OPTIMISATION OF VITAMIN C EXTRACTION AND PHYSICOCHEMICAL PROPERTIES OF *ADANSONIA DIGITATA, PARINARI CURATELLIFOLIA, STRYCHNOS COCCULOIDES* AND *ZIZIPHUS MAURITIANA* FRUITS OF MALAWI

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DECLARATION BY THE CANDIDATE

I hereby declare that no part of this work has been submitted for any degree in any university or institution of learning and either is submitted concurrently. Acknowledgement has been made where other sources of information have been used.

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CERTIFICATE OF APPROVAL

We hereby declare that this thesis is from the student's own effort and that it has been submitted this day with our approval.

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DEDICATION

I dedicate this thesis to my parents, Tryson and Tryness Tembo. With their love and support, I have reached this far with my education. I also dedicate this thesis to my wife Nthandosi, and son Joel for their patience. During the two years of this research work, I gave them little attention.

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ABSTRACT

This study was undertaken to optimise the extraction of ascorbic acid and investigate physicochemical properties of *Adansonia digitata, Parinari curatellifolia, Strychnos cocculoides* and *Ziziphus mauritiana* fruits. The factors assessed were: (i) Provenance, (ii) ripening stage (iii) harvesting time (iv) fruit tree maturity (v) storage condition and (vi) processing. The physicochemical properties [ascorbic acid (vitamin C), retinoic acid (vitamin A), reducing sugars, total soluble solids, dry matter and minerals] were determined by standard methods described in Association of Official Analytical Chemists (AOAC). Alternative extracting solvents to the most expensive metaphosphoric acid were investigated and used in the determination of ascorbic acid in this study. Different concentrations of metaphosphoric acid, orthophosphoric acid, acetic acid and their mixtures were evaluated in extracting ascorbic acid from fruit samples. The performance of a solvent system was determined by yield, percent recovery, and stability of the extracted ascorbic acid.

The dilute solvent system (0.05 M H_3PO_4 + 0.025 M HOAc) extracted significantly higher ascorbic acid ($p<0.05$) from high ascorbic acid containing fruit (fruits with high level of vitamin C), *A. digitata* and gave higher recoveries (90.44 ± 4.19%) than the standard metaphosphoric acetic acid (0.38 M HPO_3 +1.38 M HOAc) (83.08 ± 15.61%). Most of formulated blends performed poorly when low ascorbic acid containing fruits were used than the standard.

To assess the effect of provenance, fresh fruits were collected from different geographical positions of Malawi. *A. digitata* fruit pulp from Chikwawa gave the highest ($p <0.001$) ascorbic acid (347.7 mg/100g) while Salima provenance the least (233.10 mg/100 g). The level of retinoic acid was highest (60.92 mg/kg) in the fruit pulp from Mangochi while Salima the least (29.86 mg/kg). Fresh *P. curatellifolia* fruits from Nkhata-Bay afforded significantly the highest ($p<0.001$) level of ascorbic acid (134.64 ± 6.59 mg/100 g). The level of ascorbic acid (88.1 mg/100 g) was highest in *Z. mauritiana* fruit pulp from Mangochi.

S. cocculoides fruits harvested in December gave significantly higher (< 0.001) levels of ascorbic acid, reducing sugars, calcium, iron, magnesium and sodium than October harvest. As fruits ripened, the levels of reducing sugars and total soluble solids increased steadily. In *Z. mauritiana* reducing sugars increased from 10.3 ± 0.00 to 13.23 ± 0.6% with ripening. Except in *Z. mauritiana,* ascorbic acid and minerals decreased with ripening in *Strychnos*. The level of ascorbic acid decreased from 311.08 to 63.7 mg/100 g in *Strychnos* pulp while it increased from 131.8 to 146.0 mg/100 g after full ripening in *Z. mauritiana*.

Strychnos fruit pulp from middle aged trees had significantly higher ($p<0.001$) level of ascorbic acid (418.56 mg/100 g) on dry matter (DM) than fruit pulp from young (311.08 mg/100g) and old aged trees (137.26 mg/100g). No significant difference ($p>0.05$) was observed in reducing sugar levels amongst fruit pulps from all tree age groups.

The total soluble solids levels were unaffected during the entire period of refrigeration. Gradual increase in TSS was observed in clay pot storage while open air afforded a steady increase. Jam processing resulted in significant loss of important ascorbic acid and minerals ($p<0.001$). Processing *Strychnos* into jam reduced the level of ascorbic acid by 90%.

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ABBREVIATIONS AND SYMBOLS

AAS	Atomic absorption spectrophotometer
AFTPs	Agroforestry tree products
DNA	Deoxyribonucleic acid
DCIP	Dichlorophenol-indophenol
DM	Dry matter
Hb	Heamoglobin
HOAc	Acetic acid
ICRAF	International Centre for Research in Agroforestry
MAS	Microwave Assisted Sonication
POD	Peroxidase
PPO	Polyphenol oxidase
PMME	Polymer monolith microextraction
RDA	Recommended daily allowance
RNA	Ribonucleic acid
SSC	Soluble solids concentration
TSS	Total soluble solids
TDS	Total dissolved solids

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CHAPTER 1: INTRODUCTION

1.1 Background

Agriculture is the main source of livelihood for approximately 80% of the rural population in Southern Africa, and this constitutes a major driving force in the recovery of regional economies. In Malawi, agriculture accounts 40% of the GDP, 85-90% of the foreign exchange earnings, and 85% of the labour force (World Bank, 1992). Agricultural production is however currently constrained by unaffordable inputs especially fertilizers, lack of access to credits, and the minimum involvement of smallholders in the market economy. Furthermore the fast growing population in the region has led to deforestation and over-cultivation. Periodic drought aggravates the situation, but even in the years of favourable rains, most farm families cannot produce enough to feed themselves (Kwesiga et al., 1999). The Southern Africa region is also greatly hit by HIV/AIDS pandemic, which has impacted negatively on household food security. Most of the breadwinners succumb to the disease and lose their income generating capabilities (AU, 2006).

Most of the agricultural poor in Eastern and Southern Africa including Malawi are food insecure and chronically malnourished. The majority of the population lives below poverty line (less than one dollar a day). Overcoming poverty remains, therefore, the most daunting development challenge in these areas (Tiisekwa et al., 2004; UN, 2006). Wild foods like indigenous fruits can reduce vulnerability to poverty (Mithofer et al., 2006). Indigenous fruits reduced vulnerability by about 30% during the critical period in Zimbabwe. Malawi, Zambia, Mozambique and Zimbabwe rank among the lowest quartile of poor countries worldwide, with poverty rates of 30% to 50% (UNDP, 1999).

Forests and homestead farms are important sources of non-timber products including indigenous fruits, which are consumed by communities and also sold to generate income. These are essential for food security, health, social and economic welfare of rural communities (Akinnifesi et al., 2000a; FAO, 1998; Maghembe et al., 1998). Fruit and products from indigenous trees are particularly important during the hunger periods of the year and also increase rural household income through sales of fresh fruits and processed products (Akinnifesi et al., 2000a; Dietz, 1999; Maghembe et al., 1998). Some of the products from indigenous fruits include jam, juice, porridge and spirit.

In Malawi smallholder farmers are aware and appreciate the importance of fruit trees and it is not surprising to find trees growing on croplands, around the homesteads and in swampy areas (Matasyoh, 1999). In the region, children particularly benefit from wild fruits and these are important source of food even when commercial fruits are available. Additionally, commercial fruits tend to be expensive and out of reach for most rural communities due to widespread poverty (Campbell, 1987).

There is sufficient evidence to show that indigenous fruits can contribute greatly to the welfare of the rural people. Nutritional studies have shown that many of the fruits are rich in sugars, pectin, essential vitamins, minerals, vegetable oils, proteins, crude fiber and total carbohydrates necessary for human nutrition (Saka and Msonthi, 1994; Kwesiga et al., 2000). Improved nutrition increases immunity and reduces the effect of HIV/AIDS (Rajabiun, 2001). The rural areas are at a greater risk of HIV/AIDS, which affect food production and economic activities of people. Indigenous fruits therefore constitute an important food sources for combating malnutrition due to major deficiencies of vitamins A and C and essential amino acids as well as minerals such as iron and zinc (Thiong'o et al., 2000). The composition of these edible parts of plants is affected by several factors such as tree age, soil type, phenotype and agronomic practices and these aspects require further research (Ladipo et al., 1996). Saka and co-researchers reported that total and reducing sugars are significantly different within and among groups of indigenous fruits and are also influenced by storage conditions (Saka et al., 2002). Indigenous fruits are mainly consumed as fresh or as limited fruit products (Kadzere et al., 2004). These are either sold locally, or sometimes transported to urban areas. Fresh fruits take time to reach towns due to poor road infrastructure in the region especially Malawi and may lose quality with ripening.

Ascorbic acid assessment in plant materials including indigenous fruits is usually done by modern methods and techniques, which are not affordable for developing countries like Malawi. The use of 2, 6- Dichlorophenolindophenol titrimetric method would have been preferred to other techniques if it were not involving metaphosphoric acid (AOAC, 1990) as an extracting solution for ascorbic acid. Metaphosphoric acid is very expensive besides two other problems during preparation, which are weighing and dissolving (Iwase et al., 1997). Hence there is need to develop an extracting solution that can be as efficient as the

metaphosphoric-acetic acid solvent system with minimal differences if any, but cheap, readily available and easy to measure and dissolve.

1.2 Problem statement

Most of the rural people in Malawi are food insecure and chronically malnourished yet there is a widespread availability of indigenous fruits, which can reduce malnutrition situation if fully exploited. Improving the productivity of staple food crops only, such as maize, will neither meet the full subsistence requirements of rural households nor provide feasible opportunities for these rural inhabitants, to escape the vicious cycle of poverty. Most indigenous fruits are rich in vitamins (A and C) and minerals necessary for human nutrition. For instance vitamin C improves the immunity of those with HIV/AIDS (Walingo, 2005) while vitamin A is essential for the normal functioning of visual system (FAO/ WHO, 1998).

Environmental factors under which trees grow and ripening stage at harvest affect the composition of the edible pulp and the resultant quality of the fruit (Kadzere et al., 2004). Poor understanding of how geographical position, tree age and harvest and post harvest handling affect nutritional attributes of fruits. Consumption of quality fruits and fruit products will reduce the effects of HIV/ AIDS and combat malnutrition due to major deficiencies of vitamin A and C and essential amino acids as well as minerals such as iron and zinc (Thiong'o et al., 2000).

The availability of a robust, cheap and readily available method of analysis is a pre-requisite to greater utilization of indigenous fruits. However, the commonly used extracting solvent (metaphosphoric acid) during the determination of vitamin C is very expensive and not readily available therefore the development of an alternative solvent is on high demand.

1.3 Objectives of the study

The study was, therefore, undertaken to optimise vitamin C extraction and determine the influence of fruit provenance, ripening, fruit tree age, storage condition and fruit jam processing on some physicochemical characteristics of *P .curatellifolia, S. cocculoides, Z. mauritiana* and *A. digitata* in Malawi.

The specific objectives of the study were three-fold:

i. To assess optimum extractants of ascorbic acid in fresh fruits and their products,
ii. To determine the effect of provenance, ripening, tree age and storage condition on some nutritional values of fruits (vitamin A, vitamin C, reducing sugars, moisture content, pH, acidity, calcium, zinc, magnesium, copper, sodium, and potassium) and
iii. To determine the effect of processing on nutritional level of *S. cocculoides* jam

Chapter 2 of the thesis provides the literature review while Chapter 3 describes the materials and methods used. In Chapter 4 results of this study are presented and discussed. Conclusions and recommendations are presented in Chapter 5.

CHAPTER 2: LITERATURE REVIEW

2.1 Food security in Southern Africa

Food insecurity is worsening and hundreds of thousands of people in Southern Africa are in need of emergency food assistance (AU, 2006). In Malawi, markets continue experiencing escalating prices of maize, the main staple food. Commercial imports and food aid deliveries have been meagre in spite of the significant amounts pledged by international donors (AU, 2006). Forest foods could offer vital insurance against famine during times of seasonal food shortages or emergencies such as drought, floods and wars (FAO, 1989). In recent years these species have become the subject of interest because of the progression of the HIV pandemic in the region, as it is likely that many rural families living with AIDS will depend increasingly on their nutritional value (Kadu et al., 2006; Lengkeek et al., 2004). Fruits and other products from indigenous fruit trees constitute the cheapest yet rich source of food on which the poor can survive. Interest has therefore grown on these fruits (Tiisekwa, et al., 2004).

2.2 Distribution and domestication of indigenous fruits

The distribution of the miombo woodlands of Southern Africa corresponds roughly to the Zambezian Regional Centre of Endemism recognized for their floristic richness and the widespread occurrence of the tree genera *Brachystegia*, *Julbernardia* and *Isoberlinia*. The woodlands cover large areas of Angola, Malawi, Mozambique, Tanzania, Zaire, Zambia and Zimbabwe and have a long time been a useful source of various forest products and services the subsistence needs of rural communities. The miombo woodlands are rich in variety and quantity of indigenous fruit trees (Malaisse and Parent, 1985). In Malawi, a survey conducted in Zomba, Mwanza, Mangochi, Dedza, Lilongwe, Mzimba, Rumphi and Nkhata Bay revealed over 200 indigenous tree species as edible (Malembo et al., 1998).

Rural communities have for many years depended on natural forests for their household requirements including fruits and medicine. The forests resource, however, is declining rapidly due to high rates of deforestation, particularly in the miombo (Brachystegia) woodlands as a result of increasing human pressure. Consequently some of the

indigenous fruits and priority medicinal plants are on the verge of extinction. Such a situation can only be corrected through domestication. Domestication is a tool to save species from extinction because according to Simonds, if wild species have to survive at all over a long period, it will probably be in cultivation (Simonds, 1984). Domestication is naturalizing a species to human conditions and it involves human-induced change in the genetics of a plant. A wild plant when cultivated to yield useful product(s) becomes a crop. Various domestication paradigms have been used in different parts of the world. Some of the domestication pathways emphasize on improvement of the selected species as sure bait to cultivators while in other cases a market driven domestication approach is preferred. Maghembe and co-researchers advocated a forestry and horticultural approach where tree germplasm is first developed before development of product and market (Maghembe et al., 1992). Akinnifesi hypothesizes that great domestication headway could be made if it is accompanied by rigorous product development and commercialization research (Akinnifesi, 2000).

Until recently there has been little effort to cultivate, improve or add value to these fruits. However, since 1989 the International Centre for Research in Agroforestry (ICRAF: now the World Agroforestry Centre) has implemented a research and development initiative to domesticate and commercialise indigenous fruit trees in five SADC countries. This is now seen as an important strategy to reduce poverty and hunger and to create employment opportunities in rural areas. The domestication of trees producing agroforestry tree products (AFTPs) should ensure that this resource is added to future national food production statistics, and that indigenous fruits supplement staple foods, so promoting food and nutritional security and moving towards the achievement of the UN Millennium Development Goals. This agroforestry initiative by ICRAF is thus targeting livelihood security problems in Southern Africa, adding to the food security achievements of other research and development communities (Akinnifesi et al., 2006). An understanding of nutritional status of priority indigenous fruit species is therefore an important component of ICRAFs objectives.

2.3 Use of miombo indigenous fruits

Trees and forests contribute in many ways to improving diets and combating hunger in local communities and rural households. They not only directly provide food and

medicines; indirectly they increase income and agricultural production, thereby improving access to food. Hunger and malnutrition would be significantly worse if it were not for the contribution of trees and forests to household food security. Miombo indigenous fruits are consumed by rural communities and also sold to generate income by the agricultural poor in the developing countries including Malawi. In Zimbabwe, the majority of rural households benefit from consumption and sale of indigenous fruits, although the extent varies among households (Mithofer et al., 2003).

