phytate levels can be reduced during certain food processing operations, such as cooking. For example, cooking reduced the phytate content of peas by 13% (Marfo et al., 1990). There was a significant ($P < .05$) difference in the phytate content of *Nymphaea petersiana* between the cooked and uncooked samples. The uncooked samples had greater phytate content than the cooked. This shows that phytic acid content was reduced during the cooking of the tubers, indicating that boiling of the tubers is the better of the two processing methods in terms of reducing the phytate level in the tubers.

Table 5.24 **Phytate Content Of Nymphaeae petersiana (Nyika) Tubers**[a]

Location	**Chigumukire**		**Kademera**		
Year	1995	1997	1995	1997	
		Processing Method 1[b]			
P Phytate (μg/g) *N. petersiana*	4.07 ± 0.296	3.77 ± 0.90	4.08± 0.91	3.79 ± 0.91	
		Processing Method 2[c]			
P Phytate (μg/g) Sorghum[d] *Vulgare Pers.*					11.7 ± 0.424
P Phytate[e] (μg/g)	5.42 ± 0.13	5.2 ± 1.73	6.67 ± 0.563	6.8 ± 2.76	

[a]All values are means and standard deviations of duplicate assays of single pooled samples
[b]Processing method 1 - boiled and freeze-dried (BFD)
[c]processing method 2 - uncooked, sun-dried (USD)
[d]Sorghum (*Vulgare Pers*.) - from Virginia Tech. biology depatment green house
[e]All values are on a dry weight basis

Trypsin And Chymotrypsin Inhibitors

The detrimental effects on animal performance of trypsin inhibitors in different foods have been extensively studied (Han et al., 1991; Herkelman et al., 1993, to name but a few). Trypsin inhibitors in raw soybeans have been found to decrease proteolytic activity in the small intestine, resulting in decreased release of free amino acids. In addition, they have been found to cause pancreatic hypertrophy in rats and chicks (Han et al., 1991). Studies by Herkelman et al., (1993) on soybeans showed that the activity of trypsin inhibitors was reduced by half by heating. Soybeans were autoclaved for 20 min at 110°C, and the trypsin inhibitor activity was reduced from 22.1 mg/g to 9.6 mg/g.

Trypsin inhibitor activity for *Nymphaea petersiana* tubers was significantly ($P < .05$) different between boiled and uncooked samples. Results in Tables 5.25 and 5.26 show both trypsin and chymotrypsin inhibitor activity to be reduced by boiling of the tubers. The trypsin inhibitor activity was reduced by a factor of seven while the chymotrypsin inhibitor activity was reduced by a factor of approximately five. The trypsin inhibitor activity was reduced to 55 from 400 TIU/g seed (for BFD and USD samples, respectively). The chymotrypsin inhibitor activity was reduced to 50 from 240 CIU/g seed (for cooked and uncooked samples respectively). These values indicate that heat treatment reduces the inhibitor activity appreciably, thereby rendering the boiledtubers nutritionally superior to the raw uncooked tubers.

Table 5.25 **Trypsin and Chymotrypsin Inhibitors Activity In Nymphaea petersiana (Nyika) Tubers**[a]

	Location				
	Chigumukire		**Kademera**		
Year	1995	1997	1997	1997	Soybean[d]
		Processing Method 1[b]			
Trypsin[e] (TIU/g of tuber)	65.4 ± 34	69.0 ± 47	46.6 ± 0.02	40 ± 38	-
Chymotrypsin[e] (CIU/g of tuber)	31.1 ± 0.61	64.9 ± 0.10	31.3 ± 0.01	71 ± 21	-
		Processing Method 2[c]			
Trypsin[e] (TIU/g of tuber)	667 ± 69	262 ± 22	717± 278	207± 192	113 ± 9.2
Chymotrypsin[e] (CIU/g of tuber)	255± 1.0	275 ± 1.2	247 ± 3.2	291± 2.4	52 ± 3.0
Soybean (CIU/g of seed)					

[a]All values are means and standard deviations of duplicate assays of single pooled samples
[b]Processing method 1 - boiled and freeze-dried (BFD)
[c]Processing method 2 - uncooked and sun-dried (USD)
[d]Soybean (uncooked) - bought from local grocery store
[e]All values are on a dry weight basis

Table 5.26 **Comparison Of Antinutrient Content Of Nymphaea petersiana Tubers[b] By Two Processing Methods**

Antinutrient[b]	Boiled and freeze-dried	Uncooked and sun-dried
Tannin (%)	1.0 ± 0.27	1.7 ± 0.56
Phytate (μg/g)*	3.93 ± 0.171	6.02 ± 0.829
Trypsin (TIU/g of seed)*	55 ± 14.3	463.0 ± 266
Chymotrypsin (CIU/g of seed)*	50 ± 21.3	267 ± 20.2

*Significant ($P < 0.05$)
[a]All values are an average of two locations in two different years (1995 and 1997)
[b]All values are on a dry weight basis

Table 5.27 **Phytate Content Of Some Foods Eaten In Malawi Compared To Nymphaea petersiana Tubers**

Food	Phytate (mg/g fresh wt.)
White maize flour	211
Cooked maize flour	55
Unrefined flour (mgaiwa)	792
Sorghum	ND[a]
Cassava	59
Sweet potato	12
	Phytate (mg/g dry wt.)
Nyika (BFD)[b]	0.0039
Nyika (USD)[b]	0.0060

(Ferguson et al., 1988)

[a]ND not detected (LOD not stated)
[b]Nyika values on a dry weight basis

CHAPTER 6
SUMMARY AND CONCLUSIONS

The main objectives of this study were to determine the content of some selected nutrients and antinutrients in the tuber *Nymphaea petersiana* and to find out if there are significant differences in nutrient and antinutrient content due to the processing methods used. A secondary objective was to find out if the nutrient and antinutrient content of this tuber was comparable to other staple foods eaten in Malawi.

Data obtained revealed that *N. petersiana* tubers are a good source of high quality protein whose only limiting amino acid is lysine. Since *Nyika* is habitually eaten with a side dish of either beef, chicken stew or fish, which are good sources of lysine, this probably compensates for the limitimg amino acid, lysine. There was no significant ($P < 0.05$) difference in the protein content due to the processing method used.

Data from the study also revealed that the protein content of *N. petersiana* was comparable to that of the staple maize. According to Fennema (1985), staple foods with protein contents below 3% do not meet the protein requirements of humans even when ingested in amounts supplying more than the caloric requirements. However, a food with an 8 - 10% protein content meets the protein requirements of adults, provided enough is eaten to supply the caloric requirements. Data from this study indicate the tuber has an 8.1% protein content. This means that when eaten in enough quantities it has adequate protein to meet the caloric requirements of an adult. This also means that this tuber is a good substitute and/or complement for maize in times of poor harvest and in times of famine. Comparison of data from the study and literature values of other root crops eaten in Malawi revealed that the *Nyika* tuber is a good substitute and/or complement for the other root crops eaten in Malawi.

Results from data analysis revealed that the content of the minerals calcium, phosphorus and the micronutrients iron and zinc were higher than those of most foods eaten in Malawi, indicating that the Nyika tuber is a good source of these minerals. There was a significantly ($P < 0.05$) lower amount of iron in the cooked samples, indicating that processing method does affect the content of some of the minerals.

Although the fatty acid profile of the tuber was very good, considering that the predominant fatty acid was linoleic acid (an essential fatty acid), the overall amount of crude fat in the tuber was very low (less than 3%). This means that this tuber would not be considered a good source of fat, but it is a good source of carbohydrate (just like most root crops). Since the amount of soluble sugars is very small, most of the carbohydrates might be be in the form of starch or other insoluble forms.

The ascorbic acid content in the tuber was very low (0.1 and 0.003 mg/100g (USD and BFD tubers respectively). Considering that the tuber had to be transported from Malawi to the USA, there is the possibility that some of the ascorbic acid might have been lost during the transportation as well as during processing. There was a significantly ($P < 0.05$) lower amount of ascorbic acid in the boiled samples than the sun-dried samples indicating that processing method does affect the content of this vitamin in the tuber. This means the tuber is not a good source of ascorbic acid, and if eaten as a main meal, it would have to be complemented with other foods like leafy green vegetables.

The antinutritional factors - tannin, phytate, trypsin and chymotrypsin inhibitors were present in *N. petersiana* tubers, but the values obtained for each one them was lower than published values of these antinutrients in other cereals and /or root tubers eaten in Malawi. The amount of tannin found in this tuber was lower than that found in three cultivars of sorghum, including DeKalb (high tannin) sorghum from the U.S., Vulgare Pers. sorghum from Virginia Tech. green house, and Kabale sorghum from Uganda. This might mean that the amount of tannin in the tuber is not high enough to be of nutritional concern, but this could only be confirmed by carrying out further studies, possibly with animals.

The amount of phytate in *N. petersiana* was much lower than that of the staple maize and that of cassava and sweet potato. Compared to the published values for the staples eaten in Malawi, the phytate content in *N. petersiana* is very low and might not affect the bioavailability of the other nutrients found in this tuber. The trypsin and chymotrypsin inhibitor activities of *N. petersiana* were significantly ($P < 0.05$) lower in the BFD samples than in the USD samples, and were lower than that of soybean.

The overall conclusion from data collected about *N. petersiana* reveals that it is a good source of a good number of nutrients such as proteins, carbohydrates and some selected minerals

such as calcium, phosphorus, zinc and iron. The amino acid profile of its protein shows that it has a well balanced amino acid content with the only limiting amino acid being lysine. Nyika has a high amount of carbohydrates, and only a small fraction of this is in the form of soluble sugars. It is not a good source of fat since it has less than 3 % crude fat.

There is a very low content of ascorbic acid in the tuber, therefore it is not a good source of this vitamin. However, since this vitamin is water soluble and also heat-labile, it is possible that some of the vitamin might have been lost during the transportation of this tuber from Malawi to Blacksburg, Virginia, and also during processing.

Determination of antinutrients showed that the tuber has some antinutrients such as tannins, phytates, tyrpsin and chymotrypsin inhibitors, but comparison of the values found in this tuber to literature values of the amounts of these antinutrients found in maize and other root tubers eaten in Malawi shows that the values found in this tuber are much lower, and perhaps not of nutritional concern. Processing method had a significant effect on the content of the antinutrients, with the cooked samples having significantly ($P < 0.05$) lower content of phytates, trypsin and chymotrypsin inhibitors.

