VARIABILITY OF IRON, ZINC AND PROTEIN CONCENTRATION IN COMMON BEAN GENOTYPES

BY

LUNJALU, JORAM OKOTSI, BSC.
(Food Science and Technology)

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DECLARATION

This is my original work and has not been presented for a degree in any other University.

JORAM LUNJALU OKOTSI — **DATE** 20-6-07

This Thesis has been submitted with our approval as University Supervisors.

PROF. P.M. KIMANI — **DATE** 12/07/07

Department of Plant Science and Crop Protection
University of Nairobi

DR. A.M. MWANGI — **DATE** 5/07/07

Department of Food Science, Nutrition and Technology
University of Nairobi

DR. J.K. SEHMI — **DATE** 26/6/07

Department of Food Science, Nutrition and Technology
University of Nairobi

DEDICATION

I would like to dedicate this work to my parents; Mr. Josephat Okotsi and Nerea Okotsi, who have always desired to see me excel in all my endeavours.

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ABSTRACT

One prerequisite for breeding micronutrient dense bean cultivars is availability of adequate genetic variation. However the iron, zinc and protein concentration of most bean cultivars and landraces widely grown in Africa is not known. The objective of this study was to screen local bean varieties for iron, zinc and protein concentration in order to identify varieties with high mineral and protein values, acceptable grain characteristics and to evaluate some factors influencing bioavailability of these nutrients.

Thirty-eight lines of the common bean *Phaseolus vulgaris* L. commonly grown in East and Central Africa were grown for their seed in Kabete field station, University of Nairobi. Leaf samples of the same varieties were sampled from farm plots grown by farmers in Marani and Suneka Divisions of Kisii Central District in Nyanza Province.

Raw and cooked seed samples were analysed for their iron, zinc and protein concentrations in the whole bean seed and the condensed tannin concentration of the raw seed coat. Water absorption, cooking time and sensory properties of the seed were also determined. Attributes evaluated included colour, flavour, texture and overall acceptability

Results showed highly significant ($P<0.001$) differences in iron, zinc, protein and tannin concentrations, water absorption capacity and cooking time of the grain. These differences were influenced by both variety and treatment. Iron concentration in raw beans ranged from 69.9 to 107.1 mg/kg with average of 87.1 mg/kg. The ten varieties

with the highest iron concentration were; Jesca, MLB-48-89A, Mwa Mafutala, TY-3396-12, VCB 81013, Nain de Kyondo, AND 620, VNB 81010, Soya Fupi and G59/1-2. Zinc concentration varied from 26.9 to 43.9 mg/kg with mean of 35.9 mg/kg. The top most promising lines for zinc concentration included AND 620, VNB 81010, Nain de Kyondo, Nakaja, MCM 2001, Masai Red, Mexican 142, GLPX 92, RWR 10 and Ituri Matata. Protein content varied between 20.4 % and 28.4 % with mean of 25.9 %. The following lines had the highest protein concentration: Lib-1, AFR-708, G59/1-2, Selian 97, Mexican 142, RWR 10, VNB 81010, MCM 2001 and Lingot Blanc. With few exceptions, nutrient concentration in cooked samples were lower than in the corresponding uncooked ones. Retention of iron, zinc and protein averaged between 87 and 78.7 %, 87 % and 72.6 % and 89 % and 86.9 % respectively in cooked and soak-cooked bean samples. Variability of these nutrients among leaf samples was more than in the grain. Iron values ranged from 236 to 1962 mg/kg with mean of 830 mg/kg. Zinc concentration ranged from 17.4 to 94.7 mg/kg with average of 48.3 mg/kg. Protein concentration varied from 23.7 % to 35.6 % with mean of 29.3 %. It was found that iron had a statistically significant ($p = 0.01$) positive correlation with zinc of ($r = 0.5$) in seed and ($r = 0.36$) in leaf across different genotypes.

Condensed tannin concentration ranged from trace to 15 %. There was significant ($p<0.001$) variation in tannin concentration among varieties. The tannin concentration were correlated with seed coat pigmentation. Dark coloured beans had more tannins than light coloured beans. Red coloured beans had a mean tannin concentration of 8.5 mg/g whole seed while white coloured beans had no detectable values.

It was also found that soaking significantly reduced bean cooking time by 33% on average. Ten varieties with the shortest cooking times were: Awash Melka, Mwamafutala, Kiangara, Lib-1, K132, Zebra, Ranjonomby, Kirundo, MLB 49-89A and Simama. There was no clear relationship between water absorption and cooking time ($p=0.717$). Sensory evaluation showed significant differences ($p<0.001$) in colour, texture and overall acceptability among bean lines. AND 620, GLP X 92, HRS 545, Jesca and Nain de Kyondo were identified as the most prefered. The lines identified for their good nutritional qualities were: AND 620, G59/1-2, GLP-2, Jesca, K132, Maasai Red, Mexican 142, MLB-49-89A, MwaMafutala, Nain DeKyondo, Roba-1, Soya fupi, TY 3396-12, VCB 81013 and VNB 81010.

The study established that several bean cultivars and landraces grown in East and Central African region had high iron, zinc and protein values and acceptable grain characteristics. Furthermore, results indicate that sufficient genetic variability exists to improve iron content by 22% and zinc content by 25%.

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ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectrophotometer
ANOVA	Analysis of Variance
ANP	Applied Nutrition Program
AOAC	Association of Official Analytical Chemists
CIAT	International Centre for Tropical Agriculture [Centro Internacional de Agricultura Tropical]
CNS	Central Nervous System
DM	Dry matter
FAO	Food and Agriculture Organization of the United Nations
FST	Food Science and Technology
GDP	Gross Domestic Product
GIT	Gastro Intestinal Tract
ha	Hectare
Hb	Haemoglobin
M-F	Male-Female
mg	Milligram
NGO	Non governmental organization
nm	Nanometre
PEM	Protein energy malnutrition
PM	Pico mole
RBC	Red blood cell
RBP	Retinol binding protein
RDA	Recommended daily allowance
µm	Micromole
UN	United Nations
UNICEF	United Nations Children Fund
WHO	World Health Organization
CIMMYT	Centro Internacional de Mejoramiento de Maiz Trigo
CIP	Centro International de la Papa
IRRI	International Rice Research Institute

CHAPTER ONE

1.0 INTRODUCTION

1.1 Statement of the Problem

Micronutrient deficiency is a global problem, affecting over 3.5 billion people, with the majority being in developing countries (UN, 2000). The most affected Regions include: South and South East Asia, Sub Sahara Africa and Latin America.

Iron deficiency anaemia has been shown to affect over 1 billion people world wide (UN, 1993). In Kenya WHO reported rates of 20 – 90 %, with the highest incidences being in Coastal and Western Kenya. These results have been corroborated by a 1999 national survey on anaemia prevalence in Kenya (Mwaniki et al., 1999). It was also found that iron deficiency was accompanied by zinc deficiency, with more than 50 % of the women and children in some regions eliciting zinc deficiency as well.

Iron and zinc deficiency in Kenya was mainly found to be as a result of low intake (Van Steenbergen et al, 1980; Mwaniki et al., 1999). This was attributed to consumption of staple diets, which are low in minerals and protein, coupled with financial limitation to purchase animal products rich in bioavailable iron and zinc, and ascorbic acid rich diets. Other causes were found to be food insecurity, high infection rates by parasites leading to recurrent secondary chronic haemolysis, morbidity factors such as sickle cell disease, malaria, respiratory diseases and malabsorption disorders. Deficiency of other nutrients such as vitamin A, vitamin B12, folate and increased physiological requirements in children and pregnant women were also cited.

Iron and zinc deficiencies are important as they have serious public health and socio-economical implications. According to WHO, iron deficiency anaemia is known to interfere with the individual sense of well being. People suffering this deficiency are often apathetic, unmotivated and fatigued. It diminishes their physical fitness leading to reduced physical work capacity and hence becoming economically unproductive. Among children, iron and zinc deficiency may lead to impaired cognitive functions of the brain, increased distractibility hence diminishing learning ability. Other than high recurrent medical expenditure, anaemia contributes to the overall mortality associated with malnutrition, while during pregnancy, it poses threat to life and health of the mother at time of delivery and contributes to low birth weight and thus low chances of survival by the infant. Zinc deficiency on the other hand has been shown to have effects on growth, metabolic rate and reduced immune system, formation of small gonads in males, impaired brain function and slow healing of wounds and burns. Such health and socio-economical issues posed by these micronutrient deficiencies coupled with recurrent expenditures in medication and supplements demands urgent solutions to reduce human suffering. These inabilities have enormous health economic and socio-economical implications to the individual and the nation as a whole. It is against this background that the government and development partners in health and related sectors have formulated strategies and pursued various approaches to ameliorate their effects and ease the deficiency burden.

1.2 Justification

Food fortification and supplementation have been the most frequently employed approaches to alleviating micronutrient deficiencies. These are, however, recurrent and require huge financial inputs and efficient management. Lack of funds due to economic and social crises makes sustainability of these programs by most African governments impossible (Smith, 2000), hence the need to explore other intervention options.

Biofortification is probably the most effective, sustainable and potentially long lasting strategy for reducing micronutrient deficiency in Kenya and other developing countries in Africa and the world at large. The underlying course and the fundamental constraint of micronutrient malnutrition problem is that non-staple foods, particularly animal products tend to be the foods richest in bioavailable micronutrients, which the poor in developing countries cannot afford. In some cases cultural beliefs and religious taboos that bar sections of the society such as women, children or their adherents from consuming certain foods especially chicken and meats aggravates the situation. For the poor, this therefore, leaves staple foods as the primary source of micronutrients, from which they obtain up to 40 – 55 % of the total iron intake. However our staples, which include cereals, root crops and vegetables, are deficient in iron, zinc and protein and aggravate the problem. Therefore, an approach that focuses on biofortification of food staples addresses the problem of the poor. This strategy thus aims to increase the production, availability and access to local foods rich in micronutrients. The consumption of these locally available micronutrient rich foods by target populations and improved bioavailability of the micronutrients in these diets is likely to improve the nutritional

status of target communities without resorting to programs that depend on behavioural change. This strategy holds promise for significantly reducing expenditures prompted by high cost, short run programs, by significantly reducing the number of people requiring treatment.

Development of mineral rich bean cultivars can contribute to the alleviation of micronutrient (iron and zinc) deficiencies in Kenya because: common bean *(Phaseolus vulgaris*) is an important pulse crop in the diets of many families in Kenya. It is cultivated on about 670,000 ha per year (Kimani, personal communication). About 253,800 tonnes were produced and consumed in 1995/6 (GOK, 2000). However, demand for beans exceeds supply. In recent years, Kenya imported from the neighbouring countries of Uganda and Tanzania to meet the deficit. The per capita consumption among the poor may reach 50 – 66 kg / year (Wortman et al., 1998). Beans are relatively cheap compared to other sources of minerals and proteins such as meat. Beans cost Kshs 30 (>$0.5) per kg in the local market compared for Kshs. 190 (>$2.5) for a kg of beef at the time of this study. Bean proteins also complement cereal/root protein in vegetarian diets. Beans fit well in many cropping systems. Other than family consumption, part of the bean crop is often commercialized, bringing in much needed income for other family needs. Beans have well recognized nutritional qualities. They are a major source of protein and minerals especially iron and zinc (Bourne, 1989). Iron concentration of beans is much higher than that of maize and other milled cereals. Zinc concentration in beans is among the highest from vegetable sources and almost equal to dairy products (Pennington and Young, 1990). Beans also provide vitamin B and significant amounts of other micronutrients that are especially important to pregnant women.

One pre-requisite for breeding micronutrient dense bean cultivars is availability of adequate genetic variation. However, iron and zinc concentration of bean cultivars and land races widely grown in African is not yet known. The purpose of this study was, therefore, to determine variability of iron, zinc and protein in some bean cultivars and land races grown in East and Central Africa in order to identify varieties with high mineral values and acceptable grain characteristics for immediate use and evaluation of some factors influencing availability of these nutrients. The specific objectives of the study were:

1. To determine iron, zinc and protein concentration of some bean lines grown in East and Central African through quantitative laboratory analysis. .
2. To determine the concentration of condensed tannins in the selected bean varieties.
3. To determine cooking time of the bean varieties.
4. Evaluate organoleptic acceptability of mineral dense bean varieties.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Approaches to Reduce Micronutrient Deficiency

The government and development partners in health and related sectors recognize consequences of anaemia and priority micronutrient deficiencies to the socio-economic development of Kenya. The socio-economic cost of these micronutrient deficiencies and their implications to the quality of life for majority of Kenyans is enormous. It is against this background that strategies have been formulated and various approaches pursued to ameliorate their detrimental effects and ease the deficiency burden.

2.1.1 Food fortification

The Codex Alimentarius Commission of the United Nations defines food fortification as, the addition of one or more essential nutrients to a food, whether or not it is normally contained in the food, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population group. The nutrients may be added as extracts or concentrates of materials of biological origin, or as products of chemical or biochemical synthesis (UN, 2000). Food fortification aims at restoring the nutrients lost during food processing by enriching a food with the depleted nutrient or increasing the level of the nutrient in the food. In both cases, fortification may increase the intake of specific nutrients identified as inadequate in a population (Nestel, 1993)

To ensure that food fortification reaches the nutritionally vulnerable groups in the population, certain requirements must be met. The food vehicle must be a staple food consumed widely throughout the year and must pass through central processing points. The level of fortification must contribute significantly to the nutritional requirements but must not exceed the safe upper limit. The fortificant must not alter the organoleptic properties, physical structure or shelf life of the vehicle. Control and monitoring procedures must be built into the manufacturing procedures to ensure that fortification levels are adequate.

2.1.1.1 Strengths

Food fortification is socially acceptable. It does not require the active participation of the consumers or any change in buying, cooking, or eating habits. Fortification, in most cases, does not affect the organoleptic properties of food products. It can be introduced quickly, and the benefits are readily visible. Of various interventions, food fortification is the least costly and the most effective way to eliminate dietary micronutrient deficiencies (Nestel, 1993).

2.1.1.2 Limitations

According to International Nutritional Anaemia Consultative Group (INACG, 1981), food fortification is less likely to benefit people who consume locally produced unprocessed food, because it relies on centrally processed and marketed food vehicle. Fortified foods are accessible to both target and non-target groups and may not be the most economical way to reach the target groups. If the cost of fortification is passed on to target consumers only, purchasing patterns among the intended beneficiaries may change

adversely. Fortification incurs additional costs for food manufacturers. Program success is ensured only when political will, legislation, and enforcement are present. This is yet to be realised in many African nations.

2.1.2 Supplementation

Supplementation is one of the intervention strategies of increasing nutrient intake. It uses high dose preparation such as vitamins or minerals in the form of capsules or tablets. Its main objectives include correcting pre-existing deficiencies, prevent deficiencies leading to nutritional disorders and preventing development of disorders.

2.1.2.1 Strengths

Although relevant data is still not available in over 40 % of the countries, supplementation has been shown to be cost effective in the short term relative to some food-based approaches as was reported by Phillips (Phillips, et al., 1996).

The other advantages of supplementation are that it can be organized relatively quickly at reasonable cost and can produce quick results so that their impact is discernable within a short period of time.

2.1.2.2 Constraints

However major constraints facing supplementation programs especially in sub-Sahara Africa are poorly functioning health infrastructures to administer supplement distribution. Transportation facilities are poor hence inefficient distribution strategy.

Data collection tools equipment and trained manpower are inadequate or lacking altogether. Lack of emphasis on iron supplementation programs for children and low compliance with tablet intake among pregnant women has hampered supplementation programs (Schultink, 1996). Lack of funds due to economic and social constraints makes sustainability of a supplementation program by the African governments difficulty. Hence the increasing calls within international development and health circles for all concerned to actively explore other intervention options (Smith, 2000)

2.1.3 Food Based Approaches (Biofortification)

Biofortification is a fundamental strategy for solving nutrient deficiency problem in any population or group on a sustainable basis by ensuring adequate intake of foods containing a range of specific nutrients. Food-based intervention strategies thus aim to increase the production, availability and accessibility to local foods rich in micronutrients. The consumption of locally available micronutrient rich foods by the target population and improved bioavailability of micronutrients in these diets will improve the nutritional status of target communities without resorting to programs that depend on behaviour change. Research in improvement of nutrient content of staple foods has gained momentum with more attention being paid to improvement of micronutrient content of various crops. The target micronutrients are iron, zinc and vitamin A. Case studies have been undertaken by various research organizations to improve micronutrient content of various staple crops namely beans, rice, sweet potatoes, and maize.

2.1.3.1 Research on trace minerals in the common bean

Food legumes in general contain appreciable qualities of iron and other minerals. Although legumes are often cited as a complement to cereals in terms of amino acid concentration, they also make a particularly important contribution to micronutrient nutrition. It has been found that increasing iron deficiency in India could be as a result of decreasing per capita consumption of legumes, illustrating their importance in the diet (UN, 1993).

The common bean (*Phaseolus vulgaris L.*) is the most important grain legume for direct human consumption especially in Eastern Africa and Kenya in particular. Consumption is particularly high among the rural poor for example in Kisii region of Kenya and the great region including Rwanda, where per capita consumption may reach as much as 50 to 66 kg / year (Wortmann, et al., 1998)

In Kenya and Africa in general, beans are important sources of nutrients for young children as they are often used as weaning foods (UN, 1993). By the sixth month about 50 % of breast feeding children receive a diet supplemented by grains. Non breast-feeding children receive grains even earlier. Local diets often include potatoes, maize meal, cassava, bananas as carbohydrate source while beans provide much of the protein (Jansen, et al., 1987). The daily per head consumption was estimated at 40-120 g (depending on the season). This makes Kenya one of the countries where higher mineral beans would make significant contribution to the diet. It is therefore important to assess the feasibility of improving the micronutrient content of common bean especially for iron

and zinc. Before this is done the following issues need to be addressed:

2.1.3.1.1 Genetic variation in iron and zinc concentration in beans

The first essential question regarding whether the micronutrient status of beans can be improved is to determine the genetic variability of species in mineral (iron and zinc) content. Work conducted in CIAT (in Colombia South America) involving evaluation of more than one thousand accessions in the cultivated common bean core collection, a mean iron concentration of 55 mg/kg was found, with a range of 34 to 89 mg/kg (Beebe, et al., 2000). A clear relationship between iron content and geographic distribution was not evident.

Evaluation of the same bean core collection revealed a range of 21 to 54 mg/kg in zinc content, with an average value of 35 mg/kg. This initial data suggests that sufficient genetic variability exists to improve iron content by 80 % and zinc content by about 50 % (Beebe et al., 2000). However an essential question for improvement of any trait through plant breading is the degree to which the trait is stable across environments. In the case of seed mineral content, one might expect an effect of varying soil types and soil chemistry over sites. Trial planting done to asses the stability of iron and zinc content over two growing environments and two seasons in one of these sites confirmed that several accessions were superior independent of sites and seasons (Beebe, et al., 2000). This offers good prospects that genotypes selected in one environment for high iron and zinc content, will express superior levels of the minerals in other environments as well, though the degree of expression of the trait may vary. Also noted during the evaluation was a

positive correlation among mineral concentrations, suggesting that improvement of one mineral may simultaneously improve the content of other minerals, thus multiplying the impact of the effort. However similar studies need to be conducted in this region to establish variability of these micronutrients in African bean germplasm.

2.1.3.1.2 Bioavailability of iron and zinc from beans

Bioavailability is the fraction of the ingested nutrient that is utilized for normal physiological function or storage (King et al., 2000). It is an important aspect, which ensures that the human subject deficient in certain nutrients in this case iron and zinc will be able to extract them from food material (beans) and utilize them within his body to ameliorate or eliminate deficiency. One of the major determinants of bioavailability of iron and zinc is the proportion that is absorbed from the gastrointestinal tract. However, tissue utilization (or lack thereof) also influences bioavailability (King, et al., 2000).

Iron and zinc deficiencies are common in populations dependent on staple diets such as roots, cereals and legumes, which are, not only low in the mineral content but also have poor bioavailability. Selective breeding of high mineral beans can improve total intake of iron and zinc. However the additional iron and zinc from these grains may not be available for absorption and subsequent utilization for normal physiological functions. Iron and zinc bioavailability therefore needs to be measured before the high mineral beans are promoted. Measurements can be done from a physiological variable, assessment of body retention, tissue or blood uptake, changes in body pool sizes or rates of absorption (King et al., 2000). Iron and zinc bioavailability from intrinsically and

extrinsically labelled normal and high mineral common bean varieties, was tested in the USA in young women with low iron stores. The absorption of intrinsically and extrinsically labelled of iron and zinc did not differ. However the bioavailability of both minerals from the two varieties was low, about 1.5% and 1.3% respectively. These results imply that there is need for development of methods to improve the bioavailability of iron and zinc from beans. The main inhibitory substances in beans that may have contributed to this low bioavailability are phytic acid and polyphenolic compounds such as tannins. Tannins are widely cited as important antinutrients that precipitate iron in food preparations or in the gut. One possible avenue for the improvement of iron nutrition is to reduce tannin content or activity (Beebe, et al., 2000). This variability was considered within the context of grain colour, since tannins are closely related to seed coat pigment. When evaluated it was actually found out that dark coloured beans had more tannins than light coloured beans. In one trial to measure iron and zinc bioavailability in rats it was shown that white beans had higher percentage of bioavailable iron: over 70% as compared to less than 50%in darker accessions. This suggests that lower tannin content in beans could be beneficial (Welch, et al., 2000). However one concern about the possibility of lowering tannin content was related to possible collateral effect, since tannins may have other functions such as resistance factors or flavour components (Beebe, et al., 2000).

Legumes also contain considerable amounts of phytic acid. Phytin is a highly charged polyanion molecule; hence a strong chelator of positively charged mineral cations such as calcium, iron and zinc preventing their absorption hence bioavailability (Raboy, 2000). A

breeding strategy of lowering the level of phytates in the grain has been suggested as a way of increasing bioavailability of minerals already consumed (Bouis, et al., 2000). However phytin is a primary storage form of phosphorous in seeds and thus contributes to the viability and vigour of the seedling produced. Selecting for grain crops with lower phytin content could have unacceptable effect on production especially in soils of low phosphorus status. Therefore the option for breeding for lower phytin content is not recommendable.