Indigenous fruits are essential for food security, health, social and economic welfare of rural communities and are particularly important during the hungry period of the year (Rajabiun et al., 2001). For example, *Sclerocarya birrea, Ziziphus mauritiana, Uapaca kirkiana, Strychnos cocculoides, Parinari curatellifolia,* and *Adansonia digitata* are important sources of food for the people. The young tender leaves of *A. digitata* are used as green or dried vegetables while the white powdery pulp of the fruit capsule is extracted and used as flavouring in a variety of cool and hot drinks (Leakey, 1999). Maghembe and co-researchers reported that up to 97% of the households who had insufficient food stocks during the 1997/98 seasons in Malawi used indigenous fruits to minimise the situation (Maghembe et al., 1992). People living in drier parts of Zimbabwe practice more indigenous fruit processing as a way of directly or indirectly improving food security at household level (Kadzere et al. 2004). In Zimbabwe some farmers in the Mukumbura area meet educational costs from money generated through sale of fruits and *Ziziphus mauritiana* gin, "Kachasu" (Kadzere et al., 2004). Likewise in Mwanza district (Southern part of Malawi), households obtain part of their annual income through the sales of the local gin, "Kachasu", brewed from *Uapaca kirkiana* (Maghembe et al., 1998). A fruit of *T. occidentalis* costed US $0.70 – 1.00 each in the year 2002 in Nigeria (Odiaka et al., 2004). Wild foods, e.g. fruits are especially important income source for poor people since entry barriers for collection and use are low. A variety of edible wild fruits are popular natural resources in Southern Africa (Maghembe et al., 1998).

2.4 Products from indigenous fruits

Fresh fruits are processed in order to (i) provide palatable products, (ii) preserve the product, and (iii) obtain products that can be converted into other by- products (Saka et al., 2004). Indigenous fruits are used as fresh fruits, juices, powder, yoghurts, sweet beer

("thobwa"), wine, porridge, oil, nut, jam and dried. The advantage of dried fruits is that they can be stored for over 18 months and thus enhance food security in times of hunger. Therefore dried fruit is an important product in Southern Africa (Kadzere et al., 2004; Saka et al, 2002). To raise income for the rural and peri-urban households, fresh fruits, and spirit appear to be the most important products (Rao et al., 2004). Preference for some products made from *Uapaca* and *Parinari* appear to be area specific. For example in Tanzania, juice making is the most preferred by women processors followed by jams and wines (Swai, 2002). This is because juice processing is simple and prices are affordable by common people in rural and urban areas. Fruits of *A. digitata, Z. mauritiana, S. cocculoides, P. curatellifolia* and *U. kirkiana* are the most frequently used for processing into different products in Malawi. An understanding of their nutritional value and factors affecting nutritional quality is therefore very important to product development.

Farmers prefer simultaneous exploitation of indigenous and exotic fruits for product development in order to expand product range and meet market needs (Saka et al., 2002). About 26% of the technical processing knowledge is learnt from agricultural and community service programmes. Empowering the community with appropriate processing techniques is thus very essential. Processing in Zimbabwe is most common in the drier parts of the country (Zambezi Valley, Chipinge and Gokwe) to supplement food requirements (Kadzere, 2004).

2.5 Nutritional value of some priority miombo indigenous fruits

Nutritional studies have shown that miombo indigenous fruits are rich in sugars, essential vitamins, minerals, oils, fats, crude fibers, carbohydrates and proteins necessary for human nutrition. For example, Saka found that the edible pulp of *P. curatellifolia* and *U. kirkiana* contains vitamin C as high as 10.4 and 16.8 mg/100 g respectively on fresh weight (Saka, 1994). World Food Summit reported that fruits of *A. digitata* far surpass the oranges famous vitamin C content of 57 mg/100 g at 360 mg/100 g and one variety of *Z. mauritiana* reaches levels as high as 1000 mg/100 g (World Food Summit, 1996). In terms of mineral content baobab leaves are excellent sources of calcium, iron, potassium, magnesium, manganese, molybdenum, phosphorus, and zinc (Yazzie et al., 1994). In separate studies it was found that the young tender leaves of *A. digitata* are rich in

vitamin A and calcium, while the fruit pulp is rich in pectin and have vitamin C content of 169 mg/100 g, at least three-fold greater than that of oranges (Booth et al., 1988). The seed kernels contain 12-15% edible oils, more protein than groundnuts and rich in lysine, thiamine, calcium and iron. *Azanza garkeana*, a valuable edible indigenous fruit tree of Botswana, is rich in vitamin C, crude protein, total carbohydrate and important minerals necessary for human nutrition (Mojeremane et al., 2004).

2.6 Factors affecting nutritional value of fruits

Nutritional quality and quantity is not uniform between and among (exotic or indigenous) fruit species. For instance, Chapman and co-researchers established a significant variation in nutritional value (proteins, fiber, digestibility, alkaloids, saponins, cyanogenic glycosides, and minerals) in primate foods among species and among individuals of the same species (Chapman et al., 2002). Waruhiu and co-researchers studied the phenotypic variation of fruit traits in 200 trees of *Dacryodes edulis* from four populations in the humid lowlands of Cameroon (Waruhiu et al., 2004). Highly significant ($p < 0.001$) differences were found in the mean fruit length (33.5 to 122.4 mm), fruit width (23.3 to 53.5 mm), flesh depth (0.6 to 11.1 mm), fruit mass (10.0 to 114.0 g) and flesh mass (12.5 to 106.0 g). Such knowledge is not available for priority miombo indigenous fruits of Malawi including *P. curatellifolia, S. cocculoides, Z. mauritiana* and *A. digitata.*

The nutritional level of plant products is affected by several factors such as environmental/provenance, period of harvest, handling/processing techniques, genetic, fruit tree age, pest and diseases (Leroy et al., 2006). An understanding of these factors is necessary to adapt practices, which are accompanied by high nutrient content in the plant parts and their products. It is also required to locate superior phenotype for cultivars development during domestication process.

2.6.1 Effect of environment and provenance

Climatic conditions, particularly temperature and light intensity, have strong effects on the nutritional quality of fruits (Mozafar, 1994). Sidibe and co-researchers assessed the tree-to-tree variation in vitamin C content of fruits from the Black, Red and Grey bark types in 2-3 trees from 4-5 villages in three areas of Mali spanning a range of rainfall zones (450-500 mm; 600-700 mm; 750-850 mm) (Sidibe et al., 1996). The vitamin C

content varied 3-fold between trees, but there were no consistent differences in vitamin C content between zones or tree types. Low temperatures favour synthesis of sugars and vitamin C (glucose being the precursor of vitamin C) and at the same time decrease the rate of ascorbic acid oxidation. Maximum β-caroteine content in tomatoes occurs at temperature range of 15 to 21^{o}C but β-caroteine content is reduced if temperatures are higher or lower than this range, principally due to the temperature sensitivity of lycopene, the precursor to β-caroteine. Lower temperature (5 ^{o}C) with high relative air humidity (95%) limit the loss of most of the fruit quality attributes such as vitamin C without resulting in chilling injuries (Widayat et al., 2003). As light intensity increases, vitamin C increases and total carotenoids (vitamin A precursors) decrease (Gross, 1991). Higher light intensity produce more sugars leading to more vitamin C. Soil type influences the water and nutrient supply to the plant, which can affect the composition and quality attribute of the harvested plant parts (Goldman et al., 1999)

Due to varied physical and climatic conditions, miombo woodlands contain a wide range of wild fruits and nuts with different characteristics that are available all through the year. Farmers need certain traits of indigenous fruits to be improved before domestication (Swai et al., 2004). For instance, a case study conducted in seven villages of Southern Tanzania revealed that sixty-six percent of the farmers preferred large fruits and nearly 40% wanted sweeter fruits from trees such as *Strychnos cocculoides* and *Vitex mombassae* for eating and making juice, wine and beer (Rao et al., 2004).The farmers (77 %) mentioned slow growth and long period for fruiting as a major limiting factor for growing indigenous fruits on farms (Rao et al., 2004). According to the forestry approach, before domestication, it is important to determine the amount, cause and nature of the variation present in the range wide germplasm in order to use it later in selection and breeding (Nienstaedt, 1975). This is because forest trees are generally genetically variable in order to survive, grow and reproduce under numerous environments. For instance, variation in *Strychons cocculoides* fruit weight of Zimbabwean provenances was greater (177-383 g) than in Tanzania (145-183 g) and Zambian provenances (158-296 g) (Mkonda et al., 2004). The Simikinyi provenance from Tanzania has the lowest fruit weight (145 g) while Chiota provenance from Zimbabwe attained the highest (383 g). The fruit diameter showed a similar trend, with the Zimbabwean provenances having

higher variation (6.7-9.0 cm) than Zambian (6.9-8.5 cm) and Tanzania (6.9-7.1 cm) provenances. Not surprisingly, Simikinyi provenance had the smallest fruit diameter (6.7 cm) while Chiota provenance had the highest (9.0 cm). Therefore, introducing material from Botswana into the three countries could improve the fruit size. The pulp weight ranged from 47% to 51% in Zambia provenances, 50% to 57% in Tanzania provenances and from 34% to 48% in Zimbabwe provenances. In fruits, higher pulp content is necessary because this constitutes the edible or raw material for processing into valuable by-products. Selecting for reduced pericarp weight or the number of seeds would increase the pulp content.

2.6.2 Effect of harvesting period, ripening and post harvest handling

Maturity at harvest influences quality and extent of physical injuries. Therefore delays between harvest and consumption may result in losses of flavour and nutritional quality. Aydin and co-researchers studied changes in the chemical composition, polyphenol oxidase (PPO) and peroxidase (POD) activities during development and ripening of medlar fruit (*Mespilus germanica L*) and established that during the early stages of fruit development, PPO activity and the level of ascorbic acid gradually decreased, whereas in the post ripening stage PPO activity increased (Aydin et al., 2001). Ascorbic acid increased in the pre-ripening stage followed by a decrease in the post-ripening period. Glucose level gradually increased during fruit development and ripening. Contents of pentoses, hexoses and soluble proteins decreased during fruit development, but increased in the stage of ripening. Ayaz and co-researchers studied the effect of fruit development on non-volatile acid composition of *D. lotus* (Ayaz et al., 1997). They established that the quantities of all acids varied significantly during fruit development. During harvesting time (on day 331), fumaric acid was the most abundant in the fruits. Quantities of malic, succinic, fumaric and citric acids were found to be the highest in September (on day 271). Fruit development stage and period of harvest therefore affect fruit quality. Widayat and co-workers studied the effect of ripening stage and storage temperature on post harvest quality of pepino (*Solanum muricatum* Ait) (Widayat et al., 2003). It was established that metabolic activities leading to the overall fruit quality changes during ripening, and were more predominantly affected by the genetically determined ripening process in comparison to storage temperatures in the range of 5 ^{o}C to 18 ^{o}C.

Fresh fruits are highly perishable and incur direct or indirect nutrient and quality losses between the field and the consumer. For instance, long distance transportation of pepino reduced their quality in terms of sensory and nutritional attributes and shelf life is often shorter when arriving at the wholesale or retail market and does not correspond with consumer requests (Huykens et al., 2000). Indigenous fruit producers/collectors encounter post harvest problems such as rapid deterioration of fresh ripe fruits, which particularly is the case with *U. kirkiana* and *Z. mauritiana* (Kadzere et al., 2004). Fruits of *Z. mauritiana* are also susceptible to the compression and pest damage (Rao and Kwesiga 2004). The major causes of losses include mechanical damage (cracking, compression, and bruising) during harvesting and transportation, insects and pest damage, and over ripening. Mechanical damage accounts for the highest loss in *U. kirkiana* whilst insect and pests and rots in *Z. mauritiana* and *P. curatellifolia* (Kadzere et al., 2004). To overcome these problems producers/collectors, marketers, consumers and vendors should be trained in proper harvesting and post harvest handling techniques (Rao and Kwesiga, 2004).

2.6.3 Effect of processing on nutritional quality of fruit products

The fresh fruits of most indigenous fruits are acidic. For instance, Saka reported that fresh *S. cocculoides* fruits and juices had pH values lower than 5 and mixing the juices with guavas or mangoes decreased the pH further (Saka, 1994). The *Strychonos* – mango juice gave the lowest pH, consistent with high acidity of *Strychnos.* The viscosities of indigenous fruits are significantly lower than those of exotic fruits ($p < 0.05$) (Rao et al., 2004). The low viscosities (10cP) are due to low total dissolved solids (TDS). Increasing the edible pulp beyond 40% w/v had resulted in a more viscous product, which was difficult to filter. The similarity ($P > 0.05$) of refractive indices of indigenous and exotic fruits would also be affected. Saka established that the edible pulp of *P. curatellifolia* contain higher iron, copper, calcium, and magnesium than *U. kirkiana;* however *P. curatellifolia* juice had only 10 – 30% of the initial values for fresh fruits (Saka, 1994). The rest was lost during processing. Better techniques are therefore required for juice preparation that could retain more minerals in the final product. Leaky reported that traditionally, baobab seeds and pulp are sun dried, roasted or fermented to extend shelf life and enhance nutritive value (Leaky, 1999). When baobab fruits from Maiduguri

(Nigeria) were treated experimentally, it was found that fermentation for six days was better than roasting with regard to the value of crude protein (36.4 vs. 32.7%), fat (43.1 vs. 32.0%) and carbohydrates (30.0 vs. 23.5%) (Obizoba and Amaechi, 1993). Further more, water-soluble vitamins such as vitamin C and folate are lost at high rates when cooking water is discarded whereas fat-soluble compounds such as vitamin A and lycopene may be stabilized or enhanced by cooking (Kader et al., 1992). Leakey reported that the ascorbic acid content of *Dacryodes edulis,* a nutritious indigenous fruit of Cameroon is lost by some forms of cooking (Leakey, 1999).

2.6.4 Effect of fruit tree age on nutritional value

A study on the evolution of phenolic compounds during aging in wood of sherry vinegar established that acidity, dry extract, and total phenolic index increased for all the vinegars studied and the model solution (Tesfaye et al., 2002). Oleksyn and co-researchers established that the average concentrations of sulphur, potassium, manganese, and cadmium in *Pinus sylvestris* were lower in pollen of 60-year-old than 15-year-old Scots pine trees (Oleksyn et al., 1998). Tree age, had strong influence on the chemical content of *C. Arabica* (Vaast et al., 2006).

Limited knowledge on the appropriate stage for harvesting, handling, storage and processing techniques and agronomic practices for most indigenous fruits, result in the loss of micronutrients necessary for human nutrition. Dietz reported that post harvest losses in fresh fruits are estimated to be 5% to 25% in developed countries and 20 to 50% in developing countries including Malawi (Dietz, 1999). Direct and indirect losses in fruits mean reduction in the quantity of food available for family consumption and sale (Rao and Kwesiga, 2004).

2.7 Importance of minerals and vitamins (A and C) in human health

2.7.1 Importance of minerals

Minerals are absolutely essential to human health because they are building blocks of all life forms. Vitamins have no function to perform in the absence of minerals and vitamins. For instance, 99% of the American people are deficient in minerals, and a marked deficiency in any one of the more important minerals actually results in diseases. Lacking vitamins, the body system can make some use of the minerals, but lacking minerals,

vitamins are useless (On line, 2006). Acting as catalysts for many biological reactions within the human body, minerals are necessary for transmission of messages through the nervous system, digestion, and metabolism of all nutrients in food. Minerals are very important in keeping the blood and tissue fluids from either becoming too acidic or too alkaline and they allow other nutrients to pass into the blood stream and aid in transporting nutrients to the cells. A slight change in the blood concentration of important minerals can rapidly endanger life. Table 1 summarises the importance of major minerals (FAO/WHO, 1998).