CHAPTER 7
SUGGESTIONS FOR FUTURE RESEARCH

Results from this study point out the need for further research which would allow us to obtain more comprehensive data on this tuber as well as additional information about edible wild foods eaten in Malawi. Suggestions for future research are as follows:

1. Bioassays with experimental animals need to be done in order to determine the amino acid availability and protein quality of this tuber by determining one or more of the following: protein efficiency ratio (PER), net protein ratio (NPR) and net protein utilisation (NPU).
2. From the results of the chemical scores, calculations can be made on the complementary foods rich in lysine which can be mixed with the tuber to improve its protein quality.
3. Sensory evaluation of the mixtures (suggested in 2 above) could be done with either children or mothers or both, to determine the acceptability of the new mixtures.
4. Data from this study show that the tuber has a well balanced amino acid content which is limiting only in lysine. Presently the predominant weaning food being used in the rural areas in Malawi, is a thin porridge made from maize flour (*phala*), whose protein content has been found to be only 0.9/100g (Courtright & Canner, 1995). *N. petersiana* flour could be used to complement or even substitute as a weaning food. Future research could concentrate on finding the best foods and best ways to complement this tuber to make up for its low lysine content and use it as a weaning food.
5. Since the vitamin C content obtained from the tuber samples in this study was very low, more research needs to be done on fresh tubers to determine the vitamin C content. Besides ascorbic acid, other vitamins (like vitamin A) could also be assayed.
6. More research needs to be done to determine the nutritional content of other species of the genus *Nymphaea* growing in Malawi. Since this work started, attention has been drawn to the existence of two other Nyika species which are sweet-tasting and which are also eaten by the people of Chikwawa and Nsanje. Determination of the nutrient content of these other species would broaden the nutrient data base for this group of wild edible root crops.

6. Wild plants provide basic energy (caloric), vitamin, and mineral needs. Despite publication of nutritional composition tables for some African wild plants (Leung, 1968), data on most are unavailable. If compositional data were available, important decisions could be made by agriculturalists and economic planners. Systematic efforts need to be initiated to develop a nutrient database to report energy, vitamin and mineral composition data as well as antinutrients in edible wild plants in Malawi, where there is a paucity of information on the nutrient content of most edible wild foods. It is also vital to develop a nutritient database for edible wild species not only in Malawi but the whole of sub-Saharan Africa. Such data could be of enormous nutritional and economic return.
7. While there is some work done to identify wild plants in Malawi (Williamson, 1975), the nutritional composition of most is unknown. There is need for sustained research on edible wild plants (cereals, root crops, vegetables, and fruits) in Malawi, research that can be set within the context of geography and rural agricultural development. Underutilized/ exploited wild plants like *Nymphaea petersiana* and others should be considered a research priority within agricultural development programs.
8. Teams of botanists, geographers, and nutritionists should be encouraged to work together to do research on systematically examining and reviewing the potential for further development of edible wild plants in Malawi .

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APPENDICES

APPENDIX A

Ash Determination (Method 7.009 AOAC 1984)

An electric muffle furnace oven was used. method used was as follows:

`1. Samples (5g) were weighed into an acid-washed and clean ashing dish.

2. Samples were placed in muffle furnace whose final temperature was brought to 575 °C, in several automated steps. The samples were incinerated overnight until a light gray ash was obtained.

3. After ashing, the samples were cooled in a dessicator and weighed soon after reaching room temperature

4. Ash values were calculated as follows:

   % Ash = [(Residue wt.) ÷ (Sample wt.)] x 100

APPENDIX B

Moisture Determination (Method 7.007 AOAC, 1984)

1. Moisture dishes and covers were dried for 1 hour at 130 °C, cooled in desiccator and were then weighed prior to use.
2. Samples (2-3g) were weighed onto the tarred moisture dishes.
3. Dishes were uncovered and placed on oven (Fisher Scientific Isotemp® Oven, Model 6555G, Fair Lawn, NJ) shelf with covers under the dishes. The shelf in the oven was at the level of the thermometer bulb.
4. Samples were heated for 2 hours after the oven temperature had reached 135 °C.
5. After 2 hours, samples were covered with lids, removed from the oven and transferred
   to dessicator quickly for cooling. Dishes were weighed after reaching
   room temperature (45-60 minutes).
6. Replicate determinations had to be within 0.2%.
7. % moisture = [A ÷ B] x 100
   A = Moisture loss
   B = Original weight of sample

APPENDIX C

Protein Determination (Method 2.057 AOAC, 1984)

Equipment Needed:

1. Kjeldahl flasks (800 ml capacity)
2. Digestion heaters
3. Buchi apparatus

Reagents needed:

1. Concentrated sulfuric acid (H_2SO_4) (93 - 98 %, (Nitrogen free)
2. Catalyst - The catalyst used was a 2% mixture of copper sulfate in sodium sulfate (copper sulfate was the catalyst and sodium sulfate was added to elevate the boiling point)
3. Antibumping agent
4. Sodium hydroxide (NaOH) solution (nitrogen free): 450 g solid NaOH in 1L water (specific gravity = 1.36 or more).
5. Methyl-red methylene blue indicator. Mix 2 parts 0.2% alcoholic methyl red solution with 1 part of 0.2% alcoholic methylene blue solution
6. Standardized H_2SO_4 (about 0.1N)
7. Boric acid-methylene blue receiver solution. Add 360 g boric acid (crystals) and 48 ml methyl-red-methylene blue indicator to 18L water.

Procedure

1. Sample (1g) was placed into a digestion flask.
2. A catalyst and 25 ml of concentrated H_2SO_4 were added to the flask. The sample was digested till a clear blue solution was obtained. It was then digested for 30 minutes longer and cooled.
3. Samples were placed under an automated Buchi system.
4. A blank was run periodically, using all reagents except sample, and values were corrected appropriately

   Protein was determined as:

$$\% N = [(V_1 - V_2)N_1.f]/ E \text{ mg} \times 1400$$

% Protein = % N x CF

Where:

V_1 = Consumption of acid from titration

V_2 = Consumption of acid, blank determination

N_1 = Normality of the acid

f = Factor of the acid

E = Quantity of the sample in mg

CF = Conversion factor (A factor of 6.25 was used to convert % N to % protein)

APPENDIX D

Total Dietary Fiber Determination (Sigma (1991) Fiber Assay Kit Method)

Apparatus Needed

1. Fritted crucible - porosity #2 (coarse 40 -60 microns)
2. A vacuum source. A vacuum pump or aspirator equipped with an in-line vacuum flask
3. An air oven capable of operating at 105 °C
4. Dessicator with desiccant
5. Muffle furnace, 525 °C
6. Boiling water bath
7. Constant temperature water bath adjustable to 60 °C with a multistation magnetic stirrer
   to provide constant agitation during enzymic hydrolysis
8. Beakers - 400 ml tall form
9. Analytical balance capable of weighing to 0.1 mg
10. pH meter

Procedure

1. Weigh out four 1-gram samples of each material to be tested, into 400 ml beakers, Sample weights should not differ by more than 20 mg
2. Add 50 ml of pH 6.0 phosphate buffer to each beaker
3. Add 0.05 ml α-amylase (Sigma Product No. A-3306) solution to each beaker and mix well.
4. Cover each beaker with aluminum foil and place in a boiling water bath for 30 minutes. Shake beakers gently at 5 minute intervals. The 30 minute incubation time starts when internal temparature of beakers reach 95 °C
5. Cool solutions to room temperature
6. Adjust pH of solutions to 7.5 ± 0.1 by adding to 10 ml of 0.171 N NaOH to each beaker. Check pH with pH meter. Adjust pH, if necessary, with either NaOH or H_3PO_4
7. Add 5 mg of protease (Sigma Product No. P-3910) to each beaker. Make a 50

mg/ml solution in phosphate buffer immediately before use and then pipet 0.1ml into each beaker

8. Cover beakers with aluminum foil and incubate for 30 minutes at 60 °C with continous agitation. The 30 minute incubation time starts when the internal temperature of beakers reach 60 °C.
9. Cool solutions to room temperature.
10. Add 10 ml of 0.205 M H_3PO_4 to each beaker to adjust pH of solutions to 4.5 ± 0.2. Check pH with pH meter. Adjust pH carefully, if necessary, with either NaOH or H_3PO_4.
11. Add 0.3 ml of Amyloglucosidase (Sigma Product No. A-9913) to each beaker.
12. Cover each beaker with aluminum foil and incubate for 30 minutes at 60 °C with continous agitation. The 30 minute incubation time starts when the internal temperature of beakers reach 60 °C.
13. Add 280 ml or 4 volumes of 95 % ethanol, preheated to 60 °C (measure volume after heating), to each beaker.
14. Precipitate at room temperature for at least 60 minutes or overnight. Precipitation time should be approximately the same for all samples and blanks
15. Filtration: Wet and redistribute the bed of celite in each crucible using 78 % ethanol. Apply suction to draw celite onto fritted glass as an even mat. Maintain gentle suction and quantitatively transfer precipitate and suspension from each beaker to its respective crucible. Wash residue with three 20-ml portions of 78 % ethanol, two 10-ml portions of 95% ethanol, and then two 10-ml portions of acetone.
16. Dry crucibles containing residues overnight in a 105 °C air oven.
17. Cool all crucibles in dessicator, weigh to nearest 0.1 mg and record this weight as 'Residue + Celite + Crucible Weight.

    Residue Weight = (Residue + Celite + Crucible Weight)

    - (Celite + Crucible Weight).
18. Analyze residue from each of two samples and two blanks for protein using Kjeldahl analysis.

    Use 6.25 as the factor in all cases to convert ammonia determined in the Kjeldahl

to protein.

19. Ash the residue in the crucibles from two samples and two blanks for 5 hours at 525 °C. Cool in desiccator, weigh to the nearest 0.1 mg and record this as 'Ash + Celite + Crucible Weight.'

