Chemical analysis to promote the use of wild fruits from Mozambique

Magaia, Telma

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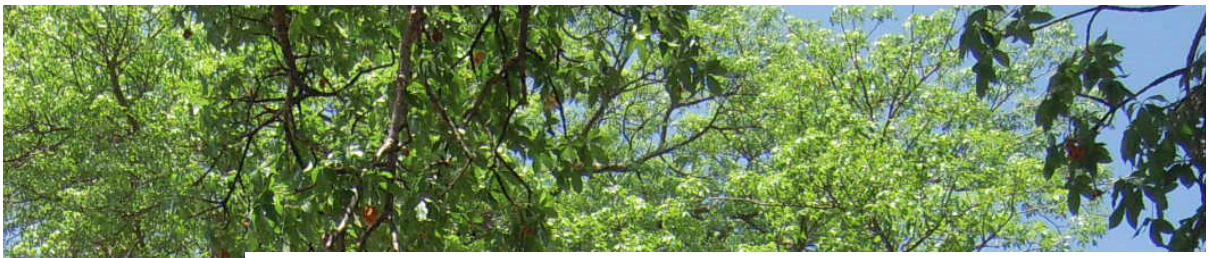

Chemical Analysis to promote the use of Wild Fruits from Mozambique

DEPARTMENT OF FOOD TECHNOLOGY, ENGINEERING & NUTRITION | LUND UNIVERSITY
TELMA MAGAIA

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Wild Fruits from Mozambique

Telma Magaia

2015

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Abstract

Wild fruit trees have significant cultural and socio-economic value in rural areas of Mozambique. Most of the wild fruits are seasonal and are available mainly in the wet season. Generally they have a short shelf-life and are eaten fresh or after minimal processing; the most common method of preservation is sun-drying. The fruits of *Adansonia digitata, Landolphia kirkii, Salacia kraussii, Sclerocarya birrea,* and *Vangueria infausta* were selected for this study.

New data on nutritional components and other characteristics have been obtained. The pH, titratable acidity and the content of soluble solids in the fruit pulps were determined. The organic acids citric, malic and succinic acids were found at various amounts in all pulps. The contents of different mono- and disaccharides were also analysed in the pulps.

The protein content was low in all the fruit pulps, as is the case in fruits in general. However, the protein content was high in the kernels of *A. digitata* and *S. birrea*, about 30 to 40% on dry matter basis. The total content and relative amounts of the different essential amino acids are with a few exceptions similar to or above that recommended by the WHO for children aged 3 to10 years. The fat content was below 2% in the fruit pulps, while the fat content in *A. digitata* kernels was almost 40%, and *S. birrea* kernels about 60%. The kernels of *A. digitata* and *S. birrea* are rich in unsaturated fatty acids, and constituted about 68 and 80%, respectively, of the total fat. The *A. digitata* kernels contained appreciable amounts of essential fatty acids; the amount of linoleic was about 30% and linolenic acid 2%. *S. birrea* kernels contained about 7% linoleic acid.

The fruits contained both insoluble and soluble dietary fibre. The pulp of *A. digitata* had the highest amount of soluble dietary fibre, around 60% (on dry matter basis), while *V. infausta* pulp had the highest amount of insoluble dietary fibre, around 40%. The kernels contained 3 to 5% phytic acid which may decrease the absorption of minerals. Treatment with phytase reduced the phytic acid content by 20 to 30% after only 15 minutes enzymatic incubation. Interestingly, almost 50% of the estimated original content of minerals was found in the supernatant after a few minutes' enzyme incubation. The amount of iron in the pulps ranged from 1 to 9 mg/100 g (on dry matter basis); the highest amount being observed in *S. kraussii*. The highest iron content, 29 g/100 g DM, was found in whole seeds of *A. digitata*, 29 mg iron/100 g. The *A. digitata* pulp contained an appreciable amount of calcium, and the kernel also had high content of calcium.

In conclusion, data from this study can be used to encourage the increased consumption of these wild fruits and kernels. In addition, the results of the analysis of the investigated fruits can form the basis for the selection of fruits for wider use, domestication, and processing to extend their shelf-life and for the manufacture of other food products.

Key words: pulps, kernels, consumption, fat, protein, minerals, dietary fibre

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Wild Fruits from Mozambique

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Cover photo: Landscape in the Changara district in Mozambique.
Back photo: A basket with wild fruits selected for the study.

*Full Name: Telma dos Anjos Leví Jamisse Magaia

Department of Food Technology, Engineering and Nutrition
Faculty of Engineering, Lund University

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“There are many people who can do big things, but there are very few people who will do the small things.” MotherTeresa

“Há muitas pessoas que podem fazer grandes coisas. Mas há muito poucas pessoas que farão as pequenas coisas.”
Madre Teresa de Calcutá

Abstract

Wild fruit trees have significant cultural and socio-economic value in rural areas of Mozambique. Most of the wild fruits are seasonal and are available mainly in the wet season. Generally they have a short shelf-life and are eaten fresh or after minimal processing; the most common method of preservation is sun-drying. The fruits of *Adansonia digitata, Landolphia kirkii, Salacia kraussii, Sclerocarya birrea,* and *Vangueria infausta* were selected for this study. These fruits are the most popular, and are consumed in different districts of Mozambique, especially by children, and form part of their normal diet. Besides from eaten fresh, the fruits are mixed with sugar, pressed to make juice, as jam or a kind of dessert, and the pulp and sometimes the kernels are dried and used as flour to make porridge or a sauce.

New data on nutritional components and other characteristics have been obtained. The pH, titratable acidity and the content of soluble solids in the fruit pulps were determined as these are of importance for the fruit processing industry. The pulp of all the fruits except *S. kraussii* had an acidic character. The organic acids citric, malic and succinic acids were found at various amounts in all pulps. The highest amounts of citric acid were found in *A. digitata* and *L. kirkii*; above 20 g/kg. Organic acids are responsible for many characteristic fruity tastes and may enhance the absorption of minerals. The contents of different mono- and disaccharides were also analysed in the pulps. The highest amounts of glucose (7.5%) and fructose (5.7%) were found in *L. kirkii*, while *A. digitata* contained the highest amount of sucrose (4.3%).

The protein content was low in all the fruit pulps, as is the case in fruits in general. However, the protein content was high in the kernels of *A. digitata* and *S. birrea*, about 30 to 40% on dry matter basis. For children aged 4 to 8 years, around 80 and 67% of the adequate intake (AI) (defined by the Food and Nutrition Board of the US Institute of Medicine) could be covered by the consumption of 40 g *A. digitata* kernels or *S. birrea* kernels, respectively. The total content and relative amounts of the different essential amino acids are with a few exceptions similar to or above that recommended by the WHO for children aged 3 to10 years.

The fat content was below 2% in the fruit pulps, while the fat content in *A. digitata* kernels was almost 40%, and *S. birrea* kernels about 60%. The kernels of *A. digitata* and *S. birrea* are rich in unsaturated fatty acids, and constituted about 68 and 80%, respectively, of the total fat. The *A. digitata* kernels contained appreciable amounts of essential fatty acids; the amount of linoleic was about 30% and linolenic acid 2%. *S. birrea* kernels contained about 7% linoleic acid. Estimations showed that 40 g *A. digitata* kernels can cover the daily intake of omega-6 fatty acids for those aged 4 to 13 years, and about 90% of the requirement for pregnant women. The

same quantity of *A. digitata* kernels can provide 60 to 93% of the daily requirement of omega-3 fatty acids for the same groups.

The fruits contained both insoluble and soluble dietary fibre. The pulp of *A. digitata* had the highest amount of soluble dietary fibre, around 60% (on dry matter basis), while *V. infausta* pulp had the highest amount of insoluble dietary fibre, around 40%. The kernels contained 3 to 5% phytic acid which may decrease the absorption of minerals. Treatment with phytase reduced the phytic acid content by 20 to 30% after only 15 minutes enzymatic incubation. Interestingly, almost 50% of the estimated original content of minerals was found in the supernatant after a few minutes' enzyme incubation.

The amount of iron in the pulps ranged from 1 to 9 mg/100 g (on dry matter basis); the highest amount being observed in *S. kraussii*. The highest iron content, 29 g/100 g DM, was found in whole seeds of *A. digitata*, 29 mg iron/100 g, and consumption of 40 g could provide more than 100% of the AI for children and 43% for pregnant women. The *A. digitata* pulp contained an appreciable amount of calcium, and the kernel also had high content of calcium. Consumption of 100 g of *A. digitata* pulp would give about 23 to 29% of the AI for children and about 37% for pregnant women.

In conclusion, data from this study can be used to encourage the increased consumption of these wild fruits and kernels. In addition, the results of the analysis of the investigated fruits can form the basis for the selection of fruits for wider use, domestication, and processing to extend their shelf-life and for the manufacture of other food products.

Popular Scientific Summary

Wild fruits trees species are widely distributed throughout the African countries. Many of these trees species produce fruits, which are used by the local communities to greater or less degrees. The importance of wild fruits in the diet depends to a large extent on the availability of the fruits, since cultivated fruit trees are not particularly common in the dry regions of the countries. The goals of this work were to perform a study on traditional utilization of wild fruits in Mozambique and to generate data on the composition and other characteristics of five wild fruits as a basis for the selection of fruits suitable for processing and increased utilisation and consumption of indigenous fruits.

Fruits of *Adansonia digitata* (n´buvu), *Landolphia kirkii* (vungwa), *Salacia kraussii* (psincha), *Sclerocarya birrea* (canhi), and *Vangueria infausta* (pfilwa), were selected for this research. When the wild fruits were collected in the different districts, local people were interviewed about the traditional use of the fruits. The fruits are eaten raw, pressed to juice, fermented to alcohol beverages, cooked, or used as flour to make porridge. The seeds and kernels are often used in times of drought when the production of peanuts is low; the seeds can be ground roasted to make a kind of coffee, and the kernels of *A. digitata* and *S. birrea* may also be roasted to be eaten as snacks or ground in a bowl, mixed with water, boiled and consumed with local plant food. In general most people know that fruits are associated with potential health benefits. Researchers has shown that a wide range of wild fruits have the potential to provide rural households with free foods to meet their nutritional and medicinal needs. The disadvantages of the selected fruits are that they grow and ripen during a very short period of the year and that normally their shelf-life is short.

Different analytical methods were used to evaluate the nutritional components and other characteristics of some wild fruits. For the fruit processing industry, data on sugar content, pH and moisture are essential characteristics. They play an important part in the perception of fruit quality indicating the possibility for future use of these wild fruits. The sugar content is important for the development of the aroma and taste, and in product development it is important to find a good balance between for example pH and sugars to receive an optimal taste. The highest total sugar content was found in *A. digitata* and *L. kirkii,* more than 10g/100 g. Organic acids such as citric and malic acid influence flavor and aroma and are responsible for many characteristic fruity tastes; they were found in all fruits in this study. The parameters above have been reported to influence shelf life, stability and microbiologic safety.

Most of the fruit pulps were juicy except of *A. digitata*, which was very dry; the moisture content was only around 10%. The fat and protein contents in the pulps

were low, which is common in fruits. The pulps of *A. digitata* and *V. infausta* contained considerable amounts of dietary fibres. The content of the mineral in the kernels such as calcium, iron and zinc were found in considerable levels and the have great contribution for many functions on the human body. Consumption for example of 100 g *A. digitata* pulp, can provide around 30% of recommended intake of calcium for pregnant women and children, and 23% of the iron for 4 to 13 years old children. Consumption of 40 g *A. digitata* kernels can provide 20 to 25% of the recommended intake of iron for children and 16 to 20% from *S. birrea* kernels. In addition the recommended intake of zinc provided 20 to 44% from *A. digitata*, and 16 to 36 % from *S. birrea* kernels for children and pregnant women.

The fat content in *A. digitata* and *S. birrea* kernels was high around 40 to 50%. Interestingly the fatty acid composition in *A. digitata* can be compared with that in peanuts, while *S. birrea* can be compared with olive oil. *A. digitata* kernels contained the essential fatty acids linoleic and linolenic acids and *S. birrea* kernels contained only the linoleic acids. Consumption of 40 g *A. digitata* kernels can cover 60 to 90% of the daily intake of essential fatty acids for those aged 4 to 13 years, and pregnant women.

Protein is one of the most crucial nutrients to the human body improving varying aspects of health. The protein content in the kernels of *A. digitata* and *S. birrea* was around 30 to 40%. In addition, the levels of essential amino acids in the kernels were comparable with the amino acid requirements stated by the World Health Organization (WHO). For children aged 4 to 8 years, 81 and 66% of the recommended intake may be covered by the consumption of 40 g *A. digitata* kernels and *S. birrea* kernels, respectively. The contribution to recommended intake for older children and pregnant women is lower, but it may be possible for these groups to increase their intake of kernels, especially if they are eaten as a snack.

The results of this study will be vital in efforts to promote use of these wild fruits. The data can be used for the estimation of dietary intakes, and for education of local communities with regard to the nutritional benefits of free sources of food in their environment. The findings indicate that the nutrient contents of the fruits may help to meet the dietary requirements for children and pregnant women in rural areas. In addition they may promote the use of wild fruits and their kernels, and can form the basis for the selection of fruits for further processing, to extend shelf-life and to manufacture new products. Initiatives should be put in place for the selection and domestication of wild fruits trees.

Resumo Científico Popular

Árvores de fruteiras silvestres encontram-se amplamente distribuídas por vários Países africanos. Muitas das espécies produzem frutas que são geralmente consumidas pelas comunidades locais. A importância das frutas silvestres na dieta das populações depende em grande medida da sua disponibilidade, tendo em conta que fruteiras cultivadas não são comuns nas regiões secas dos Países.
Os objectivos deste trabalho consistiram em realizar um estudo sobre a utilização tradicional das frutas silvestres de Moçambique e produzir dados sobre a composição e demais características de cinco frutas silvestres, como uma base para a selecção de frutas adequadas para o processamento, uso e consumo intensivo.

Frutas de *Adansonia digitata* (n´buvu), *Landolphia kirkii* (vungwa), *Salacia kraussii* (psincha), *Sclerocarya birrea* (canhi), e *Vangueria infausta* (pfilwa), foram seleccionadas para o estudo. Durante a colheita das frutas em diferentes distritos foram realizadas entrevistas as populações locais sobre a uso tradicional das frutas silvestres. Este tipo de fruta é consumido ao natural, sob forma de sumo, como bebida alcoólica fermentada, utilizado como farinha para a preparação de papas. As sementes e amêndoas são muito usadas em épocas secas quando a produção de amendoim é reduzida. As sementes podem ser moídas para preparar um tipo de café e as amêndoas de *A. digitata* e *S. birrea* podem ser torradas para ser consumidas em lanches ou misturadas com água, cozidas e consumidas acompanhando vegetais locais. Em geral a maioria da população conhece a associação das frutas aos possíveis benefícios para a saúde. Investigadores demonstraram que uma grande diversidade de frutas silvestres têm o potencial de fornecer a famílias rurais alimentos para satisfazer as suas necessidades nutricionais e medicinais. A desvantagem destas frutas é o seu crescimento e amadurecimento num período muito curto do ano e o seu reduzido tempo de prateleira.

Diferentes métodos analíticos foram usados para a avaliação dos parâmetros nutricionais e demais características das frutas silvestres. Para a indústria de processamento de fruta, dados sobre o teor de açúcares, pH e humidade são parâmetros essenciais. Eles desempenham um papel essencial para a compreensão da qualidade da fruta, indicando possibilidades futuras de utilização. O teor de açúcares é importante para o desenvolvimento do aroma e sabor e no desenvolvimento de produtos é essencial encontrar um bom equilíbrio entre, por exemplo, o pH e os açúcares para obter o melhor sabor. O teor mais alto de açúcares foi encontrado em *A. digitata* e *L. kirkii,* 10.3 e 14.4 g/100 g respectivamente. Ácidos orgânicos tais como cítrico e málico influenciam o sabor e o aroma e são responsáveis por muitos sabores característicos das frutas; foram encontrados em todas frutas estudadas.

Os parâmetros acima descritos têm sido reportados como tendo influencia no tempo de prateleira, estabilidade e segurança microbiológica.

As polpas das frutas são em geral suculentas excepto da *A. digitata*, que é muito seca; o teor de humidade encontrado foi de apenas 10%. O teor de gordura e proteínas nas polpas foi baixo, o que é comum em frutas. As polpas de *A. digitata* e *V. infausta* contem quantidades razoáveis de fibra alimentar. Minerais tais como cálcio, ferro e zinco foram encontrados em quantidades consideráveis nas amêndoas e eles possuem grande contributo para muitas funções do organismo humano. A ingestão de 100 g de polpa de *A. digitata* pode suprir cerca de 30 % do consumo recomendado de cálcio para mulheres grávidas e crianças, e 23% de ferro para crianças de 4 a 13 anos. A ingestão de 40 g de amêndoa de *A. digitata* pode suprir 20 a 25% do consumo recomendado de ferro para crianças e 16 a 20% se a amêndoa for de *S. birrea.* Ademais, o consumo recomendado de zinco para crianças e mulheres grávidas pode ser suprido em 20 a 44% por amêndoas de *A. digitata*, e 16 a 36 % de *S. birrea.*

O teor de gorduras em amêndoas de *A. digitata* e *S. birrea* foi elevado, cerca de 40 a 50%. Interessante notar que a composição de ácidos gordos em *A. digitata* pode ser comparada com a de amendoim, enquanto da *S. birrea* pode ser comparada a do azeite de oliveira. As amêndoas de *A. digitata* contêm os ácidos gordos essenciais linoléico e linolênico e de *S. birrea* apenas o ácido linoléico. A ingestão de 40g de amêndoas de *A. digitata* pode suprir 60 a 90% do consumo diário recomendado de ácidos gordos essenciais para crianças de 4 a 13 anos e mulheres grávidas.

Proteínas são nutrientes cruciais para o organismo humano melhorar diferentes aspectos da saúde. O teor de proteínas em amêndoas de *A. digitata* e *S. birrea* foi de 30 to 40%. Além disso, os teores de aminoácidos essenciais nas amêndoas foram comparáveis as exigências de aminoácidos estabelecidas pela Organização Mundial da Saúde (OMS). Para crianças dos 4 aos 8 anos, 81 e 66% do consumo recomendado pode ser suprido pelo consumo de 40 g de amêndoas de *A. digitata* e *S. birrea,* respectivamente. A contribuição para o consumo recomendado para crianças com idades acima e mulheres grávidas é baixa, mas seria possível para estes grupos aumentar a sua ingestão, especialmente se as amêndoas forem consumidas regularmente como refeição ligeira.

Os resultados deste estudo são essenciais no esforço para a promoção do uso das frutas silvestres. Os dados podem ser usados para estimar o consumo diário e para a educação das comunidades locais sobre os benefícios nutricionais de fontes gratuitas de alimento abundantes no seu meio ambiente. Os resultados indicam que o teor de nutrientes das frutas pode ajudar a suprir as necessidades nutricionais de crianças e mulheres grávidas em áreas rurais. Adicionalmente a promoção da utilização das frutas silvestres e suas amêndoas pode constituir a base para a selecção de frutas para

processamento adicional, assim como para aumentar o tempo de prateleira e para a preparação de novos produtos. O estudo poderá incentivar iniciativas para a selecção e domesticação de fruteiras silvestres.

List of papers

This thesis is based on the results presented in the following papers, referred to in the text by their respective Roman numerals.

I. Proximate Analysis of Five Wild Fruits of Mozambique
Telma Magaia, Amália Uamusse, Ingegerd Sjöholm and Kerstin Skog
The Scientific World Journal
http://dx.doi.org/10.1155/2013/601435 (2013)

II. Dietary Fiber, Organic Acids and Minerals in Selected Wild Edible Fruits of Mozambique
Telma Magaia, Amália Uamusse, Ingegerd Sjöholm and Kerstin Skog
SpringerPlus 2013, 2:88 (2013)

III. Composition of Amino Acids, Fatty Acids and Dietary Fibre Monomers in Kernels of *Adansonia digitata* and *Sclerocarya birrea*
Telma Magaia and Kerstin Skog
Submitted

IV. Effect of Heating, Lactobacillus Fermentation and Enzyme Treatment on the Content of Phytate in Kernels of *Adansonia digitata* and *Sclerocarya birrea.*
Telma Magaia, Ingegerd Sjöholm, Estera Dey and Kerstin Skog
Submitted

The author's contributions to the papers

I. The author participated in the study design, and carried out the field work, which involved the collection of fruit and information about the traditional use of the fruits. The author carried out the experimental analysis (apart from the sugar analysis), took an active part in data evaluation and wrote the first draft of the manuscript, which was finalized with the contributions of the co-authors.

II. The author participated in the study design. The author carried out the experimental analysis (apart from the mineral analysis), took an active part in data evaluation and wrote the first draft of the manuscript, which was finalized with the contributions of the co-authors.

III. The author participated in the study design. The author carried out the field work, which involved the collection of seeds. The author carried out the experimental analysis (apart from the fatty acids), took an active part in data evaluation and wrote the first draft of the manuscript, which was finalized with the contributions of the co-authors.

IV. The author participated in the study design. The author carried out the experimental analysis (apart from the mineral analysis), the phytic acid analysis was carried out in collaboration with the Department of Food Technology and Nutrition at Chalmers University of Technology in Gothenburg, took an active part in data evaluation and wrote the first draft of the manuscript, which was finalized with the contributions of the co-authors.

Related publication

Edible Wild Fruits of Mozambique
T. Magaia, J. da Cruz Francisco, A. Uamusse, I. Sjöholm, K. Skog (Proceedings), Acta Horticulturae 948, ISHS, 31, 223-228. 1st International Symposium on Wild Relatives of Subtropical and Temperate Fruit and Nut Crops, 19-23 March, 2011, Davis, California, USA

Contents

1. Introduction

The African continent is blessed with a rich tropical flora. A large number of edible wild plants are distributed throughout Mozambique and local residents are allowed to gather the fruits from the forests. These fruits have significant cultural and socio-economic value, and are important supplemental sources of food in rural areas. The rural population, with a close relationship to the forest, has traditional knowledge on the preparation, consumption and storage of these wild fruits. Some well-known wild fruits in Mozambique are *Adansonia digitata* (n´buvu), *Landolphia kirkii* (vungwa), *Salacia kraussii* (psincha), *Sclerocarya birrea* (canhi) and *Vangueria infausta* (pfilwa). These fruits are consumed in different ways, for example, eaten fresh, mixed with sugar, pressed to make juice, as jam or a kind of desert, and the fruit pulp is often dried and used as flour to make porridge. The kernels are often eaten fresh or roasted as snacks. The disadvantage of these fruits is that they grow and ripen during a very short period of the year. They are generally consumed locally, due to their short shelf-life. Transporting the fruits to markets leads to an overabundance, lowering the price and thus income.

Since many wild fruits and tubers are underutilized in Mozambique, a programme called "Technology Processing of Natural Resources for Sustainable Development" (TecPro) was initiated in collaboration between the Eduardo Mondlane University in Maputo, Mozambique and Swedish universities. Most wild fruits can be consumed in several ways, but there is little information in the literature regarding the nutritional values of these wild fruits. Thus, there is a need for nutritional data on the wild fruits found in Mozambique in order to increase processing and increase their consumption. The work described in this thesis is part of this programme, and the aim was to determine the nutritional components and other characteristics of selected wild fruits commonly consumed in Mozambique.

2. Objectives

The overall aims of this work were to study the traditional use of wild fruits in Mozambique, and to obtain data on the chemical composition and other characteristics of five wild fruits as a basis for the selection of fruits suitable for processing. The long-term goal was to promote and increase the use and consumption of indigenous fruits. The fruits studied were: of *Adansonia digitata* (nbuvu), *Landolphia kirkii* (vungwa), *Salacia kraussii* (psincha), *Sclerocarya birrea* (canhi), and *Vangueria infausta* (pfilwa). These fruits are popular in Mozambique, where they play an important role in the diet, particularly in rural areas.