A second option to minimize the effect of phytins on mineral bioavailability is to genetically increase sulphur containing amino acids as promoters of iron and zinc uptake. Though legumes are limited in sulphur amino acids i.e. methionine and cystein, they have abundant lysine which has also been reported to improve iron uptake. The question remains whether lysine can supplant sulphur amino acids in uptake promoter role (Beebe, et al., 2000). Never the less since amino acids are essential nutrients for plants as well as humans, the option appears to be a more plausible route.

The other option of improving iron and zinc availability from beans is through nutrition education to the consumer. This should include: encouraging adoption of food preparation methods that reduce phytate content and its effect on iron and zinc bioavailability. These include soaking, precooking, germination and fermentation (Reddy, et al., 1989).

Promoting consumption of bean or legume food preparations together with absorption enhancers such as ascorbic acid, citric acid and lactic acid from fruits and milk. Diet

supplementation with small amounts of animal tissue, which has meat, fish and protein (MFP) factor, will also promote absorption of iron and zinc from non-haem sources (Whitney and Rolfs, 1999). People should be discouraged from consumption of bean food preparations together with other foods that may be high in iron and zinc absorption inhibitors, such as tannic acid and other polyphenols in tea and coffee. Other minerals like calcium and phosphorous in milk which compete with iron and zinc for absorption in the gut should also not be consumed at the same time. A point to note here is that when evaluating bioavailability of iron and zinc from food items in humans, it is important to remember that a number of factors unrelated to characteristics of food influence the proportion absorbed. These factors include previous intake of the nutrient, body status of the nutrient, gut transit time and gastrointestinal function (Kings, et al., 2000).

2.1.3.1.3 Acceptability

Finally the other consideration to be put into effect is whether the foods will be organoleptically acceptable. Though not documented there is suspicion that genetically engineered foods could have shortcomings with product appearance, texture and taste.

However micronutrients comprise a tiny fraction (approximately 10 mg/kg) of the physical mass of the seed in case of cereals. Legume seed may contain up to 100 mg/kg of micronutrients. It is therefore not expected that these small amounts will alter the appearance taste and texture or cooking quality of the food. However, even if it did, nutrition education could turn this obstacle to an advantage. This brings up a primary characteristic of food-based approach, which is the need to link with other programs.

Inputs from these programs are necessary for the food-based approach to be successful. Nevertheless, a major shortcoming of food-based intervention is the lack of quantifiable and convincing data demonstrating impact on the target population or group (Smith, 2000).

2.2 Iron

Iron is an essential nutrient vital to many of the cell activities. Most of the body iron is found in two proteins; haemoglobin in red blood cells and myoglobin in muscle cells. Because iron exists in ferrous and ferric forms it can serve as a cofactor to enzymes involved in oxidation - reduction reactions.

2.2.1 Iron absorption and metabolism

Iron is found in one of two forms in foods, haem and non-haem. Haem iron is derived mainly from haemoglobin and partly from myoglobin found in animal flesh (meat, fish and poultry). About 40 % of the iron in meat, fish and poultry is haem and approximately 60 % is non-haem. Haem iron must be hydrolysed from the globin portion of haemoglobin or myoglobin prior to absorption. The digestion is accomplished by protease in the stomach and small intestines. The haem containing the iron and porphyrin ring is then absorbed intact as metalloporphyrin into the mucosa cells of small intestines. Non-haem iron is found in both plant and animal derived foods. These include nuts, fruits, vegetables, grains and in dairy products (milk, cheese) and eggs. It is usually bound to components of food and must be enzymatically liberated in order for absorption to occur.

On average approximately 10 % of iron consumed in a day comes from haem iron, even though this accounts for only a small proportion of the total intake. Approximately 23 % of this iron is absorbed as compared to 2 to 20 % of non-haem iron. Absorption however depends on other dietary factors and the body's iron stores. People with severe iron deficiency absorb both haem and non-haem iron more efficiently and are more sensitive to absorption enhancing factors than people with better iron status.

Mucosal ferritin receives iron from gastro intestinal tract (GIT) and stores it in mucosal cells of small intestines. When the body needs iron, mucosal ferritin releases it to mucosal transferrin, which transfers it to blood transferrin, which then transports it to the rest of the body.

2.2.2 Absorption enhancers and inhibitors

Meat, fish and poultry (MFP) contain not only well-absorbed haem iron but also a factor (MFP factor) that promotes the absorption of non-haem iron from other foods eaten at the same meal. Vitamin C also enhances non-haem iron absorption from foods eaten in the same meal by capturing iron and keeping it in the reduced ferrous form ready for absorption. Some acids such as citric from fruits, lactic acid from milk and hydrochloric acid from the stomach also enhance iron absorption. Reducing sugars also promote absorption. However some dietary factors bind with non-haem iron inhibiting absorption. These include phytates and fibres in legumes, whole cereals, nuts and vegetables, calcium and phosphorus in milk, EDTA in food additives and tannic acids and other polyphenols in tea, coffee, nuts and fruits and vegetables.

2.2.3 Adaptability of absorption

Generally only about 10 – 15 % of dietary iron is absorbed. However, absorption can be as low as 2 % in a person with GIT diseases or as high as 35 % in rapidly growing health children. Absorption increases when iron intake falls short or when the need increases as in pregnancy. The body makes more mucosal and blood transferrin to absorb and transport more iron around the body.

During destruction of red blood cells, which normally occurs after four months, iron is salvaged by the liver attaching it to blood transferrin, which transports it back to the bone marrow to be used in making new red blood cells.

There is however a daily loss of iron through the gastro intestinal tract, bleeding through menses and tiny amounts through urine, sweat and shed skin. Men lose approximately 0.9 - 1.0 mg per day. Women have a basal loss of 0.7 - 0.8 mg per day due to their small surface area. This is in addition to an average of 0.5 mg per day from menstruation.

2.2.4 Calculation of iron absorbed from meals

Three factors influence absorption of iron from food (Whitney and Rolfes, 1999). These are:

i. The amount of haem and non-haem iron in the food.

ii. The amount of reducing factors such as vitamin C, lactic acid, reducing sugar and citric acid in the meal.

iii. The amount of meat, fish and poultry (MFP) that was consumed.

Then calculations proceed as follows:

1. Amount of iron from meat, fish and poultry = x mg.

2. Amount of haem iron =Step 1 X 40 % = o.4x mg.

3. Amount of iron from other sources = y mg.

4. Amount of iron from non-haem sources = amount in step 3 + step 1 X 60 % = y + 0.6 x.

Amount of vitamin C in the meal: The amount of vitamin C in a meal is considered low if the total content is less than 25 mg, moderate if the content is 25-75 mg and high if the vitamin C content is greater than 75 mg.

Amount of MFP in the meal: Like vitamin C the amount of MFP present in a meal is considered low if the content is less than 23 g, medium if the amount is 23-46 g and high if the amount is greater than 69 g. (Monsen 1978).

7. Haem iron absorbed = 23 % of haem iron

= Step 2 X 23 % mg = (0.4 x) x 0.23 = 0.092 x mg

8. Now taking the best score from step 5 and 6 if either vitamin C or MFP was high or if both were medium the availability of non-haem iron was high. If neither was high the availability of none haem iron was medium. If both were low, non-haem iron had poor availability. When availability is:

a) High, 8 % of non-haem iron is absorbed

b) Medium, 5 % of the non-haem iron is absorbed

c) Poor, 3 % of the non-haem is absorbed

Total non-haem iron absorbed then = step 4 x z % where z is the percentage corresponding to availability level of non-haem iron in step 8.

Then total iron absorbed (Ti) = mg haem iron absorbed + mg non-haem iron absorbed.

This can be given by the equation

$Ti = 1/100 (zy+0.6xz + 9.2x)$

Assumptions

The recommended daily allowances are as shown in Table 1.

Table 1. Recommended daily allowances (RDA) of iron and zinc

	Age (yr.)	Iron (mg)	Zinc (mg)
Infants	0 – 0.5	6	5
	0.5+ -1.0	10	5
Children	1.0+ - 10	10	10
Males	11 – 18	12	15
	19 – 50+	10	15
Females	11 – 50	15	12
	51+	10	12
Pregnant women		30	15
Lactating women	First six months	15	19
	Second six months	15	16

Source: (Whitney and Rolfes, 1999)

The RDA assumes absorption of 10 % of iron ingested. Thus for a man of any age or a woman greater than 50 years of age (RDA 10 mg) needs to absorb 10 mg / day. A woman 11 - 50 years old (RDA 15mg) will need to absorb 15 mg / day. Those with higher menstrual losses than an average woman may need more.

2.2.5 Physiological Functions of Iron in the Body

The major role of iron is to permit transfer of oxygen and carbon dioxide from one tissue to another (Guthrie, 1995). Iron carries out this role primarily as part of both haemoglobin in the blood and myoglobin in the muscles, but also as part of several tissue enzymes essential in cell respiration .The exchanges are involved primarily in the release of energy within the cell. Haemoglobin is an essential component of erythrocytes formed

in the bone marrow. When the numbers of red blood cells drop, the level of erythropoetin, a hormone produced in the kidneys increases, stimulating erythropoesis (the production of more red blood cells). Erythrocytes begin in the bone marrow as erythroblasts (immature cells containing a nucleus). As these cells mature, they synthesize haem, an iron containing protein from amino acid glycine and iron along with the help of vitamin B6 and mineral copper. The haem unites with another protein called globin to form haemoglobin. Haemoglobin containing immature red blood cells known as reticlocytes are formed and released into the blood stream where they lose their nuclei to become erythrocytes capable of functioning as carriers of oxygen and carbon dioxide.

Studies of role of iron as an anti-infective agent suggest two conflicting roles. On the one hand since iron is essential for growth of micro-organism, potential harmful bacteria will be able to grow if it is available and will not thrive as well in iron deficient people. On the other hand if iron is not available there is a decrease in production of iron containing enzymes and other immune substances needed to destroy infectious organisms. In this case iron deficient people are less able to fight infections. Lactoferrin in human milk is an iron containing substance that is effective against *E. coli*. It binds iron making it unavailable for bacterial growth.

Iron performs many other important functions including: catalysing the conversion of beta-carotene to vitamin A. It is also involved in the synthesis of purines that form an integral part of nucleic acid, and in synthesis of carnitine that is essential in transportation of fatty acids. Iron is also involved in production of antibodies and collagen. Like zinc,

iron is important in detoxification of drugs and heavy metal poisoning in the liver (Guthrie, 1995).

2.2.6 Iron deficiency and vulnerable stages

Iron deficiency is the most common nutrient deficiency affecting over 1 billion people worldwide (UN, 2000). In developing countries 33% of children and women of childbearing age suffer from iron deficiency anaemia. Vulnerable stages include women in their reproductive years because of repeated blood losses during menstruation. Pregnancy demands additional iron to support added blood volume, growth of foetus and blood loss during childbirth. Infants and children receive little iron to support their high milk diets yet need extra iron to support their rapid growth. Teenagers' rapid growth for males, menstrual losses of females also demand extra iron that a typical teenage diet may not provide. An adequate iron intake is therefore important during these stages of life. Blood losses through bleeding from any site, menstruation, parasitic infection, or blood donation imply loss of iron, which has to be replaced.

2.2.7 Iron deficiency and anaemia

Iron deficiency refers to depleted body iron stores without regard to the degree of depletion or presence of anaemia. Iron deficiency anaemia refers to severe depletion of iron stores that result in low haemoglobin concentration (Whitney and Rolfes, 1999). In iron-deficiency anaemia red blood cells are pale and small, they cannot carry enough oxygen from the lungs to the tissue hence energy metabolism falters. The result is fatigue, weakness, headache, apathy and poor resistance to cold temperature. This shows

up in pale skin and eye lining. Long before diagnosing anaemia, iron deficiency affects behaviour, impairs complete oxidation of pyruvate reducing physical work capacity and productivity. Iron deficiency anaemia leads to reduced physical fitness, weakness, fatigue and reduced available energy to work. With no obvious deficiency symptoms, most people just appear unmotivated, apathetic and less physically fit.

Another change in behaviour seen in low-income iron-deficiency women and children is pica (an appetite for non-food substances) such as soil (geophagia) and ice (pagophagia). Other deficiency symptoms that have been documented include resistance to infections due to lowered immunity, body itching, impaired wound healing, and eye membrane and palm creases. Nails may appear concave with pale nail beds. Children show signs of impaired cognitive function, inability to pay attention and increased distractibility leading to reduce learning ability. Infants on the other hand display impaired visual discrimination, reactivity and coordination.

2.2.8 Iron toxicity

Iron overload (hemochromatosis) is usually caused by genetic disorder that enhances iron absorption. It also comes as a result of repeated blood transfusion and also due to massive doses of supplementary iron.

Long term over consumption of iron may cause hemosiderosis,a condition characterized by large deposits of iron in storage protein hemosiderin in liver and other tissues. Since some of the symptoms of overload are similar to deficiency symptoms such as apathy,

lethargy and fatigue, taking iron supplements before assessing iron status is unwise.

Iron overload is characterized by tissue damage especially in iron-storing organs such as liver. There is also increased bacterial infections because of iron rich blood a conducive nutrient media on which bacteria thrive. It is severe in alcoholics because alcohol damages the intestines impairing its defence against absorbing excess iron. Untreated hemochromatosis aggravates the risk of diabetes, liver cancer, heart disease and arthritis. Iron overload is twice prevalent among men than women. In children iron poisoning may occur due to intoxication from high iron supplements. Symptoms include nausea, vomiting, diarrhoea, rapid heart beat, weak pulse, dizziness, and confusion. However this is rare in developing countries due to low consumption of supplements.

2.2.9 Iron recommended dietary levels and sources

To obtain enough iron people must select iron rich foods. Foods rich in iron include meat, fish and poultry. Others are legumes, eggs, whole grains, and cereals. Dark green vegetables and some fruits also contribute some iron to diet. Iron enriched foods through fortification though not readily absorbed as well as natural occurring iron can make a big difference when eaten with absorption enhancers. However these are costly, hence not affordable by most people and are also likely to lead to iron overload if not appropriately used. Many physicians also routinely recommend iron supplements to pregnant women, infants and young children as a source of iron besides the diet. Iron from supplement is less well absorbed than that from food. Absorption improves when supplements are taken between meals or at bedtime on an empty stomach and with liquids other than milk, tea, and coffee, which inhibit absorption. However taking iron supplements with orange

juice does not confer any benefit, as vitamin C does not enhance iron absorption from supplements as it does for dietary iron since the supplemental iron is already in ferrous form. Supplementation is however expensive and therefore not sustainable in poor countries like Kenya. They are also not readily absorbed and cause constipation as a common side effect. Another inadvertent source of iron is contamination iron. This is iron found in foods as a result of contamination by inorganic iron salts from iron cookware, drying ware and iron containing soils.

2.3 Zinc

Zinc is a versatile trace element required as a co-factor by more than 100 enzymes in the human body (Guthrie, 1995). Virtually all cells contain zinc but the highest concentration is in bone, prostrate gland and eyes. Muscles contain approximately 60 % of total body zinc due to its high proportion of body mass. Tissues do not readily give up zinc when blood levels fall so a person must eat zinc-rich foods frequently.

2.3.1 Physiological role of zinc in the body

According to Guthrie, (1995) zinc supports the work of numerous proteins in the body including metalloenzymes involved in a variety of metabolic processes such as carbonic anhydrase, deoxythymidine kinase, DNA and RNA polymerase and alkaline phosphatase. Zinc also assists in immune function and growth and development reflecting a role in protein synthesis. Zinc associates with the hormone insulin in the pancreas hence affect glucose tolerance. It interacts with platelets in blood clotting, affects thyroid hormone function and influence behaviour and learning performance. It is necessary to produce the

active form of Vitamin A (retinol) in visual pigment and the retinol-binding proteins that transports vitamin A from liver stores. Zinc is essential to normal taste perception, as low blood levels of zinc have been associated with hypogeusia (loss of sense of taste), which is also accompanied by hyposemia (loss of sense of smell). These conditions occur under the stress of burns, fractures and infections. This partly explains poor appetite shown by hospitalised patients. Zinc concentrates in wound tissues and plays a role in incorporation of amino acid methionine into the protein of the skin. In humans zinc is critical in the latter parts of the healing process when the epidermal layers of the skin are healing. It is also essential in making of the sperms, synthesis of RNA and DNA necessary for cell reproduction. It is necessary for many digestive enzymes such as pancreatic lipase, necessary in alcohol metabolism and protection of the body from heavy metal poisoning of such elements like cadmium and lead (Guthrie, 1995).

2.3.2 Zinc deficiency

Human zinc deficiency was first reported in the 1960's in children and adolescent boys in Egypt, Iran and Turkey (Whitney and Rolfes, 1999). Children have especially high zinc needs because of rapid growth rates and synthesis of many zinc-containing proteins. When zinc intake falls short of body requirement deficiency symptoms begins to manifest. These are characterized by severe growth retardation and arrested sexual maturation. In addition, zinc deficiency hinders digestion and absorption, causing diarrhoea, which worsen nutrition of zinc and all other nutrients. It impairs immune response making infections among deficient subjects very likely. Impaired immune response also leads to infections of the gastro intestinal tract, which worsens malnutrition.

Chronic zinc deficiency damages the central nervous system and brain functioning leading to anorexia, mental lethargy and irritability. Because zinc deficiency directly impairs vitamin A metabolism, vitamin A deficiency symptoms (night blindness) do appear. Zinc deficiency also disturbs thyroid function, reduces synthesis of adrenocortical hormones and the metabolic rate. It alters taste, causes anorexia and shows wound healing. In fact the symptoms are so all pervasive that generalized malnutrition and sickness are more likely to be the diagnosis than simple zinc deficiency (Whitney and Rolfes, 1999).

2.3.3 Vulnerable stages of life

Most susceptible to the effect of zinc deficiency are pregnant and lactating women, young children, the elderly, the poor in the society and people living with HIV and AIDS.

2.3.4 Zinc sources

Zinc is highest in protein rich foods such as meat, poultry, liver, and fish. Legumes and whole grains are good sources if eaten in large quantities. Vegetables also provide some zinc. However their contents vary depending on the zinc situation of the soils in which they were grown.

2.4 Causes of Iron and Zinc Deficiency

The main cause of iron and zinc deficiencies in Kenya has been attributed to low intake. Van Steenbergen, et al., (1980) reported figures of 0.7-9.7 mg of iron/day, which is far below the recommended daily allowance of 10-15 mg/day (Whitney & Rolfes, 1999).

This is because majority of Kenyans consume staple diets primarily of low mineral content or with low bioavailable minerals. The amounts of anti nutrient factor in most of these foods such as fibre, tannins and polyphenolic compounds bind the minerals making then unavailable.

Poverty situation in the country where more than 50 % of the population live below poverty line (GOK, 1999), limits disposable income to purchase and consume non staple foods rich in iron and zinc such as animal products, fortified products and absorption enhances. Reduced food security results in intra household food restriction during food shortage seasons. The 1999 national survey reported parasitemia due to malaria, schistosoma and hookworm infection as contributory factors to iron and zinc deficiency. Similarly sickle cell disease, respiratory illnesses, diarrhoea loss of appetite, malabsorption and febrile episodes are major morbidity factors for hypozincaemia and anaemia. Increased requirements due to physiological changes during pregnancy and high growth rates during childhood and adolescent further exacerbate the risk of deficiency (Mwaniki et al., 1999).

Other micro nutrient deficiencies such as vitamin A, folic acid, and vitamin B12 are significant risk factors for anaemia. From available literature HIV and AIDS are closely linked with financial limitations, as the bulk of the finances is focused on medication rather than proper nutrition.

2.5 Proteins

The importance of protein in nutrition and health cannot be overemphasized. It is appropriate that the Greek word chosen as a name for this nutrient is "proteos" meaning primary or "taking first place." Proteins are essential nutritionally because of their constituent amino acids, which the body must have in order to synthesize its own variety of proteins and nitrogen containing molecules that make life possible (Groff, 1995). Each body protein is unique in the characteristics and pattern of the amino acid comprising its structure.

2.5.1 Protein synthesis

Proteins are synthesized from amino acids. They can be synthesized by both plant and animal cells if the plant has available nitrogen through the soil from chemical fertilizers, nitrogen containing organic matter by action of bacteria while some plants such as legumes are capable of fixing nitrogen from the atmosphere. Some nitrogen from the atmosphere is also made available through the action of lightning.

Animals obtain most of their nitrogen in the form of amino acids from either plants or other animal sources. They are also able to synthesize a few amino acids in their GIT using dietary nitrogen.

In both plants and animals protein synthesis involves the formation of peptide chains because of peptide bonds formation between adjacent amino acids at their amino acids carbonyl groups. The characteristic of a particular protein are determined by the type of amino acids used, the number of times they are repeated and the order in which they are

formed together. This sequencing of amino acids occurring as the polypeptide chain is synthesized on the ribosome determines the primary structure. The second level of protein organization, the secondary structure is achieved through weaker bonding such as hydrogen bonding that form coiled structures, e.g. αhelix, pleted sheets or B-conformation structure. The stretched polypeptide can also fold back on itself. These two structures are quite stable and provide strength and rigidity to proteins. The random coil is the third type if secondary structure with limited stability due to large side chains or two side chains with same charge repelling each other hence forming loops or twists in the chain. The secondary structure or shape of the amino acid chain influences the properties of the resulting protein and the possibilities of the number of proteins. The third level organization is the tertiary structure, resulting from clustering together of hydrophilic amino acid electrostatic attraction of positively charged amino acid residues and formation of strong covalent bonds between cystein residues. These interactions determine protein overall shape and hence its function (Groff, 1995). The shape of a protein molecule and therefore its function can be changed by heat, light or acid. The changed (denatured) protein has different physical and physiological properties. The final level of protein reorganization, the quaternary structure involves either two or four polypeptide chain (sub units) bound together by hydrogen bonds and electrostatic bridges to form oligomeric proteins. Oligomers are particularly important in intracellular regulations because the sub units can assume different special orientation relative to each other and in so doing change the properties of the oligomer (Groff, 1995) e.g. haemoglobin.