Ash Weight = (Ash + Celite + Crucible Weight) - (Celite + Crucible Weight)

% Total Dietary Fiber

$$\frac{\text{Av. wt. of sample residues (mg)} - \left[\left(\text{Av. \% protein in samples} + \text{Av. \% ash in sample residues}\right) \times \text{(Av. wt. of sample residues)} - \text{Blank}\right] 100}{100 \times \text{Average weight of samples (mg)}}$$

$$\text{Blank} = \text{Av. wt. of blank residues} - \left[\left(\text{Av. \% protein in blank residues} + \text{Av. \% ash in blank residues}\right) \times \text{Av. wt. of blank residues (mg)}\right]$$

APPENDIX E

SUMMARY OF STATISTICAL DATA ANALYSIS

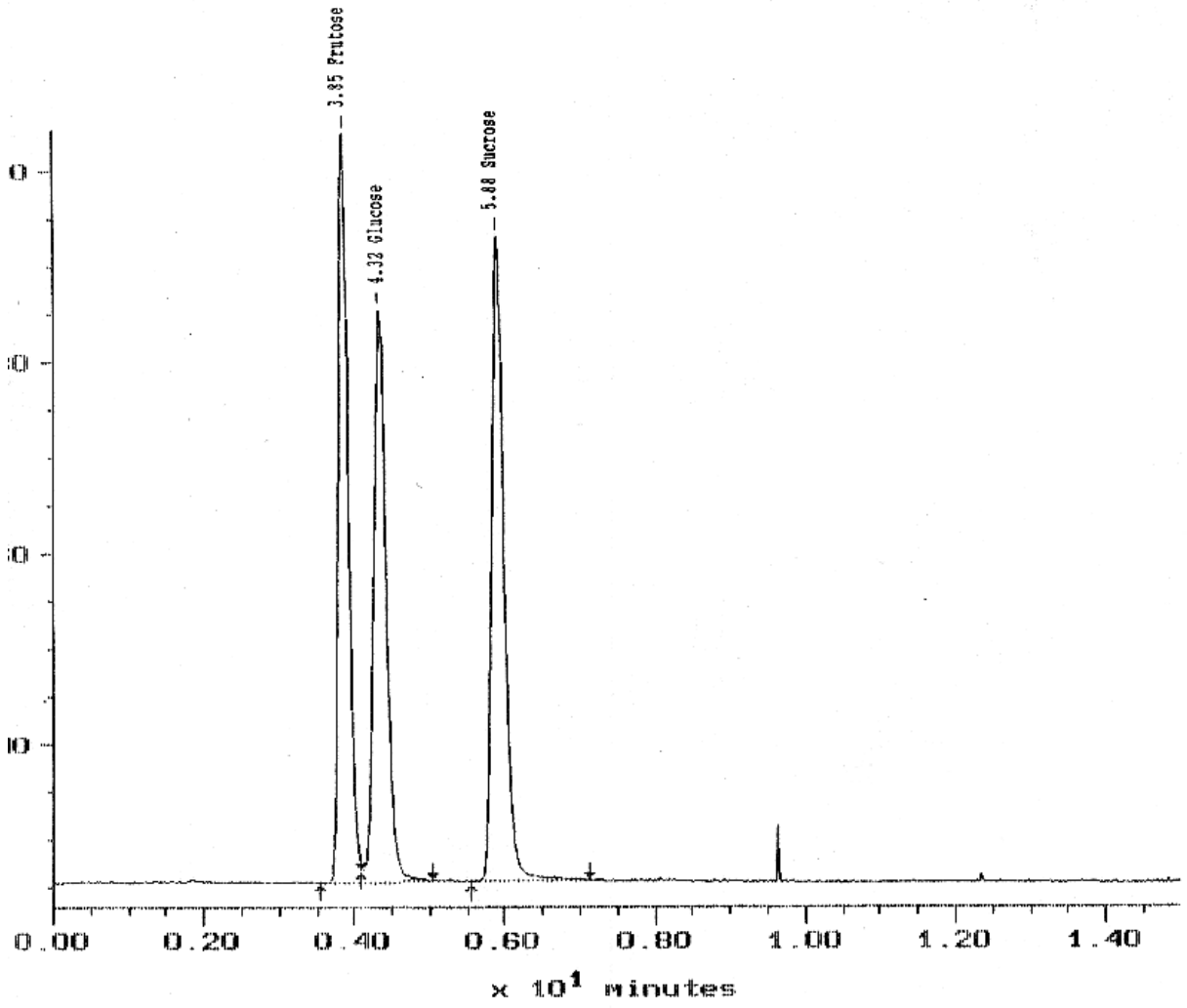
The Results Are Based On Value Of P $\leq$ 0.05

Proximate Analysis

Variable	**P-value**	**Result**
Moisture	0.0001	Significant
Protein	0.6748	Not Significant
Ash	0.0266	Significant
Fat	0.0464	Significant
Carbohydrates	0.0027	Significant
Minerals		
Calcium	0.2021	Not Significant
Phosphorus	0.1448	Not Significant
Zinc	0.1063	Not Significant
Iron	0.0271	Significant
Essential Amino Acids		
Threonine	0.1817	Not Significant
Cys + Met	0.1411	Not Signicant
Valine	0.3910	Not significant
Leucine	0.0163	Significant
Isoleucine	0.8361	Not Signicant
Phe + Tyr	0.0663	Not Signicant
Lysine	0.0220	Significant
Tryp	0.3955	Not Significant

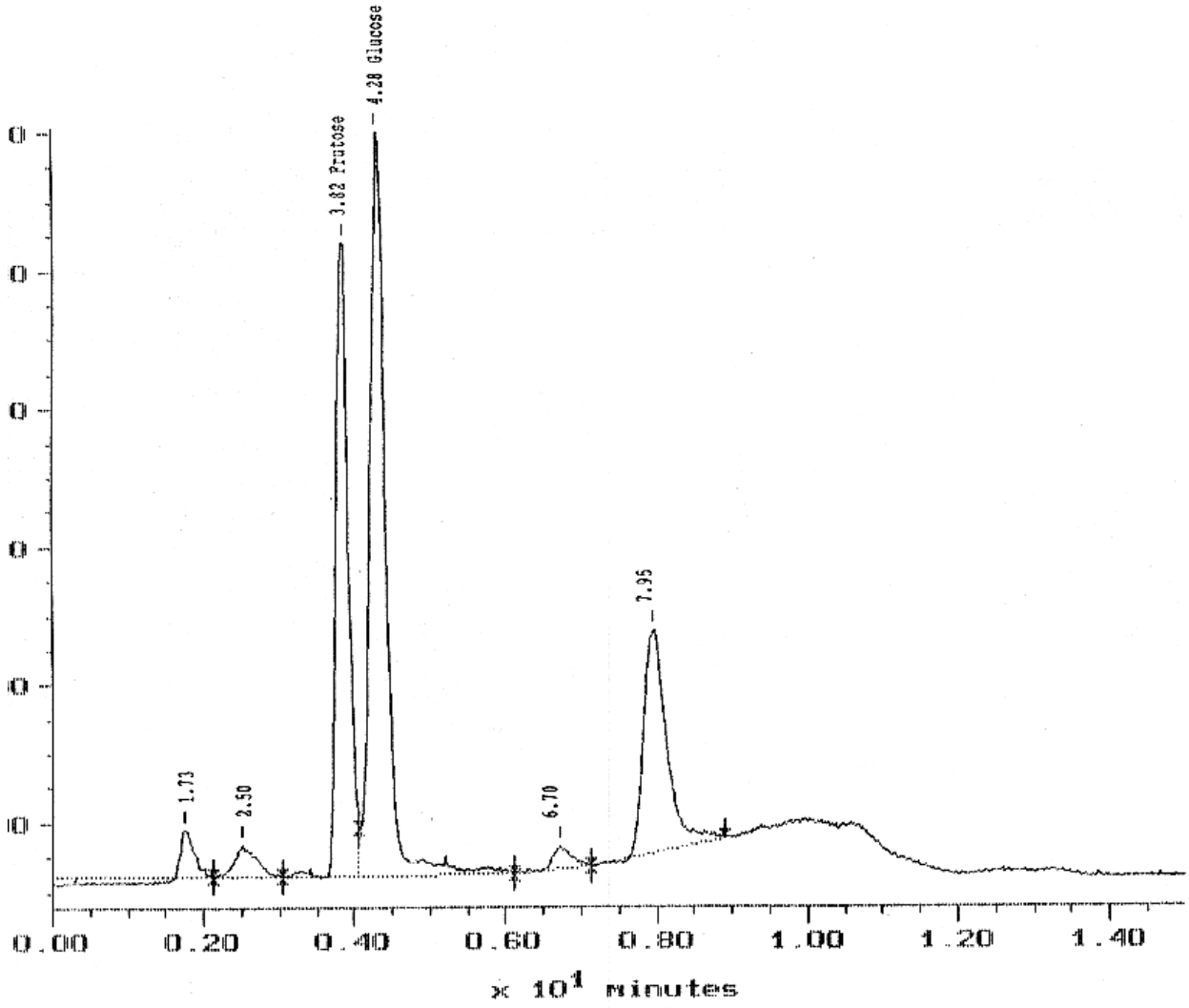
Variable	P-value	Result
Soluble Sugars		
Fructose	0.0240	Significant
Glucose	0.0404	Significant
Sucrose	0.0917	Not Significant
Fatty Acids		
Palmitic Acid	0.0397	Significant
Stearic Acid	0.4216	Not Significant
Oleic Acid	0.5823	Not Significant
Linoleic Acid	0.7434	Not Significant
Linolenic Acid	0.0604	Not Significant
Vitamins		
Ascorbic Acid	0.0447	Significant
Antinutrients		
Tannin	0.2483	Not Significant
Phytate	0.0308	Significant
Trypsin	0.0592	Not significant
Chymotrypsin	0.0001	Significant