The specific goals were:

- to collect information concerning knowledge on the occurrence of these fruits, and traditional habits regarding consumption and storage,
- to obtain data on the contents of dry matter, fat, protein, ash, minerals, insoluble and soluble dietary fibre in the pulps and kernels of the selected fruits,
- to determine the pH and titratable acidity in the pulps of the selected fruits, as well as the contents of soluble solids, sugars and organic acids,
- to analyse the amino acid composition and the fatty acid composition of the kernels of *A. digitata* and *S birrea*,
- to use enzymes or lactobacillus species to reduce the phytic acid content in the kernels of *A. digitata* and *S birrea*, and
- to compare the results with dietary recommendations.

3. Literature Review

3.1 Mozambique and wild fruits

Mozambique is located on the eastern coast of Southern Africa on the Indian Ocean (see Figure 1). It has a population of over 26 million, with 68% of the inhabitants living in rural areas (FAO, 2014; FAOSTAT., 2014). The total area of the country is around 800 000 square kilometres, of which 2% is inland water (Mangue and Oreste, 1999). The country stretches in a north-south direction from the Rovuma River to the Ponta d´Ouro. The coastline runs from the tropical to subtropical humid northern and central regions, to the semi-arid/arid subtropical southern region. The climate is characterized by a dry winter season from April to September and a rainy summer season from October to March (FAOSTAT, 2014). The mean annual rainfall ranges from 800 to 1000 mm along the coast and 1200 mm in the central region, to 1000 to 2000 mm in the northern region (Coughlin, 2006; Queface, 2009). An increase in the frequency of droughts, floods and cyclones has had a devastating effect on crops, forest, livestock and fishing in rural and coastal areas (FAO, 2012).

Figure 1. Map of Mozambique showing provinces and cities.

The provinces in which the fruits were harvested are indicated by red dots.

Many wild fruits are found in Mozambique, which are valuable to the diets and incomes of local communities across sub-Saharan Africa, particularly during times of potential food insecurity. Wild fruits and vegetables play an important role in combating malnutrition and poverty in the African continent. The consumption pattern in Mozambique generally includes three daily meals, such as breakfast, lunch and dinner. However, the number of meals can sometimes be reduced to two or even one when there is a lack of food. Plant foods may provide almost all vitamins and essential minerals, as well as a number of other health-promoting compounds (FAO, 2011). However, nutrient deficiency continues to be a major health problem in developing countries, and has far-reaching consequences on growth, development and health, especially among children, who are most vulnerable to diseases caused by dietary deficiencies (Aphane et al., 2002). Wild fruits can provide an alternative crop when cereal production fails due to lack of rain (FAO, 2010).

According to the Food and Agriculture Organization of the United Nations (FAO), "Food security is achieved when everyone has physical, social and economic access to sufficient, safe and nutritious food that meets dietary needs and food preferences for an active and healthy life." (FAO, 2012). Estimates from 2010 show that about 456 000 people lacked food security in Mozambique, most of them in the Tete province, followed by the provinces of Sofala, Inhambane, Manica, Gaza, Maputo and Nampula. Increased food production and improvements in food security remain high on the list of priorities for Mozambique, whilst simultaneously ensuring sustainable management of natural resources (FAO, 2012).

According to a report by the National Directorate of Land and Forests in 2007 (the Ministry of Agriculture in Mozambique), there is great diversity of species of forest products. About 4000 plants have been recognized as being edible plants, most of which are seasonal. The harvesting of these plants and fruits occurs mostly in the rainy season, which coincides with the lean season for agricultural products, thus contributing to the food security of households (Albano and Nhamirre, 2007).

Preferences for wild fruits in Mozambique vary according to the region and their cultural value, or the way in which different foods can be prepared from them. In the north, the most commonly found wild fruits are *Julbernardia globiflora, Markhamia obtusifolia, Sclerocarya birrea* and *Tamarindus indica*. In the centre of the country, *Adansonia digitata, Sclerocarya birrea, Uapaca kirkiana* and *Ziziphus mucronata,* are commonly found, and in the south, *Dialium schlechteri, Landolphia kirkii, Sclerocarya birrea, Strychnos madagascariensis, Strychnos spinosa, Trichilia emetica* and *Vangueria infausta* (Mangue and Oreste, 1999).

3.2 General descriptions of fruits

Botanically, fruits are the reproductive structures formed by plants, which enclose the seeds and help with their dispersal. Fruits can be categorized as stone fruits or drupes, pip fruits, berries, citrus and aril fruits (Wyk, 2005). A fruit is composed of the skin or peel, the pulp, which is the soft edible part, and seeds that consist of a hard shell and a kernel which is sometimes edible. This is illustrated for *Adansonia digitata* in Figure 2.

a) 

b) 

Figure 2. A whole *Adansonia digitata* fruit showing the skin a), and in b) the pulp with seeds embedded (A), the pulp (B), whole seeds, i.e. the hard shell + kernel (C), and kernels (D).

Fruits are widespread in nature, and are classified according to climate: (i) temperate, e.g. apples and grapes, (ii) subtropical, e.g. oranges and mandarins, and (iii) tropical, e.g. guava and pineapples (Hoe and Siong, 1999; Hernández et al., 2006; Prasanna et al., 2007). Fruits may contribute significantly to the daily nutrient needs of the individual, however, the amount of each nutrient required by the human body depends on factors such as age, weight, gender, health and level of physical activity (Prasanna et al., 2007). In addition, fruits offer a great variety of aromas, tastes, colour, and texture. Nutritional data for some fruits are presented in Table 1.

Table 1. Literature data on the nutritional composition of some common fruits (based on wet weight)

Climate Fruit	Energy[1]	Dry matter[2]	Fat	Protein	Carbo-hydrate	Dietary fibre	Ash	Vitamin C	Ca	Fe	K	P
	(kJ/100 g)	(g/100 g)						(mg/100 g)				
Temperate												
Apple	251.4	15.4	0.4	0.6	136	0.6	0.3	7.7	9	2.1	-	16
Grape	284.9	18.5	0	0.8	16.3	1.8	0.4	7.6	21	0.5	640	66
Subtropical												
Mandarin	205.3	9.3	0.5	0.7	10.4	0.8	0.5	39.6	40	2.1	42	19
Orange	146.7	10.2	0.1	1.5	7.1	0.5	0.1	71	11	0.7	16	3
Tropical												
Guava	192.7	12.9	0.2	1	10	6.8	0.7	152	33	1.2	12	15
Pineapple	205.3	12.9	0.2	1	10.9	0.4	0.4	28	28	0.3	1490	21

Data from Hoe and Siong, 1999 and Hernández et al., 2006

[1] Recalculated from kcal/100 g; [2] Recalculated from moisture %; - = No information given.

3.3 Wild fruits selected for this study

The fruits selected for this study are presented below, with photographs of the trees, fruits and seeds, together with general descriptions of the growth locations, the fruit and their consumption and processing.

Adansonia digitata (*A. digitata*)

English name: baobab, local name: n´buvu or malambe

a)  b) 

Figure 3. a) The *Adansonia digitata* tree, and b) the fresh pulp with seeds embedded.

A. digitata is the most widespread of the *Adansonia* species on the African continent, and is found in the hot, dry savannahs of sub-Saharan Africa. The largest areas of *Adansonia* are found in Mozambique, South Africa, Malawi and Zimbabwe (PhytoTrade Africa, 2008). In Mozambique, the tree is found in the provinces of Gaza, Inhambane, Maputo, Manica, Nampula, Niassa, Sofala and Tete (De Carvalho, 1968; Da Silva et al., 2004). The trees thrive in woodlands and savannah areas up to 1500 m above sea level and with an annual rainfall of 300 - 500 mm. It is known as the "tree of life", and has traditionally been a source of food and medicine.

Family: *Bombacaceae*
Tree: very large, 18-25 m tall
Harvest time: April to July
Shape and size: oval to elliptic, up to 200 mm in length
Colour: yellowish grey hairs
Pulp: powdery
Seed: bean-shaped, numerous, dark-brown containing one small kernel.

The pulp has a sweet taste and can be eaten as a snack. It can be mixed with sorghum paste to make the latter more acidic, and the acidic paste is diluted with water to obtain a thick drink. The pulp can be soaked in water or fresh milk adding sugar to form drinks, like juice or full-cream milk, or a kind of yogurt, or frozen to form "ice lollies". The pulp can also be boiled and mixed with sugar to make porridge (Sidibe and Williams, 2002; Jama et al., 2008; De Caluwé et al., 2009). The pulp serves to enrich homemade soups and sauces, or couscous, and some tribes use it to coagulate milk to make a kind of cheese (Policy Briefing, 2007; Buchmann et al., 2010).

The seeds can be crushed and the shells and kernels roasted and finely ground to provide a good substitute for coffee (De Caluwé et al., 2009). The kernels are commonly eaten fresh or roasted as a snack. The kernels can also be ground to flour, which can be added to soups and stews as a thickener, or boiled for a long time, fermented and then dried for later use (Tredgold et al., 1986; Nnam and Obiakor, 2003; Research Council, 2008; De Caluwé et al., 2009; National Buchmann et al., 2010). The kernels can be ground to a paste and added as flavouring to boiled leaves to make a sauce (Jama et al., 2008; Manfredini et al., 2002), or mixed with the pulp to form sweet "milk", which is boiled and flavoured with honey.

In 2008, the EU authorised the use of dried baobab fruit pulp (*Adansonia digitata*) as a novel food ingredient (Commission Decision, 2008), and in 2009 it was granted GRAS (Generally Regarded as Safe) status in the United States (FDA, 2009). Products containing baobab ingredients are already available in Italy, France, Switzerland, Spain, the UK, Canada and the US (MBB Consulting, 2006).

The pulp is considered a good source of vitamin C (approximately six times more than the content in an orange, based on wet weight), it is rich in pectin, making it an attractive "novel food" and beverage thickening agent, minerals (Ca, Mg and P), antioxidants (Jama et al., 2008; Kabore et al., 2011), organic acids (citric, tartaric) and dietary fibre (Maundu et al., 1999; Sidibe and Williams, 2002). The kernels have high contents of protein and fat, and have been found to be a good source of palmitic, oleic and linoleic acid (Osman, 2004).

Landolphia kirkii (*L. kirkii*)

English name: wild peach, local name: vungwa.

a)  b) 

Figure 4. a) The *Landolphia kirkii* climbing shrub, and b) the fruit.

Landolphia kirkii is a species of liana shrubs that can be found in the Democratic Republic of Congo, Malawi, Mozambique, Tanzania, Zambia, Zimbabwe and South Africa. In Mozambique, they can be found in the provinces of Gaza, Inhambane, Manica, Nampula, Niassa and Zambézia (De Carvalho, 1968; Da Silva et al., 2004). *Landolphia* plants contain milky latex, and *L. kirkii* was historically the primary source of African rubber. The extracted latex is of good quality with a high content of rubber (www.naturalhub.com, National Research Council, 2008).

Family: *Apocynaceae*
Tree: climbing shrub
Harvest time: November to February
Shape and size; round, 4 - 90 mm diameter
Colour: yellow, darkening to yellowish brown skin
Pulp: juicy, pleasant and stringy
Seeds: numerous seeds embedded in the pulp.

The fruit is commonly eaten fresh together with the seeds, or the seeds are discarded (National Research Council, 2008). Some people use a finger, knife or other utensil to remove the seeds and eat only the pulp (National Research Council, 2008). The fruit can also be used for the production of spirits (www.cde.unibe.ch). No data on its nutrient content were found in the literature.

Salacia kraussii (*S. kraussii*)

English name: not found, local name: psincha

a)  b) 

Figure 5. a) The *Salacia kraussii* shrub and b) the whole fruit and the pulp.

Salacia species are shrubs or small trees that can be found in Mozambique in sandy soils along the coast, in provinces such as Gaza, Inhambane and Maputo (De Carvalho, 1968; Schmidt et al., 2002; Da Silva et al., 2004).

Family: *Celastraceae*
Tree: shrubs, 0.5 m tall
Harvest time: December to February
Shape and size: round, 50 mm diameter
Colour: bright red to orange
Pulp: juicy, pleasant and sweet
Seeds: 1 - 3 seeds

The fruits are easily accessible to children who are the main consumers, eating the fruits on their way to and from school, or while tending grazing cattle. The fruit is commonly eaten fresh, and the kernels can be dried and eaten (Maundu et al., 1999; De Carvalho, 1968). Several *Salacia* species have been used in traditional medicine, such as the Ayurvedic system from India (www.ispotnature.org). No data on its nutrient content were found in the literature.

Sclerocarya birrea (*S. birrea*)

English name: marula, local name: canhi

a)  b)  c) 

Figure 6. a) The *Sclerocarya birrea* tree, b) the fruit, and c) the seeds embedded in the pulp.

Sclerocarya birrea trees are common in the lower-lying areas of Southern Africa, and are found in all provinces of Mozambique except for the Nampula province (De Carvalho, 1968; Da Silva et al., 2004). In Mozambique, the fruits are not commonly harvested from the tree as they fall to the ground when mature. Some fruits are sweet and others sour, depending on the tree.

Family: *Anacardiaceae*
Tree: large, 9 - 18 m tall
Harvest time: January to March
Shape and size: plum-shaped, 5 - 35 mm diameter
Colour: greenish-yellow
Pulp: juicy, tart with a strong, distinctive flavour
Seeds: a single large seed containing 2 - 3 hard kernels.

The pulp is commonly eaten fresh as a snack. When children are playing and find a tree with sweet fruits, they suck the juice and discard the seed and peel. The fruits can be made into a delicious jelly, sweet juice, syrups, jam or marmalade, or mixed with cereal or maize porridge (Tredgold et al., 1986; Van Wyk et al., 2002). The fruits can also be used to produce vinegar or fermented to traditional beer or an alcoholic drink called "*Ucanhe or Bucanhe*" (De Carvalho, 1968; Mangue and Oreste, 1999). Industrially "Amarula", a cream liqueur, is produced (Wyk, 2005).

The seeds are very hard and need careful cracking, but the delicious kernels are highly appreciated and they contain valuable oil. The kernels are widely eaten and very tasty; they are eaten fresh as a snack or ground to a paste and added to vegetables as flavouring. They can be dried or cooked and eaten together with a mixture of dried

peanut extracts, red pepper, salt and other spices in the form of a "leaf bundle" (Glew et al., 1997) The oil is used to treat sliced meat to preserve it as biltong (Tredgold et al., 1986).

The pulp is rich in vitamin C, and the amount is higher than in oranges (Wyk, 2005; Borochov-neori et al., 2008; Hillman et al., 2008). The pulp contains small amounts of citric acid and sucrose (Leakey, 1999), and is rich in minerals, the most abundant being calcium, magnesium, potassium and phosphor (Eromosele et al., 1991; Glew et al., 2004).

The kernels have a high fat content. They have been reported to be a source of oleic and linoleic acid (Glew et al., 2004), while another report shows the dominating fatty acids to be palmitic, stearic and arachidonic acid (Leakey, 1999). The protein content is around 30% (Leakey, 1999). In addition, the kernels contain compounds with anti-oxidant activity (Glew et al., 2004; Mariod et al., 2004, Bennet, 2006; Kleiman et al., 2008).

Vangueria infausta (*V. infausta*)

English name: wild medlar, local name: pfilwa

a)  b)  c) 

Figure 7. a) The *Vangueria infausta* tree, and b) the fruit, and c) the seeds embedded in the pulp.

V. infausta trees occur in abundance in woodlands, valleys and sandy dunes throughout Southern and Eastern Africa, including Madagascar (National Research Council, 2008). In Mozambique, they are found in the provinces of Gaza, Manica, Maputo, Niassa, Sofala, Tete and Zambézia (Da Silva et al., 2004). There are two varieties of the fruit; sweet and sour (www.gutsamba.co.mz).

Family: *Rubiaceae*
Tree: large, 4 - 6 m tall
Harvest time: January to May
Shape and size: round, up to 20 mm diameter
Colour: pale green to brownish-yellow
Pulp: juicy, soft with a strong, distinctive flavour
Seeds: 2 - 3 large seed hard kernels.

The fruits are mostly eaten fresh as a snack or soaked in water and mixed with sugar. The pulp can be boiled to make a kind of porridge, or mixed with milk for deserts, or mixed with a little water and sugar to produce an acceptable substitute for apple sauce. The pulp is also fermented to a strong alcoholic drink or brandy (Tredgold et al., 1986; Maundu et al., 1999; Motlhanka et al., 2008,). Industrially, the fruit is processed to make liquors, jams and marmalades (www.gutsamba.co.mz).

The whole fruit can be sun-dried and stored for use in times of food scarcity. The most common method of preservation is fermentation to produce alcoholic beverages (Boletim IIAM, 2008; www.cde.unibe.ch; Ibnouf, 2012). The pulp has high contents of dietary fibre, potassium and vitamin C (Amarteifio and Mosase, 2006).

3.4 Nutritional data on the selected fruits

Many wild fruits and nuts are good sources of fat, protein, carbohydrates, dietary fibre, vitamins and minerals, but literature data are scarce. Nutritional data for the wild fruits included in this study, are compiled in Tables 2 to 6. The literature data regarding energy, dry matter, fat, protein, carbohydrates, dietary fibre, ash and vitamin C are given in Table 2a for the pulps and Table 2b for the seeds and kernels.

Lack of energy in the diet is a major problem in many countries. Energy is supplied by carbohydrates, proteins, fats and sometimes alcohol in the diet (Kennedy et al., 2003). Fat is an important component in the diet as it provides the body with energy in a concentrated form. In addition, dietary fats provide essential fatty acids and fat-soluble vitamins (Insel, et al., 2011). Protein is another important component in the diet. Proteins are involved in the growth and repair of tissues, as enzymes in the metabolism, as hormones, transporters, antibodies, and in many other ways (Whitney and Rolfes, 1996). An inadequate supply of protein is considered to be responsible for malnutrition among people living in developing countries.

Dietary fibre alters the water content, viscosity and microbial mass of the intestinal contents (Elleuch et al., 2011). Dietary fibre reduces the risk of heart disease, improves glucose tolerance, by delaying the transport of carbohydrates into the small intestine (Anderson et al., 1994; Rodríguez et al., 2006). Insoluble fibre increases faecal bulk and decreases intestinal transit time (Hamilton et al., 1992; Al-Farsi et al., 2005). Soluble fibre increases viscosity, and reduces the glycaemic response and plasma cholesterol (Abdul-Hamid and Luan, 2000). Although dietary fibre provides many health benefits, it may reduce mineral absorption due to the ability of fibre to bind cations (López and Martos, 2004).

Vitamin C has many functions in the body. It is involved in collagen synthesis and amino acid metabolism, it strengthens resistance to infection, and helps in the absorption of iron (Whitney and Rolfes, 1996).

Table 2a. Literature data on the nutrients in the wild fruit pulps included in this study

Fruit	Energy	Dry matter	Fat	Protein	Carbo-hydrate	Dietary fibre	Ash	Vitamin C	References
Based on	(kJ/100 g)	(g/100 g)						(mg/100g)	
A. digitata									
FW	-	93.0	0.3	3.2	75.9	8.7	5.1	213	Wehmeyer, 1996
DW	1480	93.3	0.2	2.6	79.3	5.7	5.3	300	Nour et al., 1980
DW	-	86.8	4.3	3.1	79.4	8.3	5.0	-	Saka and Msonthi, 1994
DW	849	89.5	0.4	2.2	70.0	11.2	5.7	-	Lockett et al., 2000
DW	1341	95.3	0.7	2.5	-	45.1	5.1	-	Murray et al., 2001
FW	-	89.6	0.3	3.2	-	5.4	4.5	-	Osman, 2004
FW	-	86.0	-	1.3	-	16.2[a]	4.6	141.3	Amateifio & Mosasee, 2006
FW	488-631	86.3-86.7	0.4-0.5	2.03-2.04	78.3-78.4	51.4-52.2	5.5	74.1-76.2	Phyto Trade Africa, 2008
FW	-	86.0-89.0	0.5-0.7	2.0-3.2	78.3-78.9	46-54	5.0-7.0	74.0-168	FDA, 2009
FW	-	88.8	0.4	3.5	74.3	6.1	4.5	-	Oyeleke et al., 2012
S. birrea									
FW	-	8.3	0.1	0.5	7.0	0.5	0.5	67.9	Wehmeyer, 1966
DW	-	7.0	-	3.6	49.9	37.7	6.8		Murray et al., 2001
FW	-	11.6	-	3.7	-	16.3 [a]	4.9	128.3	Amarteifio & Mosase, 2006
FW	17.92	12.7	3.4	3.6	84.1	6.4	3.5	190.0	Wairagu et al., 2013
V. infausta									
DW	1445	26.5	2.6	5.7	78.1	10.2	3.4	-	Saka & Msonthi, 1994
FW	-	23.5	-	3.0	-	39.4[a]	-	67.7	Amarteifio & Mosase, 2006
FW	-	93.7	1.2	3.0	77.1	10.3	3.4	11.5	Emmanuel et al., 2011
FW	-	19.3-80.9	-	5.4-5.8	-	-	-	-	Mothapo et al., 2014

FW = fresh weight; DW = dry weight; - = no information given; [a] Dietary fibre analysed as acid detergent fibre.

Table 2b. Literature data on the nutrients in the wild fruit seeds and kernels included in this study

Fruit part	Energy	Dry matter	Fat	Protein	Dietary fibre	Carbo-hydrate	Ash	Vitamin C	References
Based on	(kJ/100 g)	(g/100 g)						(mg/100g)	
Seeds of *A. digitata*									
DW	-	93.9	16.1	14.4	56.8	-	6.1	-	Proll et al., 1998
DW	-	8.2	11.6	15.1	17.8	49.7	5.8	-	Lockett et al., 2000
FW	1525	95.7	12.2	18.4	45.1	16.1	3.8	-	Osman, 2004
DW	1871	95.3	12.7	21.7	52.5	6.7	5.0	6.7	Nkafamiya et al., 2007
DW	2169	95.0	38.0	38.2	6.4	10.0	7.5	-	Mitchikpe et al., 2008
FW	1820	95.8	6.9	48.3	21.9	4.8	3.8	-	Adubiaro et al., 2011
FW	-	88.8	3.9	2.5	70.7	9.9	5.4	57.3	Emmanuel et al., 2011
FW	-	96.5	13.4	19.5	44.6	15.6	3.1	-	Oyeleke et al., 2012
Kernels of *A. digitata*									
DW	1898	95.2	29.3	36.3	-	14.1	9.1	-	Murray et al., 2001
FW	1966	93.6	23.5	26.7	37.9	-	5.5	-	Igboeli et al., 1997
DW	-	-	18.9	25.5	48.1	-	7.6	-	Nnam & Obiakor, 2003
FW	-	91.9	34.1	32.7	30.0	-	5.0	-	Obizoba & Amaechi, 1993
Kernels* of *S. birrea									
FW	-	96.0	57.0	30.9	1.5	2.4	4.2	-	Wehmeyer, 1966
DW	-	97.5	11.0	36.7	17.2	3.4	11.7	-	Ogbobe, 1992
DW	-	93.9	42.0	30.8	17.3		3.8	-	Eromosele & Eromosele 1993
DW	665.7	89.5	58.9	31.0	3.0	2.5	4.7	-	Muhammad et al., 2011

FW = fresh weight; DW = dry weight; - = no information given.

Tables 3a and b list the contents of some minerals in the fruits studied. No data could be found for *L. kirkii* and *S. kraussii*. The minerals included in the Tables are of great importance in the diet. Some minerals or macro-elements are required in amounts greater than 100 mg per day (Insel et al., 2011; Imelouane et al., 2011); these include calcium, phosphorus, magnesium, potassium and sodium. Essential trace elements such as zinc, iron, iodine and selenium are normally required in amounts of less than 100 mg per day (Imelouane et al., 2011).

Calcium, together with potassium, phosphor and magnesium, is important for the growth and maintenance of bone and teeth (Insel et al., 2011). Calcium is also involved in muscle contraction and relaxation, nerve function and the immune defence (Whitney and Rolfes, 1996). Iron is necessary for the transport of oxygen in the blood to the cells, for immune and nerve function, and it is a cofactor in numerous enzyme reactions (Insel et al., 2011). Magnesium is involved in more than 300 biochemical reactions in the body, for example, in energy metabolism, protein synthesis, muscle contraction and bone mineralization (Insel et al., 2011). Zinc increases the affinity of haemoglobin for oxygen, participates in the transport of vitamin A, plays a role in taste perception, and interacts with a number of hormones. In addition, the body needs zinc to grow and develop properly during pregnancy, infancy and childhood (Brown et al., 1999; Insel et al., 2011).