2.5.2 Functional categories

Protein has been recognised as a dietary essential for many years.

This nutrient plays an important role in growth and maintenance of tissues. Before new protein is synthesized, all essential amino acids must be available, plus sufficient indispensable nitrogen to form the non-essential amino acids. Growth is only possible when there is an appropriate mixture of amino acids over and above those needed for the maintenance and repair of tissues. In addition some tissues call for large amounts of specific amino acids, e.g. the sulphur amino acids in hair, nails and skin.

Cell division and growth is dependant on availability of proteins as is the synthesis of the structural material of the body. These include the contractile proteins of muscles (action and myosin) and fibrous tissues (collagen) protein found in connective tissues, skin, hair and nails.

Proteins are also involved in the formation of essential body components. These include peptide hormones, which control body functions by regulating synthesis and activity of enzymes. Some peptide hormones that have particular significance for nutrition and human metabolism include: insulin, glucagons, parathyroid and thyroid hormone, Adrenocorticotropic hormone, sematotropin and vasopressin (Groff, 1995). Enzyme systems are protein structures that combine selectively with other molecules in cells thereby catalysing all human physiological changes, chemical reactions such as digestion, blood coagulation, tissue energy production excitability and contractibility of neuromuscular tissue.

Proteins combine with other substances (minerals, oxygen, carbon dioxide, vitamins) in the blood to provide a mode of transport for the substances. Important among these are albumin, transthyretin, haemoglobin, ceruplasmin, transfferin and retinal binding protein.

Provision of a defence system against invading organisms such as bacteria, viruses, is another important function of proteins. Immunoglobulin or antibodies are produced by B-lymphocytes. These function by binding with and in activating antigens. The immunoglobulin-antigen complex is easily recognized and destroyed through reactions with complement proteins produced in the liver, or cytokines produced by T-Cells. In addition the process of phagocytosis by macrophages and neutraphiles also destroy foreign antigens. Lowered resistance of injections that accounts for high infant mortality rate among malnourished children is attributed to their failure to produce adequate antibodies due to protein deficiency (Groff, 1995). Similarly, in protein depletion the body detoxication ability is reduced.

Proteins also play an important role in regulation of water balance in the body. The distribution of fluid in the intravascular, intercellular and intracellular space by the orcortic pressure and hydrostatic pressure involving both protein and electrolytes primarily sodium and potassium. Oedema, which is the accumulation of fluid in the tissue, is recognized as an early sign of protein deficiency.

Proteins in the blood serve as buffers, neutralizing excess acid or base. This is important because most body tissues cannot function when the pH of the blood and intercellular fluids deviates even by a small amount (Guthirie, 1995).

2.5.3 Factors affecting protein utilization

The utilization of amino acids in the body depends on a variety of conditions. The amino acid score, the caloric inadequacy of the body, i.e. when the caloric intake drops below a critical point, proteins will be deaminated and used as a source of energy. Immobility greatly reduces synthesis of new proteins. This is a problem experienced by the aged, bed ridden and astronauts due to stagnation and weightlessness. An increase in nitrogen loss after injury is also well documented. Emotional stress such as, fear, anxiety, anger, paid, extreme cold, also result in loss of nitrogen. Growth and body size also affects protein utilization (Guthirie, 1995).

Table 2. Safe protein intakes for selected age groups and physiological states

Selected group	Age	Intake g/kg/day
Infant	9 – 12 Months	1.4
Child	1 – 2 Years	1.2
	5 – 6 Years	1.03
	9 – 10 Years	1.00
Adolescent Girls		0.94
Boys	13 – 14 Years	0.97
Adult	19 +	0.8
Pregnant:	2^{nd} Trimester	6+
	3^{rd} Trimester	11 +
Lactating	0 – 6 months	≈ 16+
	6 – 12 months	≈ 11+

Source: Groff, 1995

2.5.4 Food sources

In assessing the amounts of protein in a food it is important to keep in mind that foods containing all essential amino acids are more useful than those with low or limiting amounts of one or more amino acids (Groff, 1995). Thus proteins from animal sources such as meat, poultry and eggs, fish and fish products, dairy and dairy products are better suited to the amino acid requirements of humans. However, proteins from vegetable sources such as grains, legumes and legume products, nuts and seeds, play an important role in developing world, where animal proteins tend to be expensive and out of reach to most families.

2.5.5 Bean protein

Bean seeds are a good source of dietary protein and energy. Energy comes mainly from carbohydrate, starch being the main component (Bourne, 1989). From various studies done proteins averaged 23.6 % with a range of 18 to 29 %. Carbohydrates averaged 62.8 % with an estimated total energy of 359 Kcal/100 g of dry beans.

2.5.5.1 Nature of the proteins

The protein content of beans is influenced by various factors like nitrogen fertilization of the soil, yield of seeds per unit area or plant and to a lesser extent, by genotype.

According to solubility properties common bean proteins are represented by albumins, soluble in deionised water and globulins soluble in diluted salt solutions. Over 80 % of the total nitrogen of common beans is extractable in a 0.25 – 0.5 N sodium chloride solution (Bourne, 1989). The remaining protein is believed to represent glutelins and

structural proteins bound to cell membranes and organells, only extractable by strong base and acid. When total nitrogen is considered, globulins account for over 50 % and albumins 10 – 20 %. On the basis of extractable nitrogen, globulins represent 70 – 80 % and albumins 10 – 20 %.

2.5.5.2 Composition and nutritive value of proteins

The nutritive value of proteins depends on its composition, digestibility and bioavailability of its essential amino acid (Bourne, 1989). Amino acid composition of common beans has been reported by many investigators. The common and important features encountered in all investigations were limiting amounts of sulphur amino acids, methionine, cysteine and cystine; fairly low concentrations of tryptophan and high concentration of lysine.

2.5.5.3 Protein digestibility

Beans can only be consumed when properly cooked (Bourne, 1989). Even in properly cooked bean protein digestibility is considered low if compared with good quality animal proteins. Low digestibility of bean protein is one of the main causes of their low nutritive value (Sgarbieri, 1979). Jeffe (1950) reported an in vitro true digestibility of 76.8, 79.5 and 84.1 % respectively for the protein of black, pink and white beans. In this study digestibility seemed to decrease as the pigment in the seed coat increased. These pigments are mainly phenalic compounds, which may interact with bean proteins, decreasing their digestibility and utilization. Recent study by Ahn, J.K., L.C. Sen and J.R. Whitaker (1987, unpublished) have identified phaseolin as the main globulin fraction

that was considerably more resistant to hydrolysis by proteolytic enzymes hence their low digestibility. However, heat treatment (cooking) greatly increases bean protein digestibility from 15.17 to 70.7 % in the whole bean seeds.

2.5.5.4 Bioavailability of essential amino acids

Another important factor that contributes to low nutritive value of beans is the low bioavailability of some of the amino acids. Bioavailability of amino acids is influenced by factors like digestibility stimulation to endogenous loss, physical and chemical modifications of the proteins during storage and industrial or domestic preparation for consumption. Sulphur amino acids bioavailability has been the subject of attention of many investigators because they are limiting amino acids in the bean protein and are likely to undergo oxidative changes during handling and processing of the bean prior to consumption.

Treatments such (a) storage under different environmental conditions, (b) soaking in water or different salt solutions, (c) cooking at normal or increased pressure, and (d) frying after cooking may also affect unfavourably the bioavailability of sulphur amino acids (Bourne, 1989). Changes occurring in bean seeds during storage have been well documented by several investigators (Antunes et al., 1979; Burr, 1973). Common findings of these studies were: (a) under certain conditions of storage beans of certain cultivars develop hard shell which results in failure to rehydrate. This phenomenon is favoured by low relative humidity in storage atmosphere and low water content in seeds, but also seems to be a characteristic of certain varieties; (b) a hardening of cotyledons with loss of cookability may occur. High storage temperatures and high humidity

accelerate the hardening. Along with studies on deterioration of commercial value, organoleptic and cooking properties, studies on loss of nutritive value of stored beans have also been done.

Antunes et al., (1979) found a decrease in rehydration capacity under one storage condition, an increase in cooking time, and a drop in protein efficiency ratio (PER). The main cause of the drop in PER was the decrease in bioavailability of sulphur containing amino acids, methionine and cystine. Therefore, prolonged or improper storage of beans can also cause a marked decrease in bioavailability of essential amino acids and protein nutritive value. Decrease in nutritive value of bean protein during storage is associated with the heat treatment necessary to cook the bean after storage and prior to consumption. Cooking dry bean is necessary not only to tenderize the seed coat and cotyledons and develop acceptable flavour and texture but also to eliminate toxic factors and to make bean protein more digestible and essential nutrients more available.

2.5.5.5 Nutritive value of bean proteins

The nutritive value of bean proteins is strictly related to its limiting amounts and low bioavailability of sulphur containing amino acids particularly methionine (Bourne, 1989), to its resistance to proteolysis resulting in low digestibility in addition to the peculiar property of bean proteins to stimulate high excretion of endogenous nitrogen. Available literature show an average protein*digestibility of 70 % with a range of 44.3 to 91 %(Bourne, 1989). Isolation of protein from the beans greatly increased its digestibility. A substantial variation in PER was also encountered with values falling in the range of 0.5 – 1.5 compared to reference casein PER of 2.5.

Addition of free methionine to the bean or mixing the bean with complementary source of sulphur containing amino acids like cereal grains greatly improves PER values as demonstrated by (Yadav and Liener, 1977). This is important since in most developing countries beans are consumed in combinations with cereal grains or flour resulting in protein mixtures more nutritive than either the bean or the cereal protein individually. The biological value index reported for bean protein is generally low. Values range from 9.21 to 79.0 %. Tobin and Carpenter (1978), reported higher values than these. These differences are likely to reflect different genetic backgrounds of bean cultivars in various parts of the world.

2.6 Protein Energy Malnutrition (PEM)

Protein malnutrition is second only to energy insufficiency in its disastrous effect on individual and society (Liepu, 1992). Protein malnutrition is often complicated by deficiencies of other nutrients, infections, parasites and the general problem of poverty. Complex interactions of economics, political decisions, cultural values and family relationships all affect each attainment of the physiological need for proteins.

Across all societies, this is a period of great risk for lack of sufficient and quality of proteins. Groups at highest risk here are infants, adolescent and pregnant mothers whose physiological demands for growth and maintenance is high.

Protein Energy Malnutrition (PEM) describes physiological condition involving deficiency of dietary calorie, dietary protein or most likely a combination to varying

degrees of both these conditions (Kies, 1992). WHO estimates that at least 500 million children across the globe show symptoms of PEM. However, PEM is also found in adults. During famine individuals of all ages may develop PEM in progressively worsening degree, which finally ends in marasmus and starvation. PEM can also occur as a secondary effect of AIDS, infectious diseases, malabsorption conditions, kidney and liver diseases, anorexia nervosa, some psychological disorders and cancer (Guthrie, 1995).

Kwashiorkor is a pure protein deficiency affecting children aged 2 – 5 years (Guthrie, 1995). It is characterized by growth failure, pitting oedema, hepatomegaly, mental changes, hair alteration, skin changes, anorexia, diarrhoea, susceptibility to infections and other nutrient deficiencies. Closely related is Marasmus, which comes from a Greek work for wasting. It is as a result of chronic lack of calories. This leads to some proteins being used to provide energy, essentially causing protein deficiency. Clinical symptoms associated with PEM include: growth failure accompanied by then, weak and wasted muscles, behavioural changes ranging from irritability of kwashiorkor to apathy of marasmus, pitting oedema, especially in lower abdomen, arms and legs, skin changes in colour, drying, peeling and eventually forming ulcers that heal slowly becoming conduits for infection. Changes in hair, which become dry, sparsed and either loses its pigmentation or takes on a reddish colour. Loss of appetite, vomiting, diarrhoea all of which result in severe dehydration and loss of electrolytes. Other symptoms include anaemia, enlargement of the liver and increased susceptibility to infections and fever (Guthrie, 1995).

2.7 Protein malnutrition in developing countries

The causes of severe under-nutrition and malnutrition in developing countries are many and varied (Guthrie, 1995). Poverty and the resulting lack of money make it impossible for many families to have enough calories. It limits their ability to purchase and consume high quality animal proteins. Lack of money makes it impossible to acquire farm inputs such as fertilizers needed to boost production. Problems of insect infestations, plant and animal diseases are all deterrent to home production of foods. High rates of population growth, large household numbers increase the pressure on the land to provide adequate food. Natural disasters such as floods, droughts, and soil erosion further limit accessibility to food. Lack of access to arable land, use of more arable land for production of cash crops and shortage of water also impact greatly on food production.

Moreover poor environmental sanitation contributes to high rates of infection and illness, which reduce the family's capacity to work or engage in activities that bring in more money or food.

Poorly developed infrastructure and marketing systems in the country makes it difficult to distribute food to those in greatest need. All these deterrents to solving the malnutrition problems are complicated further by intra-house hold food distribution, cultural beliefs and practices that prohibit or discourage the use of certain foods making young children, pregnant and lactating women more vulnerable.

Efforts being directed towards finding solutions to these problems include, attempts to slow population growth hence family dependants. However, this is a slow and long-term solution. Research to develop new strains of food crops with higher yields, resistance to pests and diseases and enhanced nutritional qualities is on going. Traditional food plants of the less affluent centre around one or a few high starch low protein foods, largely derived from cereals and root crops. These include wheat, rice, maize, sorghum, rye, barley, oats, potatoes, cassava and yams. Foods based in these single sources become the primary food for that particular population.

These primary foods are often relatively low in protein quality and have less than ideal amino acid composition pattern in relation to the needs of humans, particularly children. However, because of large quantities ingested, primary food does become an important supplier of proteins and calories. Hence efforts to improve the protein status of impoverished population have often been directed to these foods.

The discovery of Opaque – 2 gene in maize resulted in improved proteins quality of the grain and gave impetus to research material on other cereal crops and grain legume to identify characteristics, which by conventional plant breeding could lead to improvement in their protein quality.

The success of these efforts will depend on the extent to which they can be used by individuals who are most in need and on the extent to which the final product is acceptable from both palatability and cultural standpoint.

Development of weaning foods based on mixtures of plant proteins, or that involve additions of small amounts of animal proteins such as fish are most likely to be acceptable. Commitment of the governments to providing health services, directed towards preventive rather than crisis management can be an important factor in prevention of PEM (Guthirie, 1995).

2.8 Breeding for trace minerals in other crops

2.8.1 Rice

Rice is not considered a major mineral source. It is the cereal lowest in iron containing 5 to 6 mg/kg after milling. However any increase in its mineral concentration could significantly help reduce iron and zinc deficiency in humans because of the high levels of rice consumption among the poor in Asia (Gregorio, et al., 2000)

In 1992 International Rice Research Institute (IRRI) began to examine the effect of certain soil characteristics on the iron content of the grain. This research was influenced by the effort of the Philippine government to eliminate iron malnutrition problem in the country through fortification. This effort was expanded in 1995 to include analysis of both iron and zinc. Since then germplasm screening has shown large variation or iron and zinc concentrations in brown rice. Among the 1,138 samples analysed the iron concentration in brown rice ranged from 6.3-24.4 mg/kg with a mean value of 12.2 mg/kg. For zinc, the range was 13.5-58.4 mg/kg with a mean of 25.4 mg/kg. When compared to the two most widely grown commercial varieties in Asia, IR36 & IR64, Jalmagna a traditional variety grown in the same soil and season had almost double the

iron concentration of IR36 & IR64. Its zinc concentration was also high, nearly 40 % more than that of IR64.

High iron and zinc traits can be combined with improved agronomic traits as has been demonstrated in the serendipitous discovery in the IRRI testing program of an aromatic variety IR68144-3B-2-2-3, a cross between high yielding IR72 and a traditional variety Zawa Bonday from India. This variety has high concentration of grain iron (about 21mg/kg in brown rice). It has good tolerance to rice tangura virus and to soils deficient in mineral such as iron, zinc and phosphorus. It also has excellent grain qualities.

Though the yields of Jalmagna are 10 % lower than IR72, this deficiency is partially compensated for by early maturity. Fifteen minutes of polishing leaves 80 % more iron than IR64. A human feeding trial is under way and it remains to be seen if this extra iron can improve the iron status of the iron deficient human subjects (Gregorio, et al., 2000).

2.8.2 Orange fleshed sweet potatoes

An action research project (Hagenimana, et al., 2000) was recently implemented by the Kenya Agricultural Research Institute (KARI) in collaboration with Centro Internacional de la Papa (CIP) and CARE International. Orange fleshed varieties of sweet potatoes that were both high yielding and rich in β-carotene were introduced to women farmers. The main objective of the study was to improve food security, nutrition and income generation for small farmers in the region. The intervention sites were in rural Western Kenya an area where farmers have traditionally produced sweet potatoes for consumption

and sale. The trial intervention worked with 20 women groups in two districts (Ndhiwa / Nyarongi and Rongo is South Nyanza Province) and compared two packages to promote the adoption and consumption of varieties of orange fleshed sweet potatoes rich in β-carotene to improve vitamin A intake, nutrition education and train in processing methods. The results demonstrated that orange-fleshed sweet potatoes were highly acceptable to producers and consumers both when eaten alone and when used as ingredients in processed foods such as chapati (flat fried bread) and in mandazi (doughnuts) commonly sold in rural markets. As typically produced with wheat flour these products contain approximately 100 mg of β-carotene equivalent per 100 g of food product. Substituting orange-fleshed sweet potatoes for wheat flour in both boiled and mashed or flour form raised the β- carotene content to 800-3200 mg / 100g. Moreover a cost analysis indicated that the profitability of selling these processed products increased principally as a result of lowering production costs. The derived income is likely to be used to meet other basic household needs.

A Helen Keller International index (HKI) was used to asses change in vitamin A intake pattern by comparing baseline scores and post intervention score among the children of women in the intervention and control groups. In Ndhiwa /Nyarongi the area of study with the higher probability of severe vitamin A deficiency, the HKI scores increased significantly in the intervention group from pre- to post- intervention while those in the control group decreased. The overall change was highly significant 93 % increase over the pre-intervention level.

These results indicated that the promotional activities in nutrition education and food processing for sale as well as home consumption were critical for significantly increasing HKI scores.In Rongo the area of the study with lower probability of inadequate vitamin A intake, the increase in HKI scores that occurred as a result of the intervention were not statistically significant. This suggested that promotional efforts were unlikely to significantly increase consumption of vitamin A rich foods in areas where the consumption is already adequate. The potential for this intervention to be sustained over time is high. First traditional dissemination of planting material coupled with organized multiplication and dissemination programs can be rapid and continual. Second the varieties are likely to be sustained in the community for agronomic reasons due to high yield and drought resistance properties. Finally because production and consumption of the potatoes was controlled by women they were able to use the crop to improve the quality of their children's diet as well as retain any income earned by selling the fresh root or processed product.

2.8.3 Maize

Many poor people in Southern and Eastern Africa subsist on a maize-based diet that is low in iron and zinc (CIMMYT, 1999). As a result 30 % of pregnant and lactating women in Zimbabwe are thought to be iron deficient (Banziger and Long, 2000). Improving the nutritional quality of maize could therefore have significant impact on the nutrition of poor women and children in Southern and Eastern Africa.

Little is known about the genetic variation of iron and zinc concentration of grain of

maize and the potential to improve it through plant breeding. The Centro Internacional de Majoramiento de Maize y Trigo (CIMMYT) maize breeding program has been focusing on identifying white-grained maize germplasm that has the potential to increase kernel iron and zinc concentration, especially in sub-Sahara Africa. In addition research at Cornell University has focused on trials with multiple aleurone layer, which can increase kernel iron and zinc concentration and low phytic acid concentration for improved iron and zinc bioavailabuility.

More than 1400 improved maize genotypes and 400 landraces from CIMMYT - Zimbabwe and 57-white grained cultivars currently grown in Southern Africa were grown and evaluated to assess grain iron and zinc concentrations.

After thorough evaluation of genetic variability of iron and zinc in a total of 13 trials in Zimbabwe and Mexico, promising genetic variability was found in both improved maize germplasm and landraces. Across trials, grain iron concentration of 9.6-63.2 mg/kg and grain zinc concentrations of between 12.9 and 57.6 mg/kg were found. These differences were probably not due to genetic differences alone but were also influenced by different location and year in which the germplasm was grown. For example trials in Mexico had much lower average values for iron and zinc that the trials in Zimbambwe.

Within individual trials, high values for grain iron and zinc concentration averaged 69 % and 49 % respectively above mean of the trial indicating considerable variation of iron and zinc in maize gain. Some germplasm re-evaluated at different locations in Zimbabwe

averaged between 13.2 and 18.5 mg/kg for iron and between 19.4 and 24.0 mg/kg for zinc, confirming that the growing conditions influenced grain iron and zinc concentration. In search for maize material that could provide even more iron and zinc, a representative sample of the white-landraces held in CIMMYT maize germplasm bank was evaluated. The kernel concentration in this material varied from 17.5 to 58.5-mg/kg iron and 14.9 to 29.7 mg/kg for zinc. The best of those materials are being evaluated in multiplication trials if the results confirm potential of the landraces they will be crossed with elite germplasm with already high iron and zinc concentrations.

One difficulty maize breeders encounter is that grain iron and zinc are often correlated negatively with grain yield, which could be due to the dilution effect of increased carbohydrate content in high yielders. The multiple aleuron trait may be a fast tract to overcome this effect. Multiple aleuron layers increase the ratio of iron and zinc cells to regular endosperm cells (Welch, et al., 2000). Multiple eleurone layer is being introduced into various materials in United States and in Southern Africa. Another avenue being explored is use of low phytic acid maize germplasm with high iron bioavaliability (Mendoza, et al., 1997).