APPENDIX F



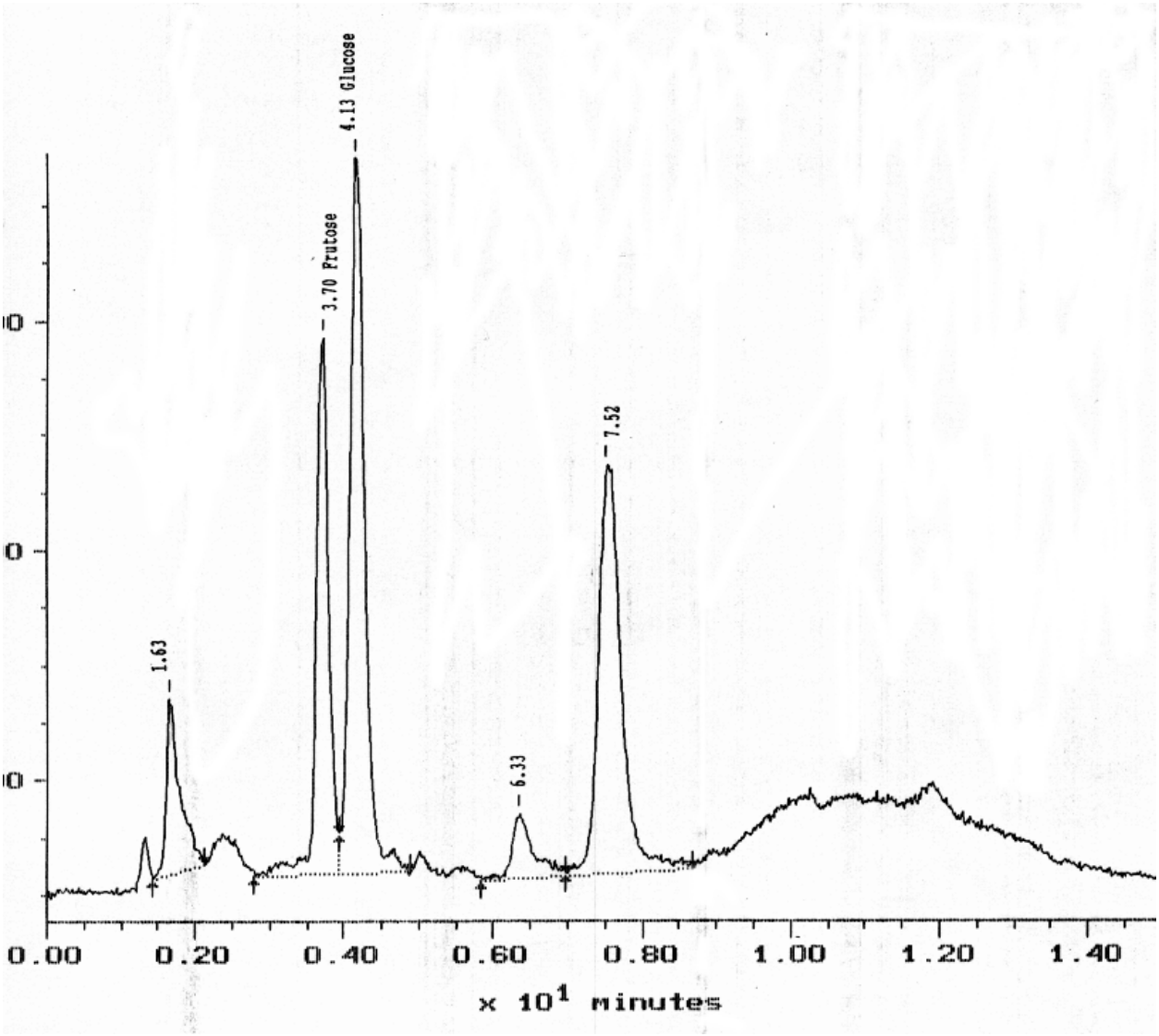
Chromatogram of Standards for the sugars - fructose, glucose and sucrose
(Johnson and Harris, 1987)

APPENDIX G



Chromatogram of the composition of soluble sugars in *Nymphaea petersiana* tubers - Boiled Sample (Chigumukire)
(Johnson and Harris, 1987)

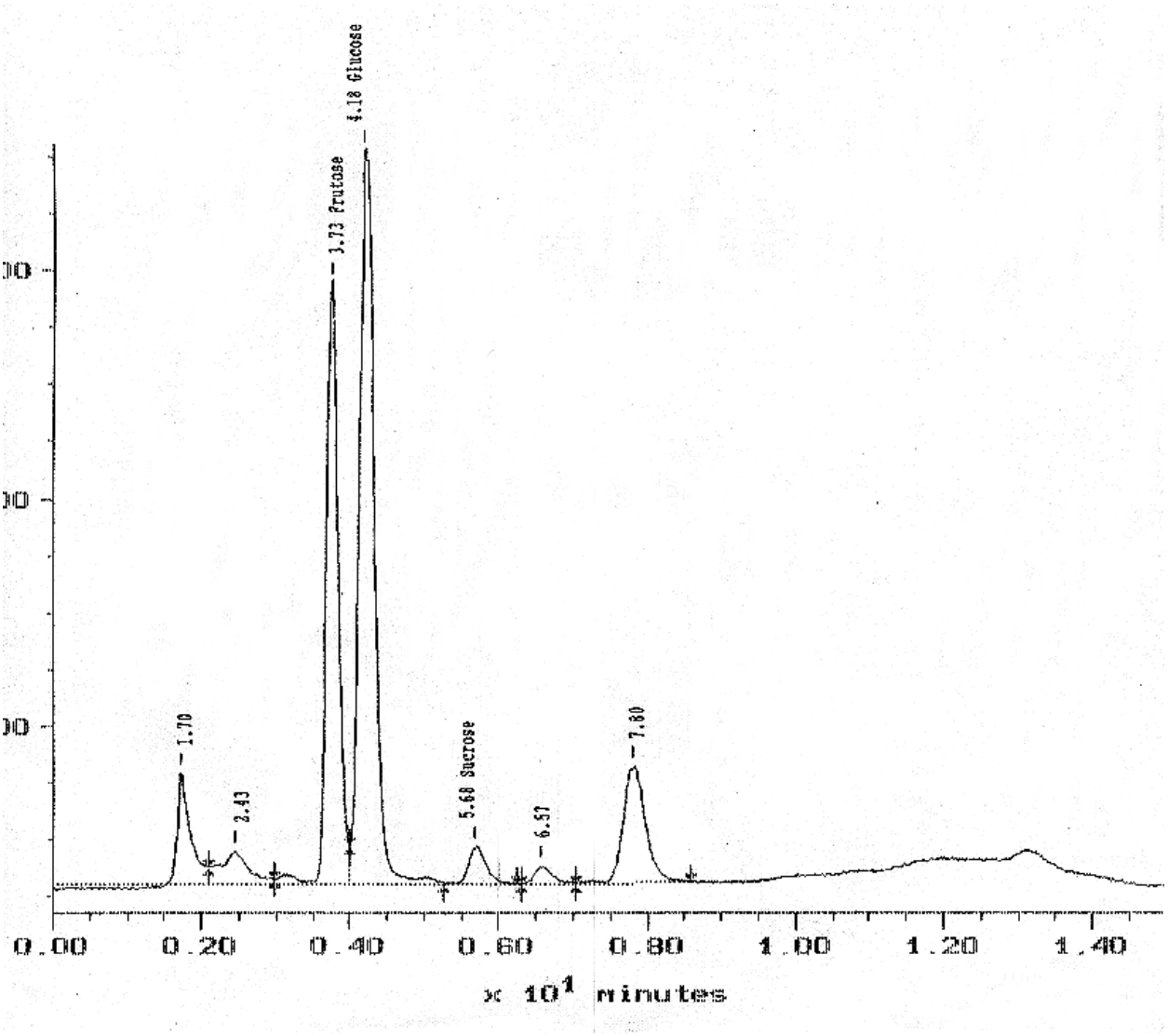
APPENDIX H



Chromatogram of the composition of soluble sugars in *Nymphaea petersiana* tubers -Boiled Sample (Kademera)

(Johnson and Harris, 1987)

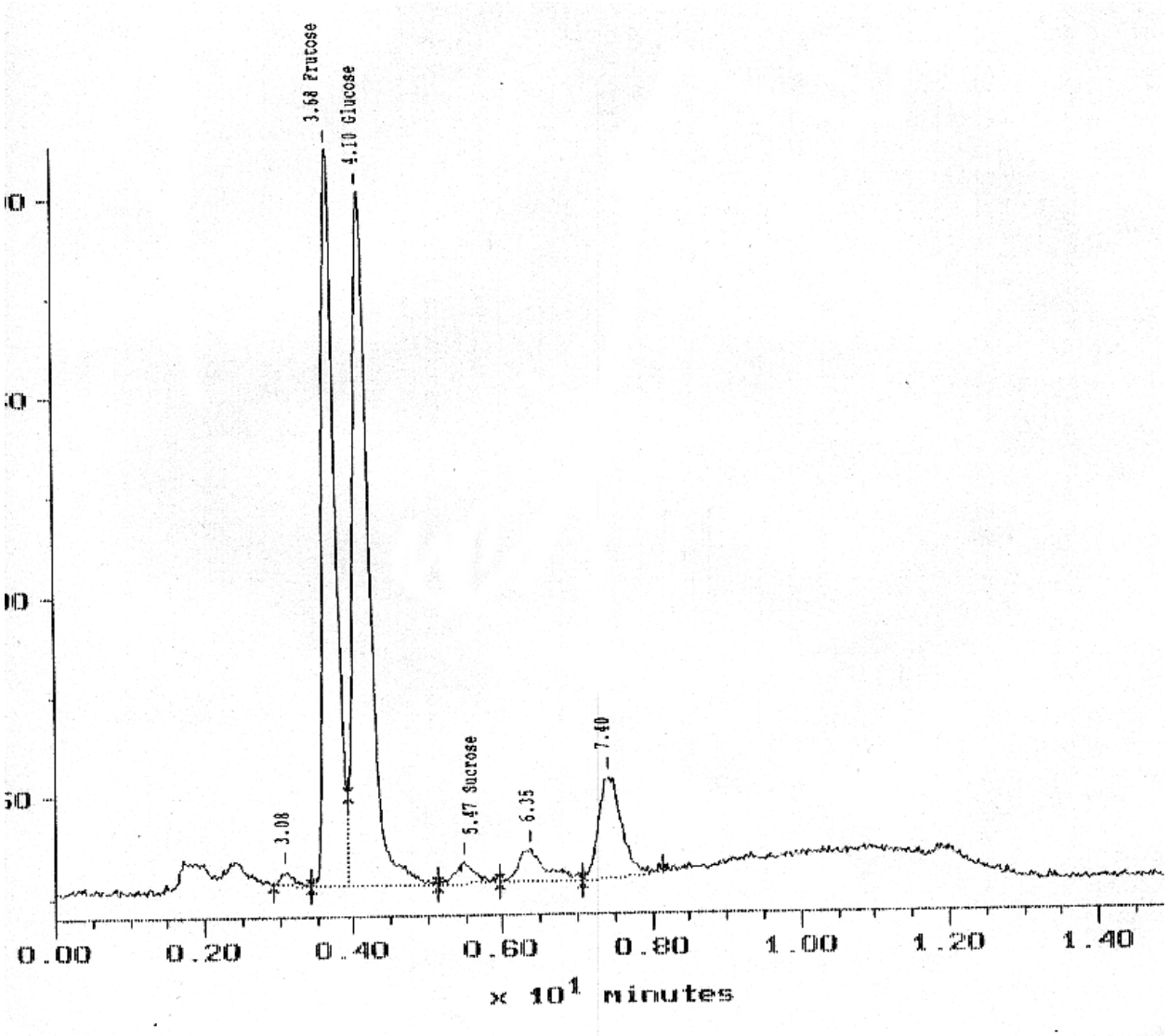
APPENDIX I



Chromatogram of the composition of soluble sugars in *Nymphaea petersiana* tubers - Uncooked Sample (Chigumukire)