Table 3a. Literature data for some minerals in *A. digitata*, *S. birrea* and *V. infausta* pulp

Fruit Based on	Ca	Fe	K	P	Mg	Na	Zn	References
	(mg/100 g)							
A. digitata								
FW	387	2.2	737	41.9	180	Trace	-	Wehmeyer, 1966
DW	655	8.6	-	50.8	-	-	-	Nour et al., 1980
DW	60	4.4	-	5.0	209	-	2.4	Eromosele et al., 1991
DW	116	5.8	2836	45	209	18.8	-	Saka & Masonthi, 1994
DW	341	1.7	-	73.3	209	5.46	1.0	Glew et al., 1997
DW	216	2.9	726	452	300	0.79	3.2	Sena et al., 1998
DW	211	4.2	-	49.8	123	-	0.5	Lockett et al., 2000
FW	295	9.3	1240	-	90	27.9	1.8	Osman, 2004
FW	128	0.1	1866	50	121	13.3	0.1	Amarteifio, Mosase, 2006
FW	330-338	9.02-9.13	2010-2030	61.1-62.1	143-146	0.82-1.21	-	PhytoTrade Africa, 2008
DW	257-370	4.0-9.1	2010-2390	56-73.3	126-179	0.7-3.1	-	FDA, 2009
FW	78.2	5.9	1410	105	69.1	35.1	ND	Oyeleke et al., 2012

FW = fresh weight; DW = dry weight; - = no information given; ND = not detected (< 10 mg/g dry weight).

Table 3a. (continued) Literature data for some minerals in *A. digitata*, *S. birrea* and *V. infausta* pulp

Based on	Ca	Fe	K	P	Mg	Na	Zn	References
	(mg/100 g)							
S. birrea								
FW	6.2	0.1	54.8	18.7	10.5	Trace	-	Wehmeyer, 1966
DW	36.2	1.1	-	18	31.9	-	0.34	Eromosele et al., 1991
DW	481	2.5	-	264	310	1.52	ND	Glew et al., 1997
FW	94	0.07	2183	69	158	13	0.13	Amarteifio, Mosase, 2006
FW	51.7	8.8	44.5	0.2	24.5	14.9	3.0	Hassan et al., 2010
FW	688	2.79	3220	146	158	-	2.3	Wairagu et al., 2013
V. infausta								
DW	13.2	28.3	181	82.3	1821	24.5	-	Saka & Masonthi, 1994
FW	124	0.09	1683	128	99	13.7	0.02	Amarteifio & Mosase, 2006
DW	186	24.4	747	86.9	18.6	161	7.1	Emmanuel et al., 2011
FW	0.2	20.1-21.6	2.2	0.2-0.3	-	-	-	Mothapo et al., 2014

FW = fresh weight; DW = dry weight; - = no information given; ND = not detected (< 10 mg/g dry weight).

Table 3b. Literature data for some minerals in the seeds and kernels of *A. digitata* and *S. birrea*

Based on	Ca	Fe	K	P	Mg	Na	Zn	References
	(mg/100 g)							
Seed*s* of *A. digitata*								
DW	395	1.8	-	614	352	1.94	2.6	Glew et al., 1997
DW	264	4.4	-	678	278	-	4.3	Lockett et al., 2000
FW	410	6.4	910	-	270	28.3	5.2	Osman, 2004
DW	58.9	6.4	280	6.0	-	6.07	3.6	Nkafamyia et al., 2007
DW	348	10.3	1029	1767	782	-	7.9	Mitchikpe et a., 2008
FW	242	22.0	536	480	352	8.4	3.4	Adubiaro et al., 2011
DW	677	21.7	1013	53.6	66.5	127	4.0	Emmanuel et al., 2011
FW	521	10.1	875	125	315	40.7	-	Oyeleke et al., 2012
Kernels of *A. digitata*								
FW	2.0	1.3	-	0.2	-	-	1.9	Obizoba & Amaechi, 1993
DW	0.5	0.6	0.6	326	-	-	1.29	Nnam & Obiakor, 2003
Kernels* of *S. birrea								
FW	106	0.42	677	836	467	338	-	Wehmeyer, 1966
DW	156	2.8	-	212	193	1.2	2.65	Glew et al., 1997
DW	403	27.5	366	2.9	206	4.8	3.3	Muhammad et al., 2011
DW	154	2.8	364	1040	421	4.3	6.24	Glew et al., 2004
FW	448.9	4.5	486.6	160.7	253.7	-	4.5	Wairagu et al., 2013

FW = fresh weight; DW = dry weight; - = no information given.

Many seeds and kernels contain phytic acid, which may have a negative influence on the uptake of minerals in the diet. Phytic acid, also known as inositol hexaphosphate, has six PO_4 groups, and is a strong chelator of divalent ions, especially zinc and iron, and to a lesser extent, also calcium and magnesium (Greiner and Konietzny, 1998). Phytic acid is the principal storage form of phosphorus in many plant tissues, especially bran and seeds (Anastasio et al., 2010). Table 4 gives the content of phytic acid reported in the literature for the fruits studied.

Table 4. Literature data on the phytic acid content of the seeds of *A. digitata* and kernels of *S. birrea*

Phytic acid (%)	Based on	Reference
A. digitata		
6.66 and 7.13	-	Adubiaro et al., 2011
4.90[a]	DW	Mitchikpe et a., 2008
1.75 and 0.62	-	Saulawa et al., 2014
1.40[a]	-	Proll et al., 1998
1.20	-	Ezeagu, 2005
0.20	-	Nkafamyia et al., 2007
0.073[a]	FW	Osman, 2004
0.18 and 0.16[b]	-	Nnam & Obiakor, 2003
S. birrea		
0.423[a]	DW	Muhammad et al., 2011

[a]= recalculated from other units; [b] = units not stated.

FW = fresh weight; DW= dry weight; - = not given.

Not only the protein content, but also the amino acid profile is important. The essential amino acids cannot be synthesized in the body and must therefore be provided through the diet. Amino acids serve as precursors for proteins, nucleic acids, hormones and other important molecules (Insel et al., 2011). The contents and ratios of essential amino acids are important in the estimation of protein quality (WHO, 2007). Table 5 gives the contents of amino acids in the seeds and kernels of *A .digitata* and *S. birrea*.

Table 5. The content of amino acids in the seeds and kernels of *A. digitata* and *S. birrea;* essential amino acids are indicated by an asterisk

Amino acid	**Glew et al., 1997**	**Osman, 2004**	**Ezeagu, 2005**	**Glew et al., 1997**	**Glew et al., 2004**	**Muhammad et al., 2011**
	***A. digitata*, whole seeds**			***S. birrea*, kernels**		
	(mg/g DM)	(g/100 g of protein)		(mg/g DM)	(g/100 g of protein)	
Cysteine*	3.6	1.5	1.9	1.9	2.5	2.4
Histidine*	5.1	2.2	2.0	1.2	2.5	2.6
Isoleucine*	8.3	3.6	3.5	2.5	3.3	3.8
Leucine*	14.0	7.0	6.5	3.8	4.8	7.4
Lysine*	11.2	5.0	3.7	1.3	2.0	5.2
Methionine*	2.3	1.0	1.3	0.7	1.6	1.7
Phenylalanine*	10.3	4.0	4.5	2.4	3.6	3.1
Threonine*	7.0	3.8	2.9	1.8	2.4	4.7
Valine*	11.6	5.9	5.0	3.0	3.9	3.9
Alanine	2.5	7.1	-	2.5	2.5	6.2
Arginine	2.2	8.0	-	6.8	14.4	15.0
Aspartic acid	21.2	10.3	-	5.2	12.7	13.4
Glycine	10.4	8.6	-	2.7	3.6	3.5
Glutamic acid	48.9	23.7	-	13.1	29.9	28.1
Proline	9.6	6.9	-	2.6	2.8	4.6
Serine	11.4	6.1	-	2.6	4.1	4.3
Tyrosine	5.6	1.5	2.7	1.5	2.0	2.6

*Essential amino acids, DM= dry matter; - = no information given.

The quality of the fat in the diet is also important, especially the content of unsaturated fatty acids. The contents of fatty acids in the kernels of *A. digitata* and *S. birrea* are given in Tables 6a and b. The essential fatty acids linoleic acid and linolenic acid are indicated by an asterisk. They are of importance, for example, for the maintenance of the immune system and cell membranes, and for the function of the brain and skin (Uauy et al., 2001). They may also reduce the risk of heart disease (Food Nutrition Board, 2002). The consumption of monounsaturated fatty acids has been associated with decreased levels of low-density lipoprotein cholesterol, and possibly with increased high-density lipoprotein cholesterol (Coultate, 2009).

Table 6a. Literature data on the fatty acids in the whole seeds of *A. digitata*

Fatty acid[1]	Glew et al., 1997	Eteshola & Oroedu, 1996	Ezeagu et al., 1998	Osman, 2004	Ezeagu, 2005	Zimba et al., 2005
	(mg/g DM)	(g/100 g of fat)				
C14:0	Trace	38.4	-	0.2	0.3	31.6
C16:0	1.4	19.7	15.5	24.2	22.1	-
C16:1	0.02	-	0.2	-	0.3	3.5
C18:0	0.2	3.2	3.1	4.6	4.0	34.5
C18:1	2.1	22.4	24.7	35.8	35.0	27.8
C18:2	1.4	16.2	19.1	30.7	26.1	-
C18:3*	0.02	-	1.6	1.0	2.0	-
C20:0	Trace	-	0.7	1.3	0.9	-
C20:1	-	-	-	0.9	0.2	-
C22:0	-	-	0.4	-	0.4	-
C24:0	-	-	0.3	0.2	-	-

[1] No. of carbon atoms and double bonds, for systematic names see Table 13.
*Essential fatty acids, DM= dry matter; - = no information given.

Table 6b. Literature data for the fatty acids in kernels of *S. birrea.* For references A-H, see footnote[1] below

Fatty acid[2]	A	B	C	D	E	F	G	H
	(mg/g DM)	(g/100 g of fat)						
C14:0	0.03	2.1	0.1	0.3	-	-	0.1	-
C16:0	2.1	22.5	15.6	14.2	10.7	8.7	5.7	12.8
C16:1	0.02	-	0.2	0.2	-	-	1.5	-
C18:0	1.1	50.7	11.1	8.8	7.2	9.5	6.0	7.2
C18:1	6.3	4.1	63.2	67.3	72.0	78.5	71.8	73.6
C18:2*	0.5	-	5.2	5.9	8.8	3.4	0.6	6.1
C18:3*	0.03	-	-	0.1	0.6	-	0.1	0.3
C20:0	0.06	8.5	1.3	0.9	-	-	3.9	-
C20:1	-	0.1	0.5	0.4	1.3	-	2.2	-
C22:0	-	5.1	0.4	0.2	0.1	-	6.6	-
C24:0	-	4.1	0.3	0.3	-	-	1.0	-

[1]References: A: Glew et al., 1997; B: Ogbobe, 1992; C: Glew et al., 2004; D: Mariod et al., 2004; E: Zharare & Dhlamine, 2004; F: Zimba et al., 2005; G: Kleiman et al., 2008; H: Robison et al., 2012.

[2] No. of carbon atoms and double bonds, for systematic names see Table 13
*Essential fatty acids, DM= dry matter; - = no information given.

4. Materials and Methods

4.1 Samples

The five wild fruit varieties included in this study were collected in different regions of Mozambique. Ripe fruits were collected in 2008 and 2009, except for the fruits from *S. birrea*, which were collected only in 2009. *A. digitata* fruits grown in the Tete province were bought at a local market in Maputo, and some fruits were collected from family orchards in the Vilanculos district. *L. kirkii, S. kraussii* and *V. infausta* fruits were collected from orchards in the Marracuene and Manhiça districts of Maputo province. *S. birrea* fruits were obtained from a garden in Maputo city, and seeds, dried for 9-10 months, from a family orchard in Manhiça. For a more detailed description, see Paper I. In addition, seeds from *A. digitata* and *S. birrea* were collected in 2013, in Tete and Maputo (see Paper III).

Unblemished fruits were selected and washed, the skin and seeds were removed and the pulp was homogenized in a blender. The seeds from *A. digitata* and *S. birrea* were crushed, the shells were removed and the kernels inside were collected.

Samples for the determination of pH, soluble solids (°Bx) and titratable acidity were kept at room temperature and the analyses were performed the day after collecting the fruit. Samples for other analyses were vacuum packed in plastic bags, frozen and stored at -18°C in a freezer. The kernels were milled before analysis.

4.2 Chemical analysis

The contents of dry matter, fat, protein, dietary fibre, ash and minerals were determined in the pulp and kernels. The pH and titratable acidity and the contents of soluble solids, sugars and organic acids were determined in the pulp. Amino acids, fatty acids and phytic acid were analysed in the kernels of *A. digitata* and *S. birrea*.

Table 7 gives an overview of the various methods and equipment used for analysis. For a more detailed description of the methods, instruments and chemicals, see Papers I - IV. Each parameter was determined at least in duplicate.

Table 7. Analytical methods and equipment

Parameter	Method/Equipment	Reference
Amino acids	Ion-exchange chromatography with post-column derivatization using ninhydrin	See Paper III
Ash	Muffle furnace, 24 h at 550°C	AOAC, 2000; 940.26
Dry matter	Vacuum oven, 24 h at 70°C Oven, 18 h at 105°C until constant weight	AOAC, 2000; 926.12 AOAC, 1980; 22.021b
Dietary fibre and their monomers	Enzymatic gravimetric method and gas chromatography	Asp et al., 1983; Theander et al., 1995; Haskå et al.,2008
Fat	Soxhlet extraction, petroleum ether	AOAC, 1996; 920.39
Fatty acids	Gas chromatography	Analysed by an authorised laboratory in Sweden
Minerals	Inductively coupled plasma-atomic emission spectrometry	Analysed by the Department of Ecology, Lund University
Organic acids	Ion-exchange chromatography	See Paper II
Phytic acid	High-performance ion-chromatography	Carlsson et al., 2001
pH	pH meter	
Protein	Flash elemental analyser	AOAC, 1980; 7.016
Soluble solids	Digital refractometer	AOAC, 2000; 932.12
Sugars	High-performance anion-exchange chromatography	Analysed by an authorised laboratory in Sweden
Titratable acidity	Titration	AOAC, 2000; 942.15

4.3 Statistical analysis

Statistical analyses were performed using SPSS (version 13). Significant differences were evaluated with one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test (Papers I and II). Microsoft Excel® was used for statistical evaluation. Student's t-test was performed to determine significant differences. (Papers III and IV). A value of $p<0.05$ was considered to indicate statistical significance.

4.4 Interviews regarding the traditional use of wild fruits

When the wild fruits were collected in the different districts, local people were interviewed about the traditional use of the fruits selected for this study. The local authority was contacted for permission for the field work. During the first period of field work, in 2008 and 2009, 3 to 10 people were interviewed about each fruit, both women and men of different ages, all of whom had grown up in the rural area. The aim of the interviews was to obtain information on knowledge concerning the occurrence of these wild fruit trees and traditional habits regarding consumption, preparation and storage of the wild fruits. The second period of field work, in 2013, was organized so as to obtain information concerning the use, consumption and preparation of the seeds and kernels. Meetings were organized with 15 to 17 people of different ages (18 to 70), including the local guide who translated from the local language to Portuguese.

The main interview questions were the following: (i) Where are the fruits collected? (ii) What is the importance of the fruits? (iii) Who are the major consumers? (iv) Apart from consumption, what is the contribution of the fruits in the household economy? (v) Which parts of the fruits are eaten? (vi) What kind of preparation is usually done? (vii) How are the fruits, seeds and kernels preserved for storage?

5. Results and Discussion

5.1 Interviews

The interviews revealed that the majority of the fruits came from the forest, or in the area surrounding their house. In periods of food scarcity, the leaves, fruits and seeds are used to provide food for everyone, as well as for medical applications. The fruits are commonly consumed by people of all ages as snacks while walking to, and working on, their grassland. However, the children are the main consumers, since they are more free to go into the forest to collect the fruits while tending grazing cattle or on their way to and from school.

The wild fruits harvested serve as a source of income for many families, depending on the amount of fruit that each family can gather. The fruits are usually sold along the roadside (Figure 9), or transported for sale early in the morning at informal markets, for example "*Bobole*" in Marracuene and "*Kwatsena*" in the Tete province, to which people come to buy fruits for later sale in the markets in the cities.

a)  b) 

Figure 9. a) *A. digitata* fruits along the roadside. b) Other common fruits along the roadside.

The pulps of all the studied fruits are consumed, and the seeds and kernels from *A. digitata* are consumed in some areas. The kernels of *S. birrea* are sometimes, but not often consumed.

Fruit pulps

In general, the pulps are eaten fresh as snacks, or squeezed to make juice. They are also fermented to produce local alcoholic drinks that are often drunk at social and traditional events. The pulps of the fruits are also mixed with water to make a juice. The juice prepared from *A. digitata* pulp is often consumed in "*tropical nhungwé*" restaurants in the Tete province. In Vilanculos, the juice is filtered and sugar is added, and then packed in small plastic bags, frozen and sold in informal markets, where it is served as a refreshment consumed by both children and adults. Pulps from fruits such as *A. digitata* and *V. infausta* are mixed with cow's milk to make yogurt or a dessert, depending on the amount of liquid added. The squeezing process is not efficient enough to obtain a high juice yield, as part of the flesh is attached to the central pit and skin. Furthermore, it is difficult to obtain consistently ripe and undamaged fruit, and using fruits at different degrees of ripeness could result in a final juice being too sour to be drinkable.

The pulps from *A. digitata* and *V. infausta* fruit are also boiled to make a porridge given to children before they go to school. This is considered a very nutritious food, aiding the development of infants and children. *A. digitata* pulp can be processed by adding a little water to obtain a kind of sauce, which is added to dried meat and then boiled. It can be used to make a chili sauce by boiling the pulp with a little water, and mixing it with fried garlic and chili powder, and cooking for a while. The mixture is left in the sun for a few days and can be eaten with any kind of food.

The preservation of the fruit is problematic, especially for the *L. kirkii, S. kraussii* and *S. birrea* fruits, since they have low dry matter contents and a short shelf-life. *V. infausta* pulp or fruit can be sun-dried and stored for up to a year for later use in periods of hunger. Whole fruits of *A. digitata* can be preserved in cool places or barns. Barns are preferred for storage because this avoids contact with the sand, and the fruits are not infested by insects causing poor quality of the fruit pulp. *A. digitata* can also be stored in special containers or in bags at room temperature for more than a year, without showing any indication of damage by insects.

The traditional alcoholic drink made from *S. birrea* can be stored in sealed clay pots and buried, allowing storage for two years. This was a traditional habit of people in southern Mozambique, particularly in the provinces of Maputo, Gaza and Inhambane, where the majority of the men work for the mining companies in neighbouring South Africa, and come home for a holiday every 18 months.

Seeds and kernels

The use and benefits of the seeds and kernels of *A. digitata* and *S. birrea* were also reported by the elders. The kernels can be eaten raw or mixed with a little salt, roasted and consumed as a snack. The seeds and kernels are often used in times of drought when the production of peanuts is low.

The seeds of *A. digitata* are generally cracked using two stones or a sharp axe, and then beaten to open the seed and separate the kernel. The seeds of *S. birrea* can be cracked using two stones, or a hammer and a piece of wood from the tree. The kernels are removed from the shell using a stick as a kind of needle to poke them out (Figure 10).

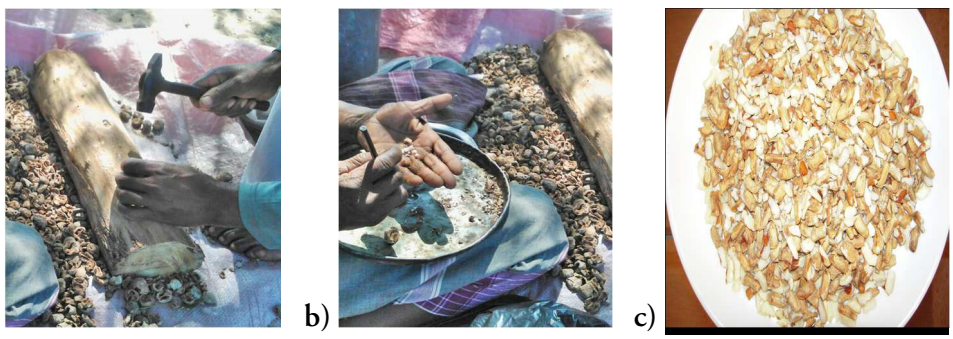

Figure 10. a) The cracking of *S. birrea* seeds, b) removing the kernels from the shell, and c) the kernels.

After squeezing *S. birrea* fruits for the preparation of beverages, the seeds can be left on the ground to dry for several months for later use. Roasted kernels of *S. birrea* can be stored in glass jars sealed with a stopper for more than two years, without any change in the taste or aroma. *S. birrea* kernels have a nice nutty flavour and pleasant smell, providing a condiment with a good taste when mixed with indigenous food plants and boiled for consumption. Due to the difficult and laborious task of obtaining these kernels, the women prepare them as special gifts for their husbands, as a gesture of their affection. In meal preparation, flour from *S. birrea* kernels is sometimes mixed with peanut flour to give the latter more flavour. The locals mentioned that the amount of *S. birrea* kernel flour needed to give a good sauce is generally smaller than the amount of peanut flour required. *S. birrea* kernels are also mixed with a little salt, roasted and consumed as a snack.

The whole seeds of *A. digitata* can be ground, mixed with corn or roasted to make a kind of coffee. The kernels may also be ground in a bowl, mixed with water in a pan with local plant food, cooked for a while, and then consumed with corn flour or

sorghum. *A. digitata* seeds are often left on the ground after consumption of the pulp, and it is not common to store them. It is not common to extract oil from either seed of the kernels, although they have a high fat content (Glew et al., 1997). The major problem related with oil extraction is a lack of sophistication in the technique to be used (MMB Consulting, 2006).

5.2 Chemical analysis

The results of the determinations of dry matter (DM) content, fat, protein, dietary fibre monomers and ash, expressed on DM basis, are given in Table 8 for the pulps and kernels investigated. For comparison with literature data, see Table 2. Generally, no significant differences were seen between the results of the analysis of the fruits collected in the two years (2008 and 2009) at different locations. The small variations in the data could be due to differences in climate, soil and weather conditions. The protein values in the kernels collected in 2013 is lower than those found in the first studies. When the amino acid composition was analysed it became obvious that the kernels had high contents of glutamic acid (with additional nitrogen groups) and thus another protein conversion factor (5.75) was used; in the first studies the common factor 6.25 was used. Also the fat content in the kernels from 2013 was somewhat lower, which may be explained by a different analytical method.

The determination of the DM content the day after collecting the fruits showed that the pulp of *A. digitata* had a very high DM content, almost 90 g/100 g. This is in accordance with results from other studies (Table 2). The result for *V. infausta* pulp was somewhat higher than the literature data. No data could be found in the literature for the other three fruits. For comparison, the DM content of apples and grapes varies between 15 – 19%, See Table 1. High DM contents in fruits indicate long shelf-life (Effiong and Udo, 2010).

In general, fruit pulps have low contents of fat and protein (Insel et al., 2011), see also Table 1. The fat contents in the pulps were below 2 g/100 g DM, in agreement with previous reports (Table 2). The protein contents were below 5 g/100 g DM. In other studies, the protein content has been reported to range from 1 to 6 g/100 g (Table 2).

The DM contents of the kernels of *A. digitata* and *S. birrea* were high, over 90 g/100 g. These results are in agreement with those reported in other studies (Table 2). The fat content of the kernels of *A. digitata* ranged from 31.9 to 39.9 g/100 g DM, and in the kernels of *S. birrea* from 49.4 to 63.1 g/100 g DM. The protein contents in the kernels of *A. digitata* and *S. birrea* were high, 29.2 to 42.7 g/100 g DM. Similar, high protein contents have been found in other studies (Table 2). This means that the kernels are good sources of fat and protein.