2.9 Anti-nutrient factors in legumes and their elimination

Anti-nutrients or anti-nutritional factors can be interpreted to mean an adverse physiological response produced in human or animals by a particular food or substance derived there from (Liener, 1962). Their effects might include interference with the bioavailability of nutrients resulting in an inhibition of growth, hypoglycemia goitrogenic response or damage to such tissues as the pancreas or liver.

Many legumes contain deleterious active factors such as trypsin inhibitor, hemagglutinins, cyanogenic glucoside, saponins and goitrogens. These influence utilization and bioavailability of nutrients in the body. Toxic compounds that cause diseases as lathyrism and favism are also present in some legumes (Liener, 1962). However for the purposes of this report only few of these factors important in common beans are considered below.

2.9.1 Flatulence factors

This is a result of undigested carbohydrate residues of oligosaccharides such as raffinose, stachyose, verbascose, and ajugose due to lack of α-galactosidase enzyme in human beings. These residues get into the colon where they are fermented by microbial enzymes producing gases such as carbon dioxide, hydrogen and methane resulting in abdominal discomfort and bloating (Murphy, 1964).

Flatulence factors can be removed by fermentation using lactic acid bacteria, rhizopus moulds and yeast. These break down complex carbohydrates considerably reducing the

quantities of stachyose and raffinose. Ruth (1989) and Murphy (1964) reported differences in the degree of gas produced following consumption of dry navy, kidney and pinto beans. Further experiments were conducted using Pikes Jacobs's cattle beans, which is gasless member of *P. vulgaris* and black Mexican variety. When fed to human test subjects the hybrid bean raised the level of intestinal gas to four times that of bland control meal compared to the expected ten times response common to *P. vulgaris* group. The significance of this is that there appears to be a genetic transfer of the degree of flatulence response. This suggests a possibility of reducing this undesirable legume trait by genetic selection (Max Milliner, 1975).

2.9.2 Inhibitors of digestive enzymes

There are two protein type inhibitors of digestive enzymes in common beans. These are the inhibitors of the serine group of proteolytic enzymes and of the α- amylases from animal and insect source (Bourne, 1989). It has been reported that (*P. vulgaris)* protease inhibitors inhibit human trypsin slightly less (60 – 80 %) while inhibitors from various cultivars have been shown to inhibit human chymotrypsin much more effectively. One important characteristic of trypsin-chymotrypsin inhibitors from *P. vulgaris* bean is their extreme high resistance to denaturation under certain conditions. It was observed that trypsin-chymotrypsin inhibitors are inactivated with a very short and fairly mild heat treatment of water soaked beans. For example 5 minutes at 100 ^{0}C boiling water. On the other hand isolated protein inhibitors resist harsh heat treatment for hours at 100 ^{0} C at pH5-6 (Bourne, 1989). Cooking the beans in order to destroy the protease inhibitor and the lectins is not without its problems because prolonged cooking decreases digestibility

and amino acid availability of the bean (Antunes et al., 1980). Various physiological effects of trypsin-chymotrypsin inhibitors on the rat include (a) hypertrophy and hyperplasia of the pancreas; (b) stimulus to pancreatic secretion, particularly pancreatic enzymes; (c) increased requirement for methionine due to elevated conversion of

methionine to cystein and large incorporation of cystein into pancreatic protein (Bourne, 1989).

Another enzymatic inhibitor identified in beans is the α - amylase inhibitor. Bean amylase inhibitor, inhibits insect amylase as well as human salivary amylase and porcine pancreatic amylase. Binding occurs away from the active site of the enzyme then a slow conformational change occurs which inactivates the catalytic power of the enzyme. Rather limited work has been done and published in the area of nutritional significance of this inhibitor. While the inhibitor is relatively stable, there is no evidence on whether it survives the cooking of beans.

2.9.3 Phytates

Phytate, the salt of phytic acid (myoinositol hexaphosphate), is a naturally occurring plant constituent (Burbano, et al 1995). The proportion of phytic acid varies between 10 and 30 g/kg of the dry matter of cereals, legumes and oil seeds and constitute the major portion of total phosphorous in the seed. Excessive amounts of phytic acid in the diet can have negative effects on mineral balance because it forms insoluble complexes with essential minerals (Cu^{2+}, Zn^{2+}, Fe^{2+} and Ca^{2+}) and consequently reduces the

bioavailability of these cations (Reddy, et al., 1989).

Moreover, phytate has also been shown to interact with the basic residues of proteins, inhibiting a number of digestive enzymes (Reddy et al., 1989). This phenomena has not been very well understood in dry beans. However, Rackis and Anderson (1977) reported that reduced availability of essential minerals by phytate or phytate protein complex in legumes is dependent on several factors such as: (a) ability of endogenous carrier in the intestinal mucosa to absorb essential minerals bound to phytate and other dietary substances; (b) concentration of phytic acid in food; (c) concentration of minerals in foods; (d) digestion or hydrolysis of phytate by phytase in the intestine; (e) phytase inhibition; and (f) method of processing the food.

During storage, fermentation, germination, food processing or digestion in the human gut, phytic acid is enzymatically hydrolysed by phytases to lower inositol phosphates such as inositol pentaphosphate (IP5), inositol tetraphosphate (IP4), inositol triphosphate (IP3) and possibly the inositol di- and monophosphates (IP2 and IP1). Only IP6 and IP5 have a negative effect on the bioavailability of mineral. The other hydrolytic products formed have a poor capacity to bind minerals, or the complexes formed are more soluble (Sanberg et al., 1989).

To distinguish between the hexaphosphate and its partially dephosphorylated analogues, the onion exchange column chromatography and ion pair HPLC methods were shown to be best suited for analysis of inostol phosphates in nutritional studies (Sandberg and

Ahderinne, 1986).

Phytins can be removed by soaking, preconditioning and heating. Preconditioning initiates germination changes whereby phytase activity is increased breaking down phytins (Reddy, et al., 1989). Yeast and lactic acid fermentation also reduces their effect. Fermentation by *Rhizopus oligosporus* produces the enzyme phytase, which breaks down phytic acid and destroys its chelating ability thereby making important minerals available as in tempeh (Ruth, 1989). Phytases are present in most grains and are active at pH 5. The ideal fermentation process provides optimal pH condition and hydration for degradation of phytates. Hence yeast and lactic acid fermentation drastically reduces their effect thereby increasing mineral availability (Reddy et al., 1989). Plant breeders are also pursuing breeding for varieties with low phytins.

2.9.4 Cynogenic glycosides

Legumes (especially lima and kidney beans) contain glycosides from which cyanide (HCN) may be released by hydrolysis (Montgomery, 1969). This could cause cyanide poisoning. Cyanide is released from glucoside (Phaseolunatin) hydrolysed by beta glucosidase producing glucose + 2 cyano-2-propanol which is then oxidized by oxynitrilase to produce acetone + HCN.

Hydrolysis is faster when soaked bean are cooked in water where most liberated HCN is volatilised. Further heating leads to eventual destruction of the enzyme. However Gabel and Kruger (1920) reported that cyanide could still be detected in urine of human subjects fed on lima beans, which had been cooked to destroy the enzyme. This has led to the

supposition that enzymes secreted in the intestinal tract or the microflora of the colon may be responsible for releasing HCN after ingestion of cooked beans (Max Milliner, 1975).

2.9.5 Polyphenols (tannins)

Legume and cereal seeds contain phenolic substances including tannins (Salunkhe, et al., 1982). Tannins are defined as naturally occurring polyphenolic compounds of high molecular weight of between 500 to 3000. They form stable complex with macromolecules (proteins, cellulose, hemicellulose, starch), minerals and vitamins, and affect their availability in man and animals (Mangan, 1988). Tannins (polyphenols) are produced via condensation of simple phenolics. They are mainly found in seed coat and more prominent in dark coloured varieties. They are generally divided into hydrolysable (galloyl and hexahydroxydiphenoyl esters and their derivatives) and condensed pro-anthocyanidins (polymers of flavan – 3-ols) (Blair, et al., 2005). Tannins are biologically active compounds and may have beneficial or adverse nutritional effects. Endogenous tannins contribute to plant protection against disease and predators and in some cases serve as flavour compounds (Beebe, et al., 2000). If present in sufficient quantities they may lower nutritional value and bioavailability of dietary proteins and minerals. According to Singleton and Kratzer (1969), tannins may exert anti-nutritional effect in the diet through:

(1) depression of the food intake; (2) complexing with dietary protein or other dietary components; (3) complexing with digestive enzymes and interfering with normal digestion; (4) complexing with endogenous proteins and amino acids, representing a

drain of high quality protein from the body; (5) complexing with or injuring part of the elementary tract itself; interfering with its function; and finally (6) tannin or its hydrolysis products may be absorbed and have toxic effects elsewhere in the body. However, not all phenolics are of nutritional concern and therefore, the method employed in tannin analysis has to distinguish between polyphenols of nutritional concern and low molecular weight fractions and flavanoid precursors suggested to have a positive effect on health through anti-oxidant activity.

The simplest way to lower or remove the effect of polyphenols is by soaking, dehusking and cooking. The metal chelating effect of tannins can be lowered by lactic acid fermentation. However, for high tannin varieties iron absorption inhibition may not be altered presumably due to production of metabolites of tannins, which enhance inhibition effect. Microbial fermentation, however, improves protein digestibility of high tannin grains. The other route being considered by breeders is manipulation of cysteine amino acid that is abundant in beans to supplant sulphur containing amino acids such as cysteine and methionine, which are deficient in beans in the iron uptake promoter role (Beebe, et al., 2000).

2.9.6 Lectins

These are also known as phytohemagglutinin due to their ability to agglutinate red blood cells. It was also found that hemagglutanins activity was specific to certain types of blood cells hence the coining of the term lectin (Latin, legere = to choose) by Boyd & Shapleigh. Besides agglutination lectins also promote cell adhesion, mitogenesis, and

inhibition of fungal growth. These effects are believed to be a manifestation of the ability of lectins to bind to specific kinds of sugars present on surface of cells. Jeff et al., (1961) suggested that lectins in a similar fashion as they combine with red blood cells, also combine with cells of intestinal mucosa and microvilli, thus causing a non-specific interference with intestinal absorption and nutrient absorption by the rat. It was also reported that lectins stimulated endogenous nitrogen secretion making a substantial contribution to the overall loss of nitrogen from the body (Bourne, 1989). It was also noted that breakdown of stored fat triglycerides and glycogen is also greatly increased in raw bean fed rats. In another rat model, high levels of lectins 0.5 % were shown to inhibit growth and higher levels shown to hasten onset of death (Pusztai and Palmer, 1977).

However, lectins of *P.vulgaris* are readily destroyed by heat treatment. Autoclaving for 5 minutes is sufficient to eliminate the hemagglutinating activity and toxicity of navy beans although 30 minutes of autoclaving may be required to produce effect on certain varieties. Dry heating however, seems to be much less effective in destroying the lectins following an observation that hemagglutinating activity was still detectable after 18 hours of dry heat (Arora, 1983).

2.10 Water uptake by beans

The water uptake (WU) process of dry legume is a complex process of diffusion accompanied by swelling, while seed coat and cotyledons display resistance against swelling. Beans that do not readily take up water during soaking are known as non-soakers (Van Loggerenberg, M. 2004). During the first stage of soaking, water is normally taken up by proteins, while starch gelatinisation play a more important role during the second phase of cooking (Deshpande and Cheran, 1986). Seed coat thickness plays an important role in water uptake during the first three hours of soaking, where after its contribution decreases and the importance of helium size increases. During the latter stage of soaking, the protein concentration becomes increasingly important in water uptake (Sefa Dedeh & Stanley, 1979). The seed coats high moisture content after soaking illustrates the influence of dry bean seed coats on water uptake, indicating a high capacity for water migration through the seed coat. White seed coats are preferentially permeable to water when compared to those of black and red beans (Del Valle et al., 1992). After water absorption the cotyledons display a moisture content of 53.8 % with both bound and free water present in the proteneous and sellulosic parts of the cells (Powrie et al., 1960).

The rate of water uptake is also related to bean size. Small seeded beans (white) take up water more rapidly than medium sized (black). Large beans (red) display the slowest water uptake rate (Del Valle et al., 1992). These results are however in contrast with those of Heinen and Van Twink (1976) who did not find a relationship between seed size and water uptake.

2.11 Influence of soaking

Convectional soaking followed by cooking was found to reduce phytate in dry beans and is more effective in reduction of phytates compared to salt soaked and cooked beans (Atunes et al., 1980). Phenolic acids are also significantly reduced during soaking. Soaking for 16 h to 20 h also significantly lowers the protein quality of beans. However digestibility is not affected (Molina et al., 1975). Losses of up to 1.8 % have been recorded (Wassimi et al., 1988). Beans such as small white beans, that imbibe water at a faster rate, also lose more solids during soaking; quantities of up to 2.5 g/100 g beans have been reported (Deshpande et al., 1984). The concentration gradient and the rate diffusion and the physical barrier (i.e. cotyledon cell wall and seed coat) might influence leaching losses. Pigment leaching in soaking water has also been observed such that beans canned without soaking pre-treatment are significantly darker in colour than those that receive soaking treatment due to better pigment retention (Bolles et al., 1990). During soaking beans undergo significant decreases in flatulence causing sugars i.e. stachyose, raffinose and vebascose. Soaking of beans also result in mineral losses especially iron, zinc, magnesium, manganese and potassium

2.12 Cooking time

2.12.1 Variability in cooking time of dry beans

Common bean is an important pulse crop for many families in Kenya (GOK, 2000). However bean consumption is limited by long time required to cook them as compared to other foodstuffs (Karuri, personal communication). Therefore it would be to the advantage of families that consume beans to choose varieties that are fast cooking.

Previous studies have shown that cooking time varies with the type of water used (Paradez, et al., 1986). Hard water which is usually rich in monovalent and divalent cations results in long cooking time when compared to soft water. Another drawback to the utilization of dry beans is their decreased cookability after storage. This phenomenon in beans known as hard to cook (HTC) is due to improperly stored beans at high temperature and humidities (Burr, et al., 1968) that do not soften sufficiently to be eaten, after having been cooked for reasonable time (Aguilera and Rivera, 1992). Increased cooking times for stored beans has been related to several factors. Morris, et al., (1950) associated it to development of hard-shell. Hard-shell is related to seed coat impermeability and cotyledon impermeability. Hardshell was defined by Boume (1967), as a condition whereby seeds fail to absorb water within reasonable soaking time. However, Molina et al., (1976) confirmed Burr's finding that water imbibition was not related to cooking time, as hard to cook beans imbibed water as quickly as normal beans, but water of HTC beans is mainly found in intercellular spaces, while that of soft beans is inside the cells (Aguilera and Rivera, 1992).Other theories that have sought to explain HTC phenomenon include loss of Na^+ during storage of beans under inadequate conditions (De Leon et al.,1992). Starch retrogradation during storage, which require more heat to break hydrogen bonds formed hence requiring longer cooking times (Deshpande and Cheryan, 1986). Jones and Boulter (1983) proposed that HTC beans could be a consequence of failure of cotyledon cells to separate during cooking. Restricted metabolism allowed when beans are stored at high temperatures and humilities causing membrane breakdown. These membranes would in turn reduce imbibitions during osmosis, and would allow bivalent cations from hydrolysed phytins to reach and

bind pectin (Jones and Boulter, 1983). The addition of Na^+ and K^+ to soaking and cooking solutions decrease cooking times of hardened beans (De Leon et al., 1992). Similarly addition of NaCl and /or NaHCO3 reduces hardness remarkably (Parades-Lopez et al., 1991). Hard beans also soften better in the presence of salts or Na/EDTA (Aguiler and Rivera, 1992). The reason for this is the disruption of cell surfaces by the salts allowing increased penetration of water into the cells, which allows gelatinisation of the starch during cooking causing softening. The EDTA binds calcium and prevents it from forming insoluble calcium pectate in the middle lamella hence promotes WU and softer beans.

The cooking process for beans involves at least two steps: water absorption to an equilibrium condition with free water, followed by softening of the texture by heat. Hence when looking at cooking time problem, it is important to consider water absorption impairment and physiochemical changes in the bean component. Electrolyte leakage from seeds has also been related to decreased cookability of stored beans. Kon (1979) showed that cooking rates of white beans paralleled the amount of organic phosphorus in bean after soaking. Another important factor related to cooking time is the bean moisture content. Jackson and Marston (1981) showed that cooking time was inversely proportional to the cotyledonary moisture content of the bean. Seed thickness (mid-point thickness (mm) of the two cotyledons of an intact seed) has also been found to correlate with optimal cooking time of beans, although the correlation coefficient ($r=0.41$) is not significant (Deshpande and Cheryan, 1986).

2.12.2 Assessment of cooking time using Mattson cooker

The cooking time of pulses is an important quality issue for pulse breeders, importers, exporters, food processors and consumers (http://www.grainscanada.gc.ca/quality). Although there are several methods for measuring cooking time, none is entirely satisfactory. Subjective sensory and tactile evaluation may not always be consistent. The Mattson cooker apparatus, which measures cooking time using weighted plungers, is more objective but requires constant attention. Also it can be difficult for the operator to take accurate notes if several seeds reach cooked state at the same time. The Canadian Grain Commission's pulse research team have modified the stand-alone Mattson cooker so that it is monitored by a computer and test results automatically recorded (http://www.ingentaconnect.com/jws/jsfa/). The advantage this has over the original stand-alone design is that (i) it does not require uninterrupted attention (ii) data is automatically recorded and (iii) it is more accurate.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Seventy-two test lines, which included genotypes, which are low and high in seed iron and zinc concentration, were evaluated. The genotypes were of diverse seed sizes and seed colours. They included landraces and released commercial cultivars from eight countries in East and central Africa (Ethiopia, Kenya, Uganda, Rwanda, DR Congo, Madagascar, Malawi, Sudan and Tanzania). These were grown under uniform conditions in a greenhouse at Kabete Field Station, University of Nairobi. Preliminary determination of iron and zinc concentration was conducted in the Food Chemistry Laboratory, Dept of Food Science and Nutrition of the University of Nairobi (CIAT, 2003). From this lot, 38 most promising high iron and zinc bean lines were selected for further analysis. These included AFR 708, AND 620, Awash Melka, G59/1-2, GLP-2, GLPX 92, Gofta, HRS 545, Ituri Matata, Jesca, K131, K132, Kiangara, Kirundo, LIB-1, Lingoti Blanc, Maharagi Soja, Maasai Red, MCM 2001, Mexican 142, MLB-49-89A, Mwamafutala, Nain de Kyondo, Nakaja, Nguaku Nguaku, PVA 8, Ranjonomby, Red Wolaita, Roba-1, RWR 10, Selian 97, Simama, Soya Fupi, TY3396-12, VCB 81013, VNB 81010 and Zebra. Each analysis was carried out according to procedures as outlined in the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC, 2000) with modifications suggested by Zarcinas, et al. (1987).

3.1.1 Seed sample preparation for analysis

Mature well-developed seeds, devoid of physical deformities were selected. This aimed at realizing full nutritional potential of the seeds. They were then cleaned by aspiration, rubbing in a clean dampened towel. They were then washed three times in distilled water, wiped in a clean dry towel and dried in air oven at 85 °C for one hour. The purpose was to remove contaminants from soil and any other foreign matter. After adequate blending (mixing), appropriate sub-samples were drawn and treated as follows: -

1. Sub sample A

This was composed of cleaned dry beans, pounded in a clean and dry ceramic motor and pestle to facilitate subsequent grinding in a mixer mill. They were then dried in an air oven at 85 ^{0}C for one hour to make the particles more brittle and flaky. This sub sample was finally ground through a mixer mill (Retsch, type MM 200, Germany) using Teflon grinding jar and zirconium grinding balls, at a frequency of 25/s for 10 minutes. This iron-free mill was used to reduce contamination associated with the conventional stainless steel mills.

2. Sub sample B

The grains were cooked in clean 1L glass beakers to readiness in distilled water and mashed. The mashed beans were then dried in an air oven at 90 ^{0}C for 2 hours. They were then pounded in motor and pestle to reduce the flake size. Finally they were ground in the mixer mill at the same frequency and time as in 1.

3. Sub sample C

These grains received same treatment as in 2 except that prior to cooking, the seeds were soaked in distilled water for 24 hours.

The ground bean powder was then put in dry stoppered bottles, labeled and stored at room temperature. The three sub-samples constituted the base material for all subsequent analysis.

3.1.2 Leaf sampling and drying

Leaves were sampled from farm plots in Marani Division of Kisii Central District. Sampling was done during flowering from healthy well-developed plants. From these plants three topmost, well developed and fully formed leaves were plucked to include the leaf petioles. These were then packed according to varietal entries in well labelled Kraft paper bags clearly showing the farmer number and variety. The samples were soaked for five minutes and washed in running tap water to remove visible and loosely held soil particles and foreign matter. The samples were then soaked in 2 % hydrochloric acid to solubilize any mineral elements from tightly attached soil particles and finally rinsed copiously three times in deionised water. The leaves were then wilted atmospherically, repacked in new well-labelled Kraft bags and transported to the Food Chemistry laboratory at University of Nairobi. Sample bags and their contents were weighed and dried at 85 ^{0}C overnight in an air oven. The final weight was then taken and the moisture content calculated as the difference in weight of the initial and final weight. However, since it took long (9 months) to commence analytical work on the dried samples, they were once again redried to get rid of any adsorbed moisture and new moisture loss

determined. This was the value used to determine the final dry matter content of the leaves used in subsequent calculations.

3.2 Methods

3.2.1 Determination of total solids

Dry matter content of both leaves and seeds were determined according to method 930.15 (Air Oven Method) (AOAC, 2000). Approximately 2 g of well-mixed test samples was weighed in aluminium dish (provided with a cover) previously dried at 130 ^{0}C ± 3° for 1h. The sample was then uncovered and dried in the oven set at 130 ^{0}C ± 3° for a period of 1 hour. Thereafter, the dish was covered and transferred to the desiccator, cooled and weighed. The loss in weight was reported as moisture content, while sample residues were calculated and reported as percent total dry matter.