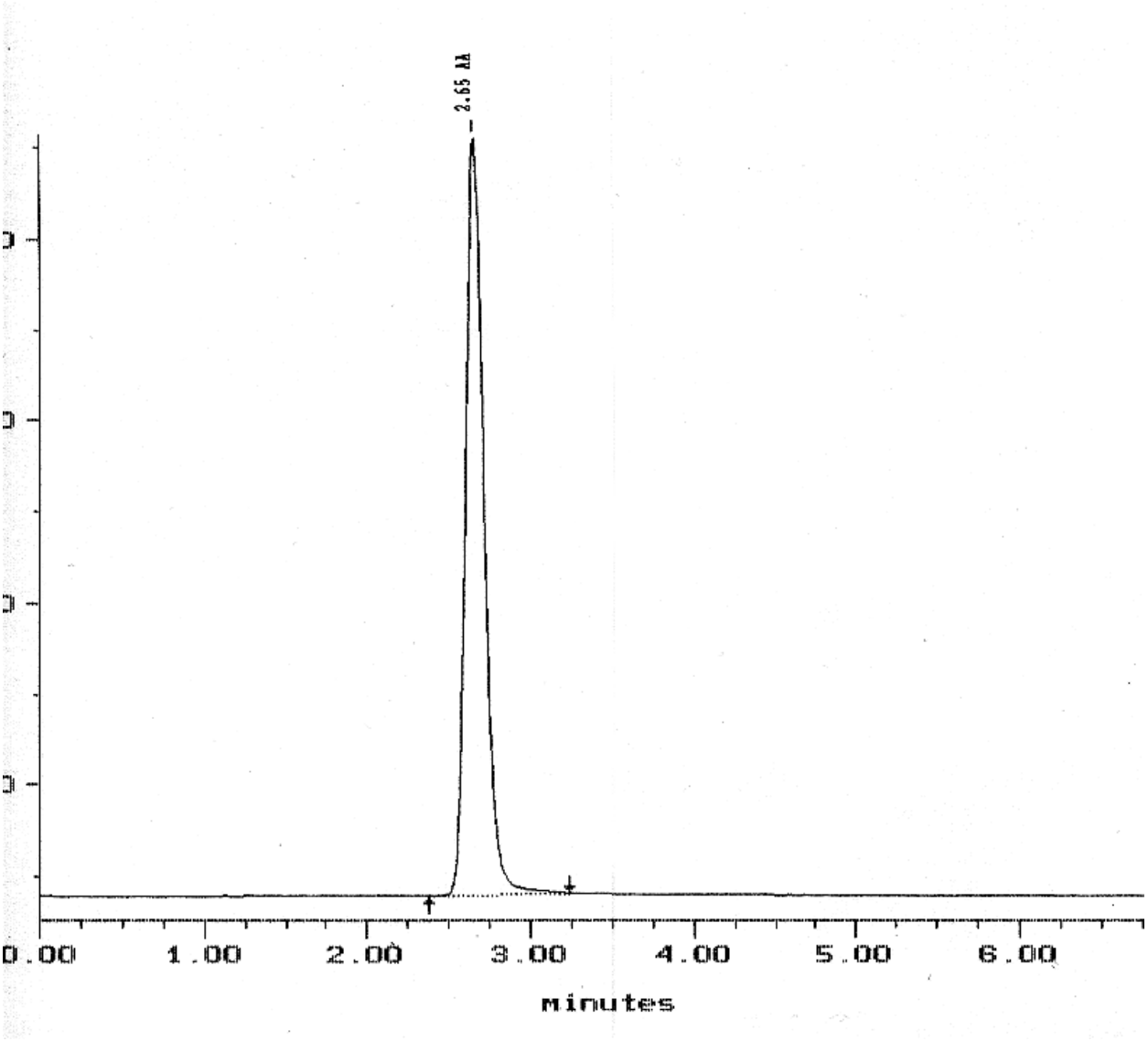
(Johnson and Harris, 1987)

APPENDIX J



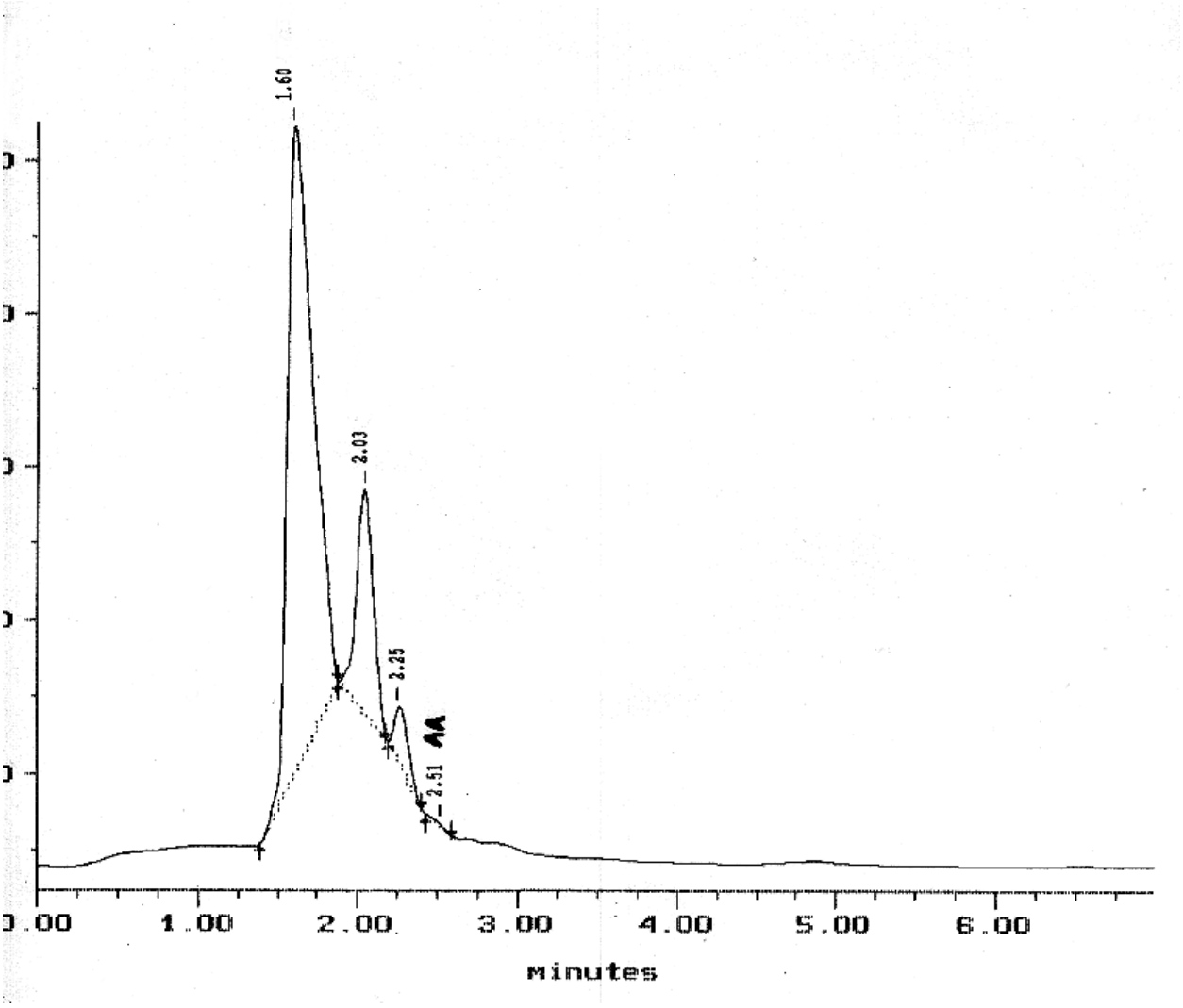
Chromatogram of the composition of soluble sugars in *Nymphaea petersiana* tubers - Uncooked Sample (Kademera)
(Johnson and Harris, 1987)

APPENDIX K



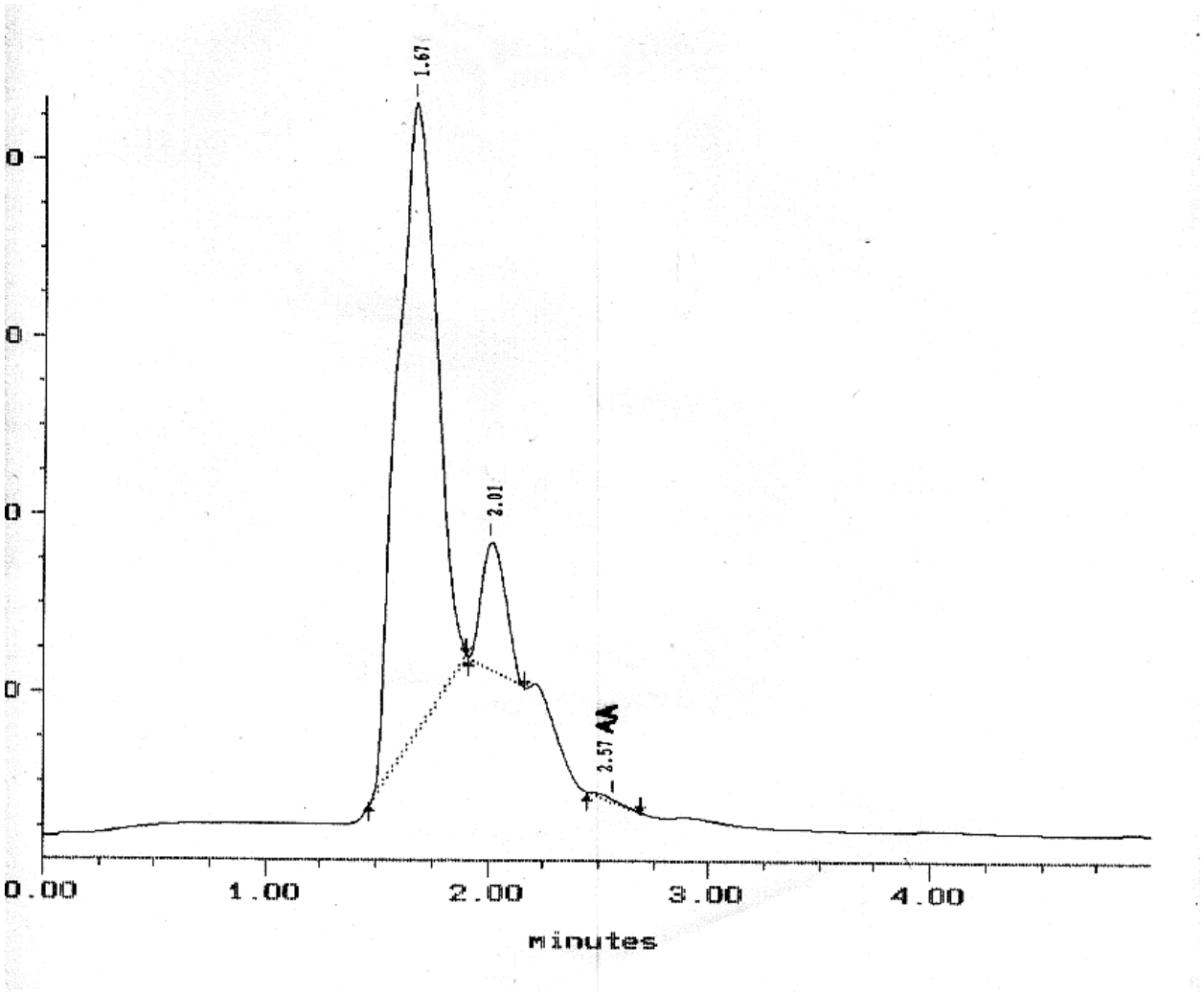
Chromatogram of ascorbic acid (AA) Standard (Wimalasiri and Wills, 1983)