Table 1: Minerals and their importance in human health (FAO/WHO, 1998)

Mineral	**Significance and deficiency symptoms**
Iron	Serves as oxygen carrier to the tissues from the lungs by red blood haemoglobin, as transport medium and as an integrated part of important enzymes in various tissues. Deficiency symptoms include anaemia and constipation.
Calcium	Is essential for bone and teeth formation. Regulates the passage of nutrients through cell walls. Muscles would not contract correctly, blood would not clot and nerves would not carry messages. Deficiency may result in muscle spasms and cramps in the short term and osteoporosis.
Magnesium	Needed for the formation of bones, proteins, and cells. Insulin secretion and function requires magnesium. It also assists in the absorption of calcium, vitamin C and potassium. Deficiency may result in fatigue, heart problems, high blood pressure, muscle weakness and cramps.
Sodium	One of the three major electrolytes of the body. Normalises glandular secretions. Deficiency may result in excessive sweating, chronic diarrhoea, impaired carbohydrate digestion and nausea.
Zinc	Identified as a co-factor for over 70 different enzymes, including alkaline phosphatase, and both RNA and DNA polymerase. Zinc helps maintain normal growth rates, normal skin hydration and senses of taste and smell.
Copper	Essential as a co-factor for serum ceruloplasmin, and oxidase necessary for proper formation of the iron carrier protein. Copper also helps maintain normal rates of red and white blood cell formation.

2.7.2 Importance of vitamin A

Vitamin A (retinol) is an essential nutrient needed in small amounts by humans for the normal functioning of the visual system, growth and development, and maintenance of epithelial cellular integrity, immune function, and reproduction. These dietary needs for vitamin A are normally provided as preformed retinol (mainly as retinyl ester) and provitamin A carotenoids. Thus retinol is an immediate active precursor to two important active metabolites: retinal, which plays a crucial role in vision and retinoic acid which serves as an intracellular messenger that affects transcription of a number of genes. Provitamin A carotenoids are abundant in darkly coloured fruits and vegetables whereas dietary contributors of vitamin A are milk, margarine, eggs, beef, liver and fortified cereals (Harrison, 2005). Carotenoids such as beta-carotene can be converted to vitamin A within the intestine and other tissues (Parker, 1996). Figure 1 shows the structures of retinol, retinal, and retinoic acid. Vitamin A deficiency is common in developing countries such as Malawi, and approximately 250,000 to 500,000 malnourished children in the developing world become blind each year (Rodrigues et al., 2004). Vitamin A is needed in increased amounts to support maternal reproductive processes, including foetal growth and development, and during lactation to replace losses in breast milk. The increased need during gestation is small and can be provided through a balanced diet and reserves from well-nourished women (National Research Council, 1989). In areas of endemic vitamin A deficiency, vitamin A supplements often must supply this need. With lactation, requirements rise to replace maternal vitamin A lost daily in breast milk and to maintain breast milk vitamin A at a level to protect the needs of rapidly growing infants during at least the first 6 months of life (Underwood, 1994).

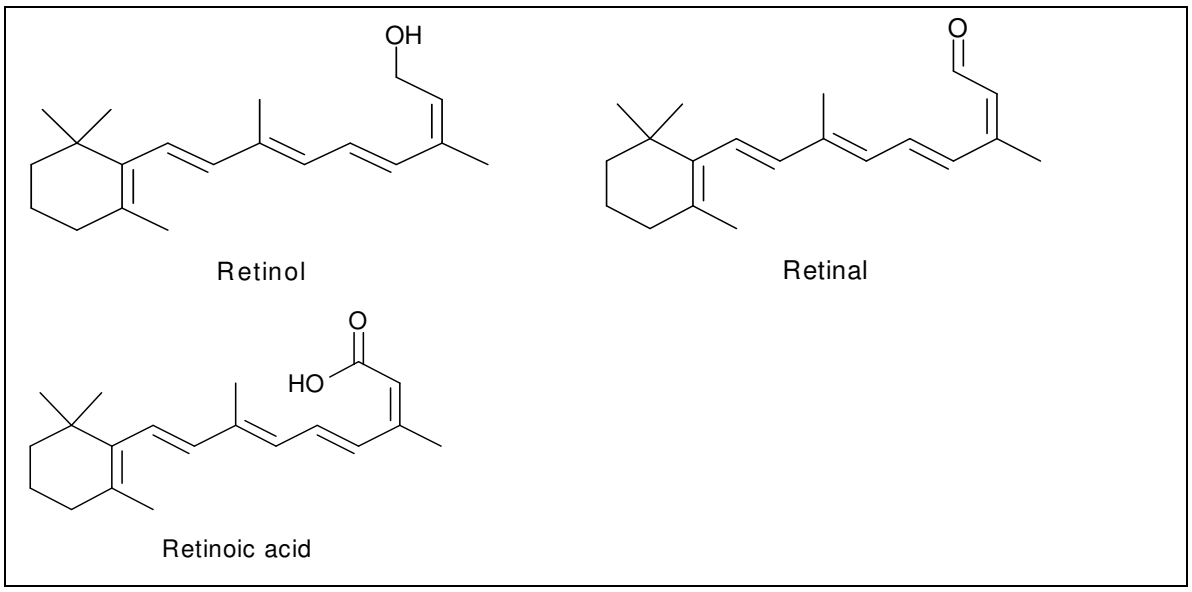


Figure 1: Chemical structures of retinol (vitamin A), retinal and retinoic acid

2.7.3 Importance of ascorbic acid (vitamin C)

Ascorbic acid is a water-soluble vitamin found in citrus fruits, tomatoes, potatoes, and leafy vegetables and is lost in large amounts during food processing (Walingo, 2005). It is an organic acid with antioxidant properties. Ascorbic acid is easily oxidised and this explains its antioxidant property, and so is used as a reductant and also as a preservative. Its oxidised form, dehydroascorbic acid, is capable of being reduced again by reductants (Conn, 1987). Exposure to oxygen, metals, such as copper and iron in the presence of oxygen, light and heat under alkaline conditions destroys ascorbic acid; therefore it must be stored in dark and cold non-metal containment (Pavan et al., 2004; Ijeri, 2004).

Ascorbic acid is very important in the human body. It is needed to develop and maintain healthy teeth, bones, gums, cartilage, vertebrae discs, joint linings, skin and blood vessels. It also does promote the healing of cuts, abrasions and wounds, helps fight infection, and reduce the effects of oxidative stress (Iqbal et al., 2004). Evidence shows that oxidative stress is implicated in several diseases, such as cancer, diabetes, sickle cell, and cardiovascular diseases. Both haemoglobin (Hb) and cell membrane appear to be principal sites of damage induced to red blood cells upon exposure to oxidative stress. Early event in oxidative stress include alteration of the mechanical properties of the red cell membrane as evidenced by a decrease in its deformability (Giakoumaki et al., 2001). Ascorbic acid lessens the risk of developing high blood pressure and heart diseases, help

regulate cholesterol levels, prevent the development of scurvy, lower the risk of developing cataracts, aid iron absorption and reduce levels of load in the blood (Walingo, 2005). It thus has got a recommended daily intake, usually given in milligrams prescribed for all stages of life ranging from babies through children to pregnant and lactating women to smokers (Conn, 1987).

Table 2 summarises recommended daily allowances (RDA) of important vitamins and minerals taking into account age and other conditions (pregnancy and lactation) (FAO/WHO, 1998). Curative dose is higher than the given dietary requirements. For instance, Walingo reported that 200 mg of ascorbic acid a day may reduce the levels of stress hormones (Walingo, 2005). Large doses of ascorbic acid decrease the symptoms of cold and flu in humans.

Table 2. Recommended Daily Allowance for vitamins and minerals (FAO/WHO, 1998)

Group	Age (years)	Vit. A (µg/day	Vit C mg/day	Ca mg/day	Fe mg/day	Mg mg/day	K mg/day	Zn mg/day
Infants and children	0- 0.5	180	25	800	6.2	36	5	6.6
	0.5-1	190	30	800	3.9	54	10	8.4
	1-3	200	30	800	4.2	60	15	8.3
	4-6	200	30	800	5.9	76	20	9.6
	7-9	250	35	800	9.7	100	25	11.2
Adolescents	10-18	340	40	1300	9.7	220	55	17.1
Adults	19-65	270	45	1300	18	220	55	14.0
	65 +	300	45	1300	18	190	60	14.0
Pregnant women		370	55	1300	*	260	55	20
Lactating women		450	70	1300	10	224	55	19

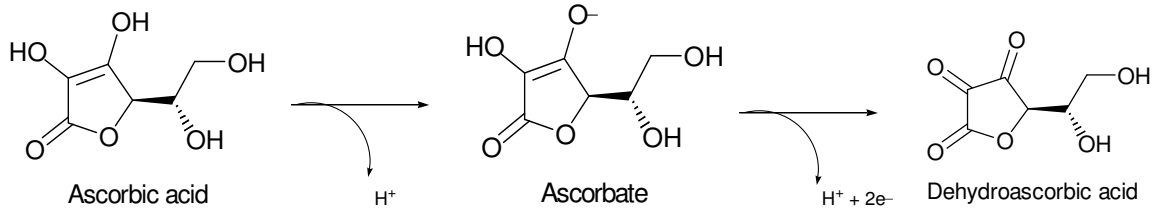
***** *Value not given because iron balance in pregnancy depends not only on diet but also on amounts of stored iron*

2.8 Extraction and analysis of ascorbic acid from plants

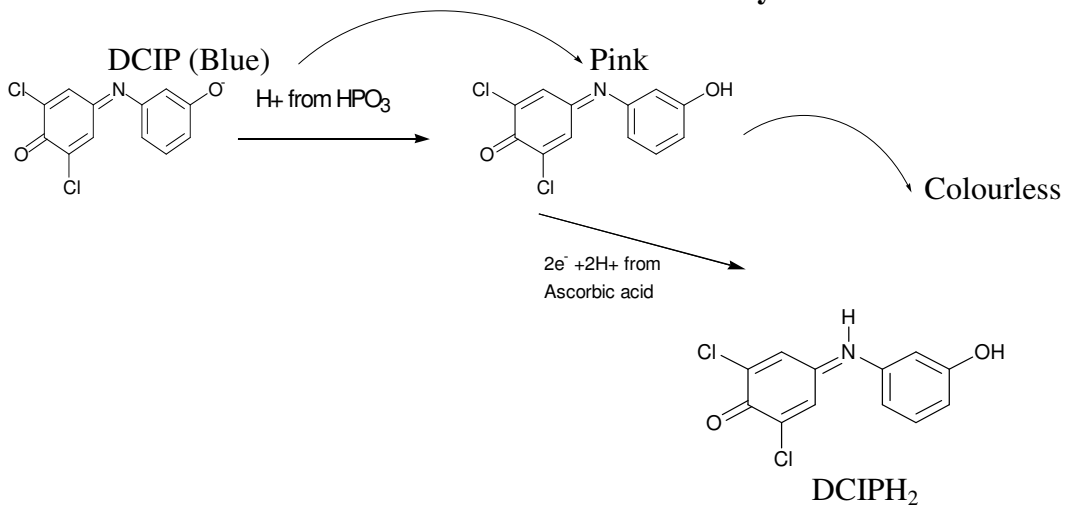
Recent advances in the food and pharmaceutical industries and the need for nutritional assessment in plant material has necessitated the development of selective, simple and accurate methods for determination of ascorbic acid. These include fluorimetric, radio enzymatic, gas chromatographic, spectrophotometer, chemiluminescent, redox titration or conventional 2,6-dichlorophenol-indophenol (DCIP) titrimetric, polarography, and High Performance Liquid Chromatography (HPLC) (AOAC, 1990; Pavan et al., 2004; Oni et al., 2003). Most of these methods require the use of metaphosphoric acid often combined with a buffer to extract ascorbic acid (Maria et al., 1995).

Moreno and co-researchers extracted ascorbic acid from orange juice by homogenizing (50 mL) with extracting solution (3% metaphosphoric acid plus 8% acetic acid). The resultant mixture was centrifuged, filtered, and adjusted to 100mL with distilled water. The concentration of ascorbic acid was then determined from the standard calibration curve obtained using HPLC system (Moreno et al., 2003). AOAC recommends the extraction of ascorbic acid in fruits as follows: Homogenise known mass or aliquot of fruit sample with extracting solution (0.38 M metaphosphoric +1.38 M acetic acid) for 2 minutes. Allow the supernatant to settle and determine the concentration of ascorbic acid extracted titrimetrically using 2, 6-DCIP dye (AOAC, 1990). Reiss extracted ascorbic acid from cabbage by homogenising using a mortar and pestle with a little clean sand in metaphosphoric acid (5%) and filtering the homogenate through Miracloth. The concentration of ascorbic acid was determined titrimetrically using DCIP (Reiss, 1996).

Scheme 1 presents oxidation of ascorbic acid to dehydroascorbic during titration with DCIP dye. The DCIP is blue in alkali, pink in acid, and can be reduced by ascorbic acid to colourless (scheme 2). If a drop of the blue dye is added to an acidified extract, it turns pink, then colourless. When all of the ascorbic acid in the extract has been converted to dehydroascorbic acid, no more electrons are available to reduce a drop of DCIP to colourless form and the solution remains pink. The amount of ascorbic acid in an extract is then measured from standard titration (Reiss, 1996).



Scheme 1: Oxidation of ascorbic acid to dehydroascorbic acid



Scheme 2: Reduction of DCIP to $DCIPH_2$

Titrimetric methods are cheap and sustainable especially in developing countries like Malawi because they do not require expensive instrumentation. However most of them involve the use of metaphosphoric acid as an extracting solution for ascorbic acid, which is very expensive besides two other problems associated with preparation, which are weighing and dissolving (Iwase et., 1997). The acid is sticky and therefore difficult to handle during quantitative transfer and it takes longer period of time to dissolve. Thus the development of a cheap, easy to formulate, and high yielding solvent with high ascorbic acid recoveries is on high demand.

2.8.1 Recoveries of ascorbic acid

The recovery of an analyte are widely used to (i) determine sensitivity and reproducibility of analytical method (ii) account for the percent loss of an analyte and (iii) test for interferences, faulty instruments and incorrect protocols (Reiss, 1996). Thus recovery experiments are essential for the evaluation of any developed or modified analytical method.

CHAPER 3: MATERIALS AND METHODS

3.1 Collection and handling of fruit samples

To assess the effect of provenance and other factors, fresh fruits and vegetables were collected as follows: *A. digitata* from Chikwawa, Dedza, Mangochi, Mwanza and Salima districts in June 2005; *P. curatellifolia* from Katope and Kaning'ina (Mzimba district), Sanga (Nkhata Bay district) and Nkhamenya (Kasungu district) in October 2005; *S. cocculoides* from Nkhamenya and Sanga in October and December 2005; *Z. mauritiana* from Dedza, Chikwawa and Mangochi districts in June 2005, *A. comosus, M. indica, M .esculenta* and *U. kirkiana* were purchased from Zomba produce market in November 2005 (Figure 2). Fruits from different provenances were kept separately. Ripe and unripe fruits of *S. cocculoides* and *Z. mauritiana* were sorted before further treatment.