Table 8. Nutritional composition of the pulp and kernels of the wild fruits from Mozambique determined in this study

Fruit part	Location year	Dry matter	Fat	Protein	Dietary fibre		Ash
					Insoluble	Soluble	
		(g/100 g)	(g/100 g DM)				
Pulp							
A. digitata	Tete, 08	89.8±0.1	0.5±0.1	2.4±0.0	14.2±0.4	60.3±1.9	5.5±0.1
	Tete, 09	89.1±0.0	0.7±0.6	2.2±0.1	14.7±0.0	65.6±0.6	7.4±0.0
	Vilan, 09	86.5±0.1	0.5±0.1	2.1±0.0	16.1±0.8	57.3±0.3	7.4±0.0
L. kirkii	Marr, 08	27.7±0.0	0.9±0.1	2.1±0.2	3.5±0.6	4.6±0.1	2.9±0.0
	Marr, 09	23.9±0.0	0.4±0.2	n.a.	4.9±0.8	4.3±0.3	3.5±0.0
	Man, 09	20.1±0.3	1.3±0.1	1.7±0.0	4.9±0.4	5.8±1.2	3.0±0.0
S. kraussii	Marr, 08	16.4±0.3	0.8±0.5	3.7±0.0	3.3±0.1	6.0±0.4	4.8±0.2
	Marr, 09	17.3±0.1	1.5±0.6	2.3±0.0	2.6±1.2	7.6±1.0	3.4±0.0
	Man, 09	16.5±0.1	0.7±0.2	2.1±0.0	5.5±0.3	7.1±0.4	3.6±0.0
S. birrea	Man, 09	16.8±0.1	0.9±0.3	1.4±0.1	7.7±1.7	10.5±0.9	3.0±0.1
V. infausta	Marr, 08	37.4±0.5	0.5±0.8	2.9±0.2	45.6±1.4	24.3±1.4	7.8±0.6
	Marr, 09	30.0±0.1	0.7±0.1	2.2±0.3	45.8±0.9	26.3±0.9	5.3±0.0
	Man, 08	37.3±0.9	0.7±0.2	4.7±0.4	41.0±0.8	10.6±0.8	5.7±0.4
	Man, 09	34.5±0.7	0.2±0.0	3.3±0.8	30.9±1.9	23.1±1.9	3.2±0.0
Kernels							
A. digitata	Tete, 08	93.6±0.0	35.0±0.2	36.7±0.9	17.4±3.4	14.0±0.6	7.7±0.0
	Tete, 09	91.7±0.1	39.9±6.2	38.6±0.8	20.9±1.3	17.0±3.4	7.2±0.0
	Vilan, 09	90.6±0.0	39.0±7.0	42.7±0.7	14.7±0.0	42.6±1.8	8.5±0.0
	Tete ,13	92.8±0.0	31.7±0.0	35.0±0.0	n.a.	n.a.	n.a.
S. birrea	Man, 08	93.6±0.0	58.3±1.3	30.1±0.2	17.6±2.9	10.5±2.7	3.8±0.0
	Man, 09	95.0±0.0	63.1±0.1	35.0±0.1	18.5±1.5	8.4±2.4	3.5±0.0
	Man, 13	95.8±0.1	49.4±3.7	29.2±0.0	n.a.	n.a.	n.a.

n.a. = Not analysed;
Vilan= Vilanculos; Marr=Marracuene, Man= Manhiça; 08 = 2008; 09 = 2009; 13 = 2013.

Table 8 also presents the results of the dietary fibre analysis. Large amounts of dietary fibre were found in the pulps of *A. digitata* and *V. infausta*. These contents are high compared with apples and guava in Table 1. The highest amount of soluble dietary fibre, around 60 g/100 g DM, was found in *A. digitata* pulp and the highest amount of insoluble dietary fibre, 31 to 46 g/100 g DM, in *V. infausta* pulp. The kernels of *A. digitata* and *S. birrea* contained large amounts of dietary fibre. The amounts found were higher than in previous studies (Table 2). However, the variations may be partly due to the use of different analytical methods in the various studies.

The analysis of the dietary fibre composition showed that the contents of monosaccharides and uronic acids in the soluble and insoluble dietary fractions of the kernels differed between the kernels (Paper III). The main constituent of the insoluble fraction of *A. digitata* kernels was glucose, followed by arabinose and uronic acids, while in the soluble fraction, arabinose was the dominating component, followed by uronic acids. In kernels of *S. birrea*, uronic acids constituted more than 90% of both dietary fibre fractions.

The ash content (Table 8), providing an indication of the mineral content, was around 3 to 9 g/100 g DM. The results generally agreed with those reported in previous studies (Table 2). The ash content of the fruits in Table 1 is below 0.7 g/100 g on wet weight.

The pH, titratable acidity and soluble solids are important parameters for the food industry in evaluating the quality and maturity of fruits (Lammertyn et al., 2000). The pH of the pulps showed an acidic character (around pH 3), apart from *S kraussii*, the pH of which was slightly above 6. The acidic character is in accordance with previous data on pulps from *A. digitata*, *S. birrea* and *V. infausta* (Amarteifo and Mosase, 2006; Mothapo et al., 2014). The pH of fruits generally varies between 2.5 and 4.5 depending on their content of organic acids (Calvacanti et al., 2006). Titratable acidity is related to the acidic or sour taste, and is expressed as the amount of organic acids. The titratable acidity of the pulp, estimated as the percentage of citric acid, ranged from 0.6 to 1.7%. Corresponding values in the literature are 7.8% for *A. digitata*, 0.9% for *S. birrea* and 1.7% for *V. infausta* (Amarteifo and Mosase, 2006). The soluble solids content, measured as °Bx (degree Brix), is often used as an approximation of the concentration of sugars in the fruits (Silva et al., 2009). However, other soluble components, for example soluble dietary fibre, will also contribute to the degree Brix. The soluble solids content in the pulps ranged from 14 to 35°Bx; the results for fruits collected in Manhica in 2009 are presented in Table 9. A lower value (3.9°Bx) has been reported in the literature for *V. infausta* (Mothapo et al., 2014).

The contents of different mono- and disaccharides in the pulps were determined and the results, expressed as g/100 g wet weight, are presented in Table 9.

Table 9. Mono- and disaccharides in the pulps from wild fruits collected in Manhiça in 2009

Sample	Glucose	Fructose	Sucrose	Lactose	Maltose	Soluble solids
			(g/100 g wet weight)			(°Brix)
A. digitata	3.0	3.0	4.3	<0.04	<0.04	n.a
L. kirkii	7.5	5.7	1.2	<0.04	<0.04	26.3
S. kraussii	3.7	3.9	<0.1	<0.04	<0.04	15.0
S. birrea	0.5	0.4	1.4	<0.04	<0.04	17.4
V. infausta	1.4	1.4	2.7	<0.04	<0.04	30.1

n.a. = not analysed.

The highest amounts of glucose and fructose were found in *L. kirkii,* 13.2 g/100 g in total, and the lowest amount in *S. birrea*, below 1 g/100 g. The highest amount of sucrose was found in *A. digitata,* 4.3 g/100 g, and the lowest amount in *S. kraussii*, below 0.1 g/100 g. Lactose and maltose were detected in all samples, but at much lower concentrations, <0.04 g/100 g. No data could be found in the literature on the sugar contents of the studied fruits. When comparing the contents of individual sugars or the sum of mono- and disaccharides, no correlation was observed between the contents of sugars and the content of soluble solids (°Brix). The high content of soluble solids in *V. infausta* (30.1 °Brix) in spite of its rather low sugar content is explained by the high content of soluble dietary fiber (Table 9).

The same pulp samples were analysed with regard to organic acids. Organic acids are not routinely determined at the Department of Food Technology, Engineering and Nutrition. Therefore, a new method was developed based on information from the manufacturer of the ion chromatograph (See Paper II). The elution solvent and the column temperature were varied to find conditions that gave reliable results. The separation of citric, malic, tartaric and succinic acids was investigated, and the optimal conditions for their separation were found to be an 85:15 (v/v) mixture of 0.5 mM sulphuric acid and acetone, and a column temperature of 30°C

The calibration curve was linear in the range studied. A chromatogram from the analysis of organic acids in *S. kraussii* is shown in Figure 8. The peaks corresponding to the different organic acids and two unknown compounds are indicated by numbers.

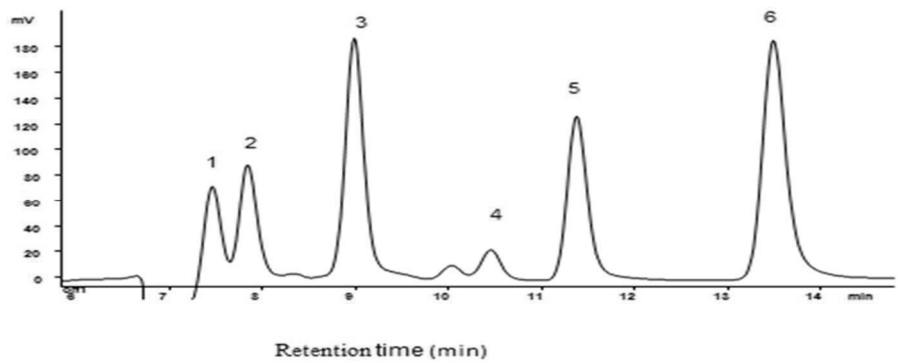

Figure 8. Chromatogram from the analysis of organic acids in *S. kraussii*.

The peaks correspond to: citric acid (1), tartaric acid (2), malic acid (3) and succinic acid (4). (Peaks 5 and 6 were not identified.)

The results of the analysis of the organic acids, expressed in g/kg wet weight, are given in Table 10. Citric, malic and succinic acids were found at various amounts in all pulps, and traces of tartaric acid in one sample.

Table 10. Organic acid content in pulps from wild fruits collected in Manhiça in 2009

Sample	Dry matter	Citric acid	Malic acid	Succinic acid	Tartaric acid
	(g/100 g)	(g/kg wet weight)			
A. digitata	88.0	25.7±2.1	1.6±0.2	0.1± 0.0	n.d.
L. kirkii	18.0	21.5±2.1	0.4±0.1	0.03±0.0	n.d.
S. kraussii	9.0	0.9±0.3	1.1±0.1	0.1±0.0	n.d.
S. birrea	8.0	8.5±1.3	1.2±0.2	0.1±0.0	Trace
V. infausta	31.0	6.2±0.5	2.1±0.2	0.1±0.0	n.d.

n.d. = Not detectable, Trace = < the determination limit (0.01 g/kg).

The maximum content of malic acid was 2.1 g/kg, and succinic acid was below 0.1 g/kg. High amounts of citric acid were found in *A. digitata* and *L. kirkii*; above 20 g/kg. When tasting the fruits, *A. digitata* and *L. kirkii* were the most acid ones. The results for *A. digitata* are in accordance with results in a previous study. Citric acid in *A. digitata* has been reported to be present at amounts around 23 to 32 g/kg, and malic acid 1.1 to 1.4 g/kg (PhytoTrade Africa, 2008). For comparison, the citric acid concentrations in some traditional fruits are: 2.2 g/ kg in pineapple, 4.5 g/ kg in oranges, 13.1 g/ kg in grapes and 41.2 g/ kg in limes (Falade et al., 2003). The presence of citric and malic acid in fruits may promote iron absorption (Gillooly et al., 1983).

The mineral contents of the fruits and kernels, expressed as mg/100 g DM, are given in Table 11. Although it is important, iodine was not included in this study as it is not commonly found in fruits (Hurrell, 1997).

Table 11. Mineral contents of the pulps, whole seeds and kernels of the studied wild fruits

Fruit, part Location, year	Dry matter	Ca	K	Fe	Mg	Na	P	S	Se	Zn
	(g/100 g)	(mg/100 g DM)								
Pulp										
A. digitata										
Tete, 08	90.1	326	2308	2.0	162	5.0	40	51	n.d.	n.a.
Tete, 09	86.4	308	2392	2.0	129	2.0	40	41	n.a.	0.5
Vilan, 09	86.5	366	2360	1.0	102	5.0	30	43	n.a.	0.6
L. kirkii										
Man, 09	20.4	28	1840	4.0	51	21	55	20	n.a.	1.4
S. kraussii										
Man, 09	8.9	127	2056	9.0	207	35	153	191	n.a.	0.6
*S. birre*a										
Man, 09	15.9	201	2753	3.0	138	30	178	90	n.a.	0.7
V. infausta										
Man, 09	34.4	90	1249	3.0	65	18	92	45	2.0	n.a.
Seeds										
A. digitata										
Tete, 08	93.6	220	1238	29	360	2.0	523	131	n.d.	n.a.
Kernels										
A. digitata										
Tete, 09	92.2	293	1416	6.0	626	1.0	1229	263	n.a.	5.7
Vilan, 09	90.6	347	1451	4.0	706	2.0	1518	269	n.a.	5.2
S. birrea										
Man, 08	92.7	60	622	4.0	436	7.0	871	284	n.d.	n.a.
Man, 09	95.0	81	531	4.0	396	6.0	719	269	n.a.	4.5

n.d. = Not detectable; n.a. = Not analysed. Vilan=Vilanculos; Man=Manhiça; 08=2008; 09=2009.

The results can be compared with the literature data given in Table 3. The discussion below focuses on some of the minerals that are often deficient in local diets, i.e. calcium, iron and zinc. The highest amount of calcium was found in *A. digitata* pulp and kernels, about 300 mg/100 g DM or more. This value generally agrees with literature data (Table 3), but is lower than that reported in a previous study (Nour et al., 1980).

The amount of iron in the pulps ranged from 1 to 9 mg/100 g DM; the highest amount being observed in *S. kraussii*. These amounts are comparable to those found in other studies (Table 3), however, higher amounts of iron have been reported in *V. infausta* pulp (24.4 and 28.3 mg/100 g DM), see Table 3. The highest iron content, 29 g/100 g DM, was found in the seeds of *A. digitata*, and was 5 to 7 times higher than in the kernels. Similar findings of higher amounts in the whole seed than in the kernels have been reported in the literature (Table 3).

The zinc content in the pulps was around 1 mg/100 g DM or below. The zinc content in the kernels of *A. digitata* and *S. birrea* was around 5 mg/100 g DM. This is in agreement with results from a previous study of *S. birrea* kernels (see Table 3).

Phytic acid may decrease the absorption of minerals from the diet. According to the literature, *A. digitata* and *S. birrea* kernels have been shown to contain phytic acid, for a literature review, see Table 4. Thus, preliminary experiments were performed to determine the content in the kernels. The results showed that kernels of *A. digitata* contained about 5.5% phytic acid and *S. birrea* kernels 3.4%. Comparing the results with the literature data given in Table 4, it can be seen that the *A. digitata* kernels had a lower content than in some previous studies (Mitchikpe et al., 2008; Adubiaro et al., 2011), and higher than in others (Osman, 2004; Ezeagu, 2005; Saulawa et al., 2014). The differences between the results may be explained to some extent by the method of analysis, which is sometimes not clearly described. The results regarding the amounts of phytic acid in *S. birrea* kernels are almost six times higher than that reported in a previous study (Muhammad et al., 2011). It is interesting to note that the phytic acid content in peanuts, which are commonly consumed in Mozambique, has been reported to be around 2 g/100 g (Lazarte et al., 2015).

Results from additional experiments showed that the presence of phytic acid in the kernels can be reduced by various processing techniques (Paper IV). For example, treatment with phytase reduced the phytic acid content by 20-30% after only 15 minutes, and after 4 hours' enzymatic incubation, the decrease was significant. Interestingly, almost 50% of the estimated original content of minerals was found in the supernatant after a few minutes' enzyme incubation. This suggests that the water

used for boiling should be included in the dishes if the kernels are to be used as a dietary supplement.

The preliminary studies showed that the kernels had high protein content, and it was thus deemed interesting to determine the amino acid content. The results of the amino acid analysis are presented in Table 12.

Table 12. Amino acid composition in kernels of *A. digitata* and *S. birrea*

Amino acid	***A. digitata***	***S. birrea***	**Peanuts**[1]
	(g/100g of protein)		
Cysteine*	0.5 ± 0.0	0.7 ± 0.0	1.3
Histidine*	2.2 ± 0.1	2.5 ± 0.2	2.5
Isoleucine*	3.4 ± 0.2	3.9 ± 0.3	3.5
Leucine*	6.0 ± 0.4	4.3 ± 1.3	6.5
Lysine*	3.4 ± 1.0	2.9 ± 0.2	3.6
Methionine*	0.8 ± 0.1	0.7 ± 0.1	1.2
Phenylalanine*	4.5 ± 0.3	4.6 ± 0.3	5.2
Threonine*	2.8 ± 0.1	2.8 ± 0.2	3.4
Valine*	4.5 ± 0.3	5.0 ± 0.4	4.2
Alanine	3.8 ± 0.3	3.7 ± 0.3	3.9
Arginine	11.4 ± 0.6	13.9 ± 1.1	12.0
Aspartic acid	8.1 ± 0.4	8.4 ± 0.5	12.2
Glycine	4.4 ± 0.3	4.9 ± 0.3	6.0
Glutamic acid	23.7 ± 1.3	25.7 ± 1.8	20.8
Proline	3.2 ± 0.2	3.5 ± 0.2	4.4
Serine	4.6 ± 0.3	2.9 ± 0.3	4.9
Tyrosine	2.5 ± 0.1	4.7 ± 0.2	4.1
Total	89.8	95.9	99.7

* Essential amino acid

[1]Data recalculated from USDA, Nutrient Database for Standard Reference, release 27, accessed at 22/11/ 2014.

All common amino acids, except tryptophan, were detected in both kernels. Under acidic hydrolysis conditions, tryptophan is totally destroyed, while asparagine and glutamine are hydrolysed quantitatively to aspartic acid and glutamic acid, respectively. Cysteine is readily oxidized to cysteic acid, and methionine to methionine sulphoxide, which is partly lost during hydrolysis. The amino acid profiles given in Table 12 generally agree with reports on amino acid contents in whole seeds and kernels (Table 5). However, the values for the S-amino acids are lower. This may be explained by partial breakdown of these amino acids during hydrolysis. Regarding the other essential amino acids, the results for lysine and valine in *A. digitata* are lower than in one previous study (Osman, 2004), and the findings regarding leucine, lysine and threonine in *S. birrea* are also lower (Muhammad et al., 2011). It is interesting to note that the amino acid composition in the two kernels studied is comparable to that in peanuts (WHO, 2007), which commonly form part of the diet in the locations from which the fruits were obtained.

The results from the first study showed that the kernels had a high fat content, and the fatty acid composition was therefore determined. The results are presented in Table 13, together with the fatty acid compositions of olive oil and peanuts for comparison.

Table 13. Fatty acid composition of kernels of *A. digitata* and *S. birrea*

Fatty acid		***A. digitata***	***S. birrea***	**Olive oil**[1]	**Peanuts**[1]
No. of carbon atoms and double bonds	**Systematic name**	(g/100 g of fat)			
C14:0	Myristic	0.2 ± 0.0	n.d.	-	0.4
C16:0	Palmitic	25.7 ± 0.2	12.1 ± 0.4	11.1	10.4
C16:1	Palmitoleic	0.2 ± 0.0	0.2 ± 0.0	1.2	0.0
C17:0	Margaric	0.2 ± 0.0	0.1 ± 0.0	-	-
C17:1	Margaroleic	0.2 ± 0.0	n.d.	-	-
C18:0	Stearic	4.6 ± 0.1	7.3 ± 0.1	2.7	2.2
C18:1	Oleic	34.9 ± 0.7	72.4 ± 0.6	70.7	48.3
C18:2	Linoleic*	29.9 ± 0.9	6.8 ± 0.4	8.1	31.7
C18:3	Linolenic*	2.1 ± 0.0	n.d.	0.7	0.0
C20:0	Arachidic	0.9 ± 0.1	0.6 ± 0.0	-	0.0
C20:1	Eicosenoic	0.2 ± 0.0	0.3 ± 0.0	-	-
C22:0	Behenic	0.3 ± 0.0	0.1 ± 0.0	-	-
C22:1	Erucic	0.1 ± 0.0	n.d.	-	-
C24:0	Lignoceric	0.2 ± 0.0	0.2 ± 0.0	-	-

[1]Data recalculated from The Swedish National Food Administration's food database, version 28/02/2014.

n.d. = Not detectable; *Essential fatty acid.

The unsaturated fatty acids constituted about 68 and 80% of the total fat content in *A. digitata* and *S. birrea* kernels, respectively. The most abundant fatty acids found in *A. digitata* kernels were oleic acid (29.9%) and linoleic acid (34.9%); the latter being an essential fatty acid. Interestingly, *A. digitata* kernels also contained the essential fatty acid linolenic acid. In *S. birrea* kernels, oleic acid constituted 72.4% of the fatty acids. The results for *A. digitata* are in agreement with some previous studies but

higher than in an older study (See Table 6). The results for *S. birrea* agree with most data in the literature (Table 6). The oils from both kernels have been reported to be very stable (Mariod et al., 2004; Zimba et al., 2005; Kleiman et al., 2008). It is interesting that the fatty acid composition in *S. birrea* is almost the same as in olive oil, with regard to the most abundant fatty acids; palmitic, oleic and the essential linoleic acid or *A. digitata*, the fatty acid composition is similar to that in peanuts, both with high contents of oleic and linoleic acid, but *A. digitata* also contains linolenic acid.

5.3 Dietary intake estimations

An adequate diet requires that the food eaten can provide all the essential nutrients in sufficient amounts to support growth and maintain health (Whitney and Rolfes, 1996). Some authorities, e.g. the Food and Nutrition Board of the US Institute of Medicine, have established adequate intake (AI) levels for a number of different nutrients. Some results from the chemical analysis of the pulps and kernels were selected for the estimation of the intake in some vulnerable age groups. A portion size of 100 g fresh fruit or 40 g kernels was assumed in these estimates.

The mineral content of the fruit pulps seemed high, but as the results are based on dry weight basis, the contribution of minerals from 100 g fresh fruit was estimated to be below 10% of the AI values. However, the *A. digitata* pulp had a high DM content, and consumption of 100 g would give around 29% of the AI of calcium for pregnant women and 30% for children and also 23% of the AI of iron for 4 to 13 years old (data not shown). The kernels, with a high DM content, may contribute considerably to the AI. Assuming a consumption of 40 g kernels, estimates were made for some selected age groups and are presented in Table 14.

Table 14. Estimated contribution of minerals through the consumption of 40 g kernels

Mineral	Life stage group	AI[1]	*A. digitata*	*S. birrea*
			Estimated contribution	
		(g/day)	(% of AI)	
Calcium	Children 4 to 8 years	0.8	16	5
	Older children 9 to 13 years	1.3	10	5
	Pregnant women	1.0	13	5
		(mg/day)		
Iron	Children 4 to 8 years	10	20	16
	Older children 9 to 13 years	8	25	20
	Pregnant women	27	7	6
Zinc	Children 4 to 8 years	5	44	36
	Older children 9 to 13 years	8	27	23
	Pregnant women	11	20	16

[1]Adequate intake according to the Food and Nutrition Board, 2002.

Calcium is important for the growth of bone and teeth (Insel et al., 2011). It can clearly be seen that consumption of 40 g *A. digitata* kernels would provide 10 to 16% of the AI of calcium for the selected groups, while the contribution from *S. birrea* would be below 5%.

Iron plays a role in the transport of oxygen in the body and has many other functions (Insel et al., 2011). Consumption of 40 g *A. digitata* kernels can provide 20 to 25% of the AI for children and *S. birrea* kernels 16 to 20%. For pregnant women, the contribution is considerably lower. Interestingly, ground whole seeds (shell + kernel) of *A. digitata* showed an extremely high iron content, and the contribution from 40 g could provide more than 100% of the AI for children and 43% for pregnant women (data not shown).

For zinc, the consumption of 40 g *A. digitata* kernels would provide 20 to 44% of the AI for the selected groups, while *S. birrea* kernels would provide slightly less, 16 to 36%. Zinc has a number of functions, for example, the body needs zinc to grow and develop properly during pregnancy, infancy and childhood (Brown et al. 1999; Insel et al. 2011).

Similar calculations were performed for protein. The protein content in the fruit pulp was low, and thus the contribution from pulp will be small. The results for the kernels are presented in Table 15.