APPENDIX L



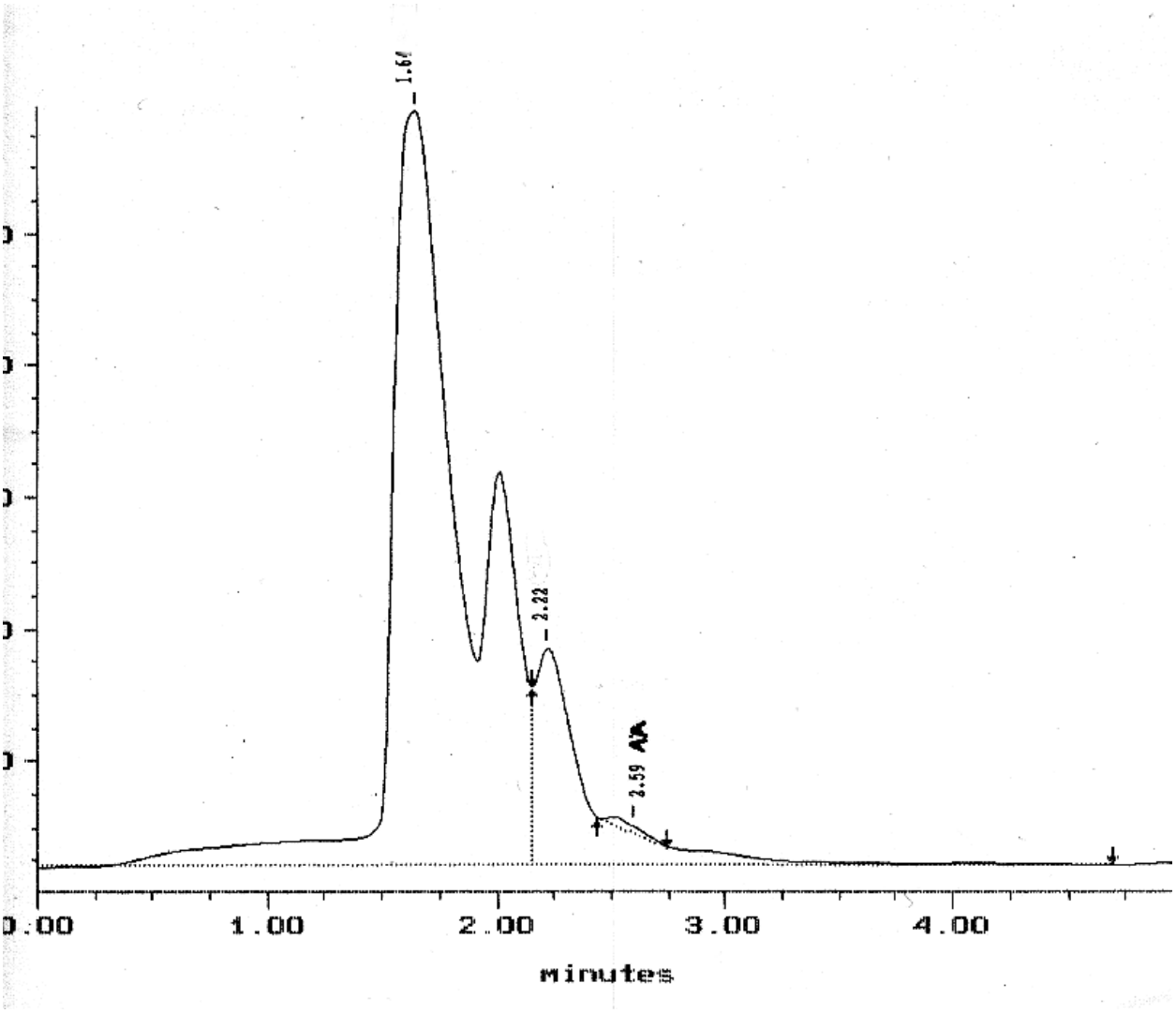
Chromatogram of ascorbic acid (AA) content in *Nymphaea petersiana* tubers - Boiled Sample (Chigumukire)
(Wimalasiri and Wills, 1983)

APPENDIX M



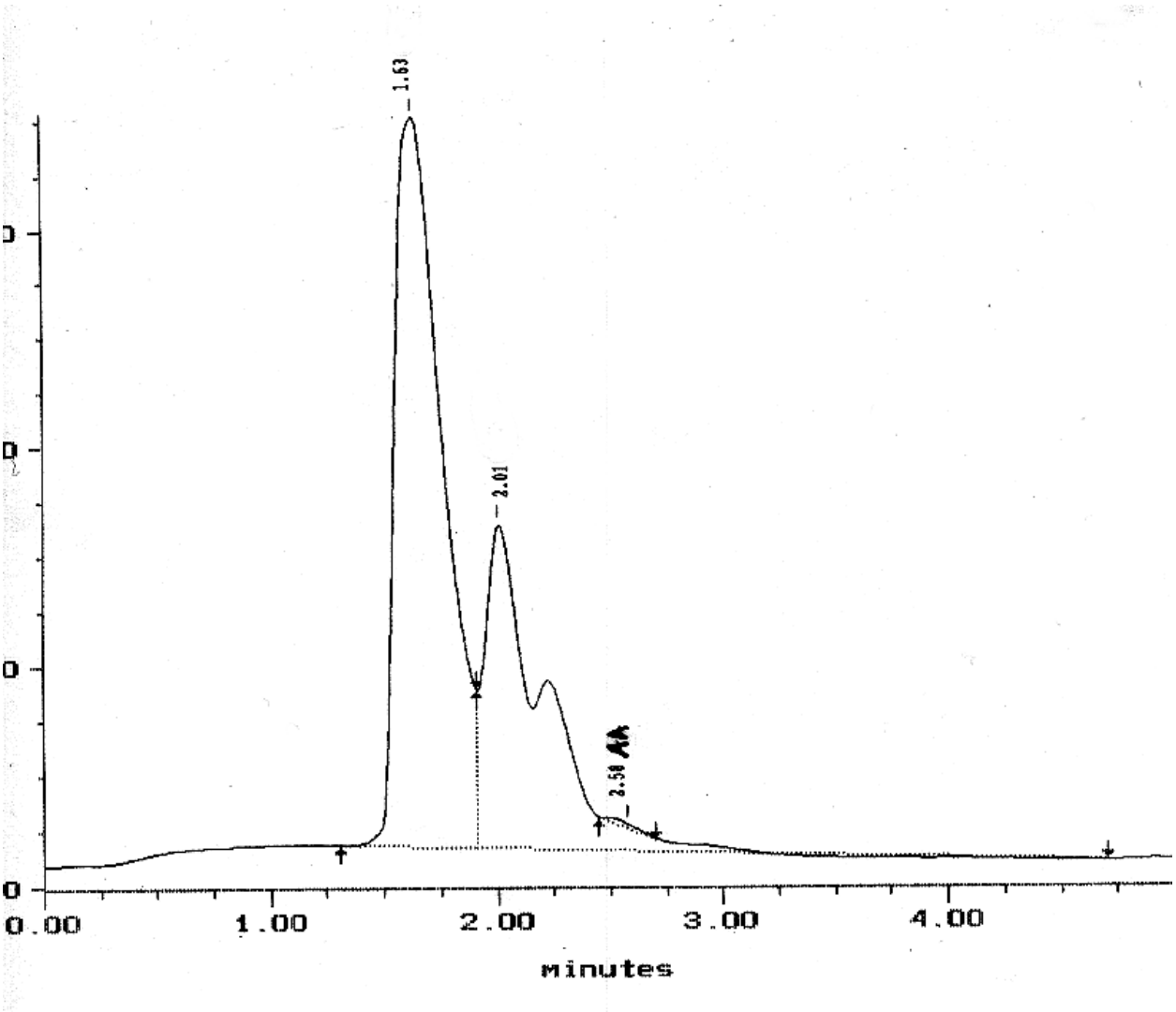
Chromatogram of ascorbic acid (AA) content in *Nymphaea petersiana* tubers - Boiled Sample (Kademera)
(Wimalasiri and Wills, 1983)