3.2.2 Determination of iron and zinc

The procedure followed was Method 975.03 (Atomic Absorption Method) (AOAC, 2000) with time/temperature modifications as described by Zarcinas et al. (1987). One gram of dried ground test sample was accurately weighed into a digestion tube to which 10 ml of 70 % nitric acid was added and left to stand overnight at room temperature. The following day the tubes were heated to release nitrous oxide fumes and cooled to room temperature. Four ml of perchloric acid was carefully added and heated slowly at 120 ^{0}C for 1 hour (Zarcinas et al., 1987). Heat was then increased and maintained at a temperature of 175 ^{0}C until the digest cleared. Digestion at this temperature was continued until about 2 ml of the content remained and was clear. Thereafter, the temperature was further raised to 225 ^{0}C for 30 minutes. The sample was then cooled

and quantitatively transferred to a 50 ml volumetric flask and diluted to volume with 1% (v/v) nitric acid. To reduce the effect of background emission, amorphous silica was separated from the digest solution by settling it overnight and filtering through ash less filter paper before aspirating into the atomic absorption spectrometer (AAS) (Chem Tech Analytical Ltd, CTA 2000, Bedford, Britain). The absorbance of iron and zinc in the sample was read at wavelength of 248.33 nm and 213.86 nm respectively. Mineral concentration in the sample was then determined as the product of the AAS concentration reading and the dilution volume of the sample on dry weight basis.

3.2.3 Determination of crude protein

The classical procedure of the semi-micro Kjeldahl method was followed (AOAC, 2000). About 0.5 g of dried, ground sample was accurately weighed into digestion tubes in a 'nitrogen free' filter paper. 5 ml distilled water, 1 tablet of Kjeldahl catalyst and 10 ml concentrated sulphuric acid in a fume cupboard were added to each tube (Fritz, 1971). The tubes were placed in a preheated (385 – 420 ^{0}C) digestion rack and digested at this temperature till the mixture cleared. At this point digestion was stopped and the tubes allowed to cool. The contents of were then diluted carefully with 75 ml of distilled water and transferred to a distillation unit (Tecator Kjeltec System, Model 1002, Hoganas, Sweden). The contents were steam distilled using 50 ml of 40% sodium hydroxide, and the liberated ammonia trapped in 25 ml of 0.1N hydrochloric acid. When about 200 ml had distilled over, the distillation was stopped and the acid in excess back-titrated with standard 0:1N sodium hydroxide with methyl orange indicator solution to an orange yellow end point. Blank determinations were similarly conducted except that they

contained no sample. The amount of ammonia liberated (hence the amount of nitrogen in the sample) was obtained from the difference between sample and blank titrations and the nitrogen content of the sample calculated using 14.01 as the equivalent weight. This value of total nitrogen was multiplied by a factor of 6.25 to convert to % protein content in the sample on dry weight basis.

3.2.4 Determination of condensed tannins (Proanthocyanidins)

Condensed tannins were determined by method suggested by Barahona, et al. (1997). Dry clean seeds were immersed in n-heptane for 12-hour to facilitate seed peeling and recovery of the seed coats. The seeds were dried at 50 ^{0}C for 1 hour and peeled by hand. The seed coats were then ground in a hammer mill to pass a mesh of 1 mm in readiness for extraction. Extraction was accomplished by the use of 70 % acetone as follows. To 10 mg of powder placed in a screw caped tube, 2.5 ml of 70 % acetone and 2.5 ml of diethyl ether was added and votexed twice for 5 minutes. The bulk of the supernatant was discarded and the process repeated. The remaining acetone was then evaporated from the tubes in air oven at 30 ^{0}C for 1 hour and thereafter the volume made up to 5 ml with distilled water. This was then mixed and centrifuged at 3500 rpm for 10 minutes The supernatant was recovered and the residues kept in a refrigerator to evaluate insoluble tannins later. To an aliquot of 0.3 ml of the supernatant, 1.8 ml of butanol-HCl (5 %) reagent was added and boiled at 95 ^{0}C for 65 minutes. The tubes were then cooled for 5 minutes in ice water and absorbance read at 550 nm in a spectrophotometer (Cecil Instruments Inc, CE 4400/UV VIS Double beam scanning spectrophotometer, Cambridge, England).

Quantification of bound or insoluble tannins: The combined residues were dried in the oven at 40 ^{0}C for 30 minutes. To this 0.7 ml of distilled water and 4.2 ml of butanol-HCl was added. This mixture was then votexed twice for 5 minutes and then boiled at 95 ^{0}C for 65 minutes and cooled in ice water for a further 5 minutes. This was followed by centrifuging at 7000 rpm for 10 minutes and absorbance read at 550 nm in a spectrophotometer (Cecil Instrument Inc. CE 4400/UV VIS Double beam scanning spectrophotometer, Cambridge, England). Blanks were treated in similar manner but instead of using butanol-HCl (5 %), butanol-water (5 %) was used as the reagent. The experiment was conducted in triplicates with a blank for each variety. The standard curves developed at CIAT for different colour categories were adopted for these experiments.

Fig.1. Calibration data for condensed tannin determination in white, cream, yellow, pinto, yellow-brown and carioca coloured beans.

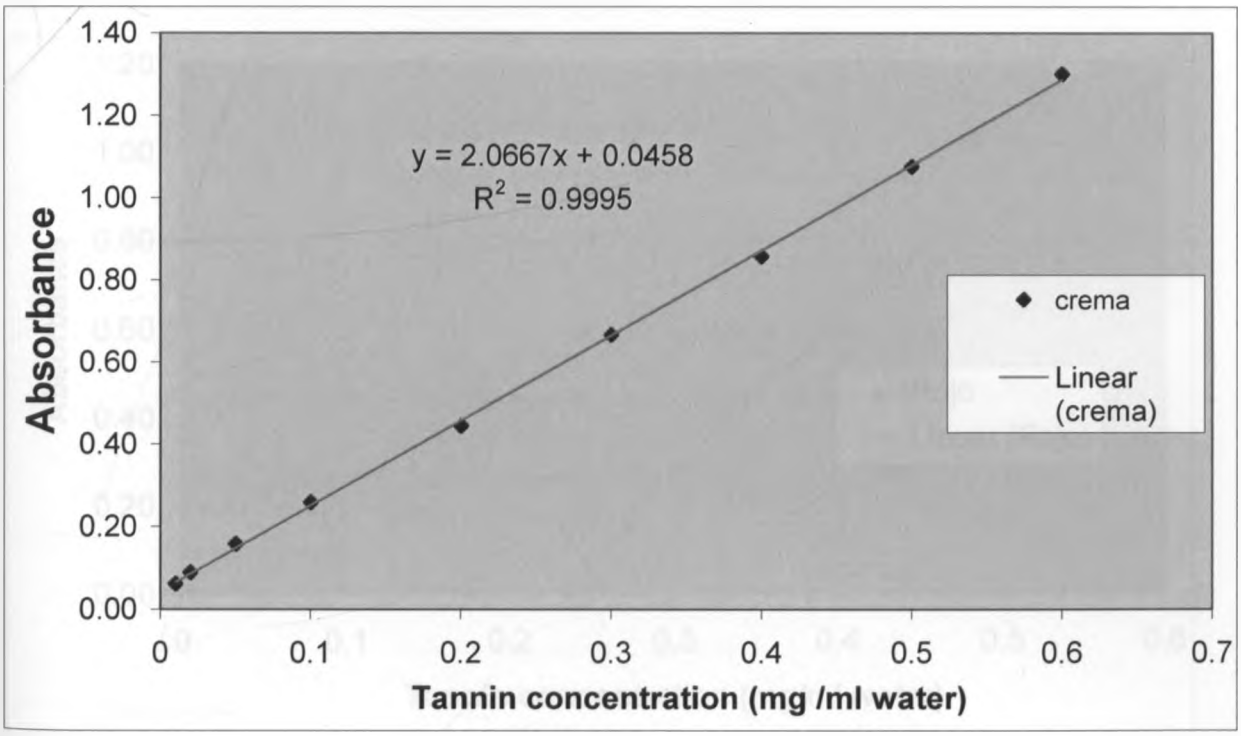


Fig. 2. Calibration data for condensed tannin determination in brown, brown-tan and brown yellow coloured beans.

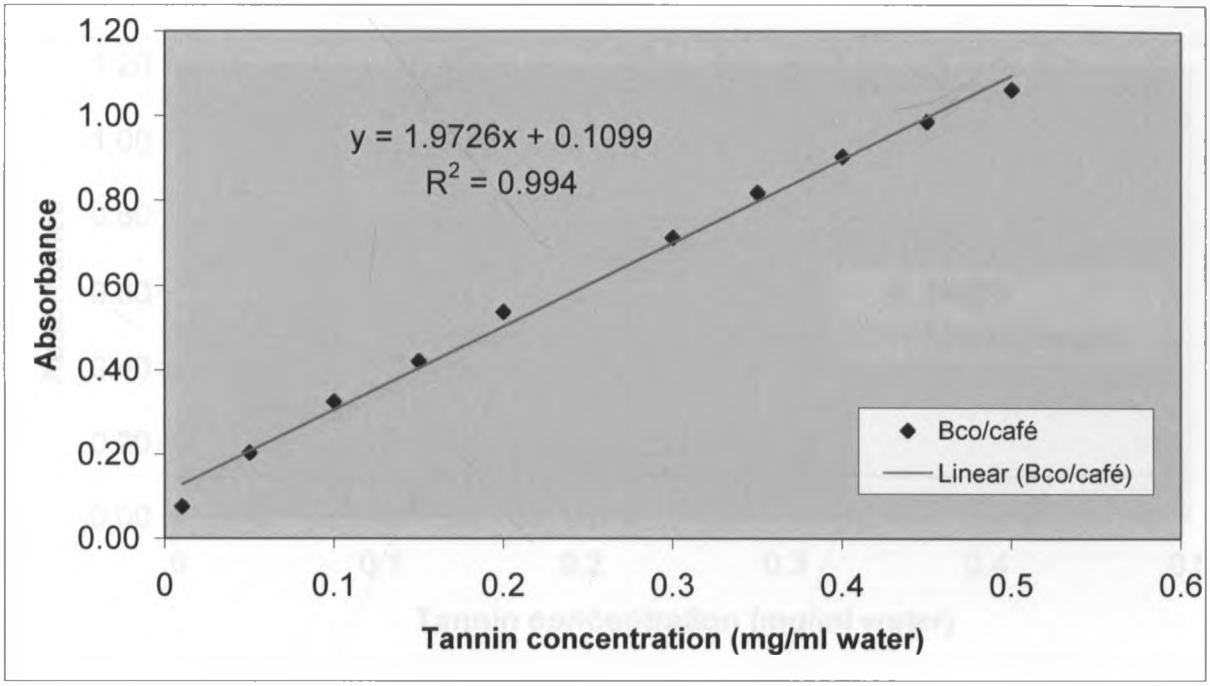


Fig 3. Calibration data for condensed tannin determination in red coloured beans.

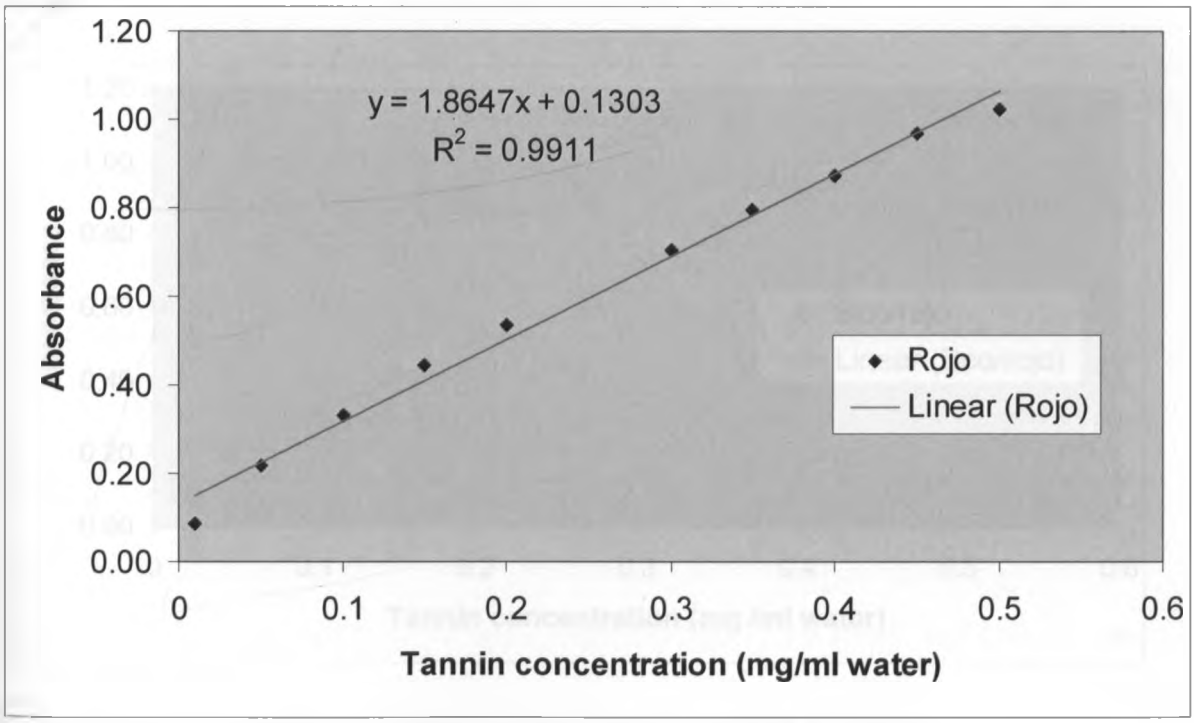


Fig 4. Calibration data for condensed tannin determination in black and purple coloured beans.

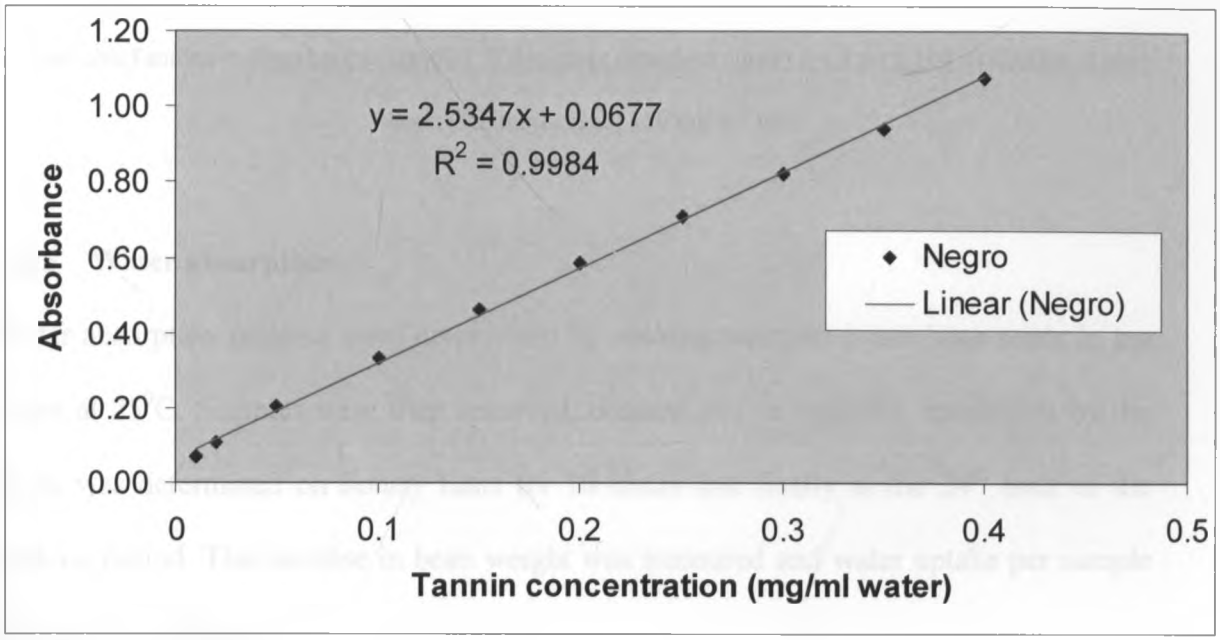
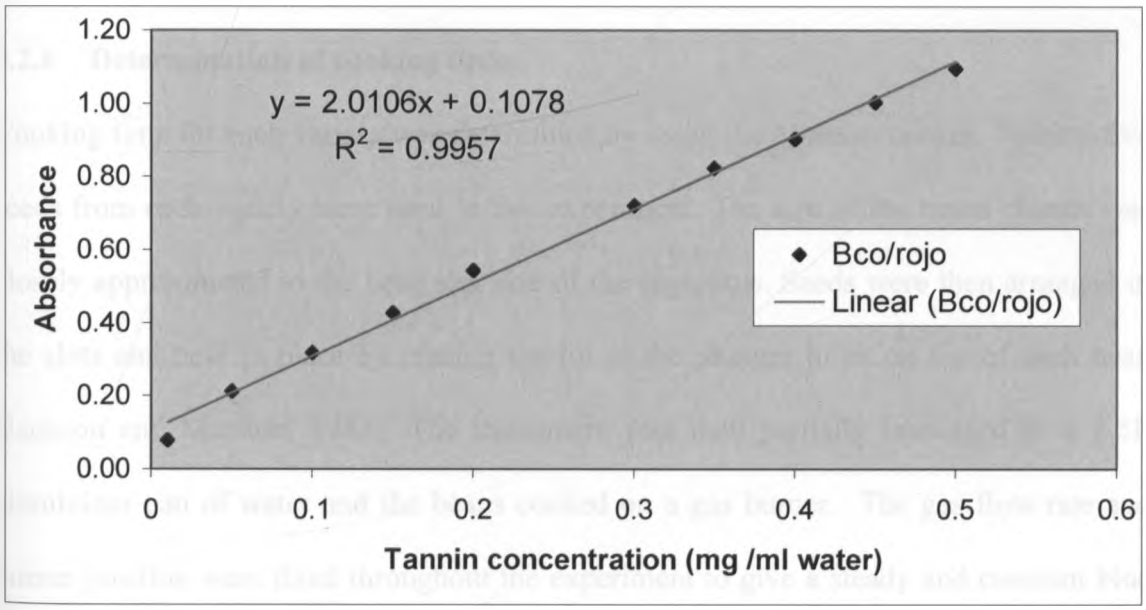


Fig 5. Calibration data for condensed tannin determination in red mottled coloured beans.



Concentration of soluble and insoluble tannins was calculated as follows:

i. Soluble tannins = Absorbance (sample) - Y-intercept (standard curve) x 5ml x 100 x (dilution if any)

Slope (standard curve) x 10 mg

ii. Insol.ubleTannins = Absorbance (sample) -Y-intercept (standard curve) x 4.9 ml x 100 x (dilution if any)

Slope (standard curve) x10 mg x 7 ml

3.2.5 Water absorption

Water absorption patterns were determined by soaking weighed intact bean seeds in tap water at 25^0C. Samples were then removed, drained and re-weighed. Imbibition by the seeds was determined on hourly basis for 10 hours and finally at the 24^{th} hour of the soaking period. The increase in bean weight was measured and water uptake per sample estimated as follows:

Water absorption = Weight of bean after soaking – Initial bean weight

Dry weight of the bean

3.2.6 Determination of cooking time

Cooking time for each variety was determined by using the Mattson cooker. Twenty-five seeds from each variety were used in this experiment. The size of the beans chosen was closely approximated to the bean slot size of the apparatus. Seeds were then arranged in the slots and held in place by placing the tip of the plunger to sit on top of each bean (Jackson and Marston, 1981). The instrument was then partially immersed in a 2.5L aluminium pan of water and the beans cooked on a gas burner. The gas flow rate and burner position were fixed throughout the experiment to give a steady and constant blue thermal flame. Tap water was used for this purpose. It was also ensured that the beans

thermal flame. Tap water was used for this purpose. It was also ensured that the beans remained under boiling water throughout the cooking process by using boiling water to top up whenever additional water was required. Finally cooking time was recorded as the time taken from the initiation of cooking until 20 of the 25 pins of the instrument had dropped and penetrated through 80% of the beans (Nin Wang, 2004).

Using the times established from the above experiments as a guide, cooking times for sub-samples B and C were determined. Cooking was done in 1L glass beakers. Except during occasional stirring, the containers were covered by means of a watch glass throughout the cooking process. Prior to cooking the beans were washed three times in distilled water.

The volume of cooking water was established in preliminary trials to be that amount that would leave about 10 ml of bean soup at the end of the cooking period. This volume ranged from 800 to 1200 ml for dry beans and 500 to 600 ml for the soaked beans. To avoid temperature fluctuations and disruption of the cooking process, any additional water to the cooking vessel was at boiling point (94^{o}C).

3.2.7 Sensory evaluation

Sensory evaluation of the fifteen varieties identified as having the highest iron and zinc concentration was conducted following standard procedures (Larmond, 1977). Sensory testing was carried out in the mid-afternoon (2.30 – 3.30 p.m.) by 12 panelists. The cooked bean samples were presented to each panelist in separate dishes labelled with a three digit random number code. The panelists were required to evaluate for the following attributes: colour, texture, flavour and overall acceptability on a 7-point hedonic scale. The lowest score of 1 represented "extremely unacceptable" while the highest score of 7 was for extreme acceptability. The value of 5 (slightly acceptable) was considered the lower limit of acceptability (Appendix 7). The panel of untrained judges were drawn from the staff and students of the Faculty of Agriculture, University of Nairobi to include men and women who are frequent bean consumers.