Table 15. Estimated contribution of protein through the consumption of 40 g kernels

Life stage group	AI[1] (g/day)	*A. digitata* Estimated contribution (% of AI)	*S. birrea* Estimated contribution (% of AI)
Children 4 to 8 years	19	81	66
Older children 9 to 13 years	34	45	37
Pregnant women	71	22	18

[1]Adequate intake according to the Food and Nutrition Board, 2002.

For children aged 4 to 8 years, 81 and 66% of the AI is covered by the consumption of 40 g *A. digitata* kernels and *S. birrea* kernels, respectively. For older children and pregnant women, the contribution is lower, but it may be possible for these groups to increase their intake of kernels, especially if they are eaten as a snack. Furthermore, these two groups generally eat larger portions of dishes containing protein.

Ideally, the protein in the diet should provide the body's requirement of all the essential amino acids, in the appropriate relative proportions. The content of essential amino acids in relation to need is a measure used to determine the protein quality. The contents of essential amino acids in the kernels were compared with the amino acid requirements stated by the World Health Organization (WHO) for children aged 3-10 years; the results are presented in Table 16. It should be noted that, according to the WHO, the sum of phenylalanine and tyrosine is important, although tyrosine is a non-essential amino acid. Histidine is regarded as being essential for children, but not for adults.

Table 16. Essential amino acids in kernels of *A. digitata* and *S. birrea* compared with the WHO protein requirement for children aged 3-10 years old

Amino acids	***A. digitata***	***S. birrea***	**WHO, 2007**
		(g/100 g protein)	
Histidine	2.2	2.5	1.6
Isoleucine	3.4	3.9	3.0
Leucine	6.0	4.3	6.1
Lysine	3.4	2.9	4.8
Methionine + cysteine	1.3	1.4	2.3
Phenylalanine + tyrosine	7.0	9.3	4.1
Threonine	2.8	2.8	2.5
Valine	4.5	4.9	4.0

The total content and relative amounts of the different essential amino acids are generally similar to or above that recommended by the WHO. However, the amounts of methionine + cysteine, and lysine in both kernels, and also leucine in *S. birrea* kernels, were lower than required. The amount of the sulphur-amino acids were lower because they are partly destroyed during acid hydrolyses in the sample prepartion. The total amino acid composition indicates that *A. digitata* and *S. birrea* kernels can provide good, cheap sources of protein, especially if combined with foods with higher contents of lysine.

Fat is another important compound in the diet. The Food and Nutrition Board has established AI values in g/day for omega-3 and omega-6 fatty acids. The European Food Safety Authority (EFSA) and the WHO, on the other hand, recommend an acceptable range of omega-3 and omega-6 fatty acid intake related to the energy intake. Based on the results of the analysis of the fatty acid composition in the two kernels, the contributions of omega-3 and omega-6 fatty acids from the consumption of 40 g kernels were estimated. The results are presented in Table 17.

Table 17. Intake of omega-6 and omega-3 fatty acids through the consumption of 40 g of kernels

Fatty acids	Life stage group	AI[1]	*A. digitata*	*S. birrea*
			Estimated contribution	
		(g/day)	(% of AI)	
Omega-6 fatty acids (Linoleic acid)	Children 4 to 8 years	10	120	27
	Males 9 to 13 years	12	100	23
	Females 9 to 13 years	10	120	27
	Pregnant women	13	92	21
Omega-3 fatty acids (α-Linolenic acid)	Children 4 to 8 years	0.9	93	-
	Males 9 to 13 years	1.2	70	-
	Females 9 to 13 years	1.0	84	-
	Pregnant women	1.4	60	-

[1]Adequate intake according to the Food and Nutrition Board, 2002.

It can be seen that 40 g *A. digitata* kernels can cover the daily intake of omega-6 fatty acids for those aged 4 to 13 years, and about 90% of the requirement for pregnant women. The same quantity of *A. digitata* kernels can provide 60 to 93% of the daily requirement of omega-3 fatty acids for the same groups. Omega-3 fatty acids are not found in peanuts, which often form a considerable part of the diet in rural areas. Thus the *A. digitata* kernels may be an important and nutritious ingredient in different dishes.

The contribution of omega-6 fatty acids from 40 g *S. birrea* kernels is 21 to 27% of the AI for selected groups, and if *S. birrea* kernels are the only source of omega-6 fatty acids, an intake of around 160 g kernels is needed. Omega-3 fatty acids were not detected in *S. birrea* kernels, however, these kernels may not be the only source of essential fatty acids in the diet.

The intake of dietary fibre has been related to various health effects, appear to be at significantly lower risk for developing coronary heart disease, diabetes, obesity, blood pressure, cholesterol levels and certain gastrointestinal diseases (Buttriss and Stokes, 2008). Assuming consumption of 100 g pulp of *V. infuasta*, 40 g of *A. digitata* pulp, 40 g of *A. digitata* and *S. birrea* kernels, estimates were made for selected life stage groups and are presented in Table 18.

Table 18. Estimated contribution of dietary fibre through the consumption 40 g kernels

Life stage group	**AI**[1]	**Estimated contribution (% of AI)**			
	(g/day)	*V. infausta* 100 g Pulp	*A. digitata* 40 g Pulp	*A. digitata* 40g Kernels	*S. birrea* 40 g Kernels
Females 19 to50 years	25	86	108	61	42
Males 14 to 50 years	35	61	71	44	30

[1]Adequate intake according to the Food and Nutrition Board, 2002.

Consumption of 100 g pulp of *V. infausta* could provide around 61 to 86% of the AI of dietary fibre for adults and consumption of 40 g *A. digitata* pulp could provide 71 to more than 100%. The contribution from 40 g kernels is lower but is estimated to cover around 30 to 61% of the AI.

6. Conclusions and Future Outlook

The results of this research present a contribution to bridge the gap between traditional understanding and scientific knowledge of the wild fruits studied. The fruits are commonly consumed by those living in most districts of Mozambique, especially by children, and form part of their normal diet. Also, the kernels of *A. digitata* and *S. birrea* are usually consumed in rural areas. Wild fruit trees have significant cultural and socio-economic value in the countryside of Mozambique. Most of the wild fruits are seasonal, and are available mainly in the wet season. They generally have a short shelf-life and are eaten fresh or after minimal processing. The most common method of preservation is sun-drying. Although there is little documented information on the composition and nutritional benefits of these fruits, rural residents believe in, and use, traditional knowledge passed down from their forefathers.

The nutritional components and other characteristics have been determined for the five fruits studied in this work. No data could be found in the literature for *L. kirkii* and *S. kraussii*, and data on *S. birrea* and *V. infausta* were scarce. Small, non-significant variations were observed between fruits gathered from different locations and in different years, which may be due to differences in climate, soil and weather conditions.

The pH, titratable acidity and the contents of soluble solids, sugars and dry matter in the fruit pulps were determined, as these are interesting for the fruit processing industry. They are of importance for taste, aroma and the prevention of spoilage by microorganisms. The *A. digitata* pulp had remarkably high dry matter content, around 85 to 90%, compared with pulps from the other fruits. Therefore, this fruit has a long shelf-life. The total amount of glucose and fructose found in *L. kirkii* was about 13 g/100 g fresh weight. The highest amount of sucrose was found in *A. digitata*, about 4 g/100 g, while the content of these sugars was below 2 g/100 g in *S. kraussii*. No substantial amounts of lactose or maltose were detected in the samples.

The protein content was low in all the fruit pulps, as is the case in fruits in general. However, the protein content was high in the kernels of *A. digitata* and *S. birrea*, around 30 to 40% on dry matter basis. The amino acid composition in the kernels was generally good according to comparison with the recommendations for amino acid requirements given by the WHO. This means that the kernels can provide good, cheap sources of protein.

The fat content was below 2% in the fruit pulps, while the fat content in *A. digitata* kernels was almost 40%, and in and *S. birrea* kernels, about 60%. The unsaturated fatty acids constituted about 70 and 80% of the total fat content in *A. digitata* and *S. birrea* kernels, respectively. Interestingly, the *A. digitata* kernels contained the essential fatty acids linoleic and linolenic acid, while the *S. birrea* kernels contained only linolenic acid.

All the studied fruit pulps and kernels contained dietary fibre. The pulp of *A. digitata* had the highest amount of soluble dietary fibre, about 60%, while the *V. infausta* pulp had the highest amount of insoluble dietary fibre, about 40%. The content of dietary fibre was lower in the kernels.

Analysis of the mineral content showed that both pulp and kernels from *A. digitata* contained an appreciable amount of calcium: more than 300 mg/100 g on dry matter basis. The whole seeds (shell + kernel) of *A. digitata* contained as much as 29 mg iron per 100 g. Interestingly, the *S. kraussii* pulp had a high iron content, 9 mg/100 g. The kernels of *A. digitata* and *S. kraussii* had high contents of magnesium, about 600 and 400 mg/100 g, respectively. Comparison with recommended adequate intake shows that the kernels may help to meet the mineral requirements of children and pregnant women in rural areas.

A new analytical method was developed for the determination of organic acids in the fruit pulps. Citric and malic acids were found at various amounts in all pulps together with small amounts of succinic acid. Tartaric acid was not detected in the samples. Large amounts of citric acid were found in *A. digitata* and *L. kirkii*, above 20 g/kg fresh weight. When tasting the fruits, *A. digitata* and *L. kirkii* were the most acid ones. *S. kraussii* had the lowest total content of these organic acids, below 2.2 g/kg.

Phytic acid, which may decrease the absorption of minerals from the diet, was detected in kernels of *A. digitata* and *S. birrea* at concentrations of about 5.5% and 3.4%, respectively. Incubation with phytase reduced the phytic acid content by 20 to 30% after only 15 minutes. Interestingly, almost 50% of the estimated original content of minerals was found in the supernatant after a few minutes' incubation. This suggests that the water used for boiling should be included in the dishes if the kernels are to be used as a dietary supplement.

The findings of this study show that *A. digitata*, *S. birrea* and *V. infausta* pulps can provide large amounts of nutrients, such as minerals and dietary fibre. The kernels of *A. digitata* and *S. birrea*, may help to supply fat, protein and various minerals. A value addition to the wild fruits could be achieved by the development of small scale methods for processing, either individually or in combination with fruits with

different nutrient content or with cereals abundant in the areas. Thus, there is still a great need to continue the chemical analysis of other type of wild fruits that may make a significant contribution to a nutritious diet.

The new data obtained from this study will be vital in efforts to promote the greater use of wild fruits and their kernels, for the estimation of dietary intakes, and the education of local communities with regard to the nutritional benefits of free sources of food in their environment. The findings can also contribute to the domestication of wild fruits trees, and the enhancement of cultural heritage and forests. In addition, the nutritional analysis of the investigated wild fruits can form the basis for the development of further processing methods to extend shelf-life and for the production of other food products.

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Research Article

Proximate Analysis of Five Wild Fruits of Mozambique

Telma Magaia,[1,2] Amália Uamusse,[3] Ingegerd Sjöholm,[4] and Kerstin Skog[2]

[1] *Department of Biological Science, Science Faculty, Eduardo Mondlane University, Praca 25 de Junho, P.O. Box 257, Maputo, Mozambique*
[2] *Division of Applied Nutrition and Food Chemistry, Lund University, P.O. Box 124, 221 00 Lund, Sweden*
[3] *Department of Chemistry, Science Faculty, Eduardo Mondlane University, Praca 25 de Junho, P.O. Box 257, Maputo, Mozambique*
[4] *Division of Food Technology, Lund University, P.O. Box 124, 221 00 Lund, Sweden*

Correspondence should be addressed to Telma Magaia; telma.magaia@food.lth.se

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Mozambique is rich in wild fruit trees, most of which produce fleshy fruits commonly consumed in rural communities, especially during dry seasons. However, information on their content of macronutrients is scarce. Five wild fruit species (*Adansonia digitata*, *Landolphia kirkii*, *Sclerocarya birrea*, *Salacia kraussii*, and *Vangueria infausta*) from different districts in Mozambique were selected for the study. The contents of dry matter, fat, protein, ash, sugars, pH, and titratable acidity were determined in the fruit pulps. Also kernels of *A. digitata* and *S. birrea* were included in the study. The protein content in the pulp was below 5 g/100 g of dry matter, but a daily intake of 100 g fresh wild fruits would provide up to 11% of the recommended daily intake for children from 4 to 8 years old. The sugar content varied between 2.3% and 14.4% fresh weight. The pH was below 3, except for *Salacia kraussii*, for which it was slightly below 7. Kernels of *A. digitata* contained, on average, 39.2% protein and 38.0% fat, and *S. birrea* kernels 32.6% protein and 60.7% fat. The collection of nutritional information may serve as a basis for increased consumption and utilization.

1. Introduction

In Mozambique, a large number of wild food plants are widely distributed throughout the country. The fruits and nuts are sold at informal markets during the harvest season and are consumed in various ways, and they are much appreciated by children [1–3]. The importance of wild fruits in the diet depends to a large extent on the availability of the fruits, since cultivated fruit trees are not particularly common in the dry regions of the country. Depending on the season, the fruits are eaten raw, pressed for juice, cooked with sugar, or used as flour to make porridge; the seeds or nuts are roasted to be eaten as snacks. The choice of fruit species varies according to region and cultural traditions [4].

Many wild fruits and nuts are good sources of carbohydrates, protein, fat, vitamins, and minerals that may be deficient in common diets [5]. There are some reports on the chemical composition of wild fruits from Southern African regions [5–9], but the literature data on the nutritional value of wild fruits in Mozambique is limited [4, 10]. People in many communities are not aware of the nutritional value of the fruits; for example, they often eat only the pulp of the fruits *Sclerocarya birrea* and *Adansonia digitata* while discarding the seeds, which contain a kernel with a higher protein and fat content than peanuts [1, 11].

The aims of this work were to perform a study on traditional utilization of wild fruits in Mozambique and to generate data on the proximate composition and other characteristics of five wild fruits as a basis for the selection of fruits suitable for processing. The long-term goal was to promote and increase the utilisation and consumption of indigenous fruits. The wild fruits selected for the present study were *Adansonia digitata, Landolphia kirkii, Salacia kraussii, Sclerocarya birrea,* and *Vangueria infausta*. These fruits are popular in Mozambique, and they play an important role in the diet, particularly in rural areas.

2. Materials and Methods

2.1. Species. Five wild fruit species were studied: *Adansonia digitata (A. digitata)* (family *Bombacaceae*, local name n'buyu or Malambe), *Landolphia kirkii (L. kirkii)* (family *Apocynaceae*, local name n'vhungwa), *Salacia kraussii (S. kraussii)* (family *Celastraceae*, local name n'phinsha), *Sclerocarya birrea (S. birrea)* (family *Anacardiaceae*, local name n'canhi), and *Vangueria infausta (V. infausta)* (family *Rubiaceae*, local name n'pfilwa). Ripe fruits were collected in 2008 and 2009, except for the fruits from *S. birrea*, which were collected only in 2009. *A. digitata* fruits, grown in the Tete district 1100 km from Maputo city, were bought at a local market in Maputo, and some fruits were collected in family orchards in the Vilankulos district, 700 km south of Maputo. *L. kirkii*, *S. kraussii,* and *V. infausta* fruits were collected in orchards in the Marracuene and Manhiça districts, 30 and 50 km south of Maputo. Fruits from *S. birrea* were obtained from a garden in Maputo city and *S. birrea* kernels, dried for 1–3 months, were obtained from a small family orchard in Manhiça. The fruits were collected in districts where there is an increased occurrence and consumption of them.

2.2. Sample Preparation. Unblemished fruits were selected and washed, the skin and seeds were removed, and the remaining parts were homogenized in a blender to obtain 100 g pulp of each type of fruit. Different numbers of fruit were used depending on fruit size and mass of pulp. The fruits from *A. digitata* had low moisture content and the pulp was ground into a fine powder and sieved (500 μm meshes). The seeds from *A. digitata* and *S. birrea* were crushed and the kernels inside were removed, milled, and sieved (500 μm meshes). Samples for determination of pH and titratable acidity were kept at room temperature and the analyses were performed on the day after collecting the fruit. The samples for the other analyses were vacuum-packed in plastic bags and stored at −18°C in a freezer.

2.3. Analysis. To determine the dry matter content, 2 g samples were dried in an oven at 105°C until constant weight [12]. The samples were weighed before and after drying and the contents of dry matter were calculated. The protein content was determined in an Elementar Analyzer (Flash EA 1112 Series, Thermo Fisher Scientific, Sweden), by means of combustion of 25 mg samples. Aspartic acid (Thermo Fisher Scientific, Delft, The Netherlands) was used as a standard. The amount of protein was calculated by converting the amount of nitrogen by a factor 6.25. The fat content was determined gravimetrically after extracting 1 g samples with petroleum ether (Sigma-Aldrich Chemicals Co., St. Louis, MO, USA) at 40–60°C for 1 hour using a Soxhlet equipment (SoxtecTM 2055, Foss, Höganäs-Helsingborg, Sweden) [13]; rapeseed oil was used as a standard. The ash content was determined by combustion of 2 g samples in silica crucibles in a muffle furnace (Carbolite, Sheffield, England) for 24 hours at 550°C [12]. The pH was determined using a pH meter (Carison GLP 21, serial no. 147012, Barcelona, Spain). Titratable acidity, expressed in percentage of citric acid, was determined after titration of 10 g samples, dissolved in 100 mL water, with 0.1 M sodium hydroxide using phenolphthalein as indicator [12]. All determinations were performed at least in triplicate; the data are expressed as means ± standard deviations. Subsamples of fruits collected in 2009 were sent to an authorized laboratory for analysis of sugar content by high performance anion exchange chromatography (Dionex) with pulsed amperometric detection (HPAEC-PAD). The variation between duplicate determinations was below 15%.

2.4. Statistical Analysis. Statistical analyses were performed using SPSS (version 13). Significant differences were evaluated with one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. A value $P < 0.05$ was considered to be significant.

2.5. Interviews Regarding the Traditional Utilization of Wild Fruits. When the wild fruits were collected in the different districts, local people were interviewed about the traditional use of the fruits. For each fruit, 3–10 people were interviewed, both women and men of different ages, all of whom had grown up in rural areas. The aim of the interviews was to obtain information on knowledge concerning the occurrence of these wild fruit trees, their owners, and traditional habits regarding consumption, processing, and storage of wild fruits.

Examples of the interview questions are as follows. (i) What kind of wild fruits exist in this area? (ii) Who owns and takes care of these fruit trees? (iii) Who are the major consumers? (iv) How are the fruits eaten? (v) What is the typical amount harvested per day? (vi) How are the fruits stored? (vii) What kind of processing can be done?

3. Results and Discussion

The results of the determinations of dry matter content and proximate composition (protein, fat, and ash) for pulp and kernels are summarised in Table 1. The values differed somewhat between the years, but the difference was not significant for any of the fruits. The dry matter content of *A. digitata* pulp was very high, on average 88.5%, while for the other fruits, it varied between 16.7% and 34.8%. The high dry matter content of *A. digitata* was in the same range as has been reported in studies in other countries, 85–95% [5, 7, 8, 14–18]. There is little literature data on the dry matter content of the other fruits, and for *S. birrea* our data agree with one report [7], while one report is showing a lower value [8]. For *V. infausta* our results are somewhat higher than reported in fruits from Malawi [5], Botswana [8], and Tanzania [17]. For *L. kirkii*, the dry matter content was comparable with the literature data [1]. The average dry matter content of *A. digitata* kernels was 92.0%, which is in agreement with other results, ranging from 85% to 97% [1, 7, 9, 14, 15, 18–21]. The average dry matter content of *S. birrea* kernels was 94.3%, which is at the same level as the results from other reports [1, 11, 15, 22, 23].

The protein content of the pulp was low in general (below 5%) for all the fruits. This is in agreement with results of other reports, [1, 5, 7, 15–18]. For *A. digitata*, however, there

Table 1: Dry matter content, protein, fat, and ash expressed in g/100 g dry matter ($n = 3$).

Sample Location_year	Dry matter (%)	Protein	Fat	Ash
Adansonia digitata pulp				
Tete_2008	89.8 ± 0.1	2.4 ± 0.0	0.5 ± 0.1	5.5 ± 0.1
Tete_2009	89.1 ± 0.0	2.2 ± 0.1	0.7 ± 0.6	7.4 ± 0.0
Vilanculos_2009	86.5 ± 0.1	2.1 ± 0.0	0.5 ± 0.1	7.4 ± 0.0
Landolphia kirkii pulp				
Marracuene_2008	27.7 ± 0.2	2.1 ± 0.2	0.9 ± 0.1	2.9 ± 0.0
Marracuene_2009	23.9 ± 0.0	NA	0.4 ± 0.2	3.5 ± 0.0
Manhiça_2009	20.1 ± 0.3	1.7 ± 0.0	1.3 ± 0.1	3.0 ± 0.0
Salacia kraussii pulp				
Marracuene_2008	16.4 ± 0.3	3.7 ± 0.0	0.8 ± 0.5	4.8 ± 0.2
Manhiça_2008	17.3 ± 0.1	2.3 ± 0.0	1.5 ± 0.6	3.4 ± 0.0
Manhiça_2009	16.5 ± 0.1	2.1 ± 0.1	0.7 ± 0.2	3.6 ± 0.0
Sclerocarya birrea pulp				
Manhiça_2009	16.8 ± 0.1	1.4 ± 0.1	0.9 ± 0.3	3.0 ± 0.1
Vangueria infausta pulp				
Marracuene_2008	37.4 ± 0.5	2.9 ± 0.2	0.5 ± 0.8	7.8 ± 0.6
Marracuene_2009	30.0 ± 0.1	2.2 ± 0.3	0.7 ± 0.1	5.3 ± 0.0
Manhiça_2008	37.3 ± 0.9	4.7 ± 0.4	0.7 ± 0.2	5.7 ± 0.4
Manhiça_2009	34.5 ± 0.7	3.3 ± 0.8	0.2 ± 0.0	3.2 ± 0.2
Adansonia digitata kernel				
Tete_2008	93.6 ± 0.0	36.7 ± 0.9	35.0 ± 0.2	7.7 ± 0.0
Tete_2009	91.7 ± 0.1	38.6 ± 0.8	39.9 ± 6.2	7.2 ± 0.0
Vilanculos_2009	90.6 ± 0.0	42.7 ± 0.7	39.0 ± 7.0	8.5 ± 0.2
Sclerocarya birrea kernel				
Manhiça_2008	93.6 ± 0.2	30.1 ± 0.2	58.3 ± 1.3	3.8 ± 0.0
Manhiça_2009	95.0 ± 0.0	35.0 ± 0.1	63.1 ± 0.1	3.5 ± 0.0

is a report showing a much higher protein content, 15.3% [24]. The protein content in the pulp was significantly lower than in the corresponding kernels ($P < 0.05$). *A. digitata* kernels contained on average 39.3% protein and *S. birrea* kernels 32.6%. For *A. digitata*, our results are in accordance with other results [7, 18], but there are also reports showing lower protein content, 13–27% [14, 15, 19, 21], and one report showing higher protein content, 48.3% [20]. The protein content in the kernels of *S. birrea* was at the same level as found in another report [23]. The high protein content in the kernels is at the same levels as reported for soya beans, around 33% [11], which means that the kernels may be a potential source of protein and can be used to improve the diet in rural communities. For example, a daily intake of 100 g of fresh pulp from the wild fruits studied here would provide around 2–11% of the recommended daily intake (RDI) for children from 4 to 8 years old, while 20 g of *A. digitata* or *S. birrea* kernels would provide 32–39% of the RDI for children of the same age [25]. The protein quality of the kernels seems to be good since high amounts of the essential amino acid lysine as well as of arginine, glutamic acid, and aspartic acid have been reported for *A. digitata* kernels [15]. *S. birrea* kernels have been shown to contain high amounts of the essential amino acids phenylalanine, lysine, and threonine [3, 6].