APPENDIX N



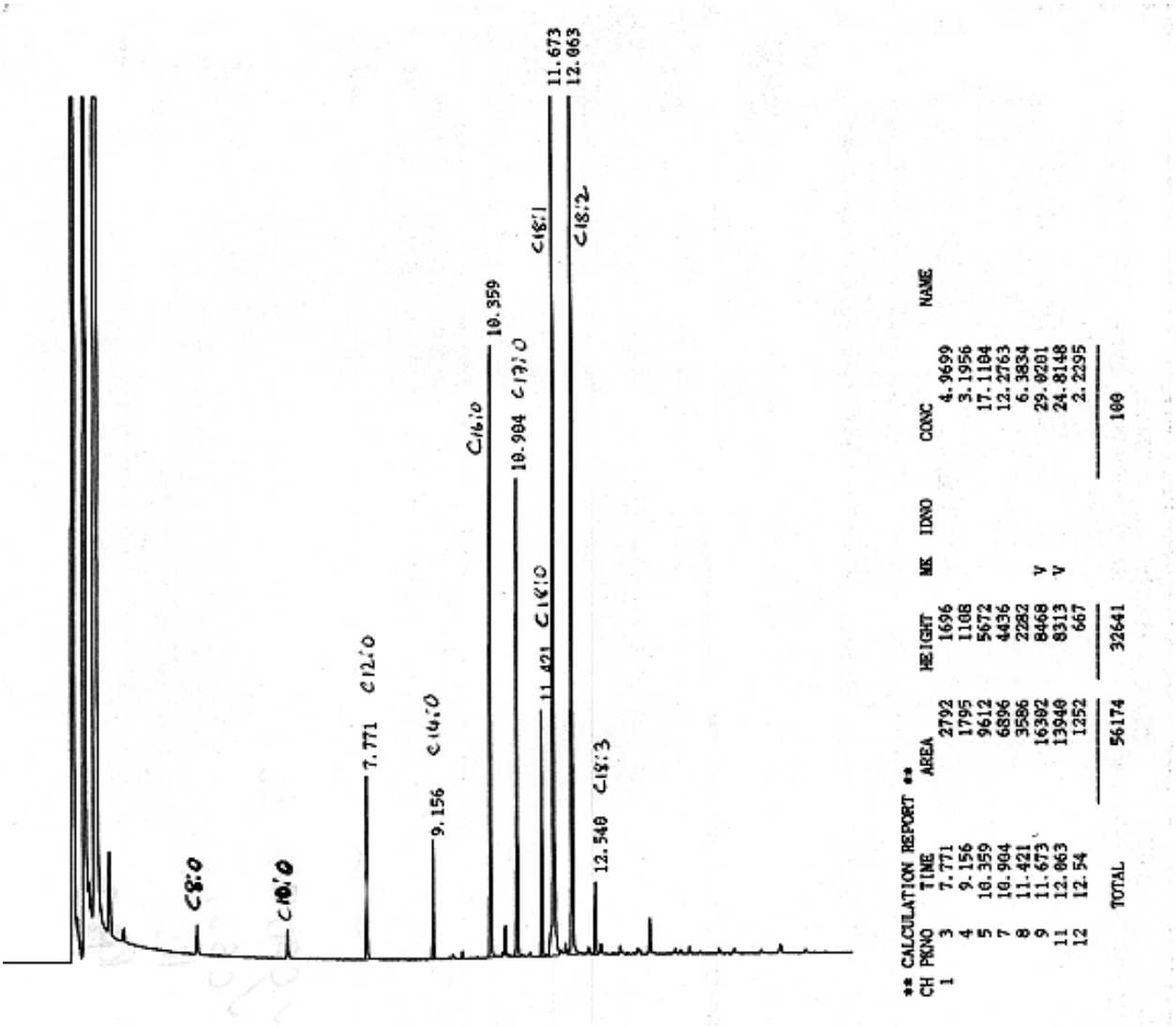
Chromatogram of ascorbic acid (AA) content in *Nymphaea petersiana* tubers - Uncooked Sample (Chigumukire)
(Wimalasiri and Wills, 1983)

APPENDIX O



Chromatogram of ascorbic acid (AA) content in *Nymphaea petersiana* tubers - Uncooked Sample (Kademera)
(Wimalasiri and Wills, 1983)

APPENDIX P

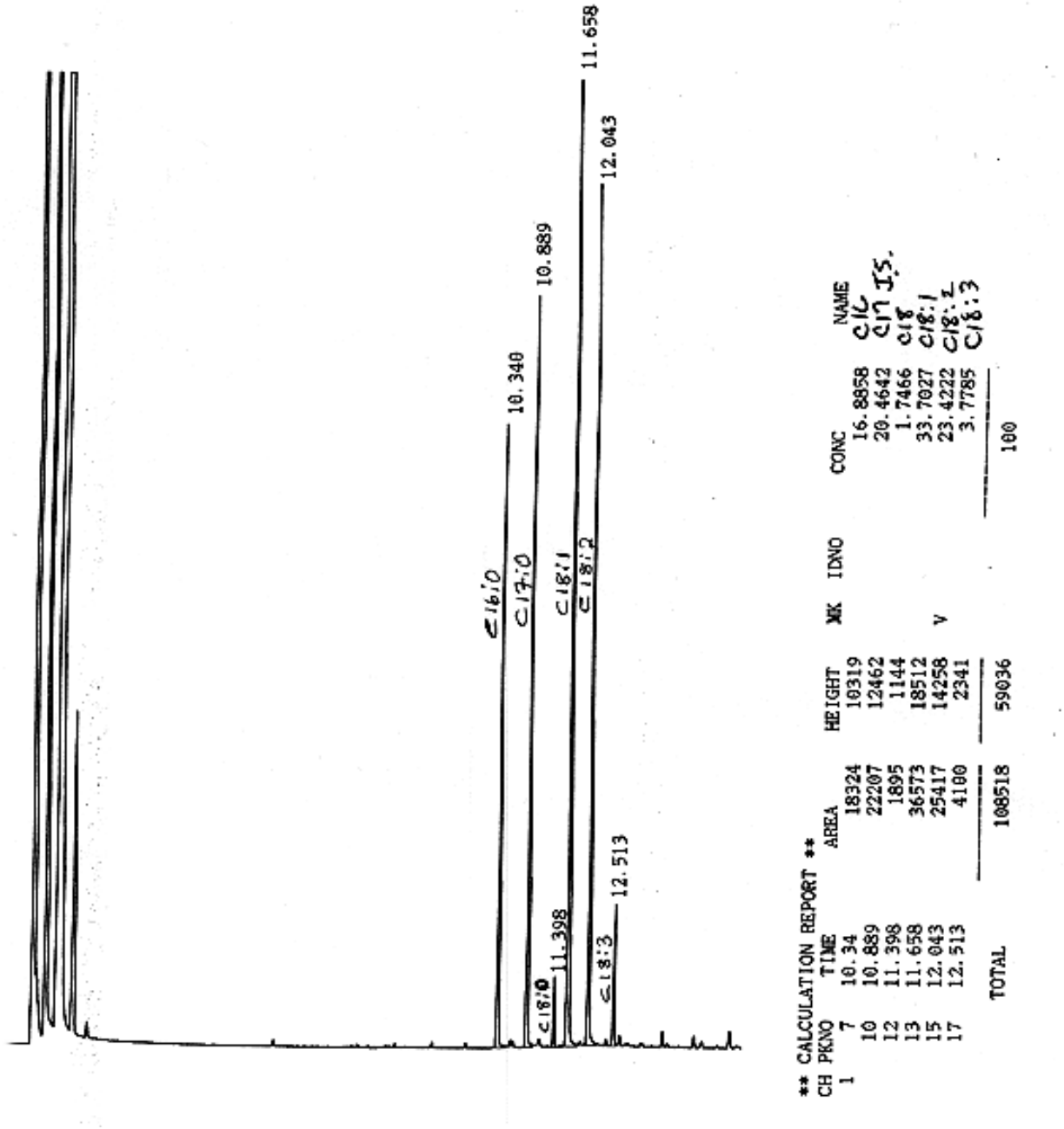


** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	3	7.771	2792	1696			4.9699	
	4	9.156	1795	1108			3.1956	
	5	10.359	9612	5672			17.1184	
	7	10.904	6896	4436			12.2763	
	8	11.421	3586	2282			6.3834	
	9	11.673	16382	8468	V		29.0201	
	11	12.863	13940	8313	V		24.8148	
	12	12.54	1252	667			2.2295	
		TOTAL	56174	32641			100	

Chromatogram of fatty acid composition in standard reference material (SRM 1544) - A frozen diet composite
(Phillips et al., 1997)

APPENDIX Q

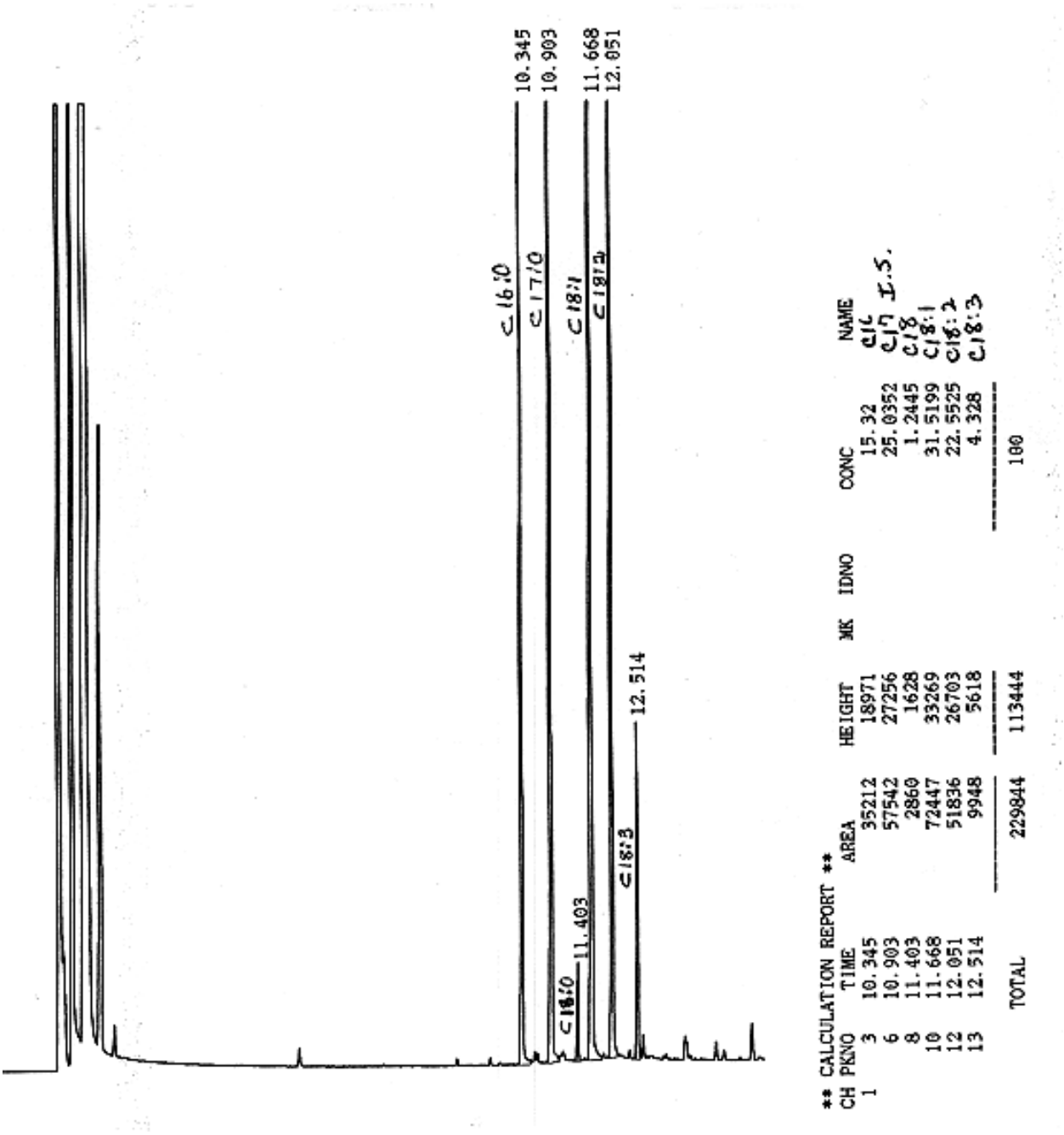


** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	7	10.34	18324	10319			16.8858	C16
	10	10.889	22207	12462			20.4642	C17 IS.
	12	11.398	1895	1144			1.7466	C18
	13	11.658	36573	18512			33.7027	C18:1
	15	12.043	25417	14258	V		23.4222	C18:2
	17	12.513	4100	2341			3.7785	C18:3
		TOTAL	108518	59036			100	