3.2.8 Data analysis

Data obtained for iron, zinc, protein, tannin concentration, water absorption, cooking time and sensory evaluation mean scores were subjected to analysis of variance (ANOVA) using Genstat Statistical package version 8, from the Biometry and Computing Centre, College of Agriculture and Veterinary Sciences, University of Nairobi. Sensory evaluation means were separated by Duncan Multiple Range Test (DMRT).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Variability in grain iron concentration of common bean varieties

Results of iron concentration in raw, cooked and soaked and cooked bean seeds were as shown in Table 3. Results showed that there were highly significant ($p<0.001$) differences in iron concentration due to genotype, treatment and genotype x treatment interaction.

Iron concentration in raw beans varied from 70.0 mg/kg in Ranjonomby to 107.1 mg/kg in Jesca, with a mean value of 87.1 mg/kg. In cooked seeds, iron concentration varied from 61.7 mg/kg in Ranjonomby to 97.1 mg/kg in Jesca. A relatively lower range was obtained from the soaked and cooked samples with Lib 1 giving the lowest value of 51.8 mg/kg up to 86.8 mg/kg in Roba 1.

Table 3. Iron concentration in raw, cooked and soaked-cooked common bean varieties and their retention values

	mg/kg			%	
Variety	Raw	Cooked	Soaked and Cooked	Retention Cooked	Retention in so and cooked
AFR 708	78.1	72.5	67.3	92.8	86.2
AND 620	101.5	79.6	72.3	78.4	71.2
Awash Melka	80.8	66.6	71.1	82.4	88.0
G59/1-2	94.8	79.5	68.9	83.9	72.7
GLP-2	92.3	82.1	70.6	88.9	76.5
GLP-92	79.1	69.4	64.6	87.7	81.7
Gofta	81.9	76.3	71.2	93.2	86.9
HRS 545	78.4	73.0	67.0	93.1	85.5
Ituri Matata	71.3	70.9	67.3	99.6	94.5
Jesca	107.1	97.1	68.6	90.7	64.0
K131	76.7	66.9	65.6	87.2	85.5
K132	94.1	71.8	70.6	76.3	75.0
Kiangara	77.0	69.7	68.8	90.5	89.3
Kirundo	84.9	66.6	67.8	78.4	79.8
LIB-1	74.5	67.4	51.8	90.4	69.4
Lingot Blanc	83.6	76.8	74.6	91.8	89.2
Maharagi Soja	90.1	70.0	61.2	77.7	67.9
Maasai Red	82.9	78.3	64.8	94.4	78.1
MCM 2001	80.7	79.3	70.3	98.3	87.1
Mexican 142	84.5	74.0	75.2	87.6	88.9
MLB-49-89A	106.9	92.6	65.6	86.6	61.4
Mwao,Mafutala	105.9	83.9	76.4	79.2	72.1
Nain de Kyondo	102.2	86.0	76.4	84.1	74.7
Nakaja	83.7	74.8	59.5	89.4	71.0
Nguaku Nguaku	76.3	68.0	69.2	89.2	90.7
PVA 8	87.7	67.8	67.3	77.3	76.8
Ranjonomby	70.0	61.9	57.3	88.4	81.8
Red Wolaita	74.1	71.9	68.2	97.0	92.0
Roba-1	93.6	84.0	86.8	89.8	92.8
RWR 10	81.1	74.5	66.8	91.8	82.3
Selian 97	80.8	77.8	69.3	96.3	85.8
Simama	90.6	84.5	67.4	93.3	74.4
Soya Fupi	96.6	87.0	60.6	90.1	62.7
TY3396-12	104.5	75.2	61.7	71.9	59.1
VCB 81013	102.6	74.5	62.9	72.6	61.3
VNB 81010	97.2	69.4	70.6	71.4	72.6
Zebra	73.4	64.1	60.4	87.3	82.2
Mean	87.1	75.3	67.7	87.0	78.7
LSD (05)	2.8				
CV (%)	3.2				

Characteristics of the top ten lines for iron concentration are shown in Table 4.

Table 4. Origin, growth habit, seed colour and iron concentration of ten bean genotypes grown in East and Central Africa.

Line	Origin	Growth habit	Seed colour	Seed size	Fe (mg/kg)
Jesca	Tanzania	bush	Purple	large	107.1
MLB-49-89A	Rwanda	bush	Black	small	106.9
Mwa Mafutala	DRC	bush	Brown	small	105.9
TY-3396-12	Ethiopia	bush	Carioca	medium	104.5
VCB 81013	DRC	climber	White	small	102.6
Nain de Kyondo	DRC	climber	White	small	102.2
AND 620	DRC	bush	red mottled	large	101.5
VNB 81010	DRC	climber	Black	medium	97.2
Soya Fupi	Tanzania	bush	Purple	medium	96.6
G59/1-2	DRC	climber	Red	large	94.8

Large (>40g/100 seeds); Medium (26 – 39g/100 seeds); small (<25g/100 seeds)

Six of the ten lines originated from Democratic Republic of Congo. Four were climbers and six were bush type. Four lines were small seeded and three were medium. Three lines had large seeds. They represented seven grain types: white, black, brown, purple, red, red mottled and carioca.

4.2 Variability in grain zinc concentration of common bean varieties

Table 5 shows zinc content of the various bean varieties analysed.

Table 5: Zinc concentration in raw, cooked and soaked-cooked common bean varieties and their retention values.

	mg/kg			%	
Variety	Raw	Cooked	Soaked and Cooked	Retention Cooked	Retention in soaked and cooked
AFR 708	35.7	30.2	28.5	84.6	80.0
AND 620	43.9	30.3	27.7	69.0	63.0
Awash Melka	33.8	31.2	24.7	92.3	73.0
G59/1-2	30.9	27.9	21.5	90.5	69.8
GLP-2	36.0	33.5	27.0	93.0	74.9
GLP-92	39.2	32.4	24.0	82.7	61.2
Gofta	36.3	34.4	28.3	94.8	78.0
HRS 545	37.5	32.3	27.9	86.2	74.5
Ituri Matata	37.9	33.2	29.6	87.7	78.1
Jesca	33.7	29.2	21.1	86.6	62.4
K131	34.9	30.9	25.1	88.3	71.9
K132	37.3	31.9	29.4	85.6	78.9
Kiangara	29.7	27.5	25.2	92.5	84.9
Kirundo	33.7	30.4	30.4	90.0	90.2
LIB-1	31.7	29.1	22.9	91.9	72.2
Lingoti Blanc	37.2	33.3	24.9	89.6	67.1
Maharagi Soja	34.2	30.4	27.2	88.8	79.3
Maasai Red	40.3	31.8	37.1	79.1	92.2
MCM 2001	41.0	36.6	27.3	89.3	66.7
Mexican 142	39.2	38.2	27.9	97.3	71.1
MLB-49-89A	31.2	25.5	24.8	81.7	79.5
Mwa,Mafutala	34.2	31.2	24.8	91.1	72.4
Nain de Kyando	41.2	33.1	25.5	80.2	61.9
Nakaja	41.0	35.7	24.9	87.0	60.8
Nguaku Nguaku	28.8	26.5	22.9	92.0	79.3
PVA 8	31.4	30.2	23.2	96.2	74.0
Ranjonomby	34.9	32.6	23.0	93.6	66.0
Red Wolaita	37.2	29.7	26.5	79.7	71.1
Roba-1	37.0	31.0	26.4	84.0	71.3
RWR 10	38.2	34.4	26.5	90.0	69.5
Selian 97	37.5	35.4	29.5	94.5	78.6
Simama	37.6	31.3	28.5	83.1	75.8
Soya Fupi	27.0	23.3	20.1	86.4	74.5
TY3396-12	33.3	27.6	22.6	82.7	68.0
VCB 81013	34.4	26.7	20.4	77.8	59.3
VNB 81010	43.4	34.8	29.3	80.3	67.5
Zebra	37.1	29.1	24.9	78.5	66.9
Mean	35.9	31.2	26.0	87.0	72.6
LSD (05)	1.6				
CV (%)	4.4				

Results indicated that there were highly significant ($p<0.001$) differences in zinc concentration due to bean genotype, treatment and the interaction between genotype and treatment. Overall variability of this micronutrient in cooked and raw beans was high. Zinc concentration in raw bean samples varied from 26.9 mg/kg in Soya Fupi, a Tanzanian purple variety, to 43.9 mg/kg in AND 620, a red mottled bush variety from DR Congo with an average of 35.9 mg/kg. Concentration in cooked beans ranged from 23.3 mg/kg in Soya Fupi, to 38.2 in Mexican 142, a small white navy canning bean grown in Kenya, Tanzania and Ethiopia. The levels were slightly lower in soaked and cooked samples, ranging between 20.1 mg/kg in Soya Fupi and 37.1 mg/kg in Maasai Red. Characteristics of the ten most promising lines for zinc concentration are presented in Table 6.

Table 6. Origin, growth habit, grain type and zinc concentration of ten bean cultivars grown in East and Central Africa.

Line	Origin	Growth habit	Grain type	seed size	Zn(mg/kg)
AND 620	D R Congo	Bush	red mottled	large	43.9
VNB 81010	D R Congo	climber	Black	medium	43.4
Nain de Kyondo	D R Congo	climber	White	small	41.2
Nakaja	D R Congo	climber	Brown	small	41.0
MCM 2001	Uganda	Bush	Red	small	40.9
Maasai Red	Tanzania	Bush	Red	small	40.3
Mexican 142	Ethiopia	Bush	White	small	39.2
GLPX 92	Kenya	Bush	Pinto	medium	39.2
RWR 10	Rwanda	Bush	Red	large	38.2
Ituri Matata	D R Congo	Bush	White	large	37.9

Large (>40 g/100 seeds); medium (26 – 39 g/100 seeds); small (<25 g/100 seeds)

These high zinc lines include both bush and climbers. Most of these lines originated from DR Congo and the East African region. Five of the lines were small seeded, three were large and two medium size. The lines belonged to six grain types: white, red, pinto,

brown, black and red mottled. AND 620, VNB 81010 and Nain de Kyondo combined both high iron and zinc levels.

Previous work conducted in CIAT (Colombia, South America) involving evaluation of more than one thousand accessions in the cultivated bean core collection revealed a range of 21 to 54 mg / kg in zinc content with a mean of 35 mg/ kg. Further trials conducted on 72 bean samples from Eastern and Central African region gave a mean zinc concentration of 31 mg /kg (CIAT, 2004) ranging from 12 to 62 mg /kg. Iron concentration in the same samples ranged from 34 to 89 mg /kg with a mean of 55 mg /kg. The mean and range values of iron and zinc concentration obtained in this study tallied well with those obtained in previous studies. These results indicated variability exists for both iron and zinc concentration in bean lines. They also indicated that a few varieties combine high levels of iron and zinc. Further evaluation showed significant positive correlation between the two elements of 0.5 in seed and 0.36 in leaf across different genotypes at 1% level (Appendix 8). This correlation probably reflects co-segregation of genes for iron and zinc such that improvement of one mineral may simultaneously improve the content of the other mineral thus multiplying the impact of the effort. This is a nutritionally important aspect to the bean consumer as he may benefit from both micronutrients. These results when compared to those obtained from Burundi (Barapama, 1993), those reported by Beebe (2000) and those reported by Kimani et al. (CIAT, 2004), may also indicate that these traits are stable over regions. This offers good prospects that genotypes selected in one environment for high iron and zinc content, will express superior levels of the minerals in other environments as well.

The differences in the range of iron and zinc concentration in the above trials suggest that the environment may influence the degree of expression of these nutrients in the seed.

4.3 Crude protein concentration in common bean varieties

The crude protein content of raw, cooked and soaked and cooked bean varieties are shown in Table 7. Results showed highly significant ($P<0.001$) differences in protein concentration among varieties (Appendix 1). The results also indicated the existence of genetic variability for protein content in seeds from different varieties. The protein concentration in raw beans varied from 20.4% in PVA 8 to 28.4% in Lib 1 with a mean value of 23.1%. These values are in agreement with those reported from previous studies (Bourn, 1987) where crude protein ranged from 18 to 29 % with a mean of 23.6 %.

The range values for the cooked and soaked and cooked samples were 17.3% in PVA 8 to 27.0% in Selian 97 and 15.9% in Simama to 26.7% in Maasai Red respectively with means of 23.1% and 22.5% respectively.

Table 7. Protein content of raw, cooked and soaked-cooked common bean varieties and their retention values

	%				
Variety	**Raw**	**Cooked**	**Soaked and Cooked**	**Retention Cooked**	**Retention soaked-cooke**
AFR 708	28.3	22.1	22.8	78.1	80.8
AND 620	22.9	22.3	21.4	97.3	93.5
Awash Melka	26.4	21.9	23.6	83.1	89.5
G59/1-2	28.0	23.7	23.5	84.8	84.1
GLP-2	26.6	26.1	24.1	98.0	90.7
GLP-92	25.1	21.8	21.6	87.1	86.3
Gofta	26.0	24.1	23.4	92.8	89.8
HRS 545	26.6	23.4	22.5	87.9	84.5
Ituri Matata	24.7	22.7	22.2	92.0	90.1
Jesca	24.5	22.0	21.2	90.1	86.7
K131	26.5	23.4	22.8	88.0	86.1
K132	26.4	23.6	23.8	89.3	90.1
Kiangara	25.8	22.3	21.6	86.5	83.7
Kirundo	25.1	22.5	22.4	89.5	89.4
LIB-1	28.4	25.5	23.7	90.0	83.6
Lingoti Blanc	27.1	24.7	24.2	91.3	89.4
Maharagi Soja	25.8	22.5	22.5	87.2	87.4
Maasai Red	27.8	25.4	26.7	91.4	96.0
MCM 2001	27.1	23.5	22.6	86.7	83.6
Mexican 142	27.8	25.6	24.1	91.9	86.6
MLB-49-89A	26.1	23.4	22.6	89.7	86.8
Mwa,Mafutala	25.9	23.1	23.6	89.3	91.0
Nain de Kyondo	24.3	21.8	20.8	89.7	85.5
Nakaja	25.8	24.4	24.4	94.3	94.6
Nguaku Nguaku	25.4	22.1	20.7	87.0	81.7
PVA 8	20.4	17.3	16.9	85.0	82.9
Ranjonomby	24.3	22.2	21.4	91.6	88.3
Red Wolaita	26.2	24.0	24.0	91.3	91.5
Roba-1	26.6	24.0	24.7	90.3	92.8
RWR 10	27.3	23.3	22.7	85.4	83.1
Selian 97	28.0	27.0	26.3	96.3	94.0
Simama	25.7	23.3	15.9	90.6	61.8
Soya Fupi	26.6	23.2	23.0	87.0	86.2
TY3396-12	24.1	20.5	19.3	85.2	80.2
VCB 81013	24.6	21.1	23.4	86.0	95.1
VNB 81010	27.3	22.7	22.7	83.2	83.3
Zebra	24.3	21.7	20.5	89.3	84.3
Mean	25.9	23.1	22.5	89.0	86.9
LSD (05)	0.4				
CV (%)	1.5				

.Results showed that average protein concentration of raw, cooked and soaked and cooked beans was >20% (Table 7). This suggested that cooked beans are a good source of dietary protein. However, the quality of bean protein is generally low due to limiting amounts of sulphur containing amino acids, low digestibility and therefore low bioavailability of the amino acids. This is an important aspect, since mixing the bean with complementary source of sulphur containing amino acids, like cereal grains, greatly improves the PER values. It also results in protein mixtures that are more nutritive than either the bean or the cereal protein individually. These mixtures are also less costly and therefore the protein needs of the poor can be met cheaply as compared to utilization of pure animal proteins.

Equally important is the fact that some varieties such as VNB 81010, G59/1-2 combine high iron and protein content, while Mexican 142, Maasai Red, MCM 2001 have both high protein and high zinc content.

4.4 Effect of cooking on iron, zinc and protein content of beans

4.4.1 Effect of cooking on iron content.

Results of iron concentration in cooked, and soaked and cooked beans are shown in Table 3. Results showed highly significant ($P<0.001$) reduction in iron concentration due to genotype, treatment and genotype x treatment interaction (Appendix 1). Iron concentration in cooked samples ranged from 61.9 mg/kg in Ranjonomby to 97.1 mg/kg in Jesca with a mean of 75.3 mg/kg. These data are slightly lower than those reported by Augustin et al. (1981). Retention values ranged between 71.4 and 99.6 % with a mean of

87 %. Results from soak-cooked samples varied from 51.8 to 86.8 % with a mean value of 67.7 %. The levels of iron retention values above could be attributed to leaching of the nutrient into the soaking and cooking water. This has also been reported by Imungi (1984).

4.4.2 Effect of cooking on zinc content

Results of zinc concentration in cooked and soaked –cooked samples are shown in Table 5. The nutrient variability in cooked samples was higher than the corresponding uncooked material. Concentration in cooked seeds ranged from 23.3 mg/kg in Soya Fupi to 38.2 in Mexican 142, with a mean of 31.2 mg/kg. A similar range was reported by Augustin et al. (1981).

Soaked-cooked samples had a mean zinc concentration of 26.0 mg/kg. Values ranged between 20.1 mg/kg in Soya Fupi and 37.1 mg/kg in Maasai Red. They were slightly lower than those obtained from cooked samples. Results indicated that cooking resulted in highly significant ($p<0.001$) reduction in zinc concentration of the beans (Appendix 1). The retention values ranged from 69.0 to 97.3 % in cooked samples with a mean of 87.0 %. The soaked and cooked samples had a lower retention values ranging between 59.3 % and 92.2 % with a mean of 72.6 %. The main route of zinc loss from cooked beans would be leaching in varying proportions of the nutrient in soaking and cooking water. These retention levels were lower than those reported by Augustin et al. (1981).

However, soaking has been shown to be beneficial in lowering antinutritional effect in

beans. Convectional soaking followed by cooking was found to reduce phytate in dry beans. It is more effective in reduction of phytate compared to salt soaked and cooked beans (Iyer et al., 1980). Phenolic acids are also significantly reduced. During soaking, beans undergo significant decreases in flatulence causing sugars (starchyose, rafinose and verbascose) (van Loggerenberg, 2004).

4.4.3 Effect of cooking on protein content

These findings were consistent with those reported by Augustin et al. (1981) and El Tinay et al. (1989). In this study as in Augustin's there was an allowance for broth loss to simulate home preparation situation. Farmers interviewed in Suneka and Marani Divisions regarding bean preparation methods, indicated that some households cooked beans without soaking while others soak them first. After soaking, the soak water is discarded and fresh water used for cooking. When the beans are cooked the remnant soup is decanted, the reason being that bean soup is associated with flatulence and stomach disturbances hence undesirable. Those who don't soak their beans prior to cooking indicated that soaked beans loose flavour. However, they too did not cherish bean soup. Therefore based on the results of this study, the maximum expected cooking loss for iron, zinc and protein are; 13 and 21%, 13 and 27%, and 11 and 13% respectively for cooked and soaked and cooked beans. However these levels could be brought down to insignificant values through appropriate nutrition education in bean preparation and utilization

One main technique for cooking bean leaves "Rikuneni" (Kigusi language) always

utilizes large volumes of water that is usually discarded in order to get rid of the bitter and toxic principles that could possibly be present in the raw leaves. In extreme cases, the cooked bean leaves are squeezed to rid them of excess cooking water and thereafter rinsed in fresh water before finally frying them.

These cooking methods often result in large losses of nutrients especially due to leaching of water soluble ones. Therefore bean grain and leaf preparation methods, which use less water to bring about acceptable softness and flavour or which utilize soaking and cooking water, could enhance recovery of nutrients. Consumption of bean broth can greatly enhance nutrient intake. However, some communities especially from Central Province, in preparation of some bean dishes do not discard cooking water. "Githeri" (Kikuyu) a dish made by cooking a mixture of maize kernels and beans is one such a dish. However, other than the loss emanating from poor preparation methods, nutrient complexing with anti-nutritional factors like phytates and tannins could have a far-reaching effect on bioavailability of these nutrients to the consumer.

4.5 Tannin concentration in common bean varieties

Results of tannin content are shown in Table 8.

Table 8. Seed colour, seed size and tannin concentration of common bean varieties.