3.1.1 Sample preparation

Fruit samples were washed thoroughly to remove dirt including plant debris and reduce microbial load. Only undamaged fruits with no symptoms of infection were selected for the study. Fruits of *P. curatellifolia* and *Z. mauritiana* were peeled and as much pulp as possible was scrapped off using a sharp knife. The pulp of *U. kirkiana* was carefully squeezed and kept. The fruits of *A. digitata* and *S. cocculoides* were initially cracked and pulp collected. *M. indica, M. esculenta* and *A. comosus* were peeled and the edible portion sliced into small pieces. The pulps and slices were kept in clean containers and stored in the deep freezer until extraction and determination.

3.2 Chemicals and reagents

Only chemicals of analytical grade were used. Ascorbic acid, sodium hydroxide, methylene blue, absolute ethanol and benzoic acid were purchased from Saarchem (Gauteng, South Africa); methanol, dichloromethane, sodium potassium tartrate tetrahydrate, metaphosphoric acid, acetic acid, orthophosphoric acid, copper sulphate pentahydrate and 2, 6-dichlorophenolindophenol dye were bought from Associated Chemical Enterprise (Southdale, South Africa); zinc acetate, pH 4 and 7 buffer tablets, and potassium hydrogen phthalate from BDH chemicals (UK). Retinoic acid and magnesium carbonate were purchased from Sigma (St. Louis, USA). Aqueous solutions

were prepared using de-ionized and distilled water prepared using portable MK 8A de-ioniser (Permutit Limited, London, United Kingdom) and 400W water distiller (BIBBY STERILIN Limited, Stafordshire, England) respectively.

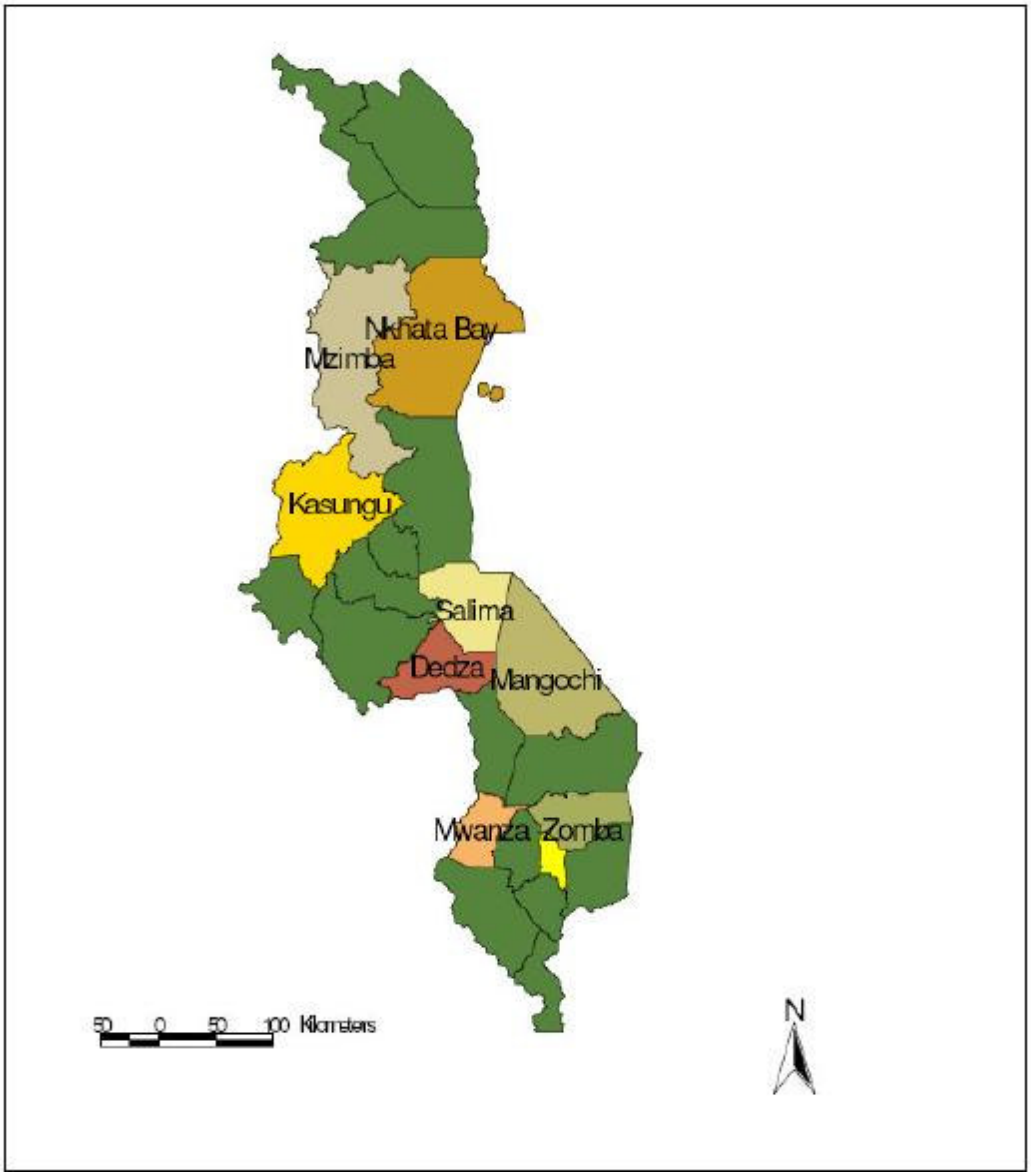


Figure 2: Map of Malawi showing districts where fresh fruits were taken

3.2.1 Preparation of extracting and standard solutions

Concentrated orthophosphoric acid (85%), acetic acid (99.5%), metaphosphoric acid chips (99.99%) and de-ionised water were used to prepare extracting solutions in a volumetric flask (2 litres). Table 3 provides the concentration and composition of extracting solvents used to extract ascorbic acid from different fruits.

Table 3. Solvent systems (SSx) for extracting ascorbic acid

0.20 M H_3PO_4 + 0.25 M HOAc (SS1)
0.20 M H_3PO_4 + 0.15 M HOAc (SS2)
0.10 M H_3PO_4 + 0.20 M HOAc (SS3)
0.10 M H_3PO_4 + 0.10 M HOAc (SS4)
0.15 M H_3PO_4 + 0.25 M HOAc (SS5)
0.10 M H_3PO_4 + 0.15 M HOAc (SS6)
0.15 M H_3PO_4 + 0.15 M HOAc (SS7)
0.25 M H_3PO_4 + 0.25 M HOAc (SS8)
0.38 M HPO_3 + 1.38 M HOAc (SS9)
0.10 M H_3PO_4 + 0.30 M HOAc (SS10)
0.10 M H_3PO_4 (SS11)
0.10 M HPO_3 (SS12)
0.10 M HOAc (SS13)
0.05 M H_3PO_4 + 0.025 M HOAc (SS14)

3.3 Extraction of ascorbic acid

3.3.1 Extraction of ascorbic acid using Pineware-PBL 404 blender

Extraction of ascorbic acid was undertaken as described in AOAC (AOAC, 1990). Fruit pulp, *A. digitata* (2.0 g), *P. curatellifolia* (5.0 g), *M. indica* (10.0 g), *A. comosus* (10.0 g), *M. esculenta* (10.0 g) or *U. kirkiana* (10.0 g) in duplicate was homogenised in extracting solvent systems (100 mL), presented in Table 3, for two minutes at medium speed, and allowed to stand for 30 minutes in a refrigerator for the supernatant to settle before determination.

3.3.2 Recoveries of ascorbic acid experiment

Table 4 provides solvents used for recovery experiment. Three formulated solvent systems (two that gave optimum yield and one which performed poorly) were evaluated against the reference (0.38 M HPO_3 +1.38 M HOAc). To one set of ascorbic acid standard (1.0, 0.8, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 and 0.0 mL; 1.00 $mgmL^{-1}$) in 100 mL solvent system, was added 2 or 5 or 10 g fresh samples. All the samples were homogenised as described in (3.3.1) to extract ascorbic acid.

Table 4. **Solvents for ascorbic acid recovery**

Fruit species	Solvent system (SS)
A. digitata	0.05 M H_3PO_4 + 0.25 M HOAc
	0.1 M HPO_3
	0.10 M H_3PO_4 + 0.10 M HOAc
P.curatellifolia	0.15 M H_3PO_4 + 0.25 M HOAc
	0.25 M H_3PO_4 + 0.25 M HOAc
	0.10 M H_3PO_4
M.indica	0.20 M H_3PO_4 + 0.25 M HOAc
	0.10 M H_3PO_4 + 0.20 M HOAc
	0.05 M H_3PO_4 + 0.025 M HOAc
A. comosus	0.25 M H_3PO_4 + 0.25 M HOAc
	0.15 M H_3PO_4 + 0.25 M HOAc
	0.15 M H_3PO_4 + 0.15 M HOAc

The ascorbic acid recoveries were calculated by using equation [1]:

$$\text{Ascorbic acid recovery (\%)} = \frac{(\text{CAEPS} - \text{CAE}) \times 100}{\text{CAS}} \quad [1]$$

where CAEPS = concentration of ascorbic acid in the extract plus standard,

CAE = concentration of ascorbic acid in the extract alone and

CAS **=** concentration of ascorbic acid standard.

3.3.3 Analysis and calculation of ascorbic acid

Ascorbic acid was determined using the 2, 6 dichlorophenol-indophenol titrimetric method described in AOAC (AOAC, 1990). The dye was initially standardized by titrating it against ascorbic acid standard (1.00 mg/mL, 2.00 mL) in a solvent system (5.00 mL) before use. Fruit or vegetable supernatant (10.00 mL) in triplicate were transferred into a well-rinsed and dried conical flask, and titrated against 2, 6-dichlorophenolindophenol until the end point (pink coloration). The concentration of ascorbic acid was calculated using equation [2]:

$$\text{Ascorbic acid (mg/100g)} = (X-B) \times (F/E) \times (V/Y) \times 100 \quad [2]$$

where X = average mL of sample titration, B = average mL for sample blank titration, F = mg ascorbic acid equivalent to 1.0 mL indophenol standard solution, E = number of grams, tablets, mL, assayed, V = initial volume of assay solution and Y = volume of sample aliquot titrated (AOAC, 1990).

3.4 Determination of retinoic acid (vitamin A)

3.4.1 Extraction of vitamin A in fruit samples

Vitamin A in fruit samples was determined by the method described in UV Methods for Micronutrient Determination (MOST, 2005). Accurately weighed fruit samples; *A. digitata* (2.00 g) or *P. curatellifolia* (5.00 g) in duplicate, were transferred into dried extraction conical centrifuge tubes (50.0 mL) and mixed with magnesium carbonate (1.0 g). Dichloromethane (8.0 mL), methanol (2.0 mL), and distilled water (12.0 mL) were added in turn with a 30-second vortexing separation after each addition. The resultant mixtures were centrifuged by a CENTRA CL2 Centrifuge (Thermo Electron Corporation, Milford, USA) for 4 minutes at 1000 revolutions per minute. Three layers were formed: organic (bottom), residues suspended in organic layer (middle) and aqueous on top. The two top layers were decanted carefully, and organic layer (5.0 mL) was diluted to 50.0 mL mark in a brown volumetric flask with dichloromethane. The solutions were kept in the dark (room temperature) for 15 minutes to equilibrate before analysis.

3.4.2 Analysis and calculation of vitamin A content

The absorbance values for standard solutions were obtained at 325 nm using a JENWAY 6405 UV/Vis spectrophotometer (ESSEX, London, UK) and plotted against actual concentration (AOAC, 1990). The absorbance of the samples was measured and the concentration of vitamin A in the fruit pulp digest determined from the standard calibration plot. The level of vitamin A (mg/kg) was calculated using the equation [3]:

$$\text{Vitamin A (mg /kg)} = \frac{\text{VPCONC x TVOL}}{\text{FNWT}} \quad [3]$$

where VPCONC = Vitamin A concentration in fruit pulp digest (mgL^{-1}),

TVOL =Total volume of diluted digest (mL) and

FNWT = Fruit sample weight (g).

3.5 Determination of reducing sugars

3.5.1 Extraction of reducing sugars

Fresh pulp (4.0 g) of *A. digitata, P. curatellifolia, S. cocculoides and Z. mauritiana* was homogenised in hot de-ionised water (75 ^{o}C, 150 mL) using a Phillips blender for 40 seconds at low speed followed by 1-minute homogenisation at high speed separated by 20 seconds rest. The supernatant was transferred into a volumetric flask (250 mL) to which Fehlings solutions (5.0 mL each) were added. The mixture was shaken and left to cool. The solution was diluted to the mark, allowed to settle for 30 minutes, filtered using a Buchner flask and kept in the refrigerator until determination as described in Pearson (1981).

3.5.2 Analysis of reducing sugars

3.5.2.1 Standardisation of Fehling's solutions

Analysis of reducing sugars was done as described in Pearson (1981). Aliquot of Fehling's solutions (5.0 mL) were transferred into a conical flask (250 mL) to which de-ionised water (15 mL) and standard invert sugar (14 mL) were added. The mixture was placed on a hot plate and at the on-set of boiling, methylene blue (5 drops) was added and invert sugar was titrated against Fehling's solution until blue colour disappeared leaving a red coloration.

3.5.2.2 Sample analysis and calculation of reducing sugars

Supernatant (20.0 mL), initially equilibrated to room temperature was mixed with standardised Fehling's solution (25 mL) in a volumetric flask, and the resultant solution treated as in 3.5.2.1. The concentration of reducing sugar was calculated using equation [4]:

$$\text{Reducing sugar (\%)} = \frac{V_o \text{ x } 25 \text{ x } f}{C \text{ x } V_t} \qquad [4]$$

where V_0 = average standardization volume, V_t= titre volume, f = correction factor and C = concentration of the sample.

3.6 Determination of acidity of fruit pulp

3.6.1 Extraction of organic acids from fruits

Fruit pulp (4.00 g) was homogenised in hot de- ionised water (75^0C) for 2 minutes using the Phillips blender. The homogenate was left to cool and kept in the refrigerator until determination (AOAC, 1990).

3.6.2 Analysis of acidity (%)

3.6.2.1 Sample analysis and calculation of acidity

Refrigerated samples were initially equilibrated at room temperature and 10.0 mL aliquot titrated in triplicate against standardized sodium hydroxide using phenolphthalein indicator (5 drops) (AOAC, 1990). The acidity was calculated using equation [5] below:

$$\text{Acidity (\%)} = \frac{MM_a \times M_b \times T_b \times V_s \times f \times 100}{V_c \times m} \quad [5]$$

where MM_a = molar mass of the organic acid in the fruit, M_b = molarity of the base, T_b = titrant volume, V_s = total volume of the sample extract, f = correction factor, V_c = analyte volume and m = mass of the sample.

3.7 Determination of total soluble solids

Total soluble solids (%) in fruit pulp were determined using a hand held PR-32 Refractometer (ATAGO, Tokyo, Japan) as per AOAC (AOAC, 1990).

3.8 Determination of the pH of fruit pulp

To 4.0 g fruit pulp was added de-ionised water (150 mL), which was previously boiled and cooled to room temperature. The contents were homogenised for 1 minute using a Phillips blender. The resultant mixture was transferred into a container and the pH measured using a 744 pH meter (Metrohm, Switzerland) (AOAC, 1990).

3.9 Determination of moisture content

To determine moisture content in the samples, the hot oven method was used (AOAC, 1990). Accurately weighed fruit pulps were enclosed in an oven (B5S 88H 113), (ELE International Limited, Hertfordshire, England) set at 105 – 110^oC overnight, cooled in a

desiccator for 30 minutes and weighed. The procedure was repeated until constant weight was obtained.