Chromatogram of fatty acid composition in *Nymphaea petersiana* tubers -Boiled Sample (Kademera)
(Phillips et al., 1997)

APPENDIX R



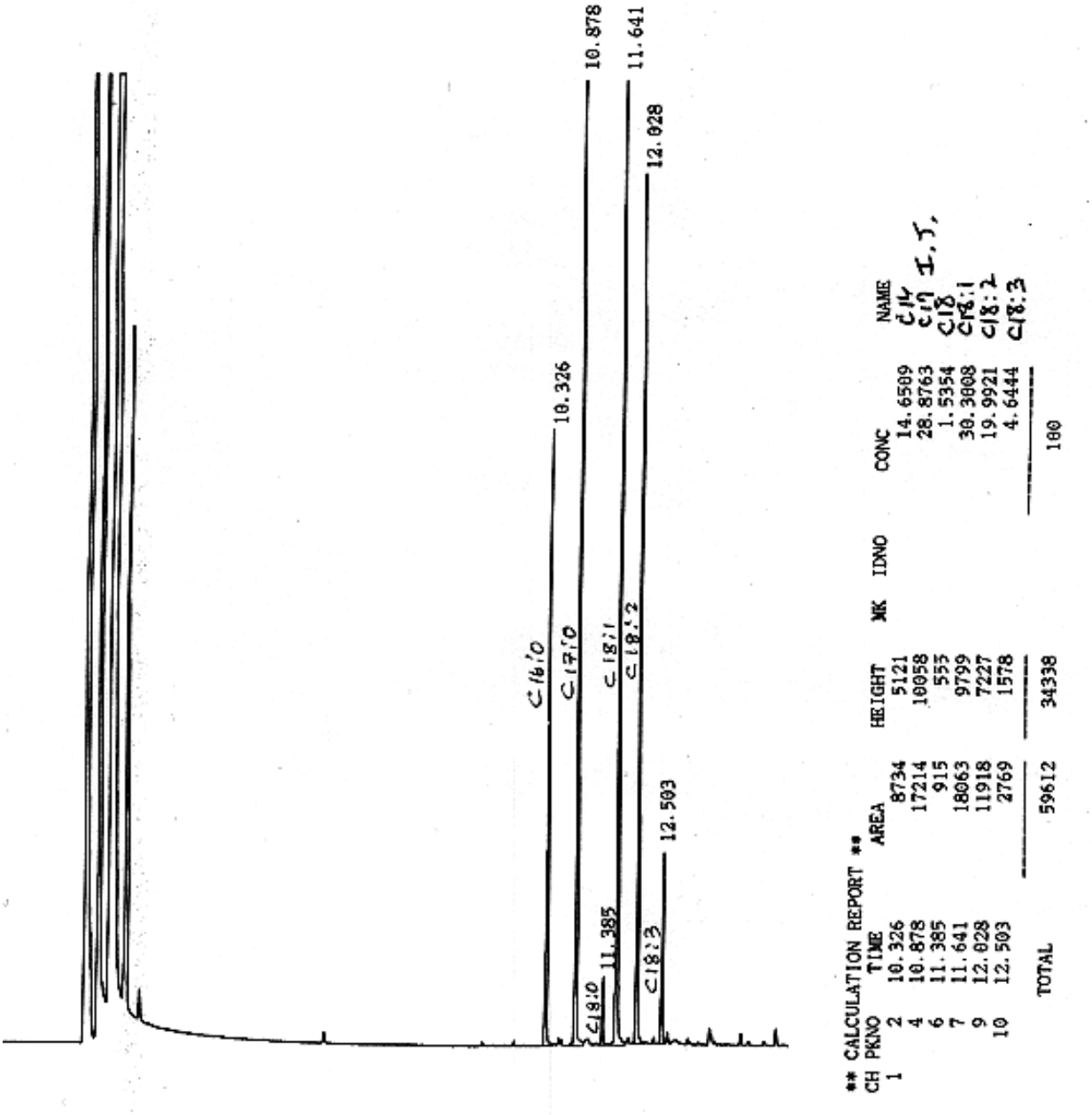
** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	3	10.345	35212	18971			15.32	C16
	6	10.903	57542	27256			25.0352	C17 I.S.
	8	11.403	2860	1628			1.2445	C18
	10	11.668	72447	33269			31.5199	C18:1
	12	12.051	51836	26703			22.5525	C18:2
	13	12.514	9948	5618			4.328	C18:3
		TOTAL	229844	113444			100	

Chromatogram of fatty acid composition in *Nymphaea petersiana* tubers - Boiled Sample (Chigumukire)

(Phillips et al., 1997)

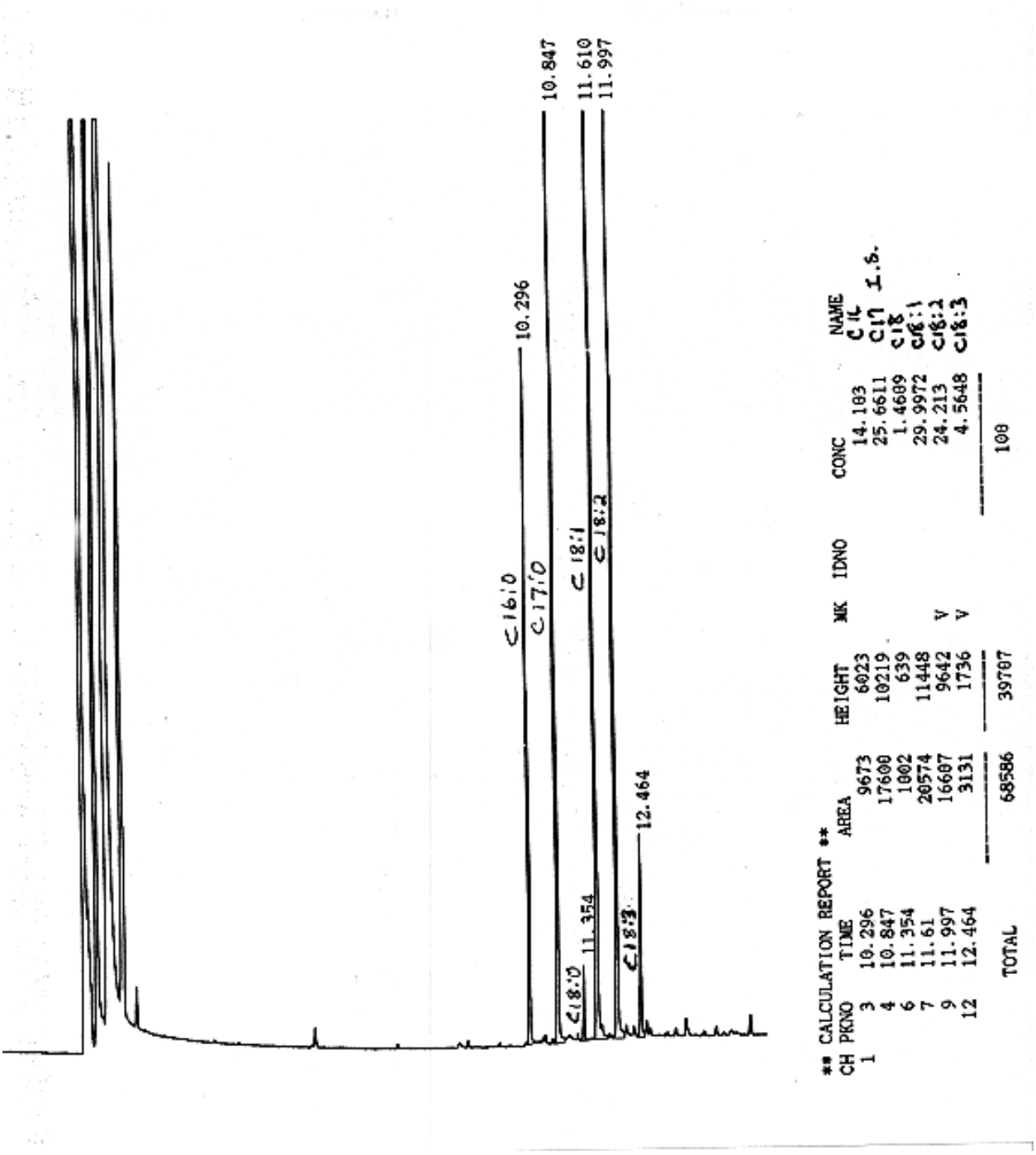
APPENDIX S



Chromatogram of fatty acid composition in *Nymphaea petersiana* tubers - Uncooked Sample (Kademera)

(Phillips et al., 1997)

APPENDIX T



** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	3	10.296	9673	6023			14.103	C16
	4	10.847	17600	10219			25.6611	C17 I.S.
	6	11.354	1002	639			1.4609	C18
	7	11.61	20574	11448	V		29.9972	C18:1
	9	11.997	16607	9642	V		24.213	C18:2
	12	12.464	3131	1736	V		4.5648	C18:3
		TOTAL	68586	39707			100	

Chromatogram of fatty acid composition in *Nymphaea petersiana* tubers - Uncooked Sample (Chigumukire)

(Phillips et al., 1997)

VITA

The author, Chrissie Maureen Chawanje is from Thyolo District, Southern Malawi, South-East Africa. She got her B.S. in biology and chemistry at Chancellor College , University of Malawi in 1974. She did her M.S. in biology at Ball State University, Muncie , Indiana USA in 1977. She is currently on a study leave from the Malawi Polytechnic, a constituent college of the University of Malawi, where she is a senior lecturer in the department of Applied Sciences.

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