			%				
Variety	**Seed size**	**Seed colour**	**Soluble tannins**	**Insoluble tannins**	**Total tannins**	**Seed coat**	**Tannin(mg/g) whole seed**
Ranjonomby	large	white	Trace	Trace	trace	9.0	trace
Ituri Matata	large	white	Trace	Trace	trace	9.0	trace
Lingot Blanc	large	white	Trace	Trace	trace	5.0	trace
Mexican 142	small	white	Trace	Trace	trace	9.0	trace
Awash Melka	small	white	Trace	Trace	trace	9.0	trace
HRS 545	small	white	Trace	Trace	trace	5.0	trace
VCB 81013	small	white	Trace	Trace	trace	6.0	trace
Nain de Kyondo	small	white	Trace	Trace	trace	9.0	trace
Red Wolaita	small	red	13.1	2.2	15.3	6.0	9.2
Maasai Red	small	red	11.9	1.3	13.2	9.0	11.9
MCM 2001	small	red	10.2	1.2	11.3	9.0	10.2
RWR 10	large	red	5.0	0.6	5.6	9.5	5.3
Selian 97	large	red	7.5	0.9	8.3	10.0	8.3
G 59/1-2	large	red	7.2	1.0	8.2	7.0	5.7
AFR 708	large	red mottled	6.1	0.8	6.9	7.0	4.8
Simama	large	red mottled	5.9	0.9	6.8	9.0	6.1
K 132	large	red mottled	5.7	1.4	7.2	8.5	6.1
AND 620	large	red mottled	5.4	1.1	6.6	9.0	5.9
GLP 2	large	red mottled	6.2	2.1	8.3	8.5	7.1
PVA 8	large	red mottled	5.9	1.2	7.1	9.0	6.3
Zebra	medium	carioca	6.5	2.0	8.5	5.0	4.3
TY 3396-12	medium	carioca	6.0	1.1	7.1	10.0	7.1
K 131	small	pinto	11.1	1.5	12.6	10.0	12.6
GLPx92	medium	pinto	4.9	1.0	5.9	10.0	5.9
Kiangara	medium	yellow/Brown	6.8	1.8	8.5	7.0	6.0
Kirundo	medium	yellow	2.1	0.9	3.0	6.0	1.8
Lib-1	medium	yellow	6.1	0.4	6.5	5.0	3.3
Gofta	medium	brown	8.1	1.0	9.0	5.0	4.5
Roba-1	small	brown/tan	3.2	1.0	4.1	10.0	4.1
Mwa Mafutala	small	brown	8.4	1.0	9.4	6.0	5.7
Maharagi Soja	small	brown/yellow	1.6	0.6	2.2	5.0	1.1
Nakaja	small	brown	6.3	1.5	7.8	5.0	3.9
Soya Fupi	medium	purple	3.9	1.4	5.3	8.0	4.3
Jesca	large	purple	1.5	1.6	3.1	8.5	2.6
MLB 49-89A	medium	black	3.3	2.2	5.5	5.0	2.7
VNB 81010	medium	black	2.9	1.5	4.3	6.0	2.6
Mean							5.8
LSD(05)							0.3
CV(%)							2.9

Results showed highly significant ($p<0.001$) differences in tannin concentration of the various bean varieties analysed. The concentration seemed to be influenced by the colour of the dry bean seed coats. Coloured bean seeds had higher tannin concentrations than those obtained in white bean seeds. White bean varieties produced no colour at all during extraction with acetone and development with butanol-HCL. Phenolic compounds were not detectable by the method used, a possible indication of low levels of tannin in the seed coat. Other colours showed some pigmentation of the solution during extraction that intensified on heating with the reagent. However, the black varieties, i.e. MLB-49-89A and VNB 81010 gave intense pink colouration during extraction but the intensity significantly decreased on heating with butanol-HCl reagent. The decreased colour intensity in the black bean extract could be due to higher degradation heat reactions than condensation reactions on anthocyanins. These observations were later verified by the final calculated tannin concentrations in the seed coat. On average tannin concentration in decreasing order was red > brown > black > yellow > white. Similar trends have been reported by other researchers (Beebe, et al., 2000; Bressani, 1993). Bressani (1993) reported an average of 12.56 mg/g of catechin equivalents in red beans, 7.8 mg/g in beans with a bronze testa colour, 6.65mg/g in beans with black testa colour and 2.31 mg/g in white beans. Similar observations were also made by Barampama and Simard (1993) and Blair et al. (2005). High tannin variabilities were also noted between bean as well as within bean colour class.

Tannin concentration is an important nutritional concern especially to those families who rely heavily on pulses for their mineral and protein requirement. According to Chung et

al. (1998), tannins are considered nutritionally undesirable because they precipitate proteins (1 mole of tannin is reported to bind 12 moles of protein), inhibit digestive enzymes and interfere with the utilization of vitamins and bioavailability of minerals. These findings may suggest that coloured bean types with high mineral and protein concentration may not benefit the consumer as would be expected if they also contain high tannin concentration. However recently, polyphenols because of their antioxidant properties are associated with health benefits of disease prevention- including cancer, diabetes, arteriosclerosis and even HIV/ AIDS. These findings also indicate that, white coloured varieties may possibly be the avenue for maximizing delivery of these nutrients to the needy populations. Recent publications (CIAT, 2005) indicate that certain bean genotypes were found to have iron bioavailability, which was associated with white seed coat colour. Very little bioavailable iron was observed in all coloured beans and the addition of ascorbate or meat did not enhance bioavailability. These results clearly indicate that phenolic compounds are predominantly inhibitors of iron using the in vitro CaCO-2 cell model. However, various processing methods such as soaking, cooking, germination and autoclaving reduce significantly ($\square$ <0.05) the levels of tannins in beans (Chau and Cheung, 1997). The observed reduction in tannin content in heat treated and germinated seeds has been attributed to decreased extractability, change in chemical reactivity and formation of hydrophobic associations (complexes of tannins with proteins and enzymes) and not due to actual loss or degradation of tannins (Buller et al., 1984; Van der Poel, 1990).

4.6 Variability of iron, zinc and protein concentration in common bean leaves

The levels of iron, zinc and crude protein concentration in dry bean leaves are presented in Table 9.

Table 9. Iron, zinc and protein concentration of dry bean leaves.

	mg/kg		%
Variety	**Iron**	**Zinc**	**Protein**
AFR 708	854.6	36.0	26.7
AND 620	939.5	34.2	24.6
Awash Melka	366.1	35.2	27.7
GLP-2	1128.5	43.7	27.8
GLP-92	516.0	33.6	24.4
HRS 545	778.3	41.8	32.3
Ituri Matata	947.3	65.1	29.0
Jesca	957.5	39.2	27.4
K131	984.7	40.1	29.9
K132	653.8	56.2	28.4
Kiangara	1044.9	94.7	27.7
Kirundo	1961.8	57.1	31.5
LIB-1	462.7	35.4	24.3
Lingoti Blanc	1043.9	44.7	30.5
Maharagi Soja	575.5	43.7	31.1
Masai Red	860.1	67.6	29.3
MCM 2001	590.6	53.1	28.8
Mexican 142	629.5	48.7	31.4
Nain de Kyondo	610.4	58.6	29.7
Nakaja	1142.6	67.1	31.8
Nguaku Nguaku	509.4	17.4	28.9
Oba-1	1201.9	49.9	26.1
PVA 8	594.3	43.0	30.1
Ranjonomby	868.8	62.1	28.7
Red Wolaita	1374.5	53.5	23.7
Roba-1	899.3	53.5	33.3
RWA 10	998.8	47.4	33.8
Simama	502.4	21.8	26.8
Soya Fupi	1051.6	37.1	29.3
TY3396-12	334.5	49.5	35.6
VCB 81013	923.3	67.6	33.2
Zebra	236.1	47.2	34.7
Mean	829.5	48.3	29.3
LSD (05)	28.5	2.1	0.8
CV (%)	1.7	2.2	1.3

Results showed highly significant ($p<0.001$) variation in concentration of iron, zinc and protein in the bean leaves (Appendix 2). The level of iron concentration was very high when compared to values obtained from the grain. A mean iron concentration of 829.5 mg/kg was found, ranging from 236.1 mg/kg in Zebra to 1961.8 mg/kg in Kirundo.

Zinc concentration ranged from 17.4 mg/kg in Nguaku Nguaku to 94.7 mg/kg in Kiangara with an average value of 48.3 mg/kg. Nguaku Nguaku and Simama had very low levels of zinc. They recorded 17.4 mg/kg and 21.8 mg/kg respectively.

Crude protein varied from 23.7% in Red Wolaita to 35.6% in TY 3396-12 with a mean of 29.3%. These values were comparable to those of seeds.

The high variability of iron, zinc and protein concentration observed in leaf samples, and the wide range of values shown, could be due to differences in soil composition in various farm plots from which these leaves were sampled. Table 9 reveals that iron concentration was higher and more variable in the leaves than in the seeds of the same variety. Similar observations were also made in the case of zinc and protein in most varieties. The magnitude was, however, not as big as that observed in iron. This difference could be an effect of varying soil type and chemistry over these two sites (Marani and Kabete). Since the leaf samples were taken at flowering it could be possible that before podding and seed formation, leaves act as the major nutrient storage organs of the bean plant. During maturation this role seems to be translocated to the seeds. The leaves retain a higher concentration of these nutrients may be as a result of

immobilization due to their high fibre content. High iron, zinc and protein content in leaves is nutritionally good news. Many of the people in both rural and urban areas of Kenya consume large quantities of vegetables (Imungi, 1984). Only very few people can afford animal products to provide the necessary minerals and proteins. For the majority of the population, therefore, the main source of protein in their predominantly starch based diets is the vegetable that are always eaten with these diets (Imbamba, 1973). Nutritional survey conducted by Applied Nutrition students of the University of Nairobi in 2003 in Marani and Suneka Division of Kisii Central District, revealed that bean leaves, locally known as "Rikuneni" is a relished vegetable frequently consumed throughout the year by all family members. Therefore, high iron, zinc and protein levels in bean leaves would be nutritionally a tremendous headway in improving nutrient intake. Since proteins of leafy vegetables are high in lysine and tryptophan they can complement cereals as much as legume seeds do (Jelliffe and Jelliffe, 1973). However, eating of leaves is not expected to meet the consumers protein and mineral requirements. This is because the high fibre content of the leaves may limit protein digestibility and mineral absorption.

4.7 Water absorption capacity of common bean seeds

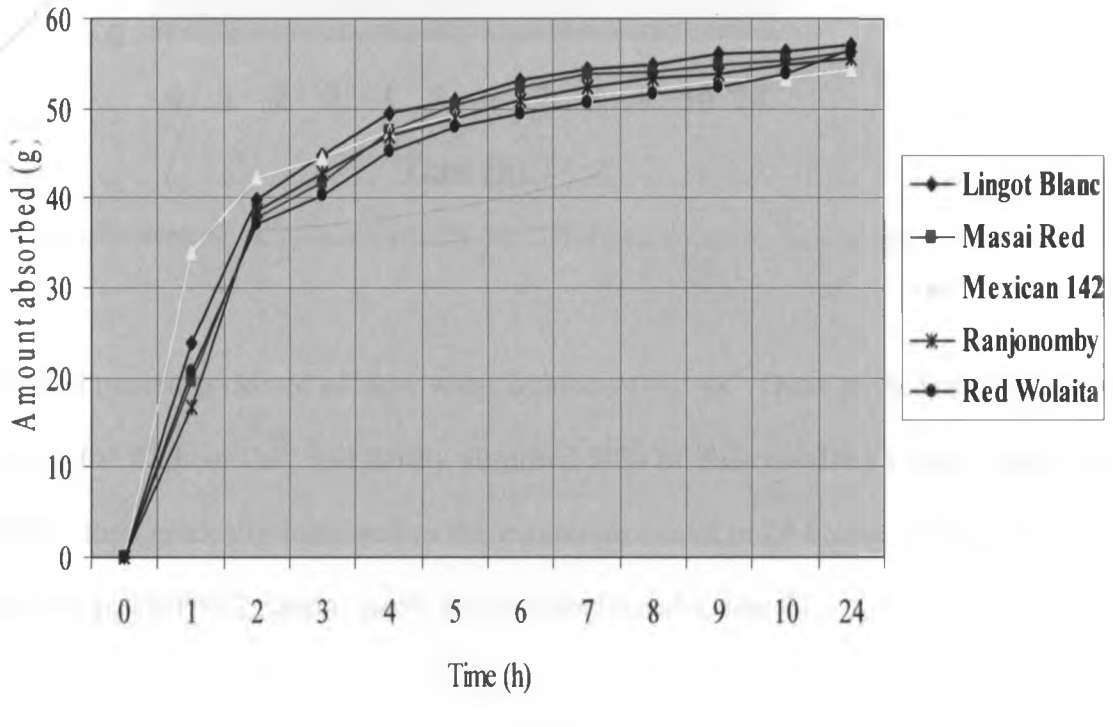
Seed water uptake is shown in Table 10. Results showed differences in water absorption rate emerging right from the first hour of soaking.

Table 10. Water absorption capacity of common bean varieties after 24 h

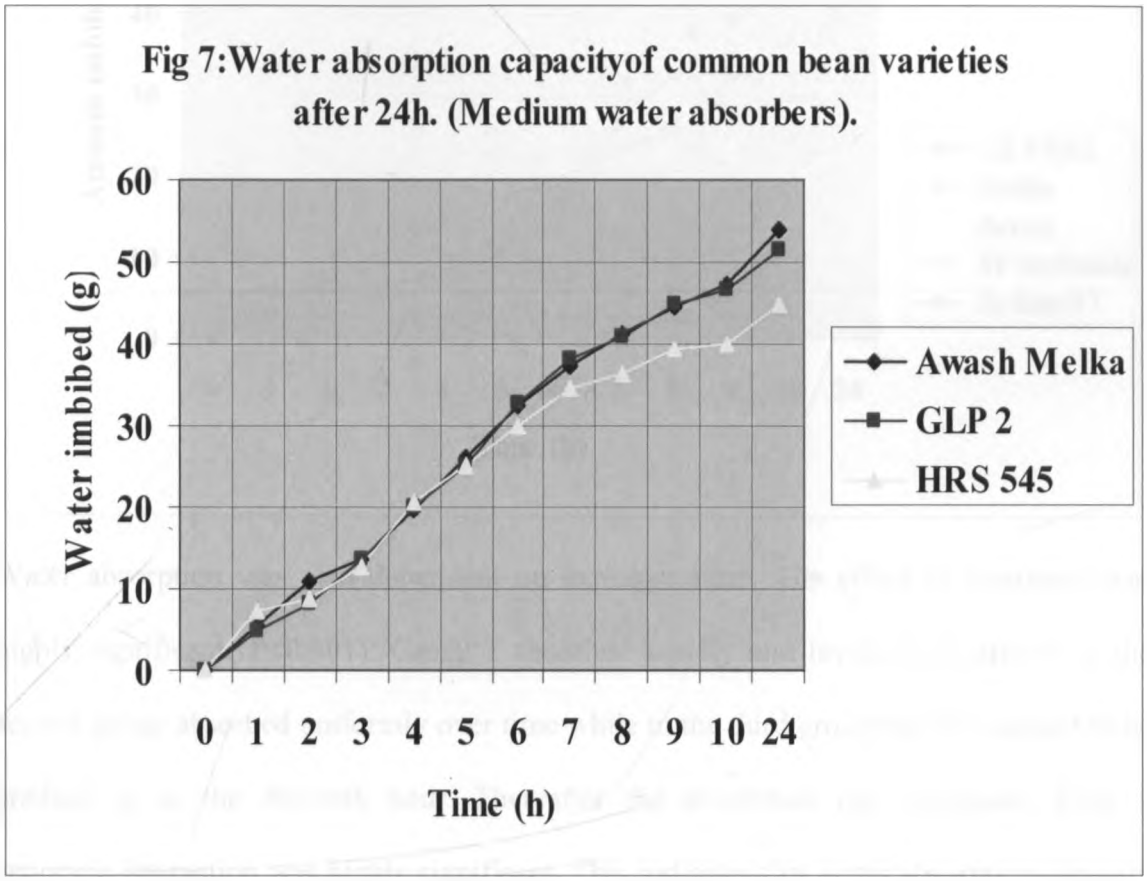
Time (h)	1	2	3	4	5	6	7	8	9	10	24
	Amount of water imbibed (g)										
Variety											
AFR 708	4.2	5.4	8.8	13.1	17.4	24.5	30.8	35.4	40.2	43.5	54.8
Awash Melka	5.4	11.0	13.6	19.8	26.1	32.4	37.4	41.3	44.7	47.4	53.9
G 59/1-2	9.3	16.2	27.9	34.1	40.0	48.8	50.9	52.6	53.7	54.2	54.8
GLP 2	5.0	8.3	13.8	20.2	25.3	32.6	38.1	41.0	44.8	46.8	51.5
GLPX92	5.5	7.2	10.8	14.8	17.3	19.7	22.9	25.7	27.3	29.8	44.0
Gofta	5.0	6.0	6.5	8.2	10.7	16.7	26.4	32.6	38.9	44.0	55.1
HRS 545	7.2	8.9	12.9	20.6	25.1	30.2	34.4	36.4	39.4	39.9	44.9
Ituri Matata	7.8	18.5	22.7	31.1	37.2	42.6	46.9	49.1	51.7	52.4	53.8
Jesca	2.9	3.7	4.8	6.3	7.8	12.0	15.9	20.0	24.1	28.2	52.5
K 131	5.0	6.0	8.5	14.9	20.5	20.8	25.7	30.7	34.8	38.9	51.6
K 132	3.6	5.0	7.7	11.4	15.3	21.0	25.9	29.9	35.2	39.1	52.4
Kiangara	8.8	12.3	17.7	23.6	30.4	32.0	34.7	36.1	38.9	41.0	49.7
Kirundo	6.1	9.7	15.4	26.6	27.6	35.3	39.6	43.1	45.4	47.9	52.7
LIB 1	3.0	6.6	10.2	22.7	26.1	27.4	29.8	32.0	34.9	37.6	49.6
Lingot Blanc	23.8	40.0	44.7	49.5	50.9	53.1	54.4	54.8	56.1	56.4	57.1
Maasai Red	19.8	37.8	41.9	47.4	50.5	52.3	53.8	54.2	54.8	55.4	56.3
MCM 2001	7.6	13.0	15.0	18.1	20.8	27.0	33.5	37.9	41.1	44.3	48.5
Mexican 142	34.0	42.2	44.5	47.5	49.3	50.6	51.3	52.3	53.1	53.4	54.4
MLB 49/89A	14.7	19.8	28.2	36.9	41.6	41.3	43.1	45.5	46.8	47.6	52.6
Mwa Mamafutala	3.1	3.9	5.9	8.2	12.7	19.4	26.8	32.7	38.4	43.9	53.3
Nain Dekyondo	18.1	27.8	32.9	42.8	43.5	44.9	46.5	47.8	48.4	49.5	49.6
Nakaja	24.5	35.2	38.8	39.5	41.0	50.5	52.2	53.6	54.2	54.9	56.2
Ranjonomby	16.7	38.5	42.9	47.0	48.9	51.0	52.4	53.4	53.9	54.9	55.7
Red Wolaita	20.8	37.2	40.4	45.3	48.1	49.4	50.7	51.6	52.5	53.9	56.5
Roba 1	4.8	5.8	7.2	10.2	13.9	21.6	29.8	34.6	40.0	43.8	55.1
RWR 10	4.1	5.4	7.4	10.8	15.1	20.3	27.3	31.9	37.0	42.0	56.1
Selian-97	2.8	3.1	4.7	7.3	10.4	16.3	22.7	26.9	31.7	37.0	55.0
Simama	4.6	5.5	7.8	11.5	15.9	21.9	28.3	33.5	39.5	43.9	57.1
Soya Fupi	4.8	7.2	10.7	15.2	19.5	27.1	34.1	38.3	42.6	45.9	51.9
TY3396-12	5.1	10.2	15.1	19.6	22.8	26.9	30.8	34.9	36.7	39.3	51.2
VCB 81013	5.4	9.9	14.2	22.5	28.1	34.6	39.7	42.7	46.6	48.5	52.0
VNB 81010	5.7	8.9	11.2	21.8	22.5	23.4	26.7	29.3	33.9	39.0	55.6
Zebra	6.8	13.6	19.5	24.9	29.2	34.2	39.9	43.7	46.1	47.3	49.9
Mean	30.9										
LSD (05)	0.3										
CV (%)	1.5										

Results indicated highly significant ($p<0.001$) differences in water absorption rate among varieties (Appendix 4). The beans also portrayed three distinctive absorption patterns. These were: relatively high absorbers (Fig. 6). These lines showed rapid water absorption. They absorbed over 70% own weight in the first two hours of soaking. The absorption rate decreased gradually after this time to a maximum water uptake of about 100 % in 6 hours and levelled out. Varieties in this group were the large and small whites: Lingot Blanc and Ranjonomby, Mexican 142, a small white variety, Nakaja, a brown variety, and two small red lines (Maasai Red and Red Wolaita).

Fig 6: Water absorption capacity of common bean varieties after 24h. (Fast water absorbers).

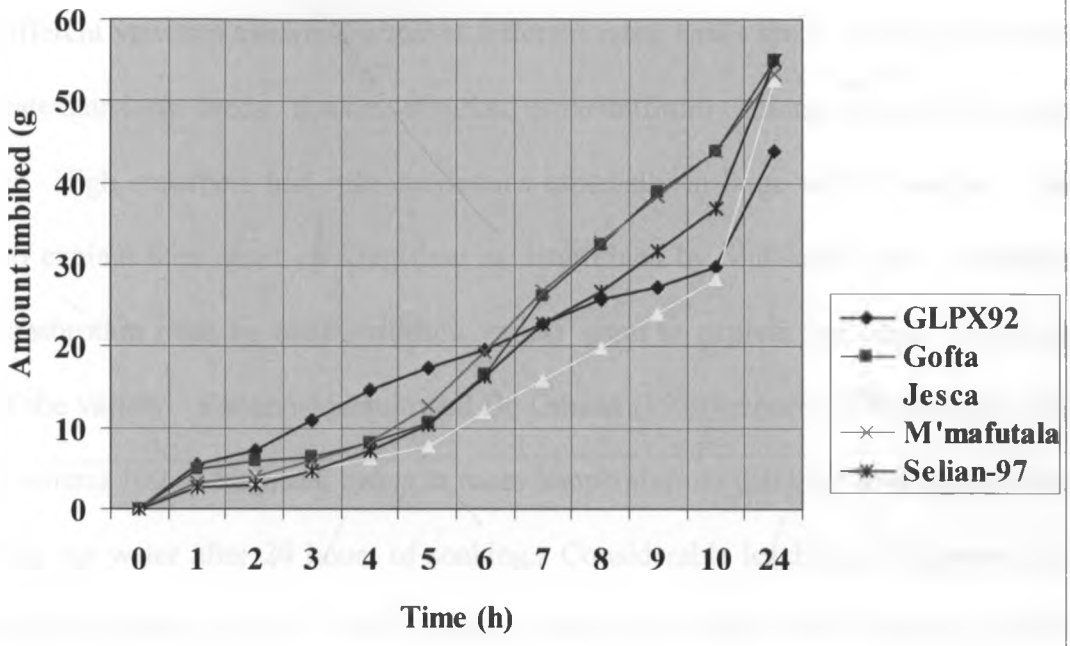


The second category illustrated in Fig. 7 is of those varieties with relatively linear water absorption. These tended to absorb uniformly over time and by the end of 6 hours, they were found to have imbibed up approximately 70 % of the water. Included in this category were Awash Melka, GLP 2 and HRS 545.



The third pattern consisted of slow water imbibers (Fig. 8). These started off slowly and even by the 6th hour they had hardly absorbed 50% of their maximum water capacities. The rate then gradually increased to the maximum intake in 24 hours. This pattern was exhibited by GLPX92, Gofta, Jesca, Mwa Mafutala and Selian 97.