The fat content in the pulp was below 2% for all the fruits. The literature data on *A. digitata* and *S. birrea* pulp generally show fat contents below 1% [1, 7, 14–16, 18], while some reports show a higher fat content in *A. digitata*, 4% [5, 17, 24]. The pulp of wild fruits is typically low in fat and protein [5], while the kernels are good sources of fat and protein [10]. In the present study, the average fat content was 38.0% in kernels of *A. digitata* and 60.7% in kernels of *S. birrea*, and the fat content in the kernels was significantly higher than that in the pulp ($P < 0.05$). Our data on kernels are in agreement with results from other studies [1, 3, 22, 23], while the fat content was lower in two reports [20, 21] and higher in one [18]. The fat quality of the kernels is good according to the literature data: *A. digitata* kernels are rich in palmitic, oleic, and linoleic acid (essential fatty acid) [15], and *S. birrea* kernels are rich in oleic and linoleic acid [3].

The average ash content ranged from 3.0% to 7.8%. For *A. digitata* pulp and kernel, the results are at the same level as in other reports [7, 8, 14–17, 21]. The ash content was somewhat lower than that in some other reports for *S. birrea* [7, 8, 23]

and *V. infausta* pulp [5]. The high ash content indicates that the fruits and kernels may be good sources of minerals.

The pH of the pulps showed an acidic character (around pH 3) except for *S. kraussii*, for which the pH was slightly above 6. The acidic character is in accordance with data on pulps from *A. digitata, S. birrea,* and *V. infausta* [8]. The pH of fruits generally varies between 2.5 and 4.5 due to their content of organic acids [26]; the low pH enhances the microbiological and physicochemical stability [27].

The titratable acidity of the pulp, which contributes to the acidity of the aroma, ranged from 0.6% to 1.7%. In another report, also using citric acid, the titratable acidity was 7.8% for *A. digitata,* 0.9% for *S. birrea,* and 1.7% for *V. infausta* [8]. Comparable data were 0.3% for mango pulp [28] and 0. 7% for orange juice [29].

Table 2 shows the sugar content of the investigated fruits expressed as g sugar/100 g pulp. The highest total sugar content was found in *A. digitata* and *L. kirkii,* 10.3 and 14.4 g/100 g, respectively. The value for *A. digitata* is much lower than that reported in another study, where the total sugar content was around 30% [16]. The highest sucrose content, 4.3 g/100 g, was found in *A. digitata*, while for the other fruits it was lower than 3 g/100 g. As expected, only very low amounts of maltose and lactose, below 0.04 g/100 g, were detected; the most abundant sugars in fruits, are glucose, fructose, and sucrose in various proportions, depending on species [30].

The sugar content, data on pH, and titratable acidity are essential characteristics, indicating the possibility for future use of these wild fruits. The sugar content is important for the development of the aroma and taste, and in product development it is important to find a good balance between pH, sugars, and titratable acidity to receive an optimal taste. The wild fruits in our study have different profiles regarding these characteristics but are in accordance with the literature data on some traditional fruits for juice production, for example, papaya, mango, pineapple, and orange [29–32].

Interviews. The interviews revealed that the majority of the fruits came from the forest and that wild fruits provide food for everyone, especially for children because they are more free to go into the forest to collect fruit. Some people said that in periods of hunger, the leaves, fruits, and seeds are used as food as well as for medical applications. Wild fruits can serve as a source of income for many families, depending on the amount of fruit that each family can reap in the forest or in the area surrounding their house. The fruit is usually sold early in the morning at informal markets to which people come to buy fruit to sell in the markets in the cities.

Different fruits are used in different ways; see Table 3. *A. digitata* fruits are sold in different forms, for example, whole fruit, pulp with seeds embedded, and fine powder made from the pulp packed in plastic bags. The seed-containing pulp is often consumed fresh and soaked in warm water to remove the seeds, and the remaining "milk" can be mixed with sugar to form juice, or boiled with maize flour or sorghum to make a porridge given to children before they go to school. Another way of using the pulp is to dilute it with warm water to prepare a juice, which is filtered, mixed with sugar and packed in small plastic bags and frozen. This sweet ice is commonly sold in informal markets, and it is served as refreshment consumed by both children and adults.

Table 2: Sugar content of selected fruits obtained in 2009 from Manhiça expressed in g sugar/100 g pulp ($n = 2$).

Sample	Dry matter (%)	Glucose	Fructose	Sucrose
Adansonia digitata	88.0	3.0	3.0	4.3
Landolphia kirkii	18.0	7.5	5.7	1.2
Salacia kraussii	9.0	3.7	3.9	<0.1
Sclerocarya birrea	8.0	0.5	0.4	1.4
Vangueria infausta	31.0	1.4	1.4	2.7

The variation between duplicate determinations was below 15%.

Table 3: Traditional consumption and use of the studied fruits.

Species	Utilisation
Adansonia digitata	Pulp: fresh, diluted, and sweetened to juice, frozen to sweet ice, cooked to porridge, and fermented to an alcoholic drink Kernels: fresh or roasted, milled and boiled to sauce or porridge
Landolphia kirkii	Fresh or fermented to an alcoholic drink
Salacia kraussii	Eaten fresh
Sclerocarya birrea	Pulp: fresh, squeezed to juice, and fermented to an alcoholic drink Kernels: fresh or roasted, cooking oil can be extracted
Vangueria infausta	Fresh, soaked, squeezed to juice, mixed with sugar, water, or milk to a porridge, and fermented to an alcoholic drink

The seeds are crushed and the kernels inside can be consumed fresh or roasted. They can be milled to powder and mixed with a small amount of water and boiled with local plant food to make a sauce consumed with boiled maize flour. The seeds are also boiled with a small amount of water or milk to make porridge for children.

People in rural communities usually eat *L. kirkii* fruits fresh, but when large amounts of these fruits are available, they are squeezed and fermented to produce a local alcoholic drink that is consumed at social gatherings. Rural people of all ages eat *L. kirkii* fruit while walking to and working on their grassland and cattle farm plots, which are sometimes far away from their homes.

The fruits from the small *S. kraussii* bushes are more easily accessible to children. Many school children eat the fresh fruits on their way to and from school or while grazing cattle.

Fresh fruit from *S. birrea* is squeezed to make juice or fermented to produce a popular alcoholic drink. After juice extraction and fermentation, the juice may be stored in sealed clay pots or plastic containers for up to a year. The kernels can be eaten fresh or roasted or ground in a mortar together

with water, boiled with local plant food to make a sauce. The kernels can also be used to produce oil for cooking.

In the southern part of Mozambique, *V. infausta* fruits are commonly consumed fresh, as juice, but they are also often fermented to produce alcoholic drinks. Fresh fruit is soaked in water, and the skin and seeds discarded before the preparation of a juice which is mixed with water and sugar or milk and served as porridge for children. Excess fruit is dried and stored for later use.

4. Conclusion

The wild fruits studied are consumed by people living in different districts of Mozambique and form a part of their normal diet. Our data on the proximate composition, pH, titratable acidity, and sugar content are consistent with the few reports available in the literature. We observed low but not significant variations between the growth locations and the harvest year, and these variations may be due to differences in climate, soil, and weather conditions. The findings of this study have shown that the analyzed fruits, and especially the kernels, are good sources of protein and fat. In Mozambique malnutrition is responsible for one-third of deaths in children under five years, and based on the above results, it may be concluded that promotion of consumption and processing of these fruits, to various products, may help to improve the diet and alleviate nutrient deficiencies.

Conflict of Interests

The authors declare that they have no conflict of interests.

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Paper II

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Dietary fiber, organic acids and minerals in selected wild edible fruits of Mozambique

Telma Magaia[1,3*], Amália Uamusse[2], Ingegerd Sjöholm[3] and Kerstin Skog[3]

Abstract

The harvesting, utilization and marketing of indigenous fruits and nuts have been central to the livelihoods of the majority of rural communities in African countries. In this study we report on the content of dietary fiber, minerals and selected organic acids in the pulps and kernels of the wild fruits most commonly consumed in southern Mozambique. The content of soluble fiber in the pulps ranged from 4.3 to 65.6 g/100 g and insoluble fiber from 2.6 to 45.8 g/100 g. In the kernels the content of soluble fiber ranged from 8.4 to 42.6 g/100 g and insoluble fiber from 14.7 to 20.9 g/100 g. Citric acid was found in all fruits up to 25.7 g/kg. The kernels of *Adansonia digitata* and *Sclerocarya birrea* were shown to be rich in calcium, iron, magnesium and zinc. The data may be useful in selecting wild fruit species appropriate for incorporation into diets.

Keywords: Wild fruits, Minerals, Citric acid, Dietary fiber, Daily intake

Background

Fruits are generally recognized as essential for health optimisation, with human health depending to a large extent on factors such as high fruit and vegetable consumption (Ibrahim 2011). Deficiencies of essential micronutrients found in fruits can increase the risk of illness or death from infectious diseases by reducing immune and non-immune defenses and by compromising normal physiology and development (Black 2003). Such nutrient deficiencies are highly prevalent in low and middle income countries.

Recent research has shown that a wide range of indigenous fruit trees have the potential to provide rural households with a means to meet their nutritional and medicinal needs (Ekesa et al. 2009). In the past decades several reports have been published on the nutritional composition of wild fruits and vegetables growing in different areas in various African countries and on the effect they could have on combating malnutrition and poverty in the continent (FAO 2011). In June 2008, the European Commission authorised the placing on the market of dried pulp of one wild fruit, Baobab (*Adansonia digitata*), as a novel food ingredient (Commission Decision 2008). Three components are of special importance for determining whether such ingredients are health-enhancing: the kind and amount of minerals, organic acids and dietary fiber.

Minerals are of great importance in the diet, although they comprise only 4–6% of human bodyweight. Some minerals or macro elements required in amounts greater than 100 mg per day represent 1% or less of bodyweight (Insel et al. 2011; Imelouane et al. 2011). The essential macro elements include calcium, phosphors, magnesium, potassium, sodium, sulfur and chloride. Essential trace elements such as zinc, iron, copper, manganese, selenium, iodine and molybdenum are normally required in amounts of less than 100 mg per day, making up less than 0.01% of the bodyweight (Imelouane et al. 2011).

Dietary fiber is increasingly viewed as an essential aspect of good nutrition. Intake of dietary fiber alters the water content, viscosity, and microbial mass of the intestinal contents, resulting in changes in the rate and ease of passage through the intestine (Elleuch et al. 2011). High intake of dietary fiber plays a significant role in weight control and the prevention of several diseases. For example, dietary fiber improves glucose tolerance, by delaying the transport of carbohydrates into the small intestine, reducing the risk of heart diseases and reduces constipation (Anderson et al. 1994; Rodríguez et al.

* Correspondence: Telma.Magaia@food.lth.se
[1]Department of Biological Science, Science Faculty, Eduardo Mondlane University, PO Box 257, Maputo, Mozambique
[3]Department of Food Technology, Engineering and Nutrition, Lund University, PO Box 124, Lund SE-221 00, Sweden
Full list of author information is available at the end of the article

2006). Organic acids are involved in human growth, maturation and senescence (Al-Farsi et al. 2005). The organic acids influence organoleptic properties such as flavor, color and aroma and are responsible for many characteristic fruity tastes. They increase shelf life, stability and microbiologic safety (Hasib et al. 2002; Loredana et al. 2006; Nour et al. 2010).

In a previous study we reported on the proximate composition of five wild fruits of Mozambique. *Adansonia digitata, Landolphia kirkii, Salacia kraussii, Sclerocarya birrea* and *Vangueria infausta* were evaluated for pH and titratable acidity and their content of dry matter, fat, protein, ash, soluble solids and sugar content (Magaia et al. submitted). The present study was carried out to further analyze the nutritional potential of these fruits. The dietary fiber, organic acids and mineral content of the five wild fruits and selected seeds were determined with the plan to highlight their potential as an effective means to combat macro and micronutrient deficiencies, especially in children.

Results

The contents of insoluble and soluble dietary fiber in the fruits are presented in Table 1. All pulps and kernels contained dietary fiber, but with large variations in concentration among the different fruits. In *S. kraussii* and in *V. infausta*, the content of insoluble dietary fiber ranged from 2.6 g/100 g to 45.8 g/100 g in the pulps, and from 14.7 to 20.9 g/100 g in the kernels, respectively. The content of insoluble dietary fiber in *A. digitata*

Table 1 Insoluble and soluble dietary fiber content expressed on the basis of dry matter (n=3)*

Fruit part, Location_year	Dry matter	Insoluble dietary fiber (g/100 g)	Soluble dietary fiber
Adansonia digitata pulp			
Tete_2008	86.4±0.5	14.2±0.4	60.3±1.9
Tete_2009	86.5±0.1	14.7±0.0	65.6±0.6
Vilankulos_2009	85.6±0.5	16.1±0.8	57.3±0.3
Adansonia digitata kernels			
Tete_2008	92.7±0.3	17.4±3.4	14.0±0.6
Tete_2009	91.7±0.1	20.9±1.3	17.0±3.4
Vilankulos_2009	90.1±0.1	14.7±0.0	42.6±1.8
Adansonia digitata whole seed			
Tete_2009	98.2±0.0	56.5±3.1	15.9±1.5
Vilankulos_2009	96.4±0.0	62.0±1.2	16.3±0.6
Landolphia kirkii pulp			
Marracuene_2008	26.9±0.1	3.5±0.6	4.6±0.1
Marracuene_2009	23.9±0.2	4.9±0.8	4.3±0.3
Manhica_2009	20.4±0.2	4.9±0.4	5.8±1.2
Salacia kraussii pulp			
Marracuene_2008	16.1±037	3.3±0.1	6.0±0.4
Manhica_2008	14.0±0.7	2.6±1.2	7.6±1.0
Manhica_2009	16.5±0.2	5.5±0.3	7.1±0.4
Sclerocarya birrea pulp			
Manhica 2009	16.7±0.0	7.7±1.7	10.5±0.9
Sclerocarya birrea kernels			
Manhiça 2008	93.6±0.2	17.6±2.9	10.5±2.7
Manhiça 2009	95.0±0.0	18.5±1.5	8.4±2.4
Vangueria infausta pulp			
Marracuene_2008	34.9±0.2	45.6±1.4	24.3±1.4
Marracuene_2009	30.0±0.1	45.8±0.9	26.3±0.9
Manhica_2008	27.9±0.2	41.0±0.8	10.6±0.8
Manhica_2009	34.5±0.7	30.9±1.9	23.1±1.9

* ± indicates standard deviation.

pulp was significantly higher than that in *L. kirkii* and *S. kraussii*. The soluble dietary fiber in the pulps ranged from 4.3 g/100 g in *L. kirkii* up to 65.6 g/100 g in *A. digitata*. The content of soluble dietary fiber in *A. digitata* pulp was significantly higher than in the other fruit pulps.

A chromatogram from the analysis of organic acids in *S. kraussii* is shown in Figure 1. The peaks corresponding to the different acids are indicated by numbers. The calibration curve was linear within the concentration ranges used for the analysis.

The results of the determination of organic acid content based on wet weight are shown in Table 2. Fruits collected in Manhiça, 2009 were used for this analysis. Citric acid was found in all fruits, with the highest contents in *A. digitata* (25.7 g/kg) and *L. kirkii* (21.5 g/kg). Malic acid was detected at concentrations between 0.4 g/kg and 2.1 g/kg. Succinic acid was found at concentrations of 0.1 g/kg or below. Tartaric acid was detected at trace levels only in *S. birrea*.

The mineral contents in the fruit pulps, kernels and seeds, expressed as mg/100 g dry matter, are presented in Table 3. The relative standard deviation of the mean values was generally below 5%. The content in different fruit samples varied considerably, from undetectable levels of selenium up to 2753 mg/100 g for potassium (*S. birrea* pulp). The richest source of calcium was *A. digitata* both for pulp (308 to 366 mg/100 g) and for kernels (293 to 347 mg/100 g.) The iron content in the pulps and kernels generally ranged from 1.0 - 4.0 mg/100 g, while *S. kraussii* pulp contained 9.0 mg/100 g, and the whole seeds of *A. digitata* contained 29 mg /100 g. The highest content of magnesium was found in the kernels of *A. digitata* (626 to 706 mg/100 g) and *S. birrea* (396 – 436 mg/100 g). Selenium was not analyzed in all fruits, but was detected in *V. infausta* (1.5 mg/100 g). The richest source of zinc was the kernels of *A. digitata* (5.2 – 5.7 mg/100 g) and *S. birrea* (4.5 mg/100 g).

Discussion

The literature provides little data on dietary fiber in the fruits investigated and we have found no data on soluble or insoluble dietary fiber. Our data thus contributes substantially to knowledge about dietary fiber content in wild fruits. The analyses conducted showed that all fruits selected for the study contained both soluble and insoluble dietary fiber, and the total amount of dietary fiber in the pulps ranged from around 10 to 80 g/100 g dry matter (Table 1), thus indicating their potential for improving nutritional content in the local diet.

The largest amount of dietary fiber was found in *A. digitata* pulp. The amount 80.3 g/100 g was much higher than in previous studies, which ranged from 5.4 g/100 g in fresh weight to 45 g/100 g in dry weight (Saka and Msonthi 1994; Lockett et al. 2000; Murray et al. 2001; Osman 2004). The high content of dietary fiber in the present study, of which 80% was soluble dietary fiber, could thus provide a substantial contribution to total dietary intake. For example, the consumption of 20 g *A. digitata* pulp can supply 42 – 52% of the recommended daily intake (RDI) for children from 4 to 13 years of age and also for pregnant women (National Research Council 2005). The high content of soluble dietary fiber could also help to control serum cholesterol levels, reduce other risk factors for cardiovascular disease (Van Duyn and Pivonka 2000), and reduce appetite and caloric intake (Anderson et al. 1994).

V. infausta pulp also contained large amounts of total dietary fiber (on average 62 g/100 g), of which more than half was insoluble dietary fiber. Several studies have shown that intake of fruits rich in insoluble dietary fiber benefits weight control and health of the large intestine; the insoluble fiber decreases intestinal transit time and increases fecal bulk (Al-Farsi et al. 2005; Hamilton et al. 1992). Consumption of 100 g *V. infausta* pulp can supply up to 40% of RDI for children and pregnant women (National Research Council 2005). In a report on die-

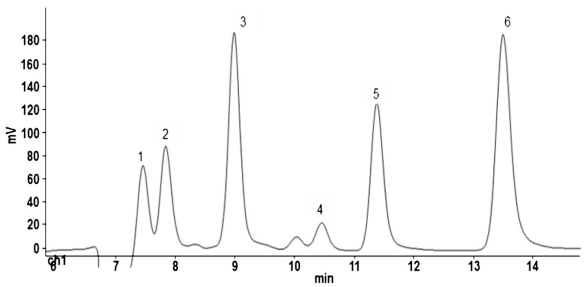

Figure 1 Chromatogram from the analysis of organic acids in *Salacia kraussii*. The peaks correspond to 1- citric acid, 2- tartaric acid, 3- malic acid, 4- succinic acid, 5 and 6 unknowns.

Table 2 Organic acid content in fruit pulp expressed on the basis of wet weight (n = 3)*

Sample	Citric acid	Tartaric acid	Malic acid (g/kg)	Succininc acid
Adansonia digitata	25.7±2.1*	ND	1.6±0.2	0.1±0.0
Landolphia kirkii	21.5±2.1	ND	0.4±0.1	0.03±0.0
Salacia kraussii	0.9±0.3	ND	1.1±0.1	0.1±0.0
Sclerocarya birrea	8.5±1.3	trace	1.2±0.2	0.1±0.0
Vangueria infausta	6.2±0.5	ND	2.1±0.2	0.1±0.0

* ± indicates standard deviation, ND= not detected.

tary fiber in *V. infausta*, the content of acid detergent lignin was reported to be 35.5% (fresh weight), acid detergent fiber 39.5% and neutral detergent fiber 39.4% (Amarteifio and Mosase 2006). The discrepancies between our findings and previous research may be due to the difficulty in comparing data on dietary fiber when it isn't clear whether figures refer to dry or fresh weight. Furthermore, different analytical methods may have been used in different studies, including different types of dietary fiber.

Our data on the amounts of dietary fiber in *S. birrea* pulp were about half of that found in another study determining dietary fiber after extraction of fat by a gravimetric method, 37.7 g/100 g (Murray et al. 2001). In a report where AOAC method was used, the amount of acid detergent lignin was 13.7% (fresh weight), acid detergent fiber 16.3% and neutral detergent fiber 16.1% (Amarteifio and Mosase 2006).

The total amount of dietary fiber in *L. kirkii* pulp was low (10 g/100 g) compared with that of the other fruits in this study, but very similar to that of *L. oweriensis*, of the same family (Effiong and Udo 2010). The amount of dietary fiber in *S. kraussii* pulp was similar to *L. kirkii*. The amounts of dietary fiber in *S. krausii*, and *L. kirkii* pulps are in the same range as in for example avocado, 6.7 g/100 g, and guava, 12.7 g/100 g (Li et al. 2002).

The whole seeds of *A. digitata* contained large amounts of insoluble dietary fiber. These seeds are often crushed into a powder in rural cooking and mixed with other ingredients to make a sauce. Increased use of this local custom may thus help to increase the intake of dietary fiber.

In the kernels of *A. digitata* the total content of dietary fiber was on average 42.2 g/100 g, which is higher than that found in other studies (Murray et al. 2001).

Table 3 Mineral content in the pulps, kernels and whole seeds of the studied wild fruits in Mozambique

Fruit part, Location_year	Dry matter g/100 g	Ca	K	Fe	Mg	Na mg/100 g	P	S	Se	Zn
Adansonia digitata pulp										
Tete_2008	90.1	326	2308	2.0	162	5.0	40	51	nd	-
Tete_2009	86.4	308	2392	2.0	129	2.0	40	41	-	0.5
Vilankulos_2009	86.5	366	2360	1.0	102	5.0	30	43	-	0.6
Adansonia digitata kernels										
Tete_2009	92.2	293	1416	6.0	626	1.0	1229	263	-	5.7
Vilankulos_2009	90.6	347	1451	4.0	706	2.0	1518	269	-	5.2
Adansonia digitata whole seed										
Tete_2008	93.6	220	1238	29	360	2.0	523	131	nd	-
Landolphia kirkii pulp										
Manhiça 2009	20.4	28	1840	4.0	51	21	55	20	-	1.4
Salacia kraussii pulp										
Manhiça 2009	8.9	127	2056	9.0	207	35	153	191	-	0.6
Sclerocarya birrea pulp										
Manhiça 2009	15.9	201	2753	3.0	138	30	178	90	-	.0.7
Sclerocarya birrea kernels										
Manhiça 2008	92.7	60	622	4.0	436	7.0	871	284	nd	-
Manhiça 2009	95.0	81	531	4.0	396	6.0	719	269	-	4.5
Vangueria infausta pulp										
Manhiça 2009	34.4	90	1249	3.0	65	18	92	45	2.0	-

The relative standard deviation was in general < 5%, nd = Not detectable; - = not analyzed.

The kernels of *S. birrea* contained similar amounts of insoluble dietary fiber as *A. digitata* kernels, but the amount of soluble dietary fiber was lower. There is no report in the literature about dietary fiber of *S. birrea* kernels. However, in cashew nuts and peanuts, which are commonly consumed Mozambique, the total dietary fiber content was 3.9 g/100 g for cashew nuts and 5.2 g/100 g for peanuts in fresh weight (de Oliveira Sousa et al. 2011).

The effects of dietary fiber cannot, however, be considered in isolation. Although dietary fiber provides many health benefits, it may affect mineral absorption negatively due to the capacity of the fibers to bind cations (López and Martos 2004). The ability of dietary fiber to interfere with iron absorption is especially negative for human nutrition (Reinhold et al. 1981). However, citric and malic acids in fruits promote iron absorption (Gillooly et al. 1983), and improve iron solubilization (López and Martos 2004).

Fortunately, citric and malic acid were found in all fruits in this study (Table 2). Citric acid ranged from 0.9 g/kg of wet weight basis in *S. Kraussii* to more than 20 g/kg in *A. digitata* and *L. kirkii.* For malic acid the results were lower and ranged from 0.4 g/kg to 2.1 g/kg. Succinic acid was found in low amounts in all fruits, around 0.1 g/kg, and tartaric acid was detected in trace levels in *S. birrea*. No data in the literature are available on organic acids in the selected wild fruits; however, one type of wild fruit called medlar (*Mespilus germanica L.)* was reported to contain around 4 g/kg fresh weight of citric acid and malic acid (Glew et al. 2003). For comparison, the citric acid concentrations in some traditional fruits are: pineapple (2.2 g/kg), orange (4.5 g/kg), grapes (13.1 g/kg) and lime (41.2 g/kg) (Falade et al. 2003).