3.10 Determination of Ca, Cu, Fe, Mg and Zn metal

3.10.1 Dry ashing of fruit samples

Accurately weighed fruit pulp (1.00 g), enclosed in a hot oven at 110^{o}C for 12.0 hours and the dried material ashed in a Muffle furnace size 1 (Gallenkamp, Loughbrough, UK) for 2 hours at 500^{o}C. The white ash was cooled and de-ionised water (10 drops) and nitric acid (5.85 M, 4 drops) added. Excess acid was evaporated in enclosed oven (110^{o}C) for 30 minutes and returned in a furnace for additional 1 hour. The samples were cooled, dissolved in hydrochloric acid (5.09 M, 10.0 mL) and diluted to 50.0 mL in a volumetric flask with de-ionised water (AOAC, 1990).

3.10.2 Analysis and calculation of metals

Metal concentration was determined as described in AOAC (AOAC, 1990). The absorbance of five standard solutions (50.0 mL) including the blank was read in duplicate using a 200A atomic absorption spectrophotometer (AAS) (BUCK Scientific, Norwalk, USA). The absorbance of samples was measured and the concentration in fruit pulp digest determined from standard calibration plot. The level of metals (mg/kg) was then calculated using the equation [6] below:

$$\text{Metal (mg/kg)} = \frac{(\text{MPCONC} - \text{MBCONC}) \text{ x } (\text{TVOL})}{\text{FNWT}} \qquad [6]$$

where MPCONC = Metal concentration in fruit pulp digest (mgL^{-1}), MBCONC = Metal concentration in blank digest (mgL^{-1}), TVOL = Total volume of diluted digest (mL) and FNWT = Fruit sample weight (g)

3.11.1 Statistical analysis of data

Analysis of variance was performed using the Genstat Discovery edition (Genstat, 1999) to test the efficiency of extracting solvent, and the effect of provenance, ripening, tree age, processing and storage condition on the levels of physicochemical properties. Statistical significance was tested at $p < 0.05$. Correlations were performed between solvent systems to ascertain relationships.

CHAPTER 4: RESULTS AND DISCUSSIONS

4.1 Optimisation of ascorbic acid extraction and determination

The yields of ascorbic acid using different extracting solvent systems are presented in Table 5.

Table 5. Effect of solvent concentration and composition on yield of ascorbic acid (mg/100g)

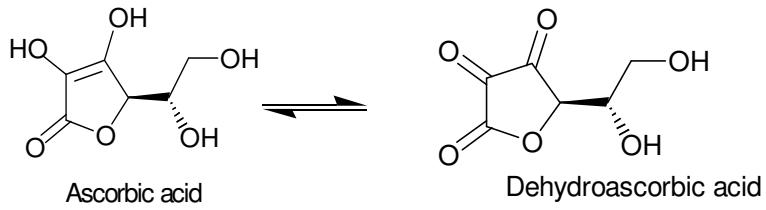
Solvent system	Fruit species				
	A. digitata	*M. indica*	*P. curatellifolia*	*A. comosus*	*U. kirkiana*
SS1	181.04 ±7.4	73.23±2.93	153.87±7.18	3.53±0.09	6.74±0.10
SS2	184 ±4.81	62.59±13.64	178.76±4.56	3.015±0.37	4.46±0.20
SS3	253.24±9.96	68.07±4.86	26.47±7.15	2.76±0.22	3.24±0.27
SS4	164.73±5.43	59.51±11.73	224.10±16.01	2.87±0.37	3.86±0.28
SS5	164.97±3.89	42.66±7.31	255.81±31.66	4.64±0.82	1.90±0.62
SS6	169.62±4.37	60.37±4.43	169.89±3.99	2.83±0.36	1.33±0.20
SS7	246.64±9.36	23.71±0.37	24.19±2.13	0.49±0.02	3.30±.51
SS8	168.11±6.22	65.48±3.33	233.24±16.63	17.10±1.30	3.15±0.28
SS9	222.31±2.76	88.34±1.31	347.60±3.36	9.40±0.02	10.60±1.8
SS10	246.64±9.36	41.26±0.01	34.36±2.67	8.67±0.00	3.27±0.59
SS11	247.64±4.68	23.29±0.76	22.48±1.74	10.23±0.00	4.01±0.11
SS12	271.51±5.07	19.97±2.30	26.30±1.54	6.67±1.09	5.41±0.64
SS13	243.53±2.54	21.33±1.14	23.12±3.06	1.73±0.01	3.62±0.66
SS14	258.04±16.9	10.36±0.02	39.58±2.33	1.73±0.01	0.03±0.00
$LSD_{0.05}$	**8.46**	**6.24**	**13.44**	**0.64**	**0.61**
CV (%)	**3.4**	**11.0**	**8.8**	**10.4**	**13.2**
P value	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**

Key: SSx, solvent system.

The results indicated that solvent concentration and composition significantly affected ($p<0.001$) the amount of ascorbic acid extracted from different fruits. In the high ascorbic acid containing fruit (A. *digitata*), dilute solvent systems like; SS12, SS14 and SS11, extracted significantly higher ($p<0.001$) ascorbic acid than most concentrated blends. The

solvents; SS12, SS14 and SS11, extracted 271.51±5.07, 258.04 ± 16.09 and 247.64 ± 4.68 mg/100 g ascorbic acid respectively while the reference SS9 gave 222.31±2.76 mg/100 g. These values are significantly higher ($p<0.001$) than those reported by Saka et al. (1994) using a more concentrated solvent SS9. Similarly, Amarteifio and co-researchers extracted ascorbic acid from *A. digitata* using the reference and reported significantly lower value (141.3 mg/100g) (Amarteifio et al., 2005).

Acidification of the sample to an appropriate range, during extraction serves to stabilize the ascorbic acid which can otherwise undergo auto oxidation and become undetectable (Scheme 3) (Reiss, 1996). However very high ascorbic acid containing fruits like *A. digitata* and *Vitex payos* are often acidic with pH 3.30 and 2.36 respectively (Saka ,1994; Amarteifio et al., 2005) and may have enhanced the pH level of the solvent system in attaining the optimum pH range for extraction. Thus diluted solvents like SS14 developed in this study can be used for the extraction of ascorbic acid in very high ascorbic acid containing fruits.



Scheme 3: Auto oxidation of ascorbic acid

Generally in the average and low ascorbic acid containing plant materials, the reference extracted significantly higher amount of ascorbic acid ($p<0.001$) than most formulated solvents. In general, the yield of extracted ascorbic acid increased with concentration of the solvent system. Minor discrepancies could be due to instability of ascorbic acid. Thus a more concentrated blend SS1 extracted significantly highest ($p<0.001$) amount of ascorbic acid (73.23 ± 2.93 mg/100 g) and competed very well with the reference SS9 (88.34 ± 1.31 mg/100 g). This could be due to the fact that average and low ascorbic acid containing fruits are generally less acidic and may require more acidic conditions during extraction. For instance, Saka reported that *Azanza garkeana* (pH 5.96), *Bauhinia petersiana* (pH 6.01) and *Syzygium guineense* (pH 6.91) gave 20.5, 13.0 and 7.7 mg/100 g ascorbic acid respectively (Saka, 1994). The reference extracted significantly higher

ascorbic acid (10.60 ±1.18 mg/100 g) from a very low ascorbic acid containing fruit (*U. kirkiana*) than all formulated solvents. Similarly, more concentrated formulated solvents extracted significantly higher ($p<0.001$) ascorbic acid than dilute counterparts. For instance, SS1, SS2, SS3, SS14 extracted 6.74 ± 0.10, 4.46 ± 0.20, 3.24 ± 0.27, and 0.03 ± 0.00 mg/100 g ascorbic acid respectively. Ascorbic acid in the average and low ascorbic acid containing plant materials is heavily complexed with other molecules like proteins therefore extraction may require very acidic or concentrated solvents (AOAC, 1990). Some least ascorbic acid containing fruits are basic and tend to neutralise extracting solvents hence the use of concentrated solvents is required to achieve the normal extracting pH (AOAC, 1990).

4.1.1 The effect of pH of solvent system on the yield of ascorbic acid in *A. digitata*

The results for the relationship between extractable ascorbic acid and pH of the solvent system are presented in Figure 3.

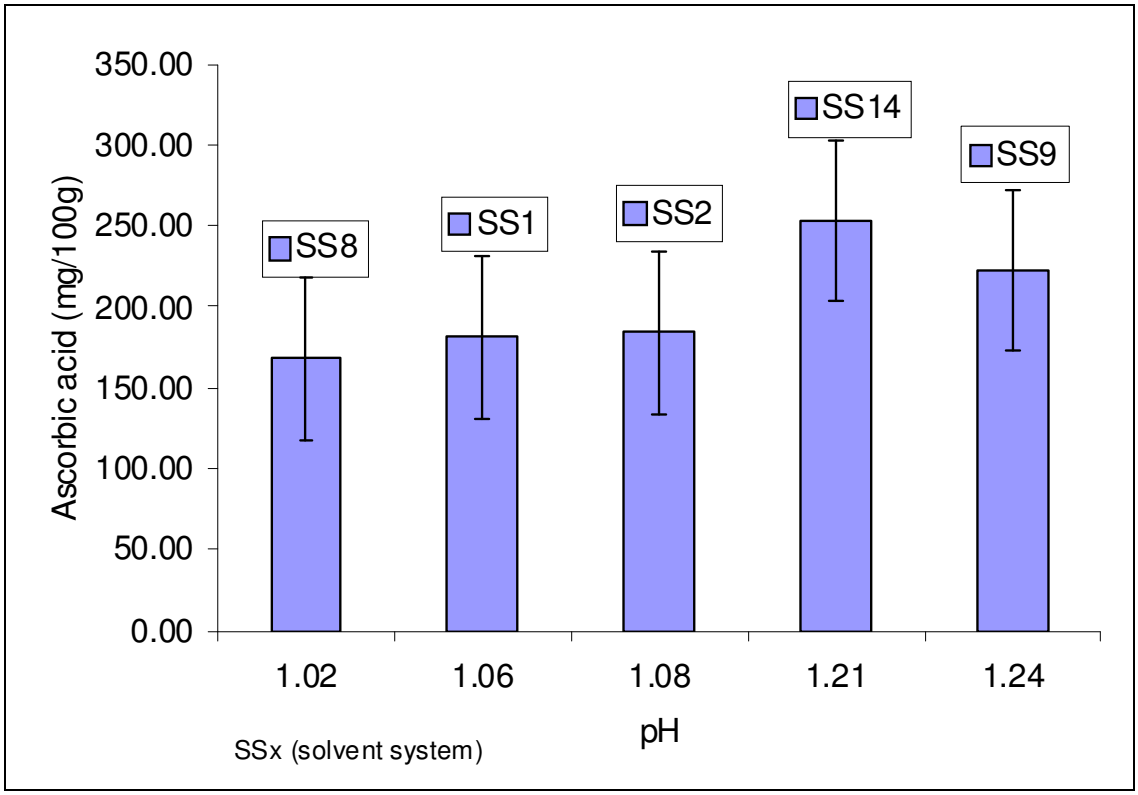


Figure 3: **Effect of pH of the solvent system on ascorbic acid**

The results showed that the yield of ascorbic acid generally increased with pH of a solvent system until pH of 1.21 was reached. For example, the solvents (SS8, SS1, SS2, SS14 and SS9) yield varied from 168.0, 181.0, and 184.1, 253.2 to 222.3 mg/100 g for the corresponding pH of 1.02, 1.06, 1.08, 1.21 and 1.24 respectively. Results further indicate that the pH of a solvent system is not proportional to concentration. For instance, the more concentrated reference SS9 had a pH value of 1.24 and gave significantly ($p< 0.05$) lower yield for *A. digitata* (222.31 mg/100 g) than SS14 (253.24 mg/100 g), pH 1.21.

4.1.2 Correlation matrix between different solvent systems

In order to ascertain the performance of solvent systems for *A. digitata*, the reference, and representatives of very high to least ascorbic acid yielding solvents were selected for correlation analysis.

Table 6. Correlation coefficients for selected solvent systems

Solv. Syst.	SS 9	SS1	SS5	SS8	SS13	SS14	SS 11	SS12
SS9	1.000	0.913*	0.997**	0.996**	0.405	0.502	0.391	0.403

**p< 0.01
*p <0.05

The results revealed that solvent systems such as SS5, SS1 and SS8 are strongly correlated with the reference SS9; r = 0.997**, 0.913* and 0.996** respectively. This means that they can extract ascorbic acid from different fruit species more proportionally to the reference than other blends. The solvents; SS11, SS12, SS13 and SS14 had very weak correlations with the reference at r = 0.391, 0.403, 0.405, and 0.502, ($p>0.05$) respectively. For the selected solvent systems, descending order of correlation with the reference was; SS5 > SS8 > SS1 > SS14 > SS13 > SS12 > SS11. Therefore, dilute orthophosphoric acetic acid blends like, SS14 can be used to extract ascorbic acid only from rich sources of ascorbic acid.

4.1.3 Effect of selected solvent systems on ascorbic acid Recoveries

The recoveries of ascorbic acid using high to least ascorbic acid yielding solvents in different fruits are provided in Figure 4. The reference solvent was included in each case. The results revealed that for high ascorbic acid containing fruit, *A. digitata,* the solvents, SS14 and SS12 gave higher average recoveries; 90.44 ± 4.19% and 85.56 ± 6.65% respectively. Similarly for *P. curatellifolia* and *M. indica,* the solvents SS6, and SS1 had significantly higher ($p < 0.001$) recoveries; (87.91 ± 2.80%) and (88.09 ± 7.19%) respectively while the reference had (85.13 ± 6.24%) and (57.53 ± 12.47%) respectively in same fruits. However the reference had highest recoveries in the least ascorbic acid containing fruit, *A. comosus*. High recoveries entail that the solvent had minimum interference with standard ascorbic acid form. This could be due to proper chemical properties like pH, of the extracting solvent in that particular fruit species.