Fig 8:Water absorption capacity of common bean varieties after 24h. (Slow water absorbers).



Water absorption was also dependant on exposure time. The effect of treatment was highly significant ($P<0.001$). Group 1 absorbed rapidly and levelled off after 6 h; the second group absorbed uniformly over time while in the third group the rate seemed to be gradual up to the eleventh hour. Thereafter the absorption rate increased. Time x genotype interaction was highly significant. This indicates that water absorption capacity of each bean variety was highly influenced by exposure time.

Variability in water imbibition was reported by Hyde (1954) to be as a result of seed differences in hilar dysfunction. This is a situation where the counter palisade layer in individual seeds is in various stages of sensitivity to the surrounding free soaking water. Hilar dysfunction is greatest for beans with low imbibition rates and vice versa.

The following observations were also made in this study during soaking.

That different varieties absorbed water at different rates, small seeds imbibed water at a faster rate than large seeds. Soakers absorbed more uniformly among the seeds than non-soakers. High absorbers had split cotyledons especially in large white varieties. This tends to explain their short cooking time as determined by Mattson Cooker. Different water absorption rates by seeds within a variety seem to explain the range in cooking time of the variety. Variano-Marston and De Omana (1979) reported a maximum water uptake around 100 % for black beans at room temperature in different soaking solutions including tap water after 24 hours of soaking. Considerable leaching of pigments was also observed during soaking. Black and red varieties had more intense leaching than the other colours. White and yellow showed the least leaching.

4.8 Cooking time for dry common bean varieties

Results of the cooking time trials are given in Table 11.

Table 11. Cooking time for dry bean varieties

	Minutes	
Variety	**Soaked**	**Unsoaked**
AFR 708	93.5	165.0
AND 620	91.0	220.0
Awash Melka	75.0	111.5
G59/1-2	107.5	155.0
GLP-2	92.5	161.0
GLP-92	132.5	163.5
Gofta	112.5	209.5
HRS 545	120.0	160.5
Ituri Matata	93.0	131.5
Jesca	112.5	161.0
K131	122.5	169.0
K132	82.5	141.0
Kiangara	80.0	125.0
Kirundo	87.5	106.5
LIB-1	80.0	147.5
Lingot Blanc	92.0	107.5
Maharagi Soja	123.0	162.5
Maasai Red	117.5	170.0
MCM 2001	102.0	155.0
Mexican 142	92.5	156.0
MLB-49-89A	90.0	175.0
Mwa Mafutala	77.5	140.0
Nain de Kyando	110.0	110.0
Nakaja	110.0	150.0
Nguaku Nguaku	112.5	132.5
PVA 8	107.1	157.5
Ranjonomby	87.5	120.0
Red Wolaita	117.0	175.0
Roba-1	145.0	150.0
RWA 10	115.0	139.5
Selian 97	105.0	127.5
Simama	90.0	152.5
Soya Fupi	102.5	197.5
TY3396-12	122.5	158.5
VCB 81013	110.0	190.0
VNB 81010	116.0	170.0
Zebra	85.0	147.5
Mean	102.8	153.3
LSD (05)	1.8	
CV (%)	4.2	

Results showed highly significant (P<0.001) variability in cooking time due to variety and treatment. Interaction between variety and treatment was also an important source of variation in cooking time (Appendix 5). Among genotypes cooking time ranged between 106.5 minutes in Kirundo to 220 min in AND 620. Ngwira et al. (2001) reported a cooking time range of 88.4 to 209 min in freshly harvested beans cooked in borehole water and a range of 61 to 132min of the same varieties cooked in treated tap water.

Soaking significantly (P< 0.001) influenced cooking time. Cooking time for soaked beans were significantly lower than unsoaked bean samples. An average reduction on cooking time of about 33% was recorded and even higher reduction on cooking time of over 50% was observed in some varieties such as AND 620. Grain cooking time was however negatively correlated with seed water absorption indicating that fast water imbibing varieties may take long to cook.

On average Awash Melka recorded the shortest cooking time while Gofta had the longest cooking time. Characteristics of ten shortest cooking varieties are shown in Table 12 and 13 below.

Table 12. Grain type and seed size of bean varieties with shortest cooking time when soaked.

Variety	Grain type	Seed size	Time (min)
Awash Melka	white	Small	75.0
Mwamafutala	brown	Small	77.5
Kiangara	yellow/brown	Small	80.0
Lib-1	yellow	Medium	80.0
K132	red mottled	Large	82.5
Zebra	caroca	Medium	85.0
Ranjonomby	white	Large	87.5
Kirundo	yellow	Medium	87.5
MLB 4989A	black	Medium	90.0
Simama	red mottled	Large	90.0

Large (>40g/100 seeds); Medium (26 – 39g/100 seeds); Small (<25g/100 seeds)

Table 13. Grain type and seed size of bean varieties with shortest cooking time when unsoaked.

Variety	Grain type	Seed size	Time (min)
Kirundo	Yellow	medium	106.5
Lingot Blank	White	large	107.5
Nain de Kyondo	White	small	110.0
Awash Melka	White	small	111.5
Ranjonomby	White	large	120.0
Kiangara	yellow/brown	medium	125.0
Selian 97	Red	large	127.5
Ituri Matata	White	large	131.5
Nguaku Nguaku	brown/Yellow	large	132.5

Large (>40g/100 seeds); Medium (26 – 39g/100 seeds); Small (<25g/100 seeds)

Dry bean varieties with longest cooking time were AND 620 and Gofta. They registered a cooking time of over 3 hours. The ten fast cooking varieties were predominantly white closely followed by the yellow type. The three sizes were all represented.

Cooking time is important for two reasons: First, long cooking time results in increased consumption of fuel (Shellie and Hosfield, 1990). Most families in Kenya use firewood, charcoal or kerosene, which are increasingly becoming scarce and expensive. Therefore, it would be to the advantage of families that consume beans to choose varieties that are fast cooking. Secondly, if beans take a long time to cook, they demand a lot of time from the person responsible for cooking. In Kenya, as in many other developing countries, women have the responsibility of meal preparation and their time could be better spent on many other chores that demand their attention.

Previous studies have shown that cooking time varies with the type of water used (Ngwira et al, 2001). Use of deionised water results in faster cooking time because it is

free of monovalent and divalent ions. Divalent cations such as calcium found in hard water get bound by pectic substances in middle lamellae of the bean cotyledon, forming calcium pectate. Calcium pectate is insoluble and resist cell separation during cooking. Therefore, beans that are cooked in water containing high divalent cations have longer cooking time. The water used in these trials was a mixture from two sources. Treated city council water and bore hole water. It had a pH 7.62 and total hardness of 0.0728 g/L $CaCO_3$. The control sample of distilled water had a total hardness of 0.0016 g/L $CaCO_3$, while treated tap water from city centre had a total hardness of 0.0304 mg/L $CaCO_3$. The borehole water is not subjected to any further chemical treatment other than chlorination. It is therefore most likely that this water contained hardness producing metallic cations such as calcium and magnesium. This explains the relatively long cooking times recorded. Before commencement of cooking trials in this study, these samples had been under storage for a period of over nine months after harvesting; it is therefore likely that the development of hard to cook characteristics was well underway in the samples. This phenomenon could have influenced the cooking times reported in this study. Water sources of most bean consuming regions of Kenya are natural springs, wells and rivers (Western and Nyanza), boreholes, dams and rivers (Eastern) and treated tap water (Urban Areas). This water with unknown cooking quality offers a possible explanation for variable cooking time.

From Table 11, the cooking time for soaked bean samples were significantly lower than that of the unsoaked samples. Similar observations were reported by Paredez et al., (1986). It has been established that cooking of unsoaked beans involves an initial period of water absorption (Jackson and Marston, 1981). Pre-soaking lengthens the hydrated

bean cooking exposure and promotes more rapid softening hence shorter cooking time. Another important factor related to cooking time is the bean moisture content. Jackson and Marston (1981) showed that cooking time was inversely proportional to the cotyledonary moisture content of the beans, which explains reduced cooking time in soaked beans when similar water is used in cooking. Cooking time is not only an important bean quality to the consumer. Its importance cascades down to the processor, the bean trader and finally to the bean breeder. Consumer demands have driven breeders and traders towards the development of and trading in higher nutritional and cooking quality varieties. The biochemical basis of bean cooking has been studied and shown to result from pectin solubilization (Liu, 1993). As a consequence of pectin solubilization, the middle lamella softens and allows separation of adjacent whole cells (Hinks and Stanley, 1986). Cell separation contributes significantly to palatability (texture and flavour) and acceptability to the consumer. Therefore, the rate of cooking might be related to the thermal solubility properties of pectic substances, which in turn depend on pectin composition (Be Miller, 1986). A major drawback to selection of beans of short cooking time is that screening a large number of bean experimental lines for cooking and/or pectin composition is time consuming and expensive.

4.9 Sensory evaluation

The panelists mean scores for colour; flavour, texture and overall acceptability of cooked bean varieties are presented in Table 14.

Table 14. Sensory evaluation mean attribute scores for some varieties of cooked beans

Variety	Colour	Flavour	Texture	Overall acceptability
AND 620	6.0[b]	5.4[a]	6.3[a]	5.8[a]
G59/1-2	4.8[b]	5.1[a]	5.4[a]	5.1[a]
GLP –2	6.0[b]	5.0[a]	5.3[a]	5.2[a]
GLPX92	5.8[b]	5.0[a]	5.5[a]	5.7[a]
Gofta	5.7[b]	4.3[a]	4.2[b]	4.8[a]
HRS-545	5.0[b]	5.3[a]	5.7[a]	5.6[a]
Jesca	5.7[b]	5.0[a]	5.3[a]	5.5[a]
Maharagi Soja	5.5[b]	4.8[a]	4.7[a]	4.8[a]
MLB-49-89A	2.7[a]	5.3[a]	5.7[a]	3.6[b]
Mwa Mafutala	4.9[b]	4.7[a]	5.2[a]	4.9[a]
Nain de Kyando	5.8[b]	5.2[a]	5.4[a]	5.7[a]
Soya Fupi	5.3[b]	5.4[a]	4.9[a]	4.8[a]
TY 3396-12	5.0[b]	4.8[a]	4.6[a]	4.6[a]
VCB 81013	5.4[b]	5.5[a]	6.0[a]	5.3[a]
VNB 81010	2.7[a]	4.7[a]	4.5[a]	4.2[a]
Mean	5.1	5.0	5.2	5.0
LSD (05)	1.1	1.0	1.0	0.9
CV (%)	25.7	24.5	23.5	22.1

Means within column superscripted by the same letter are not significantly different from each other at ($p<0.001$)

There were significant ($p<0.001$) differences among the mean hedonic scores for the fifteen bean varieties evaluated (Appendix 6). MLB-49-89A and VNB-81010 were significantly different in colour when cooked from the rest of the varieties. However all other varieties were not significantly different from each other.

AND 620 had the best texture when cooked. It was significantly better than the cooked texture of Gofta at 0.1% level. However, there was no significant difference in cooked

texture among the other varieties. At 0.1% level AND 620, GLPX92, HRS 545, Jesca and Nain de Kyondo were significantly more acceptable to the panellists than MLB 49-89A (Table 14). The rest of the varieties were not significantly different from each other. Also noted was the presence of significant (p=0.005) panelist effect. However, panelists could not detect any differences in flavour of the cooked beans among cultivars. Generally in all attributes, the samples had a grand mean score of 5, the score for like slightly on a 7-point hedonic rating scale. AND 620 was consistently rated among the top two varieties in all attributes while VNB 81010 was among the two least rated variety.

Bean characteristics that are usually considered as important in determining consumer acceptability include: reduced cooking time, high volume expansion after cooking, integrity of the product, broth characteristics, colour, softness of texture, flavour, aroma and digestibility (Ngwira et al., 2001). In a study of 176 farming families in September of 1992 in Dedza and Ntchisi districts in Malawi, the quality characteristics of beans were rated by the participants. Cooking time was considered the most important factor (80%) to consider when choosing beans for consumption, taste was rated second (69%), flavor third (64%), and broth thickness was fourth (63%).

However, the ultimate test for bean quality is sensory acceptability (Silva, et al., 1981). Acceptability of a food product is paramount as it indicates actual use of the product (purchase/growing and eating). Although most of the named characteristics above were not evaluated, the effect of colour, flavour and texture on overall acceptability was evident. With the exception of G59/1-2, any other variety with a score of less than 5 in any of the three attributes tested had low acceptability. Black pigmentation of MLB 49-89A and VNB 81010 contributed to the low acceptability scores. The two varieties had

an average colour score of 2.7, which is far below the expected minimum score of 4 on a 7-point hedonic scale. The main comment for the score was unappealing colour. This may be because black coloured beans are relatively new to most Kenyan households hence their rejection.

The presence of significant panelist effect was expected since this was untrained in-house consumer panel. The relative acceptability of these beans as indicated by this pilot consumer panel could improve in a true consumer testing. This is because in-house panels may not be representative of the actual consuming population who may be influenced by other characteristics besides the three evaluated attributes. Sample preparation also influences acceptability, a home preparation will appeal more to the consumer than a standardized laboratory preparation generally with a number of ingredients purposefully excluded, making the dish relatively bland.

Other factor crucial to acceptability is the agronomic performance of the variety in terms of yield, resistance to diseases and maturity period. Other than organanoleptic acceptability of some of these beans, on farm survey conducted in Marani and Suneka Divisions of Central Kisii District revealed that farmers were happy with the seed and vegetative yield of these varieties. The maturity period was also satisfactory.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMEMNDATIONS

5.1 Conclusions

1. These results indicate the existence of variability for iron, zinc and protein concentration in native bean germplasm and bred lines grown in this region. Statistical analyses of data confirmed that nutrient content; tannins and other factors investigated were influenced by both variety and treatment. These findings can assist breeders to further improve the concentration of these nutrients in the bean varieties. However, since the data represented only one growing season and location, caution is exercised in making this a universal conclusion.

2. Similar variability existed in tannin concentration. It was observed that tannins were closely related to seed coat pigmentation. Dark coloured beans had more tannin than light coloured types.

3. Soaking significantly decreased cooking time by up to 33% on average. Therefore, in the interest of time and fuel economy, beans should always be soaked. There was, however, no clear relationship between water absorption and cooking time.

4. Although cooking resulted in reduced levels of iron, zinc and protein, their overall retention value of over 70% was considerably high. This, therefore, means that cooked beans are a good source of iron, zinc and protein for those individuals

who heavily rely on them for their nutritional requirements.

5. Results from bean leaves do confirm the existence of variability in iron, zinc and protein concentration in bean genotypes analysed. The high levels of these nutrients in leaves does bear promise for significantly increasing iron and zinc intake in communities that consume bean leaf as a vegetable. This however needs to be coupled with nutrition education to improve bioavailability.

6. In-house consumer evaluation and on farm survey showed that most of the beans were organoleptically acceptable, with a grand mean score of 5 for each attribute evaluated. Furthermore, agronomic evaluation revealed that farmers were satisfied with the yields and early maturity of the varieties. This has the potential of enhancing acceptability and adoption of these varieties by farmers when selected for advancement and delivery of iron and zinc among deficient groups.

7. Sixteen outstanding lines were identified for their good nutritional qualities. These were: AND 620, G59/1-2, GLP-2, Jesca, K132, Maasai Red, Mexican 142, MLB-49-89A, Mwamafutala, Nain de Kyondo, Roba-1, Soya fupi, TY 3396-12, VCB 81013 and VNB 81010. These had iron concentration (>80 mg/kg), zinc concentration (>31mg/kg) with the exception of Soys Fupi and G59/1-2 and protein concentration (>24 %) with the exception of AND 620. Most of them were organoleptically acceptable except for MLB-49-89A due to its black pigmentation.

5.2 Further work

1. Samples evaluated in this research were all grown in one location over one season. It may, therefore be necessary to evaluate the same genotypes over seasons in different environments. This will enable the researcher to examine the effect of soil and climatic factors on the iron, zinc and protein concentrations in grain and leaves. This is an important issue for plant breeders, since the ultimate goal is to obtain nutrient traits that are stable across environments. Furthermore, the number of accessions screened so far is limited. Full diversity of this germplasm should be determined.

2. Further study on anti-nutritional factors especially phytates is needed to establish their concentration and effect on nutritional aspects of the bean in relation to the consumer.

3. Owing to the high protein content of the bean varieties, it is imperative to assess the amino acid profiles especially the sulphur containing amino acids as a measure of protein quality.

4. Investigations on condensed tannin concentration of raw, soaked and cooked bean samples need to be conducted to establish the effect of processing on tannin concentration. These trials could be extended to dehulled bean seeds, which are used by some mothers to feed their children.

5. There is need to study in vitro protein digestibility trends and iron and zinc extractability in high and low tannin bean varieties to give an indication of bioavailability of these nutrients to the consumer.

6. Further breeding work is recommended for improvement of cooking quality in all genotypes. Consumer acceptability can also be enhanced by improving on the black pigmentation of MLB-49-89A.Other areas that require more work are; elevation of protein concentration in AND 620 and improvement on zinc concentration of Soya Fupi and G59/1-2.

7. For the consumer to realize maximum nutritional benefit from the beans, appropriate nutrition education in preparation, proper cooking method and utilization of bean seed and leaves is highly recommended.

8. Since it has been established that cooking time of beans depends on the type of cooking water used, it may be necessary to test the cooking time of bean breeding lines using several types of water used in different bean consuming regions before the line can be classified as fast cooking.

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7.0 APPENDICES

Appendix 1. Analysis of variance for seed iron, zinc and protein concentration

		Iron	Zinc	Protein
Source of variation	Df	MS		
Replication	1	0.18	0.03	0.04
Genotype (G)	36	281.1 **	54.31**	16.02**
Treatment (T)	2	7036.8 **	1823.44 **	246.80 **
Genotype x Treatment	72	81.4	9.77	1.89
Residual	110	5.95	1.84	0.12
S.E.		2.44	1.36	0.12
C.V.		3.20	4.40	1.50
LSD (G)		2.79	1.55	0.40
(T)		0.79	0.44	0.11

NS = Not significant, ** significant at 0.1% level.

Appendix 2. Analysis of variance for leaf iron, zinc and protein concentration.

		Protein	Iron	Zinc
Source of variation	Df	MS		
Replication	1	0.05	143.90	0.10
Variety	31	18.53 **	239441 **	452.89 **
Residual	31	0.14	195.20	1.10
S.E.		0.37	13.97	1.05
C.V.		1.30	1.70	2.20
LSD.		0.75	28.50	2.14

NS = Not significant, ** significant at 0.1% level.

Appendix 3. Analysis of variance for seed coat tannin concentration.

Source of variation	Df	MS
Replication	2	0.04
Variety	35	60.88 **
Residual	70	0.03
S.E.		0.17
% C.V		2.90
LSD		0.28

NS = Not significant, ** significant at 0.1% level.

Appendix 4. Analysis of variance for seed water absorption

Source of variation	Df	MS
Replication	1	2.05
Variety (V)	32	2.06**
Time (h)(T)	23	1.34 **
Variety x Time		8.26**
Residual		2.04
S.E.		0.45
C.V.		1.50
L S D (V)		0.27
(T)		0.16

NS = Not significant, ** significant at 0.1% level.

Appendix 5. Analysis of variance for seed cooking time

Source of variation	Df	MS
Replications	1	8.33
Treatment (T)	1	94450.74 **
Varieties (V)	36	1238.77 **
Treatment x Variety	35(1)	735.03 **
Residual	72(1)	29.53
S.E.		5.43
C.V.		4.20
L S D (T)		1.78
(V)		7.66

NS = Not significant, ** significant at 0.1% level.

Appendix 6. Analysis of variance for sensory evaluation scores.

		Acceptance	**Colour**	**Flavour**	**Texture**
Source of variation	Df	MS			
Variety (V)	14	4.42 **	13.30**	1.38 NS	4.18**
Panelist (P)	11	3.16	4.23	1.98	5.15
Residual	152(2)	1.24	1.71	1.51	1.51
S.E		1.11	1.31	1.23	1.23
C.V		22.10	25.70	24.50	23.50
L S D (V)		0.90	1.05	0.99	0.99
(P)		0.80	0.95	0.89	0.89

NS = Not significant, ** significant at 0.1% level.

Appendix 7. Regression analysis for water absorption verses cooking time

Source of variation	Df	MS	F pr.
Regression	1	38.8	0.717 NS
Residual	64	293.0	
S.E		0.675	

NS = Not significant

Appendix 8. Correlation between seed and leaf iron and zinc concentration.

	Mineral	Iron	Zinc
Seed	Iron	1	0.496**
Leaf	Zinc	0.356**	1

**Significant at 0.01 level

Appendix 9 Score sheet for cooked bean sensory evaluation

SELECTED HIGH MINERAL BEANS HEDONICS CORING QUESTIONAIRE

NAME .....

DATE .....

Please evaluate each of the bean samples presented for the following quality attributes:-

(a) Colour

(b) Texture

(c) Flavour

(d) Overall Acceptability

Do one attribute at a time for all the samples according to the following scale:-

7 - Like very much

6 - Like moderately

5 - Like slightly

4 - Neither like nor dislike

3 - Dislike slightly

2 - Dislike moderately

1 - Dislike very much

We are asking you to say how much you like or dislike each of the listed quality attributes in the tables given. Write the Code number of the sample and the number that corresponds to the phrase that best describes your assessment of the attribute. Please feel free to make any comment on the listed attribute and/or the sample you are testing.

Remember you are the judge, the only one who can tell what you like or dislike about the product and why you dislike. Nobody knows whether these products should be considered good, bad or indifferent. Therefore, a honest expression of your assessment will help us decide.

Thank you.

COLOUR SCORE BOARD

Sample code	Score given	Comments

TEXTURE SCOREBOARD

Sample code	Score given	Comments

FLAVOUR SCOREBOARD

Sample code	Score given	Comments

OVERALL ACCEPTABILITY

Sample code	Score given	Comments