Although there were large variations in the mineral content in the different fruits (Table 3), we did not observe any pronounced difference between growth place or harvest year, and the small differences found may be explained by soil, climate and weather conditions.

The highest amounts of calcium from the fruits selected in the present study were found in *A. digitata* pulp and kernel, around 300 mg/100 g dry matter. The amounts are at the same levels as in other reports (Osman 2004; Glew et al. 1997) but higher than in some studies (Saka and Msonthi 1994; Lockett et al. 2000; Amarteifio and Mosase 2006; Eromosele et al. 1991; Sena et al. 1998). *S. birrea* pulp contained 201 mg/100 g, which is about half the amount reported in the literature (Glew et al. 1997), but higher than that in other reports (Amarteifio and Mosase 2006; Eromosele et al. 1991). Calcium is an important factor in bone health, and a high intake is recommended particularly during pregnancy and infancy (Insel et al. 2011) calcium together with phosphor, magnesium and potassium is important for growth and maintenance of bone, teeth and muscle (Insel et al. 2011) and bone metabolism (Ilich et al. 2003; New 2003; Bonjour et al. 2009).

High amounts of phosphor were found in the kernels: *A. digitata* kernels contained up to 1500 mg/100 g, which is four times higher than that in another study (Nnam and Obiakor 2003). Regarding the pulp, there are reports showing higher phosphor content than in our study, for example 452 mg/100 g in *A. digitata* (Sena et al. 1998) and 264 mg/100 g in *S. birrea* (Glew et al. 1997).

High content of potassium, more than 2000 mg/100 g, was found in pulps from *A. digitata, S. kraussii* and *S. birrea*. For *A. digitata*, this is in agreement with results from other studies (Saka and Msonthi 1994; Amarteifio and Mosase 2006), while some reports show lower values. For *S. birrea*, our data agree with other results (Amarteifio and Mosase 2006). The content of potassium in *V. infausta* pulp was on the same level as in one report (Amarteifio and Mosase 2006) but almost 7 times greater than in another report (Saka and Msonthi 1994). The potassium content in kernels of *S. birrea* was somewhat higher than that in another report (Glew et al. 2004). In the whole seeds of *A. digitata,* potassium is higher than in other reports (Osman 2004; Nkafamiya et al. 2007). Potassium, together with sodium, regulates muscle contraction and nerve impulse transmission, and a high potassium/sodium ratio may assist the excretion of excessive salt and water (Arthey et al. 2001). All fruits in our study had low amounts of sodium and thus the potassium/sodium ratio was high in the fruits.

The whole seeds of *A. digitata* had extremely high iron content, 29 mg/100 g, while data in the literature range from 1.83 to 6.36 mg /100 g (Lockett et al. 2000; Osman 2004; Glew et al. 1997; Nkafamiya et al. 2007). *S. kraussii* had the highest iron content of the pulp, 9 mg/100 g. In the other pulps it was 2 to 4 mg/100 g. In the literature, the amounts of iron in *A. digitata* ranged from 0.1 to 9.3 mg/100 g (Saka and Msonthi 1994; Lockett et al. 2000; Amarteifio and Mosase 2006; Glew et al. 1997; Eromosele et al. 1991; Sena et al. 1998; Glew et al. 2004) and in *S. birrea* from 0.07 to 2.49 mg/100 g (Amarteifio and Mosase 2006; Glew et al. 1997; Eromosele et al. 1991). For *V. infausta* there is one report showing 0.09 mg /100 g (Amarteifio and Mosase 2006) and one showing 28.3 mg/100 g (Saka and Msonthi 1994). Very high iron content, 129 mg/100 g dry matter, was reported in *L. oweriensis*, which is a fruit in the same family as *L. kirkii* (Effiong and Udo 2010). Iron is necessary for the transport of oxygen in the blood to the cells and for supplying the body with energy, for immune function and nerve health and in addition, it is a cofactor in numerous reactions (Insel et al. 2011).

The kernels of *A. digitata* had the highest magnesium content, around 600 – 700 mg/100 g. This is higher than the magnesium content in other kernels or seeds. For example dried pumpkin seeds contain 540 mg/100 g, linseed 392 mg/100 g and sunflower seeds 355 mg/100 g (National Food Agency 2012). Our data on whole seeds are at the same level as in other reports for *A. digitata* (Lockett et al. 2000; Osman 2004; Glew et al. 1997) and for *S. birrea* (Glew et al. 2004). Magnesium is of great importance for cardiac and nerve function, is involved in more than 300 biochemical reactions in the body and is involved in energy metabolism and protein synthesis (Insel et al. 2011).

The zinc content in the kernels of *A. digitata* and *S. birrea* was around 5 mg/100 g, which is in agreement with literature for *S. birrea* (Glew et al. 2004) and higher than previous data for *A. digitata* (Nnam and Obiakor 2003). Zinc increases the affinity of hemoglobin for oxygen, participates in taste perception and interacts with a number of hormones. In addition, the body needs zinc to grow and develop properly during pregnancy, infancy, and childhood (Brown et al. 1999; Insel et al. 2011).

The results of the mineral analysis can be compared with the RDI for children 4–13 years of age and pregnant women 19–30 years of age (National Research Council 2005). For example, 100 g of fresh *A. digitata* pulp can contribute on average 23% of the iron and 30% of the calcium RDI for children (4–13 years) and almost 29% of the calcium for pregnant women. Furthermore, 100 g *S. birrea* pulp can contribute 13% of the magnesium RDI for children (4–13 years) and 33% for pregnant women, and almost 41% of zinc requirements. Consumption of 60 g *A. digitata* kernels can supply around 30% of iron, almost 50% of zinc and more than 100% of magnesium RDI for children.

Conclusion

New data on dietary fiber, organic acids and mineral content have been obtained for five wild fruits and selected seeds. The highest content of dietary fiber was found in *A. digitata* pulp. It was also found that fresh *A. digitata* pulp can contribute a large amount of the iron and calcium RDI for children and that kernel of *A. digitata* and *S. birrea* can contribute significantly to the magnesium and zinc requirements for pregnant women. Thus some of the fruits and kernels studied show large potential to reduce mineral deficiencies in local diet especially in children. The research results highlight the significance of wild edible fruits as a cheap source of nutrients and the benefits of increasing the use these species as dietary supplements. Initiatives should be put in place to promote consumption and domestication of edible indigenous fruit: to improve the nutritional and health status of women and children, contributing to income generation and stimulating rural economic development.

Materials and methods

Fruit samples

Five fruits were used for the analysis: *Adansonia digitata (A. digitata)* (Fam. Bombaceae, local name n'buvu or malambe), *Landolphia kirkii (L.kirkii)* (Fam. Apocynaceae, local name wungwa), *Salacia kraussii (S. kraussii)* (Fam. Celastraceae, local name phinsha), *Sclerocarya birrea (S. birrea)* (Fam. Anacardeaceae, local name canhi) and *Vangueria infausta (V. infausta)* (Fam. Rubiaceae, local name pfilwa). Approximately 5 kg of each fruits were collected in January to July in 2008 and 2009 in four districts of Mozambique, with the exception of the fruits from *Sclerocarya birrea*, which were collected only in 2009. Unblemished fruits were selected and washed, the skin and seeds were removed and the remaining parts (pulp) were homogenized in a blender to obtain 100 g pulp of each type of fruit. Different numbers of fruit were used, depending on size and mass of pulp. Seeds from *Adansonia digitata* and *Sclerocarya birrea* were crushed and the kernels were removed, milled and sieved. The dry matter content in the pulps was determined immediately. Fruit pulps and kernels for analysis of dietary fibre, organic acids and minerals were vacuum packed in plastic bags and stored at −18°C.

Chemicals

Chemicals and solvents were of analytical grade. Sulphuric acid, nitric acid, hydrochloric acid, sodium di-hydrogen phosphate, di-sodium hydrogen phosphate; sodium hydroxide, ethanol and acetone were from Fluka (Sigma-Aldrich, Steinheim, Germany). Pepsin (2000FIP U/g) obtained from Merck (Darmstadt, Germany), pancreatin from Fluka (Sigma-Aldrich, Steinheim, Germany), and celite were used for the analysis of dietary fibre. Lithium chloride, organic acid standards (citric, malic, tartaric and succinic) were from Merck (Darmstadt, Germany).

Analysis

Dry matter

To determine the dry matter content, 2 g samples were dried in an oven at 105°C until constant weight (AOAC 2000 method 920.151). The samples were weighed before and after drying and the contents of dry matter were calculated. All determinations were performed in triplicate.

Dietary fiber

The content of total dietary fiber was first analyzed using an enzymatic gravimetric method, and then divided into fractions of either soluble dietary fiber (SDF) or insoluble dietary fiber (IDF) (Asp et al. 1983). All experiments were

performed in triplicate. The sample (0.5-1.0 g) was suspended in a phosphate buffer and hydrochloric acid was added to adjust the pH to 1.5. The sample was digested by pepsin for 60 min at +4°C and then the pH was adjusted to 6.8. Pancreatin was added and the sample was incubated for 60 minutes at 40°C and the pH was adjusted to 4.5. The solution was filtered and the insoluble residue was washed with distilled water, 95% and 99% of ethanol and then dried overnight at 105°C, cooled and weighed (IDF). The filtrate was precipitated with hot 95% ethanol and filtered, washed with ethanol (78%, 95% and 99%), dried, cooled and weighed (SDF). The results were corrected for protein and ash contents.

Organic acids

Citric, malic, succinic and tartaric acids were analysed using ion exchange chromatography (Metrohm International, CH-9101, Herisau, Switzerland) with inverse suppression and conductivity detection. Reference samples of the organic acids (Merck, Darmstadt, Germany) were injected via a 20 μl loop onto a column (250 mm × 7.8 mm, 9 μm, polystyrene/divinylbenzene copolymer, Metrosep 6.1005.200, Switzerland) and eluted isocratically at a flow rate of 0.5 mL min-1 and a pressure 3.8 MPa. The suppressor system was regenerated by pumping a solution of 10 mM LiCl, together with Millipore water, through the system. Different ratios of the mobile phase (0.5 mM sulphuric acid and acetone) were tested, as well as different column temperatures (30, 40 and 50°C). The optimal conditions for separation of the organic acids were 0.5 mM sulphuric acid and acetone (85:15, v/v) and 30°C. Stock standard solutions (1 mg/mL) of the organic acids were prepared and kept at +4°C. Different dilutions of the stock solutions were used for the calibration curves. Fruits collected in 2009 in Manhiça were used for this analysis. About 500 mg fruit pulps were mixed with 5 ml sulphuric acid (Ultra Turrax TP18/10) for 2 minutes and then centrifuged at 2000 rpm for 15 minutes. The supernatants were filtered through 0.45 μm membrane filters and diluted with Millipore water to appropriate concentrations. The retention time and peak areas were compared with reference samples run under the same conditions and used to calculate the concentration of the organic acids in the fruit samples. All experiments were performed at least in triplicates.

Minerals

Selected samples of the pulps, seeds and kernels were prepared for determination of mineral content. The samples were digested in a microwave oven with concentrated nitric acid and then analysed by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES, Perkin Elmer, OPTIMA 3000 DV). The experiments were performed in duplicate.

Statistical analyses

The results of the dietary fibre were subjected to analysis of variance. Differences between means were tested at 5% probability by Turkey's test, using SPSS program (version 13).

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

TM performed most of the experimental work, (apart from the mineral analysis), took part in the evaluation of the results and wrote the manuscript. All authors participated in the design of the study, supervised the field work and data collection and took part in the evaluation of the results. All authors read and approved the final manuscript.

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Author details

[1]Department of Biological Science, Science Faculty, Eduardo Mondlane University, PO Box 257, Maputo, Mozambique. [2]Department of Chemistry, Science Faculty, Eduardo Mondlane University, PO Box 257, Maputo, Mozambique. [3]Department of Food Technology, Engineering and Nutrition, Lund University, PO Box 124, Lund SE-221 00, Sweden.

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Paper III

Composition of amino acids, fatty acids and dietary fibre monomers in kernels of *Adansonia digitata* and *Sclerocarya birrea*

Telma Magaia[1,2] and Kerstin Skog[1]

[1] Department of Food Technology, Engineering and Nutrition, Lund University, PO Box 124, SE-221 00 Lund, Sweden
[2] Department of Biological Science, Science Faculty, Eduardo Mondlane University, PO Box 257, Maputo, Mozambique
Corresponding author: Telma Magaia, e-mail: Telma.Magaia@food.lth.se

Abstract

There is increasing worldwide demand for sources of energy and non-meat protein with balanced amino acid profiles. Nuts are generally rich in protein and essential amino acids, and have a high energy value due to their high fat content. Kernels from two wild fruits in Mozambique, *Adansonia digitata* and *Sclerocarya birrea*, were selected for this study based on their amino acid and fatty acid composition, as well as the monomeric composition of their dietary fibre. Information on these compounds in the kernels of these fruits is scarce in the literature. The fatty acid composition differed between the two kernels, but both are rich in unsaturated fatty acids. The dominating fatty acids in *Adansonia digitata* kernels were palmitic, linoleic and oleic acid; their contents varying from 25.7% to 34.9% of the total fatty acids. In *Sclerocarya birrea* kernels the main fatty acid was oleic acid (72.4%), found together with small amounts of linoleic, stearic and palmitic acid (6.8 to 12.1%). All common amino acids were detected in both types of kernels; glutamic acid being the most abundant amino acid, comprising more than 20% of the protein. The contents of essential amino acids in the kernels were compared with the amino acid requirements stated by the WHO for children aged 3-10 years. The findings indicate that *A. digitata* and *S. birrea* kernels can provide good, cheap sources of protein, especially when combined with foods with high lysine content. The results of this study show

that the intake of these kernels can help provide the fatty acids and amino acids required in the daily diet, especially for people living in rural areas. The results can be used for intake estimates, and to encourage increased consumption and utilization of these kernels.

Key words: Wild fruit, nutrients, consumption, Mozambique, seeds

Introduction

Wild fruits in Mozambique are an important source of food for rural people. Especially the seeds and kernels add essential nutrients to the diet, and are available when other foods are scarce [1]. The main staple foods in Mozambique are cassava, maize, beans, sorghum and rice. Cereals and starchy roots provide almost 80% of the dietary energy supply, while the contribution from pulses (mainly beans), nuts and oil crops is 5%. However, the diet in rural areas is poor in fat, protein and micronutrients, and does not supply enough energy to meet nutritional requirements [2].

Fat is an important component in the diet as it provides the body with energy in a concentrated form. In addition, dietary fats provide essential fatty acids and fat-soluble vitamins. Linoleic acid (an omega-6 fatty acid) and alpha-linolenic acid (an omega-3 fatty acid) are the essential fatty acids required in the diet [3]. Among the food sources of essential fatty acids are fish, soya oil, seed oils, kernels and nuts. Protein is another important component in the diet. Inadequate supply of protein is considered to be responsible for malnutrition among people living in developing countries. The content of essential amino acids determines the protein quality and the extent to which the protein can satisfy the amino acid requirements [4].

Kernels from wild fruits are commonly consumed in the southern and central parts of Mozambique, where a variety of edible fruits can be found. The kernels offer a convenient and cheap means of providing fat, protein, minerals and other health-promoting components to people living in rural areas [5]. We recently studied the content of fat, protein, dietary fibre and minerals in some wild fruits and kernels of Mozambique, and found that kernels of *Adansonia digitata* (baobab) and *Sclerocarya birrea* (marula) had high contents of fat, protein and dietary fibre [6]. Kernels of *Adansonia digitata* are highly appreciated in the diet, and are eaten roasted as a snack, or used for oil extraction. They are also used in soups or mixed with wild spinach or other food [7]. Kernels of *Sclerocarya birrea* can be dried and eaten alone, or cooked and, for example, eaten together with a mixture of dried peanut extracts, red pepper,

salt and other spices [8]. Literature data on the nutritional components of the kernels of *Adansonia digitata* and *Sclerocarya birrea* are scarce. The aims of the present study were, therefore, to interview a number of people to obtain information on how these kernels are used, and to measure the contents of amino acids, fatty acids and dietary fibre components in the kernels. It is important to have information on the nutritional composition of the kernels to be able to estimate adequate nutrient intake and to promote increased utilization and consumption of the kernels.

Materials and Methods

Samples

Kernels from two wild fruit species were studied: *Adansonia digitata (A. digitata)* (Fam. *Bombacaceae*, local name n´buyu or malambe), and *Sclerocarya birrea (S. birrea)* (Fam. *Anacardiaceae,* local name n´canhi). Seeds were collected in 2013 in districts where they are commonly consumed. Seeds (5 kg) from *A. digitata* were collected in family orchards in the Changara district, 95 km from the city of Tete. *S. birrea* seeds (1 kg), dried for two to three months, were obtained from a small family orchard in the Manhiça district, 50 km from Maputo. The seeds were crushed and the shells removed and the kernels inside were vacuum-packed in plastic bags and stored at -18ºC in a freezer. Before analysis, *A. digitata* kernels were milled in a coffee grinder (TEFAL, Type 8100, Prep'Line, China) and sieved (500 µm mesh). *S. birrea* kernels were ground with a mortar and pestle.

Chemicals

All chemicals were of analytical grade. Water was passed through a Milli-Q purification system. Amino acid standards, DL-norleucine (internal standard) and phenol, were purchased from Sigma Chemical Co. (St. Louis, USA), and lithium buffers and ninhydrin from Biochrom Ltd (Cambridge, UK). Sodium di-hydrogen phosphate, di-sodium hydrogen phosphate, sodium hydroxide, ethanol and acetone were purchased from Fluka (Sigma-Aldrich, Steinem, Germany). For the analysis of dietary fibre, pepsin was obtained from Merck (Darmstadt, Germany), pancreatin from Fluka (Sigma-Aldrich), and galacturonic acid, chloroform, monohydrate and myo-inositol (internal standard) from Sigma Chemical Co. The standards rhamnose, fucose, arabinose, xylose, mannose, galactose and glucose were obtained from Merck,

together with hydrochloric acid, sulphuric acid, acetic acid, acetic acid anhydride, ammonium hydroxide, potassium hydroxide, sodium sulphate, potassium borohydride and 1-methylimidazol.

Analysis

Fatty acids in the samples were analysed by an authorised laboratory using gas chromatography [9]. Determinations were performed in duplicate, and the difference between the determinations was generally below 3%. The results are expressed in g/100 g fat.

About 5 g of each type of kernel was used for the analysis of amino acids. The samples were defatted using acetone in the proportions 1:10 (w/v), stirred for one hour and ultra-centrifuged at 18 000 rpm for 20 minutes. The samples were left to evaporate overnight at room temperature. Triplicate samples (35 mg) of kernels were put into glass tubes, 5 ml 6 N HCl containing 0.1% phenol was added, and the samples were hydrolysed at 110°C for 24 hours [10, 11]. The samples were then centrifuged at 4000 rpm for 10 minutes, after which 2 ml of the supernatant was transferred to a round-bottomed flask and 100 µl of the internal standard (DL-norleucine 22.9 µmol/ml) was added. The samples were evaporated at 40°C to dryness. Lithium citrate buffer (5 ml 0.2 M, pH 2.2) was then added, and the solutions were mixed for a few minutes, transferred to Eppendorf tubes with a 0.45 micron inner vial, and centrifuged at 13 000 rpm for 10 minutes. The samples were analysed using an amino acid analyser (Biochrom 30 series Amino Acid Analyser, Biochrom Ltd). The method is based on ion-exchange chromatography with post-column derivatization using ninhydrin. The injection volume was 20 µl. The column (Physiological Fluid High Performance, 200 x 4.6 mm, Biochrom Ltd) was eluted with lithium citrate buffers of pH 2.80, 3.00, 3.15, 3.50 and 3.55 at a flow rate of 25 ml/hour. Post-column derivatization was performed with ninhydrin at a flow rate 20 ml/hour. The temperature of the reaction coil was 135°C. In the reaction coil, ninhydrin reacts with the amino acid present in the eluent and forms conjugated compounds with absorbance maxima at 570 and 440 nm. The internal standard was used to calibrate the amino acid response. Identification was confirmed by comparing the retention times of the peaks with those of a standard solution of amino acids run under the same conditions. All determinations were made in triplicate.

The protein content in the defatted and untreated samples was determined using an elemental analyser (Flash EA 1112 Series, Thermo Fisher Scientific, Sweden) by means of combustion of 25 mg samples. Aspartic acid was used as a standard. The amount of protein was calculated by multiplying the amount of nitrogen by a factor 5.7. Determinations were performed in duplicate. The results of the amino acid analysis are reported as g amino acid/100 g protein.

The dry matter content was determined after drying 2 g samples in an oven at 105 °C until constant weight [9]. The determinations were performed in triplicate, and the

dry matter content was found to be 92.8 ± 0.0% for *A. digitata* and 95.8 ± 0.1% for *S. birrea*.

The dietary fibre in the kernels was separated into soluble and insoluble fractions, and their monosaccharide compositions were analysed with gas chromatography (HP6890 Hewlett Packard, with a flame ionization detector) and the uronic acids with Visible spectrophotometry (Pharmacia Biotech Novaspec II model 80-2088-64, Sweden) using D-galacturonic acid monohydrate as a reference [12, 13].

Statistical analysis

Microsoft® Excel was used for the statistical evaluation. Student's t-test was performed to determine significant differences. A value of $p<0.05$ was considered to indicate statistical significance.

Interviews regarding traditional use of the kernels studied

When the seeds and kernels were collected in the different districts, local people were interviewed about common uses. The aim of the interviews was to obtain information on major consumers, how often the kernels were used for consumption, and examples of food preparation methods.

Results

The total fat content in the kernels was 31.7 ± 0.0% for *A. digitata* and 49.4 ± 3.7% for *S. birrea*. The results of the determination of the fatty acid compositions of the kernels are presented in Table 1, together with the fatty acid compositions of olive oil and peanuts for comparison. The unsaturated fatty acids constituted about 68 and 80% of the total fat content in *A. digitata* and *S. birrea* kernels, respectively. The most abundant fatty acids in *A. digitata* kernels were palmitic acid, linoleic acid and oleic acid; their contents varying from 25.7 to 34.9%. In *S. birrea* kernels, oleic acid constituted 72.4% of the fatty acids and linoleic, stearic and palmitic acid (6.8 to 12.1%). The difference in fatty acid composition between the kernels was significant for palmitic acid, oleic acid and linoleic acid ($p<0.05$).

A chromatogram from the analysis of amino acids *in A. digitata* is shown in Figure 1.

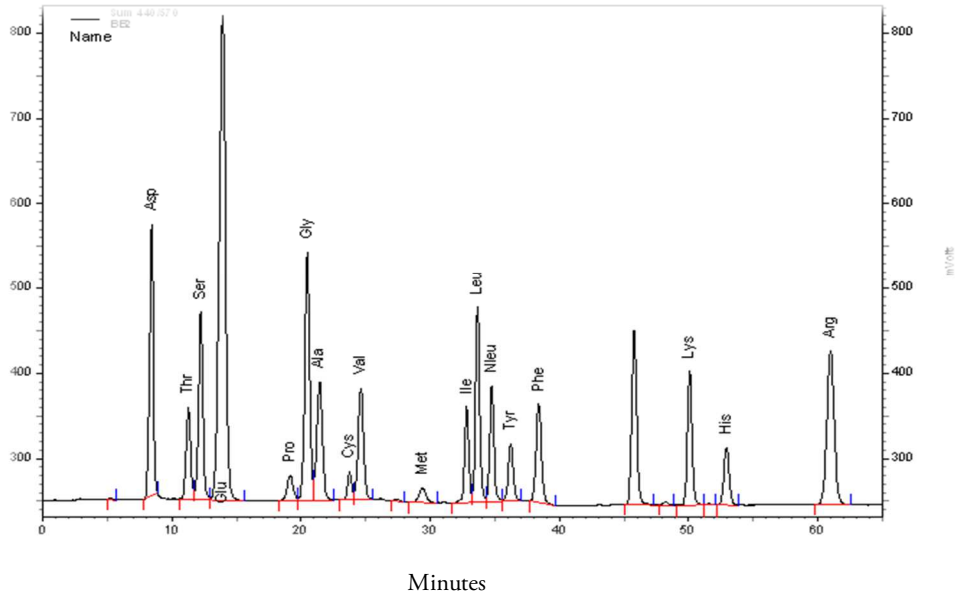

Figure 1: Chromatogram from amino acid analysis of *Adansonia digitata* kernels. The peaks corresponding to the different amino acids are indicated.