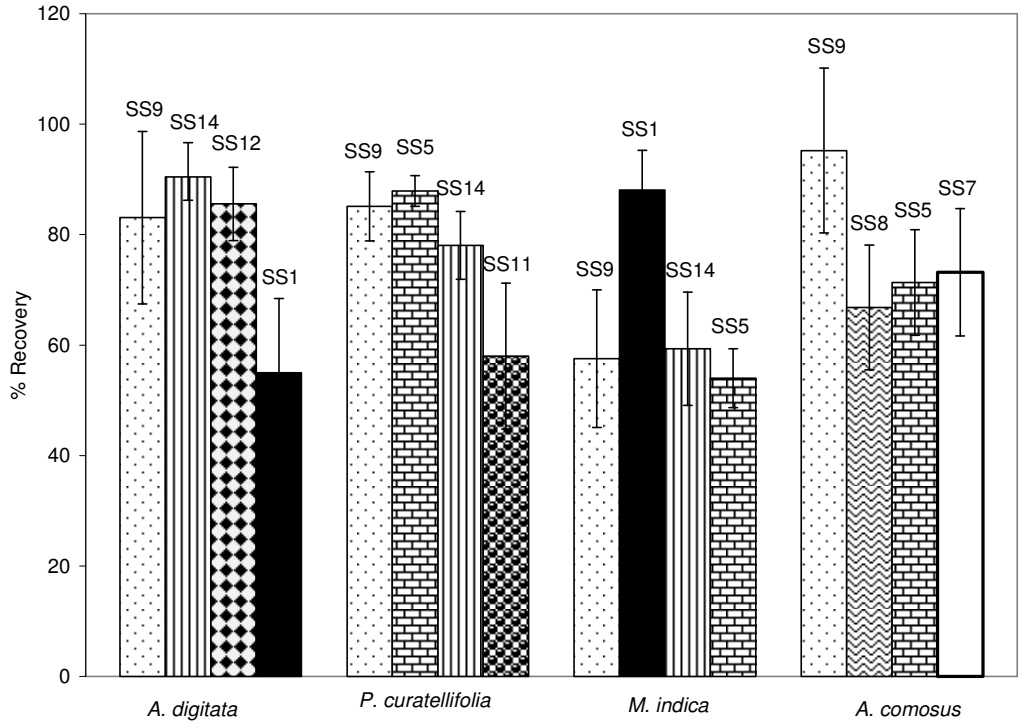


Figure 4: Recoveries of ascorbic acid using high to least ascorbic acid fruit sources

Iwase and co-workers recommended the use of ethylenediaminetetraacetic acid disodiumdihydrate as an extracting solvent for ascorbic acid because it had high recovery (> 90%) (Iwase et al., 1997). Chukwumah and co-researchers evaluated four extraction methods and solvents of phytochemicals (biochanin A and trans-resveratrol) in peanut

using recoveries (Chukwumah et al., 2007). MAS method extracted significantly higher ($p<0.05$) phytochemicals than Stirring, Sonication and Soxtec methods from hexane-defatted and mechanically defatted peanuts compared to the nondefatted peanuts. The recoveries were 85.52 ±5.84%, 93.16 ±2.10%, and 98.34 ±2.20% for biochanin A and 87.91 ±6.09%, 78.76 ±4.19%, and 93.89 ±3.24% for trans-resveratrol) and thus may be used for total isoflavone content quantitation. Mhone used recoveries to evaluate the performance of microdiffusion picrate method against the Cooke's enzymatic and the improved acid hydrolysis for the determination of cyanogens in cassava and reported that significantly high recovery (98%) meant the improved solid-state picrate method was as quantitative as the Cooke's method (Mhone, 2001). Huang and co-workers recommended the extraction of Chloramphenicol from food (milk, honey, and eggs) using Polymer Monolith Microextraction (PMME) followed by Liquid Chromatograghy-Mass Spectrometry determination due to high recoveries (85-102%) besides being inexpensive, simple and fast (Huang et al., 2007). Thus the protocol and recommendations of this study regarding selection of extracting solvents are consistent with those reported by others. Solvent systems with higher recovery (>85%) are thus sufficiently good for extraction and determination of ascorbic acid in fruits and vegetables.

4.1.4 Effect of selected solvent systems on stability of ascorbic acid

Ascorbic acid in fresh plant materials, food products and sample extract is easily oxidized to dehydroascorbic acid under most storage conditions (Pavan et al., 2004).

The effect of two selected solvent systems SS14 and SS3 on the retention of ascorbic acid extracted from *A. digitata* is presented in Figure 5. This was performed by determining the amount of ascorbic acid in the sample extract stored in the refrigerator after every 24 hours for 96 hours. The two solvents extracted significant amount of ascorbic acid (Table 5). The results indicate that the content of ascorbic acid decreased from 100% to 84 and 75% in SS14 and SS3 respectively after 96 hours of storage. After 24 hours, the solvents, SS14 and SS3 showed 96% and 95% retention respectively. After 72 hours, the solvent system, SS14 still exhibited significantly higher ($p< 0.001$) ascorbic acid (>90%) than SS3 (Figure 5). Thus 0.05M H_3PO_4 + 0.025M HOAc (SS14) achieves greater stability of the extracted ascorbic acid in *A. digitata.*

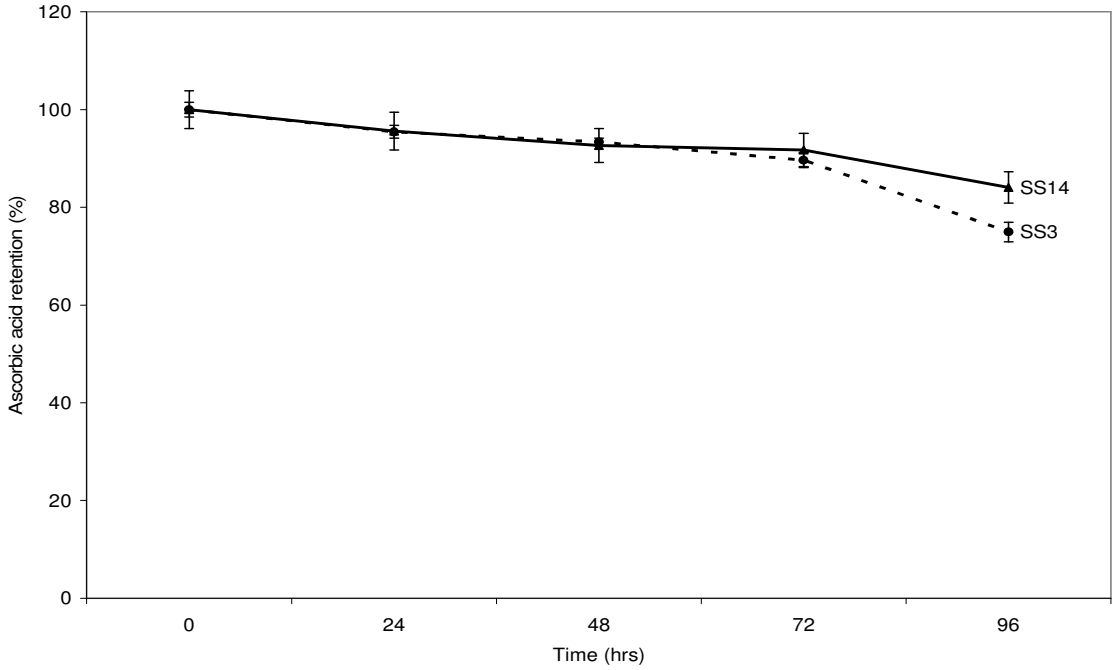


Figure 5: **Effect of formulated solvents on ascorbic acid retention**

4.2 Effect of provenance on some physicochemical properties of fruits

The effect of provenance on some important physicochemical properties/nutritional attributes of three fruits is provided in Tables 7.

Provenance significantly affects the levels of nutrients in *P. curatellifolia* ($p<0.05$). For instance, fruits from Katope (Mzimba) had significantly highest ($p<0.001$) levels of vitamin A and reducing sugars compared to all. The least vitamin A levels ($p<0.001$) were observed in the Kaning'ina fruit provenance. No significant difference existed in vitamin A levels for the sample fruits from Nkhata Bay and Nkhamenya ($p >0.05$). *P. curatellifolia* from Nkhata Bay provenance exhibited significantly highest levels of ascorbic acid and total soluble solids ($p<0.001$) than other provenances. This is probably due to differences in soil types. Ascorbic acid content in Nkhata Bay fruits was five fold more than Katope and Kaning'ina fruit provenances. All fruits were acidic. Nkhata Bay samples gave higher acidities ($p<0.001$) than the rest.

Acidic conditions favour starch hydrolysis into monosaccharides (glucose and fructose) (Goldman et al., 1999) thus high levels of TSS for Nkhata Bay fruit provenance. The total soluble solids varied from 23.17±2.27 to 26.12±4.70% for all provenances. No significant

variation existed in TSS levels ($p>0.05$) amongst fruits from Katope, Kaning'ina and Nkhamenya. The reducing sugar levels were significantly different amongst all provenances ($p<0.001$). The Katope provenance gave significantly highest levels of reducing sugars (25.17±4.37%) while Nkhamenya afforded the least (3.92±0.05%). The variations in nutrient content could be genotypic, environmental (soil property) or geographical attributes. Physicochemical properties of Hard Spring Wheat were significantly different Mpofu et al. (2006). For example, total phenolic content, antioxidant activities, and concentrations of all the phenolic acids measured were significantly different with genotype and environment.

Table 7. Effect of provenance on physicochemical properties of three fruit species

Provenance	***Adansonia digitata* (Analyte)**													
	Vit. A (mg/kg)	Vit.C (mg/100g)	R.sugar (%)	Acidity (%)	pH	DM (%)	TSS (%)	Ca (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	K (mg/kg)	Mg (mg/k)	Na/ mg/kg	Zn/ mg/kg
Chikwawa	35.83	347.70	11.27	2.58	3.19	32.03	21.80	1956	2.48	19.60	979	20.30	166.90	3.36
Dedza	31.08	259.70	8.75	2.20	3.25	32.47	32.50	2786	5.02	15.30	766	10.40	172.30	2.37
Mangochi	60.92	239.30	10.18	2.86	3.14	32.53	32.03	1958	1.68	22.90	1172	26.20	252.90	5.18
Mwanza	54.71	317.00	10.22	2.40	3.16	32.50	30.63	2269	4.14	17.60	530	21.20	282.30	2.02
Salima	29.86	233.10	9.01	2.68	3.43	33.80	31.87	1978	3.58	16.40	1142	33.60	248.20	3.40
LSD (0.05)	1.25	12.30	0.19	0.15	0.01	1.27	5.29	1.46	7.46	4.82	10.61	58.84	1.74	0.01
CV (%)	1.60	2.30	1.00	3.10	0.20	1.00	5.10	12.80	22.90	21.60	25.00	25.20	13.90	0.30
Sign. level	0.001	0.001	0.001	0.001	0.001	0.092	0.001	0.027	0.005	0.241	0.007	0.011	0.006	0.022

	***Parinari curatellifolia* (Analyte)**							***Ziziphus mauritiana* (Analyte)**				
Provenance	Vit. A (mg/kg)	Vit.C (mg/100g)	R.sugar (%)	Acidity (%)	pH	DM (%)	TSS (%)	**Provenance**	Vitamin C (mg/100g)	R. sugar (%)	Acidity (%)	TSS (%)
Katope	32.39	25.17	25.17	0.08	3.54	25.64	25.10					
Kaning'ina	9.80	23.51	10.20	0.05	3.48	28.17	23.20	Chikwawa	37.19	15.94	0.19	19.17
Nkhatabay	12.31	134.64	8.43	0.10	4.07	24.10	26.10	Dedza	73.38	15.48	0.36	15.08
Nkhamenya	11.64	41.38	3.92	0.07	3.65	28.29	23.80	Mangochi	88.05	15.11	0.48	13.95
LSD (0.05)	1.16	5.68	0.16	0.02	0.03	0.98	4.0		3.80	0.75	0.04	2.10
CV (%)	5.80	8.20	2.00	24.40	0.70	3.30	13.00		21.20	3.80	8.50	10.20
Sign. level	0.001	0.001	0.001	0.001	0.001	0.001	0.38		0.001	0.092	0.001	0.001

The significant effect ($p<0.001$) of provenance on physicochemical properties has also been observed in *Z. mauritiana* fruits (Table 7). Mangochi fruit provenance gave significantly higher vitamin C levels ($p<0.001$) than Chikwawa and Dedza. Further, Mangochi fruits exhibited significantly higher ($p<0.001$) acidity than Chikwawa and Dedza. In contrast, the significantly higher total soluble solids and reducing sugar levels ($p<0.001$) were obtained in *Z. mauritiana* fruits from Chikwawa provenance.

Further, provenance significantly affected physicochemical properties of *A. digitata* fruits ($p<0.001$)**.** For instance, Chikwawa fruits had significantly highest ($p<0.001$) ascorbic acid and reducing sugar levels. Values as high as 347.7 mg/100 g and 11.27 % respectively were obtained. Fruits from Salima had significantly least levels of vitamin A and C ($p<0.001$). Other studies have also established that differences in nutritional quality of same fruit genotype may be due to variation in soil types, altitude, soil pH and climate (Goldman et al., 1999 and Gross, 1991). Gross (1991) reported that light intensity is proportional to sugar synthesis by the fruit (glucose being the precursor of vitamin C). Generally, fruits from all sites were acidic. However, minor differences within provenances were observed (Table 7). Mangochi fruits were the most acidic ($p<0.05$). Osman attributed this as due to the presence of high amino acids content (glutamic and aspartic) as well as ascorbic acid in *A. digitata* fruit pulp (Osman, 2004). The total soluble solids ranged from 21.80 to 32.50% and the Dedza provenance afforded significantly highest levels while Chikwawa the least ($p<0.001$).

The fruit pulp of *A. digitata* from Mangochi and Mwanza had significantly highest levels of vitamin A ($p<0.001$); 60.92 and 54.71 mg/kg respectively. Chikwawa and Salima fruits afforded only 35.83 and 29.86 mg/kg vitamin A respectively. Gross reported that plant materials in areas of high light intensity are associated with low vitamin A (Gross, 1991).

Analysis of the mineral content in *A. digitata* pulp indicated that provenance significantly affects their levels. For example, Mwanza and Dedza provenances showed significantly higher ($p<0.05$) calcium content than fruits from the other three sites. Mangochi and Salima had significantly highest ($p<0.05$) potassium level. This is possibly due to differences and similarities in soil types and rainfall patterns from other sites. Mangochi and Salima are

along the lake shore (Lake Malawi) hence may share similar rainfall patterns and soil properties.

4.3 Effect of harvesting period and fruit condition on some physicochemical properties of fruits

The effects of harvesting time and fruit condition on some physicochemical properties of *S. cocculoides* are presented in Table 8.

Table 8. Nutritional attributes of *S. cocculoides* harvested in October and December

Analyte/ Nutrient	Fruit condition				**LSD (0.05)**		**Sign. Level**	
	Mature (unripe)		Mature (ripe)					
	Oct	Dec	Oct	Dec	Oct	Dec	Oct	Dec
Vit C (mg/100g)	20.20	25.95	15.53	20.02	0.32	1.22	0.001	0.001
R. Sugars (%)	3.42	9.41	7.69	10.90	7.68	0.04	0.001	0.001
Acidity (%)	0.94	0.23	1.56	0.35	0.09	0.04	0.001	0.001
Ca (mg/kg)	18.14	22.76	7.95	25.60	0.75	2.59	0.001	0.001
Cu (mg/kg)	0.95	0.76	0.60	0.77	0.07	0.14	0.001	0.85
Fe (mg/kg)	15.70	15.71	8.80	12.39	4.60	1.58	0.004	0.001
K (mg/kg)	32.09	38.10	26.99	41.44	0.85	2.88	0.001	0.001
Mg (mg/kg)	5.70	7.70	4.24	5.20	0.12	6.52	0.001	0.42
Na (mg/kg)	48.51	109.40	44.00	81.90	0.33	17.41	0.001	0.004
Zn (mg/kg)	4.03	3.72	2.77	3.06	0.25	0.70	0.001	0.065

The results showed that both period of harvest and fruit condition have strong influence on nutritional level of plant material. Significant differences existed in physicochemical properties of *Strychnos* at the two harvesting times ($p<0.001$). Figure 6 shows clearly that levels of vitamin C, reducing sugars, calcium, iron, magnesium and sodium levels were significantly higher ($p<0.001$) in fruits harvested in December than October.

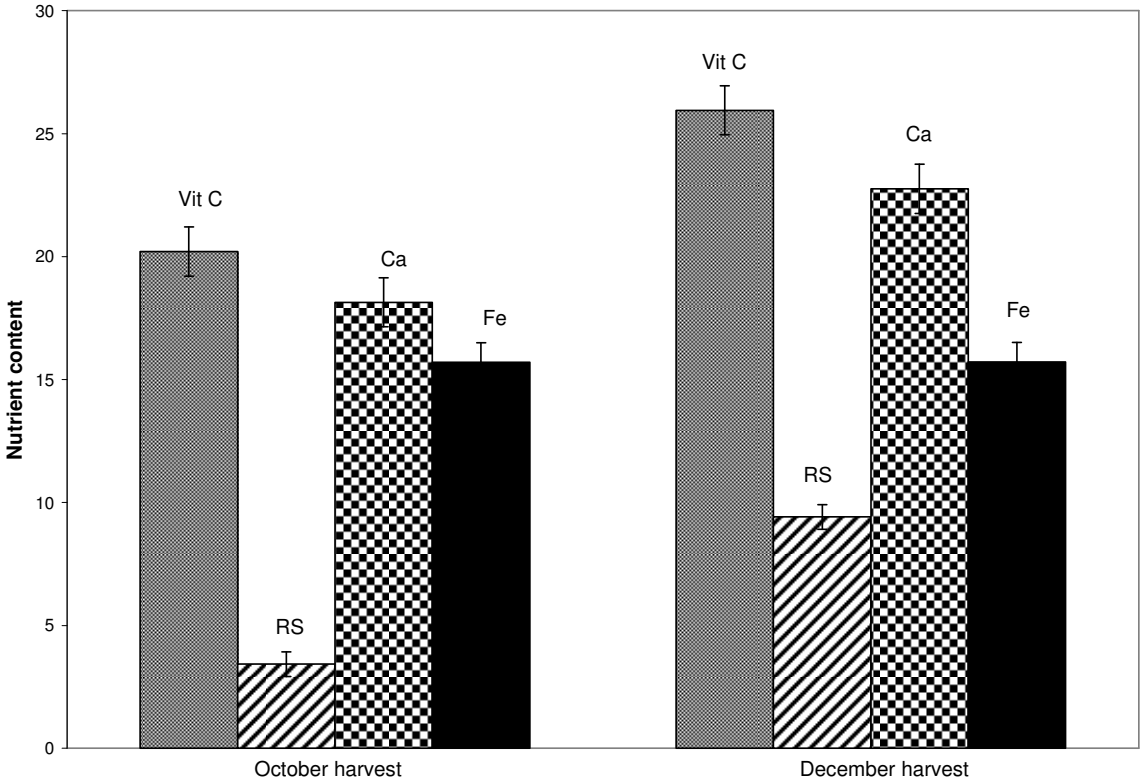


Figure 6: Nutrient levels of *S. cocculoides* fruit pulp for two harvesting times

In contrast, acidity levels were significantly higher ($p<0.001$) in October than December harvest. This is possibly due to large amount of organic acids like citric acid which are later (December) converted to other organic compounds. High levels of reducing sugars in December fruits entails high glucose level, the raw material for vitamin C. In recent studies, Kadzere and co-researchers reported that delaying harvesting of *Uapaca kirkiana* fruits help to improve quality attributes such as skin colour at harvest and during storage, to reduce weight loss and obtain higher soluble solids concentration (SSC) (Kadzere et al., 2006 and 2007). For instance, late harvested fruits had SSC varying from 11.8% in greenish-yellow fruit to 14.4% in browner fruits versus 6.7% to 13.8% for the early harvested fruits. Reducing sugars and total soluble solids levels were significantly higher ($p<0.001$) in ripe fruits than unripe fruits for both harvesting seasons. The two variables (reducing sugars and total soluble solids) were significantly higher ($p<0.001$) in ripe fruits than unripe counterparts. Iron, magnesium, sodium and zinc levels were significantly decreased ($p<0.001$) in ripe fruits. Thus harvesting of *S. cocculoides* in December assures quality fruits in terms vitamins and sugar levels.

4.3.1 Effect of ripening on some physicochemical properties of *S. cocculoides*

Fruit developmental stage affects fruit quality and the resultant products. Although fruits are much appreciated when harvested after full ripening, pre-ripe harvesting for some fruits, including mangoes, can otherwise be recommended (Kansci et al., 2003). The importance of state of ripening at harvest is primordial as notable changes occur in the fruit during ripening which improved its physicochemical and sensory characteristics (appearance, smell and flavor).

The results for changes of nutrient levels with storage time (ambient air storage condition) in both mature and ripe fruit pulps of *S. cocculoides* are presented in Table 9.

Table 9. Changes of nutritional attributes with storage time in mature unripe and ripe *S. cocculoides* fruit pulps

Fruit condition	Time (hours)	Physicochemical properties				
		Vit.C (mg/100g)	Red. sugar (%)	TSS (%)	Acidity (%)	Dry matter (%)
Mature (unripe)	0.00	306.25	2.42	16.64	0.94	25.11
	72.00	276.11	3.46	18.92	1.43	25.28
	144.00	285.07	3.05	18.14	1.04	26.52
Ripe	0.00	53.91	7.69	16.11	1.56	22.63
	72.00	93.66	5.85	17.96	1.87	23.49
	144.00	35.24	4.10	17.43	1.46	23.55
LSD(0.05)		**24.51**	**0.62**	**1.82**	**0.25**	**2.71**

The results showed that generally reducing sugars and total soluble solids were increasing until peak ripening when these variables started decreasing (over ripened fruits). Such a decrease could be due to dilution effect evident from a decrease in dry matter content with ripening (Table 9). The levels of vitamin C were decreasing with ripening. Cell wall components undergo changes after harvest as a consequence of the action of various enzymes and all these account for the increase in TSS and reducing sugars with ripening (Aydin et al., 2001). McWilliams reported that the pectic substances in cell walls and middle lamella undergo degradation as a result of the increasing levels of two types of

enzymes: pectinesterases and polygalacturonases (McWilliams, 1993). The action of pectinesterases is valued in making apple and grape juices because of the increased solubility of the degraded pectic substances, notably pectic acid, promotes a less cloudy beverage and increases the visual appeal of the juices. Other enzymes include hemicellulase and cellulase. As a consequence of the reactions catalysed by these enzymes, some sugars are released from the complex polysaccharides constituting the cell walls. The result is that ripening fruits increase in sweetness despite the fact that they may have little or no starch to serve as a potential source of sugar. Another possible route of increasing sugar levels during ripening in some fruits is by the conversion of starch to sugars. This reaction is catalysed by amylase. Invertase is the enzyme effective in converting sucrose in fruits to its component sugars, glucose and fructose (McWilliams, 1993).

The increase in acidity with ripening could be due to oxidation of monosaccharides, those with an aldehyde group (reducing sugars) to carboxylic acid under the action of enzymes. In general a significant increase in acidity with time of ripe fruits is accompanied by a significant decrease in reducing sugars. Green harvested tomatoes ripened at 20^0C were found to contain less vitamin C than those harvested at ripe stage (George et al., 2006). At breaker stage of maturity, tomatoes contained only 69% of the vitamin C of their ripe counterparts. Further, Martinez and co-researchers found that vitamin C content in sweet pepper increased with ripening (Martinez et al., 2005). Total sugar content of ripe pulp of all the *Mangifera indica* varieties was significantly higher ($p<0.05$) than that of the pre-ripe pulps at 9.4-15.2 and 4.0- 7.6 % w/w respectively (Kansci et al., 2003). The starch content of pre-ripe pulp varied from 4.4 to 11.1 g/100 g and higher than the ripe pulp (0.8 – 1.1% w/w). Germain and co-workers attributed this to the transformation of starch into soluble sugars under the action of phosphorylase enzyme during ripening (Germain et al., 1981). Therefore results of the present study are consistent with previous findings.

4.3.2 Effect of ripening stage on some physicochemical properties of *Z. mauritiana*

The results (Table 10) has also revealed that ripening significantly ($p<0.001$) affect the chemical composition of *Z. mauritiana* fruit pulp. In general, total soluble sugars, reducing sugars and vitamin C levels increased with ripening. In some fruits, ascorbic acid level increases greatly while in others, it remains unchanged or decrease. For example, papaya

presented about 4- fold increase in ascorbic acid level from the mature green to ripe stage (Barata et al., 2004). On the other hand, strawberry did not show significant changes from the mature green to the intermediate stages. The increase or decrease of the ascorbic acid and dehydroascorbic acid levels from precursors also affect both enzymatic and non-enzymatic factors. The balance between these factors assures the final content and underlies the variation of ascorbic acid level during ripening. Chapman and Horvat found that the total ascorbic acid level in *Mespilus germanica* fruits increased in the pre-ripening stage followed by a decrease in the post- ripening stages and the level of glucose gradually increased during fruit development and ripening (Chapman and Horvat, 1993). For some fruits including *P. curatellifolia,* and *S. cocculoides* many reactions still occur during fruit ripening, such as colour transformation, sugar synthesis and cell wall degradation. All these phenomena may cause tissue stresses, which would require antioxidant action especially by ascorbate, preventing cell damage. Due to these stresses ascorbic acid levels would invariably decrease during fruit ripening. Ascorbic acid acts as a free radical scavenger in animal and plant tissues (Foyer, 1993).

Table 10. Effect of fruit condition on some physicochemical properties of *Z. mauritiana*

Sample	DM (%)	TSS (%)	pH	Acidity (%)	RS (%)	AA (mg/100 g)
UR	17.3±0.2 16-18	14.0 ±0.5 13.4-14.1	3.41±0.1 3.32-3.58	0.24±0.1 0.18-0.27	10.3 ±0.0 0.0	131.79± 2.0 130.60-134.10
SR	21.30±0.3 20-22	16.6 ±0.3 16.3-16.8	3.35 ±0.0 3.34-3.84	0.12 ±0.0 0.0	12± 0.0 0.0	126.90±3.8 125.35-130.05
R	21.3±0.3 20-22	19.93±0.5 19.6-20.5	3.39 ±0.0 3.34-3.42	0.15±0.0 0.14-0.15	13.23±0.6 13.2-13.3	146.01±2.8 144.13-149.30
VR	20.7±0.3 20-22	18.07±0.2 7.9-18.2	3.31±0.1 3.26-3.38	0.26±0.0 0.25-0.26	13.77±0.9 13.6-14.1	89.37±0.0 0.0

Key: UR, Unripe; SR, Slightly ripe; R; Ripe, VR, Very ripe.

4.4 Effect of fruit tree age on some physicochemical properties of *S. cocculoides*

The results (Table 11) indicated that fruit tree age significantly affect the chemical composition of fruit pulp ($p < 0.001$). Slightly ripe (SR) fruit pulp from middle-aged fruit

trees gave significantly higher vitamin C (418.56 mg/100 g) on dry matter basis (DM) than SR fruit pulp from young and old aged fruit trees ($p<0.001$). However, SR fruit pulp from old aged trees exhibited significantly higher acidity than the rest. Reducing sugars were significantly higher in SR pulp from old aged fruit trees ($p <0.001$). Very ripe (VR) fruit pulps from all tree age groups had lower total soluble solids. This could be due to dilution effect.

Table 11. Effect of fruit tree age on some physicochemical properties of *S. cocculoides* pulp

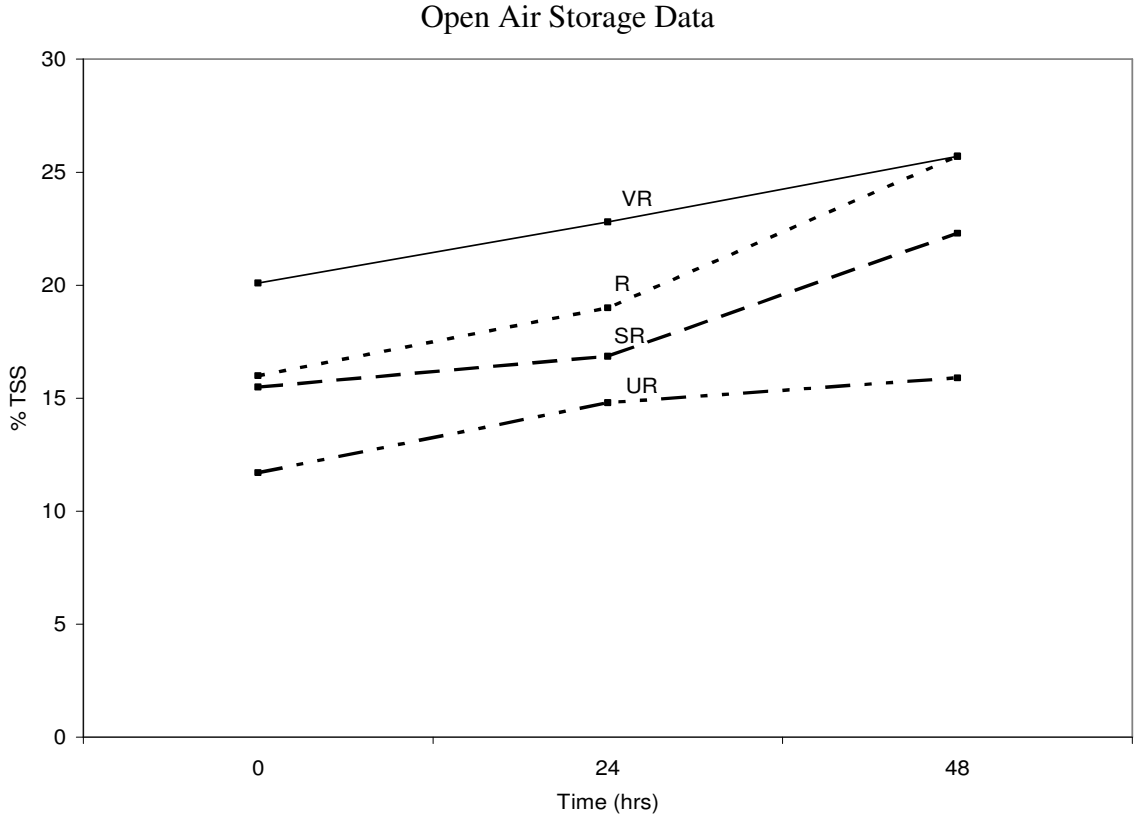
	Physicochemical properties				
Tree age (Fruit condition)	Vit (mg/100g)	Red. Sugar (%)	TSS (%)	Acidity (%)	DM (%)
Young (SR) (5-9 years)	311.08	2.90	18.24	1.39	26.52
Young (R) (5-9 years)	63.69	5.96	17.29	1.39	23.55
Middle(SR) (10-14 years)	418.56	3.39	16.07	0.986	25.11
Middle (R) (10-14 years)	71.14	5.83	16.35	1.72	22.63
Old (SR) (≥15 years)	137.26	3.34	19.39	1.035	25.28
Old (R) (≥15 years)	48.53	5.85	17.86	1.789	23.49
LSD (0.05)	**38.6**	**1.08**	**1.73**	**0.07**	**2.75**

Key: SR, Slightly ripe; R, Ripe.

4.5 Effect of storage condition on some physicochemical properties of *Z. mauritiana*

The data (Figures 7a, 7b and 7c) revealed that storage conditions significantly affected the change in total soluble solids (TSS) with time in *Z. mauritiana* fruits ($p<0.001$). Fruits kept in open air showed significantly increased TSS levels ($p<0.001$). For instance, % TSS for initially unripe (UR) fruits increased from 11.71% to 15.5% over a 48 hour period of

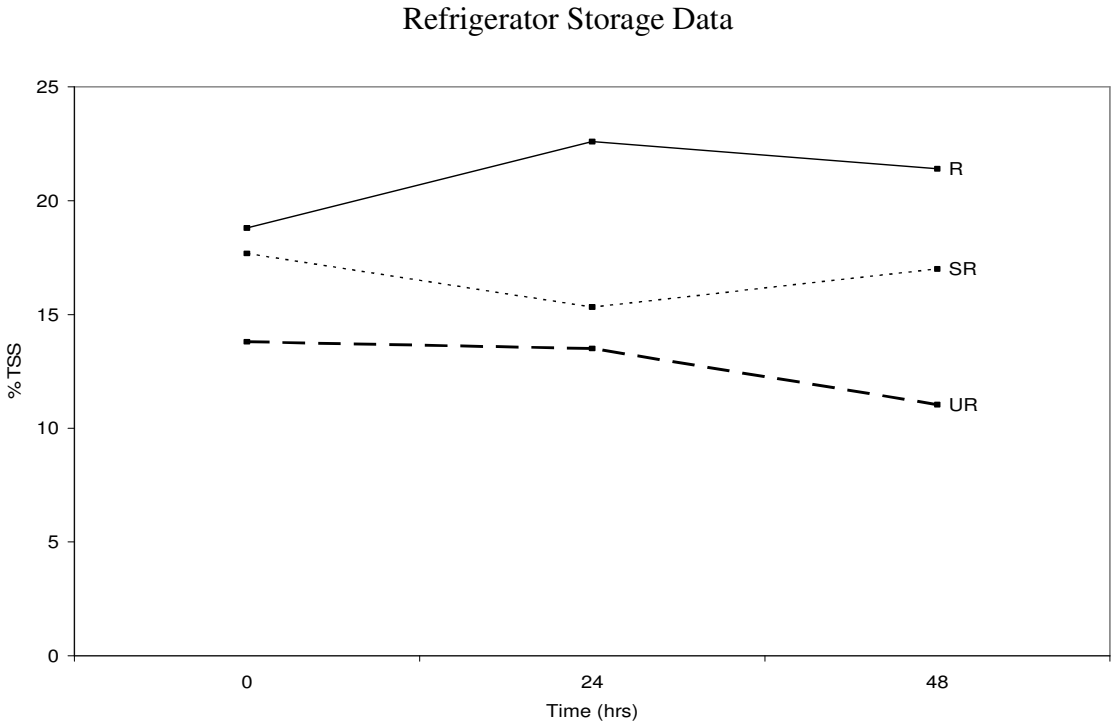
storage. Similarly TSS levels for slightly (SR) ripe (R) and very ripe fruits (VR) increased steadily under ambient air storage condition (Figure 7a). Higher temperatures under open air provided favourable conditions to enzymes for metabolic processes like cell wall degradation leading to the release of soluble sugars hence increase in TSS levels (Aydin et al., 2001).



Key: VR, very ripe; R, ripe; SR, slightly ripe; UR, unripe

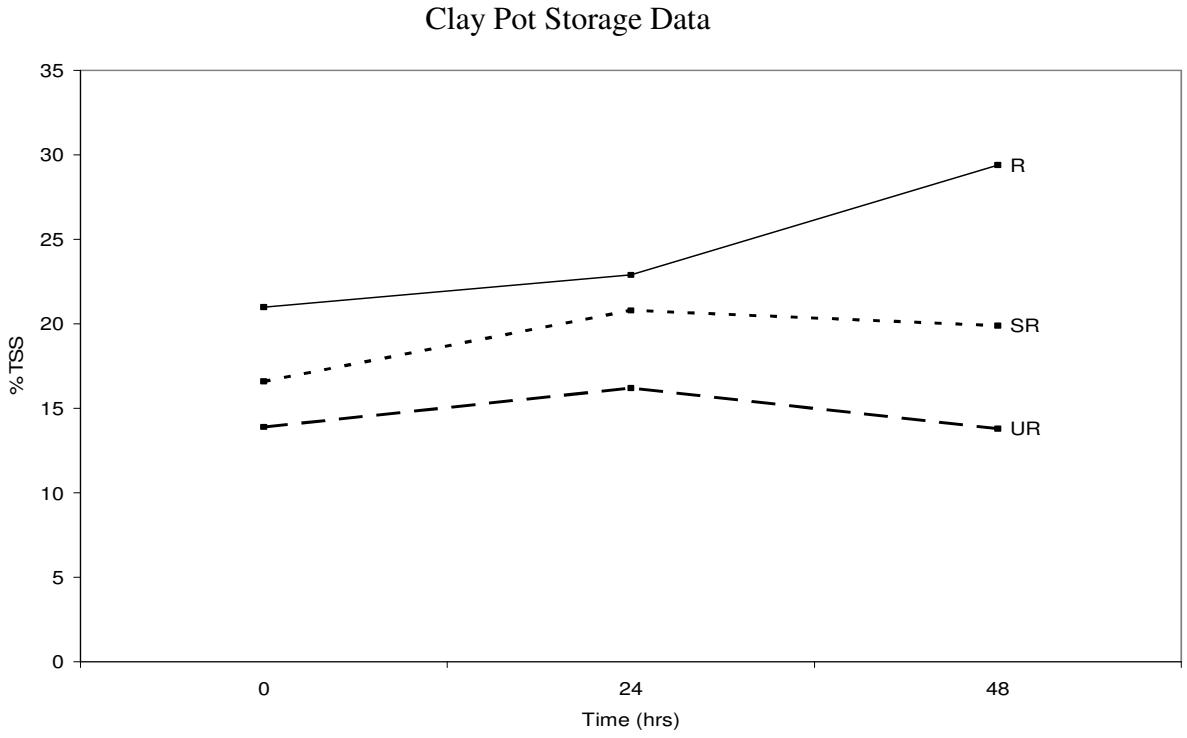
Figure 7a: Effect of ambient air storage conditions on % TSS of *Z. mauritiana*

Negligible and gradual changes in TSS levels were observed for fruits kept in the refrigerator and clay pots respectively. The levels under refrigerator storage condition were nearly constant. For instance, SR fruits gave 17.68% of TSS at time zero while 17.0% was determined after 48 hours of refrigeration (Figure 7b). For clay pot storage the % TSS for the unripe fruits increased for the first 24 hour period from 13.9% to 16.2% but dropped to 13.8 after the next 24 hours (Figure 7c).



Key: R, ripe; SR, slightly ripe; UR, unripe

Figure 7b: Effect of refrigerator storage conditions on % TSS of *Z. mauritiana*



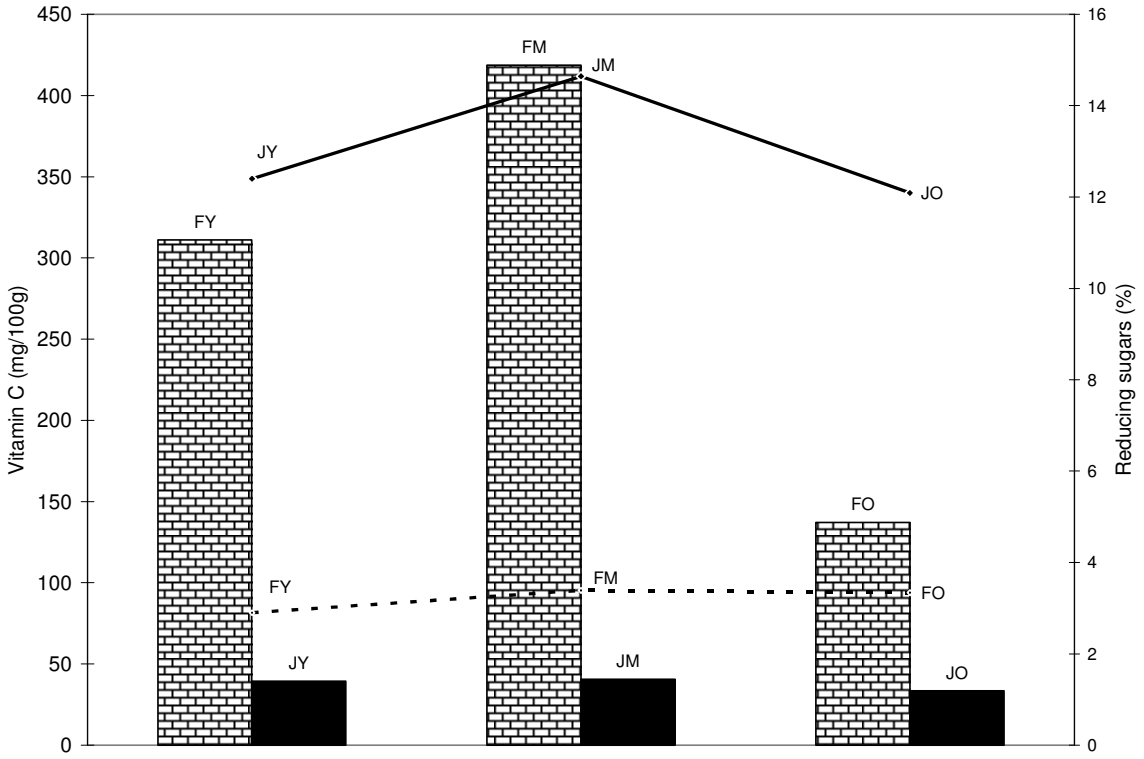
Key: R, ripe; SR, slightly ripe; UR, unripe

Figure 7c: Effect of clay pot storage conditions on % TSS of *Z. mauritiana*

The lower temperatures in the clay pots accounted for significantly lower change with time ($p<0.05$) in TSS levels than fruits under open air. The physiological activities were inhibited by very low refrigerator temperatures while normal metabolic processes were being carried on under open air storage hence the observed effects. Similar results for the effect of storage temperature on the post harvest quality were reported in *Solanum muricatum* (Pepino) (Widayat et al., 2003). Results revealed that pigments and carbohydrates including monosaccharide and disaccharides (total soluble solids) vary to some extent depending on the storage temperature, specifically at high temperatures of 18 ^{0}C. Ascorbic acid content for green mature pepper and breaker pepper stored at room temperature (20 ^{0}C) increased up to 10 days of storage, reaching similar values as those obtained for red pepper direct from plant (Martinez et al., 2005). However, stored red ripe peppers (20 ^{0}C) showed a significant loss (around 25%) in vitamin C content. On the other hand refrigeration at 4 ^{0}C did not change ascorbic acid content for green mature pepper. This indicates that a pronounced temperature affect the dynamic of fruit quality. Storing fruits at a lower temperature (5 ^{0}C) appear to limit the loss and change of most of the fruit nutritional quality attributes.

4.6.1 Effect of jam processing on some physicochemical characteristics of *S. cocculoides* fruits from young, middle and old aged trees

Jam prepared from different fruit ripening stages and tree age groups of *S. cocculoides* had their physicochemical properties analysed then compared with fresh edible pulp. Vitamin C and reducing sugar levels of fresh fruits (FY, FM, FO) and their jams (JY, JM, JO) from young, middle and old fruit trees including reducing sugars are summarised in Figure 8.



Key: YF, Fresh fruits from young aged trees and their jam (YJ); MF, Fresh fruits from middle aged trees and their jam (JM); OF, Fresh fruits from old aged trees and their jam (OJ)

Figure 8: Effect of jam processing on nutritional levels of *S. cocculoides*

Processing affected the composition of the final produced jam from *Strychnos* fruits from three tree age groups. Ascorbic acid decreased significantly during the production of jam while reducing sugars increased ($p<0.001$). For example, unprocessed *Strychnos* fruit pulp from middle aged fruit trees (MF) showed significantly higher ascorbic acid level (418.56 mg/100 g) but only 40. 66 mg/100 g was retained after jam processing (Figure 8). This represented more than 90% loss in the final product. On the contrary, reducing sugar was 14.39% in the jam representing more than 320 % increase. Significant losses of vitamin C could be due to pressing and heating during jam preparation. Vitamin C might have passed into the liquid phase during processing. Further to that, vitamin C, which is unstable to heat and oxygen was easily oxidised to nonantioxidant effective substances. Increase in reducing sugar content was due to extra sucrose added during jam production which undergoes hydrolysis to fructose and glucose during heating. An acidic medium provided

by the application of *Citrus limonium* juice favoured degradation reaction. Similarly, a decrease of all investigated parameters with processing of strawberries to different products was observed (Klopotek et al., 2005).

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The solvent systems (**0.05 M $H_3P\ O_4$ + 0.025 M HOAc**) and (**0.20 M H_3PO_4 + 0.25 M HOAc**) are as efficient as **(0.38 M HPO_3 + 1.38 M HOAc)** for high and average vitamin C containing fruits respectively. Recoveries of vitamin C were high, 90.44 ± 4.19% and 88.09 ± 7.19% respectively. The extracted ascorbic acid was stable over a 96 hour period. Increasing the concentration of orthophosphoric and acetic acid improved the performance of the extracting system **(0.25 M H_3PO_4 + 0.25 M HOAc)** in low vitamin C containing fruits.

Provenance significantly ($p< 0.001$) affected the nutritional quantity of fruits. For instance, *A. digitata* from Chikwawa exhibited significantly ($p< 0.001$) higher vitamin C level (347.70 mg/100 g) on fresh weight basis than those from Mangochi, Salima, Dedza, and Mwanza. *A. digitata* fruits from Mangochi are very rich in vitamin A. *P. curatellifolia* from Nkhata Bay had highest level of vitamin C. *Z. mauritiana* fruits from Chikwawa were richer in vitamin C than those from Mangochi and Dedza. In general provenances richer in vitamin C had low levels of vitamin A. It is therefore, necessary to have information about superior provenances for a particular nutrient sought.

The period of harvest significantly affected nutritional quantity of *S. cocculoides* fruit pulps. Important nutrients such as vitamin C and iron were significantly higher ($p<0.001$) for December than October harvest. Thus *Strychnos* fruits harvested late in the season ensure consumption of quality fruits and derived products. Fruit tree age significantly affected nutritional quantity of *Strychnos* pulp. For instance, fruits from middle aged trees had highest levels of vitamin C while those from old aged trees gave highest acidity.

Ripening affected nutritional levels of fruit pulps. For *S. cocculoides*, vitamin C content decreased with ripening while for Z. *maurutiana* it was increasing. In both fruit species, reducing sugars and total soluble solids increased with ripening. Over-ripened *Strychnos* and *Ziziphus* pulps had lower vitamin C and sugar levels due to dilution effect and transformation to other organic compounds as a result of oxidation and stress.

The change of fruit chemical properties was gradual over a 46 hour period for fruits kept in clay pot while a steady change took place under ambient air storage. Fruits in refrigerator afforded negligible change in total soluble solids due to very low temperature. Thus elevated temperatures reduce shelf life and nutritional quality of fresh fruits and derived products. *Strychnos* jam processing reduces the level of ascorbic acid significantly in the final product.

5.2 Recommendations

Nutritional assessment like vitamin C is very important in food industry and health but the commonly used solvent, (0.38 M HPO_3+1.38 M HOAc) is very expensive. It is important that alternative extracting solvents for ascorbic acid be explored. Dilute and cheaper solvents, (0.05 M H_3PO_4+0.025 M HOAc) and (0.20 M H_3PO_4+0.25 M HOAc) developed in this study, extract significantly higher ascorbic acid in very high and average vitamin C containing fruits respectively and can therefore be used.

Indigenous fruits that are widely distributed in forests and homestead farms of Malawi are rich in vitamins and minerals. However these nutritional attributes are affected by several pre and post harvest factors including processing. It is therefore, recommended that

- nutritional assessment should be undertaken for priority indigenous fruits of Malawi in order to adapt practices that would ensure high nutritional levels
- *S. cocculoides* fruits should be harvested late in the season (December)
- consumption of *S. cocculoides* from middle aged fruit trees be promoted for those suffering from HIV/AIDS as they provide high levels of vitamin C
- fresh fruits like *Z. mauritiana* should be stored in clay pots in the absence of a refrigerator

Further research should be undertaken to establish (i) the relationship between pK_a of solvent system and ascorbic acid yield, (ii) the effect of extraction time on ascorbic acid yield, (iii) the relationship between soil properties and nutritional levels of *A. digitata*, *P. curatellifolia* and *Z. mauritiana* and (iv) alternative techniques for the production of *Strychnos* jam with high nutrient retention.

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