The calibration curve was linear for the concentration range used in the analysis. Under acidic hydrolysis conditions, tryptophan is totally destroyed, while asparagine and glutamine are hydrolysed quantitatively to aspartic acid and glutamic acid, respectively. Cysteine is readily oxidized to cysteic acid, and methionine to methionine sulphoxide, which is partly lost during hydrolysis.

Table 2 gives the amino acid compositions of the *A. digitata* and *S. birrea* kernels. All common amino acids except tryptophan were detected. Glutamic acid was the most abundant amino acid in both types of kernels, comprising more than 20% of the total amino acid content, followed by arginine. The differences between the contents of the various amino acids in the kernels were not significant. The protein content was 35.0 ± 0.0% in *A. digitata* and 29.2 ± 0.0% in *S. birrea*.

After separation of the dietary fibre into soluble and insoluble fractions, the contents of monosaccharides and uronic acids were determined. The calibration curves for both components were linear over the concentration range used in the analysis. The results are given in Table 3. The compositions of the monomers (neutral

sugars) in the dietary fibre fractions differed between the kernels. The main constituent in the insoluble fraction of A. digitata kernels was glucose, followed by arabinose and uronic acids, while in the soluble fraction arabinose was the dominating component, followed by uronic acids. In kernels of S. birrea, uronic acids constituted more than 90% of both dietary fibre fractions.

From the interviews, it was found that traditionally, the seeds of *A. digitata* are crushed, boiled and mixed with local plant food to make a sauce consumed with boiled maize flour. The seeds are also boiled with a small amount of water or milk to make porridge for children. The kernels of *S. birrea* were said to be delicious, adding flavour to the dishes. They are eaten as a snack, sometimes roasted, ground, mixed with water and local plant food and boiled to make a sauce. The kernels are used instead of peanuts in time of drought or in combination with peanuts. The kernels can also be crushed to produce oil for cooking.

Discussion

New nutritional data have been obtained for two commonly consumed kernels of wild fruits in Mozambique. Both types of kernels form part of the diet in rural areas and are highly appreciated, crushed, boiled and mixed with local plant foods. There are few reports on the nutritional value of these kernels, but our data and literature data show that the quality of both fat and protein is good. Differences between reported analytical data are probably due to the use of different analytical methods, and variability in the raw materials in terms of growth location, weather conditions, maturity, handling and storage.

Our study shows that the fatty acid composition differed between the two kernels, in agreement with previous reports. For *A. digitata* kernels our results are in agreement with some recent reports [14, 15], but somewhat higher than in older reports [16, 17]. For *S. birrea* our results agree with literature data [18-21]. Both *A. digitata* oil and *S. birrea* oil are rich in monounsaturated fatty acids, and have been reported to be very stable [18, 20, 21]. *A. digitata* oil is very stable with a shelf life estimated to be between 2 and 5 years [22].

Interestingly, the fatty acid compositions of *S. birrea* kernels and olive oil are comparable; both having high contents of the monounsaturated fatty acid oleic acid, (>70%). Olive oil is generally regarded as having a good fat quality [3]. The consumption of monounsaturated fatty acids has been associated with decreased levels of low-density lipoprotein cholesterol and possibly increased high-density lipoprotein

cholesterol [23]. However, its ability to raise high-density lipoprotein cholesterol is still being debated.

The fatty acid compositions of *A. digitata* kernels and peanuts correspond well, especially with regard to oleic acid and the essential linoleic acid. In addition, kernels of *A. digitata* contained low amounts, ~2%, of the essential linolenic acid. These essential fatty acids have many functions in the body, and are of importance, for example, in the immune system, cell membranes and for the function of the brain and skin [24]. They may also reduce the risk of heart disease [25].

Some authorities, e.g. the Food and Nutrition Board of the US Institute of Medicine, have established adequate intake (AI) levels, in grams, for omega-6 and omega-3 fatty acids [25], while the European Food Safety Authority (EFSA) and the World Health Organization (WHO) recommend an acceptable range of omega-3 and omega-6 fatty acid intake related to the energy intake [26, 27]. Table 4 gives the estimated contribution to the AI for omega-6 and omega-3 fatty acids from the consumption of 40 g kernels. It can be seen that 40 g *A. digitata* kernels can cover the daily intake of omega-6 fatty acids for those aged 4 to 13 years, and provide about 90% of the requirement for pregnant women. The same quantity of *A. digitata* kernels provides 60-93% of the AI of omega-3 fatty acids for the same groups. The contribution from 40 g *S. birrea* kernels is lower, and to reach the AI levels an intake of around 160 g kernels is needed. However, these kernels may not be the only source of essential fatty acids in the diet.

Table 4 also gives the AI levels of protein and the contribution from the intake of 40 g kernels. For children aged 4 to 8 years, 74 and 61% of the AI is covered by *A. digitata* kernels and *S. birrea* kernels, respectively. For older children and pregnant women, the contribution is less, but it may be possible for these groups to increase their intake of kernels, especially if they are eaten as a snack. Furthermore, these two groups generally eat larger portions of dishes containing protein.

The amino acid profiles given in Table 2 generally agree with reports on amino acids in whole seeds of *A. digitata* [14, 15] and kernels of *S. birrea* [28, 29]. However, our data for the S-amino acids are lower. This may be explained by partial breakdown of these amino acids during hydrolysis. Regarding the other essential amino acids, our results for lysine and valine in *A. digitata* are lower than in one previous report [14], and our findings regarding leucine, lysine and threonine in *S. birrea* are also lower [29]. It is interesting to note that the amino acid composition in the two kernels studied is comparable to that in peanuts and walnuts [30], which commonly form part of the diet.

Ideally, the protein in the diet should provide the body's requirement of all the essential amino acids, in the same relative proportions. The contents of essential amino acids in the kernels are compared with the amino acid requirement stated by

the WHO for children aged 3-10 years [31] in Table 5. Histidine is regarded as being essential for children, but not for adults. It should be noted that, according to the WHO, the sum of phenylalanine and tyrosine is important, although tyrosine is a non-essential amino acid. The amounts of methionine + cysteine were lower than the requirement in both kernels, but it is reasonable to believe that the real content is higher than the analytical value, due to losses during sample preparation, as described above. The amount of lysine is lower than that required in both *A. digitata* and *S. birrea* kernels, while in *S. birrea* kernels leucine is also lower than required. However, the total amino acid composition indicates that *A. digitata* and *S. birrea* kernels can provide good, cheap sources of protein, especially if combined with foods with higher contents of lysine.

In conclusion, our findings indicate that kernels of *A. digitata* and *S. birrea* form part of the diet in rural areas in Mozambique, and that they are good sources of protein and essential amino acids, as well as fat and essential fatty acids. They are rich in unsaturated fatty acid, and *A. digitata* kernels contain appreciable amounts of essential fatty acids, while the content in *S. birrea* is lower. We also conclude that the kernels of *A. digitata* and *S. birrea* have a potential as sources of essential nutrients and can contribute to the nutritional needs of various communities. Furthermore, the results can be used for the estimation of dietary intake and to encourage increased consumption and use of these wild fruit kernels.

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Table 1. Fatty acid composition in kernels of *A. digitata* and *S. birrea* (mean ± standard deviation, n=2). The contents in olive oil and peanuts are given for comparison

Fatty acid		***A. digitata***	***S. birrea***	**Olive oil**[1]	**Peanuts**[1]
No. of carbon atoms and double bonds	**Systematic name**	(g/100 g fat)			
C14:0	Myristic	0.2 ± 0.0	n.d.[2]	-	0.4
C16:0	Palmitic	25.7 ± 0.2	12.1 ± 0.4	11.1	10.4
C16:1	Palmitoleic	0.2 ± 0.0	0.2 ± 0.0	1.2	0.0
C17:0	Margaric	0.2 ± 0.0	0.1 ± 0.0	-	-
C17:1	Margaroleic	0.2 ± 0.0	n.d.	-	-
C18:0	Stearic	4.6 ± 0.1	7.3 ± 0.1	2.7	2.2
C18:1	Oleic	34.9 ± 0.7	72.4 ± 0.6	70.7	48.3
C18:2	Linoleic[3]	29.9 ± 0.9	6.8 ± 0.4	8.1	31.7
C18:3	Linolenic[3]	2.1 ± 0.0	n.d.	0.7	0.0
C20:0	Arachidic	0.9 ± 0.1	0.6 ± 0.0	-	0.0
C20:1	Eicosenoic	0.2 ± 0.0	0.3 ± 0.0	-	-
C22:0	Behenic	0.3 ± 0.0	0.1 ± 0.0	-	-
C22:1	Erucic	0.1 ± 0.0	n.d.	-	-
C24:0	Lignoceric	0.2 ± 0.0	0.2 ± 0.0	-	-

[1] Data recalculated from The Swedish National Food Administration's food database, version 28/02/2014.

[2] n.d. = not detected

[3]Essential fatty acid

Table 2. Amino acid composition in kernels of *A. digitata* and *S. birrea*
(mean ± standard deviation, n=3). The contents in peanuts and walnuts are given for comparison

Amino acid	*A. digitata*	*S. birrea*	Peanuts[1]	Walnuts[1]
		(g/100 g protein)		
Cysteine*	0.5 ± 0.0	0.7 ± 0.0	1.3	2.4
Histidine*	2.2 ± 0.1	2.5 ± 0.2	2.5	2.5
Isoleucine*	3.4 ± 0.2	3.9 ± 0.3	3.5	3.9
Leucine*	6.0 ± 0.4	4.3 ± 1.3	6.5	6.4
Lysine*	3.4 ± 1.0	2.9 ± 0.2	3.6	2.7
Methionine*	0.8 ± 0.1	0.7 ± 0.1	1.2	1.9
Phenylalanine*	4.5 ± 0.3	4.6 ± 0.3	5.2	4.4
Threonine*	2.8 ± 0.1	2.8 ± 0.2	3.4	3.4
Valine*	4.5 ± 0.3	5.0 ± 0.4	4.2	5.0
Alanine	3.8 ± 0.3	3.7 ± 0.3	3.9	4.2
Arginine	11.4 ± 0.6	13.9 ± 1.1	12.0	14.6
Aspartic acid	8.1 ± 0.4	8.4 ± 0.5	12.2	10.2
Glycine	4.4 ± 0.3	4.9 ± 0.3	6.0	5.2
Glutamic acid	23.7 ± 1.3	25.7 ± 1.8	20.8	19.5
Proline	3.2 ± 0.2	3.5 ± 0.2	4.4	3.8
Serine	4.6 ± 0.3	2.9 ± 0.3	4.9	5.4
Tyrosine	2.5 ± 0.1	4.7 ± 0.2	4.1	3.0
Total	89.8	95.9	99.7	98.5

[1] Data recalculated from USDA, Nutrient Database for Standard Reference, release 27, accessed on 22/11/ 2014.
* Essential amino acid

Table 3. Monomeric composition of dietary fibre in kernels of *A. digitata* and *S. birrea* (mean ± standard deviation, n=2)

	A. digitata		***S. birrea***	
	(%)			
	Insoluble dietary fibre	Soluble dietary fibre	Insoluble dietary fibre	Soluble dietary fibre
Rhamnose	1.9	2.6	n.d.	n.d.
Fucose	n.d.	n.d.	n.d.	n.d.
Arabinose	25.9	37.6	3.9	1.3
Xylose	11.1	5.5	3.2	1.3
Mannose	3.0	0.4	n.d.	n.d.
Galactose	5.6	9.8	n.d.	1.2
Glucose	37.6	11.1	10.5	n.d.
Uronic acids	14.8	33.0	82.4	96.2

n.d. = not detected

Table 4. Adequate intakes (AI) of essential fatty acids and protein for different life stage groups and contribution to the AI from the consumption of 40 g *A. digitata* or *S. birrea* kernels

			A. digitata	*S. birrea*
Nutrient	Life stage group	AI (g/day)[1]	Contribution (% of AI)	
Omega-6 fatty acids	Children 4 to 8 years	10	120	27
	Males 9 to 13 years	12	100	23
	Females 9 to 13 years	10	120	27
	Pregnant women	13	92	21
Omega-3 fatty acids	Children 4 to 8 years	0.9	93	-
	Males 9 to 13 years	1.2	70	-
	Females 9 to 13 years	1.0	84	-
	Pregnant women	1.4	60	-
		19	74	61
Protein	Children 4 to 8 years			
	9 to 13 years	34	41	34
	Pregnant women	71	20	16

[1] Data from Food and Nutrition Board, 2002 [25]

Table 5. Essential amino acids in kernels of *Adansonia digitata* and *Sclerocarya birrea* compared with the WHO protein requirement for children aged 3-10 years old.

Amino acid	***A. digitata***	***S. birrea***	**WHO**[1]
		(g/100 g protein)	
Histidine	2.2	2.5	1.6
Isoleucine	3.4	3.9	3.0
Leucine	6.0	4.3	6.1
Lysine	3.4	2.9	4.8
Methionine + cysteine	1.3	1.4	2.3
Phenylalanine + tyrosine	7.0	9.3	4.1
Threonine	2.8	2.8	2.5
Valine	4.5	4.9	4.0

[1] Data from WHO, 2007 [31]

Paper IV

Effect of heating, lactobacillus fermentation and enzyme treatment on the content of phytic acid in kernels of *Adansonia digitata* and *Sclerocarya birrea*

Telma Magaia[1,2], Ingegerd Sjöholm[1], Estera Dey[3] and Kerstin Skog[1]

[1] Department of Food Technology, Engineering and Nutrition, Lund University, PO Box 124, SE-221 00 Lund, Sweden
[2] Department of Biological Science, Science Faculty, Eduardo Mondlane University, PO Box 257, Maputo, Mozambique
[3] Division of Pure and Applied Biochemistry, Lund University, PO Box 124 SE-221 00 Lund, Sweden

Corresponding author: Telma Magaia e-mail: Telma.Magaia@food.lth.se

Abstract

Adansonia digitata and *Sclerocarya birrea* are wild trees found in many African countries, the leaves, fruits and kernels of which are commonly eaten in rural areas of Mozambique. The kernels contain high amounts of several minerals, for example iron, magnesium and zinc. However, these kernels have been shown to contain phytic acid, which may decrease the absorption of minerals from the diet, especially zinc and iron, and to a lesser extent, also calcium and magnesium. In this study we have analysed the content of phytic acid in kernels from *Adansonia digitata* and *Sclerocarya birrea* using high-performance ion chromatography. Our results give the first data on contents of phytic acid in these kernels from Mozambique. The results show that the amount of phytic acid in the kernels can be reduced by various processing techniques such as boiling or autoclaving followed by incubation with *Lactobacillus plantarum* or by enzyme (phytase) treatment. The content of phytic acid was lower after autoclaving than after boiling. Treatment with phytase reduced the phytic acid

content by 20-30% after only 15 minutes. Around half the content of Fe, Mg and Zn in the kernels was found in the supernatant after 15 minutes' enzyme treatment.

Key words: Wild fruits, Mozambique, phytase, high-performance ion chromatography

1.0 Introduction

Kernels from wild fruits are commonly eaten in rural areas of Mozambique. They provide essential nutrients to the diet and are available in the dry season when other foods are scarce (Jama et al., 2008; Mangue and Oreste, 1999). The kernels from the *Adansonia digitata* (*A. digitata*) fruit are commonly eaten fresh or roasted, or they can be dried and ground into flour which can be added to soups and stews as a thickener, roasted and ground into a paste, or boiled for a long time, fermented and then dried for use (De Caluwé et al., 2009; Nnam and Obiakor, 2003). Kernel sauce is prepared by (optional) roasting of the kernels followed by grinding, and the resulting product is used as protein concentrates in tomato sauces or other spiced sauces (Chadare et al., 2009). The kernels from the *Sclerocarya birrea* fruit (*S. birrea*) are very tasty and widely eaten. They can be dried and eaten alone, or cooked and eaten together with a mixture of dried peanut extract, red pepper, salt and other spices in the form of a "meat bundle" (Glew et al., 1997).

In a previous study (Magaia et al., 2013), we found that kernels of *A. digitata* and *S. birrea* contained high amounts of several minerals, for example, iron, magnesium and zinc, as has also been reported in other studies (Emmanuel et al., 2011; Glew et al., 2004; Oyeleke et al., 2012; Wairagu et al., 2013). However, these kernels have been shown to contain phytic acid (for literature references, see Table 4), which may decrease the absorption of minerals from the diet (Holzapfel and Schillinger, 2002). Phytic acid, also known as inositol hexaphosphate, has six PO_4 groups and is a strong chelator to divalent ions, especially zinc and iron, and to a lesser extent, also calcium and magnesium (Greiner and Konietzny, 1998). Phytic acid is the principal storage form of phosphorus in many plant tissues, especially bran and seeds (Anastasio et al., 2010).

Removal of phosphate groups from the inositol ring decreases the mineral binding strength of phytic acid and thus improves the nutritional value (Kumar et al., 2010). Domestic food preparation techniques, such as boiling, can reduce the phytic acid content to some extent, but soaking in an acid medium, lactic acid fermentation, sprouting, and the use of enzymes (phytase) are more effective methods (Greiner and

Konietzny, 2006; Liang et al., 2008; Mahgoub and Elhag, 1998; Sharma and Sehgal, 1992). Fermentation, either spontaneous or with a starter culture, is a simple and efficient means of reducing the phytic acid content in foods. At suitable pH (4.8 to 5.6) native plant phytase is activated, which removes phosphate groups from the phytic acid (Reale et al., 2007). Microbial phytase can be produced by *Lactobacillus* bacteria and the optimal pH is 5 to 6 (Greiner and Konietzny, 2006; Sandberg and Svanberg, 1991). *Lactobacillus* strains have been reported to produce extracellular phytase (Sreeramulu et al., 1996).

Results from preliminary experiments (data not shown) indicated that the content of phytic acid was about 5.5% in kernels of *A. digitata* and 3.4% in *S. birrea* kernels. This prompted us to study how the content of phytic acid in kernels of *A. digitata* and *S. birrea* could be influenced by heating, fermentation with *Lactobacillus* bacteria or incubation with phytase. Furthermore, we determined the content of some minerals in the water solution after the enzyme treatment.

2.0 Materials and Methods

2.1 Samples

Kernels from two wild fruit species were studied: *A. digitata* (Fam. *Bombacaceae*, local name n´buvu or malambe), and *S. birrea* (Fam. *Anacardiaceae*, local name n´canhi). The kernels, collected in 2013, were sub-samples from another study (Magaia et al, Unpublished results). The hard shell of the *A. digitata* fruit was removed by crushing and the kernels were collected, vacuum-packed in different batches and stored at -18°C in a freezer. Before analysis, *A. digitata* kernels were milled in a coffee grinder (TEFAL, Type 8100, PreP'Line, China) and sieved (500 µm mesh), and *S. birrea* kernels were ground with a mortar and pestle.

2.2 Chemicals

All chemicals were of analytical grade and de-ionised water was used for all experiments. Hydrochloric acid and $Fe(NO_3)_3 \cdot 9H_2O$ were obtained from Fluka (Sigma-Aldrich, Steinheim, Germany) and sodium phytate from BDH Chemicals Ltd (UK). Wheat phytase (0.01-0.004 units/mg) was obtained from Sigma Chemical Co. (Stockholm, Sweden). For the experiments with *Lactobacillus* bacteria, the chemicals were obtained from Merck (Darmstadt, Germany), Sigma-Aldrich Co. (Steinheim,

Germany), and VWR-International (Stockholm, Sweden), and *Lactobacillus plantarum* 299v (DSM 9843, Probi AB, Lund, Sweden) from the local pharmacy.

2.3 Processing

The samples were subjected to boiling or autoclaving followed by fermentation with *Lactobacillus* bacteria or incubation with phytase without pre-treatment.

In the first series of experiments, one capsule of *Lactobacillus plantarum* 299v was suspended in sterile MRS medium and incubated for 24 hours at 37°C. The cells were washed 3 times with small portions of 0.9% (w/v) NaCl and centrifuged at 4000 rpm for 10 min. *L. plantarum* cells were suspended in 50 ml 0.9% (w/v) NaCl and subjected to serial dilution. From each dilution tube 0.1 ml was plated onto MRS plate count agar. After incubation for 48 hours at 37°C, the number of colonies was counted to evaluate the cell level in the undiluted suspension; the result being 12 x 10^8 colony-forming units (CFU) per ml. To test the influence of *L. plantarum* on the phytic acid level in the kernels, 1 g sample was dissolved in 5 ml distilled water, and the samples were first either boiled or autoclaved for 15 minutes to inactivate possible intrinsic phytase. When the samples had cooled down, they were inoculated with 1 ml bacteria solution (12 x 10^8 CFU/ml) and fermented for 48 hours at 37°C. After fermentation the pH had decreased slightly to between 4 and 5. The samples were freeze-dried for later phytic acid analysis.

In the second series of experiments, a stock solution of phytase (10 mg/ml) was prepared. Samples of 1 g kernel were suspended in 9 ml water and the pH adjusted to 5 (optimal pH for phytase). Then 1 ml of the stock solution was added and the samples were incubated in a water bath at 55°C for 2, 15, 30, 60 and 240 minutes. In addition, one sample was dissolved in 5 ml water and 5 ml of the stock solution. After enzymatic incubation, the samples were immediately boiled for 5 minutes. As a control, a sample without phytase was boiled for 5 minutes. The samples were cooled and half of the samples were freeze-dried and stored at -18°C until phytic acid analysis. The other samples were centrifuged at 18000 rpm for 20 minutes, and selected supernatants were sent to the department of Ecology at Lund University, Sweden for determination of the mineral content.

2.4 Analysis

The dry matter content was determined after drying 2 g samples in an oven at 105°C until constant weight (AOAC, 2000). The determination was performed in triplicate.

Phytic acid was analysed as inositol hexaphosphate (Carlsson et al., 2001). Duplicate samples (0.5 g) were extracted with 20 ml 0.5 M HCl for 3 hours at 20°C under magnetic stirring. The extracts were frozen overnight, thawed and centrifuged at 12000 rpm for 10 minutes, and an aliquot (300 µl) of the supernatant was injected into a high-performance ion chromatography (HPIC) instrument (Waters Associates Inc, Milford, MA) equipped with a guard-column (PA-100, 4 x 50 mm i.d., Dionex Corp., Sunnyvale, CA, USA) and an analytical column (HPIC OmmiPac PA-100, 4 x 250 mm i.d.). The column was eluted isocratically with 80% HCl (1 M) and 20% water. Inositol hexaphosphate was identified and quantified after post-column reaction with $Fe(NO_3)_3 \cdot 9H_2O$, and the absorbance was monitored at 290 nm (Waters 486, tunable absorbance detector). Sodium phytate was used as an external standard. The determinations were performed in duplicate.

The mineral content in the supernatant was determined after the addition of 65% nitric acid, corresponding to 1% of the sample volume. The samples were filtered and an aliquot was analysed using inductively coupled plasma-atomic emission spectrometry (ICP-AES, Perkin Elmer, OPTIMA 3000 DV). The experiments were performed in duplicate.

2.5 Statistical analysis

Microsoft® Excel was used for statistical evaluation. Student's t-test was performed to determine significant differences. A value of $p<0.05$ was considered to indicate statistical significance.

3.0 Results

A chromatogram from the analysis of phytic acid in *A. digitata* kernels after phytase treatment is shown in Figure 1. The retention time for the peak corresponding to the inositol hexaphosphate was around 5 minutes. The calibration curve was linear for the concentration range used in the analysis.

The results of the determinations of phytic acid in the first series of experiments with boiling and autoclaving followed by *Lactobacillus* fermentation are given in Table 1, where it can be seen that the content of phytic acid is lower after autoclaving than after boiling. The reduction in the amount of phytic acid after fermentation was significant for the autoclaved samples, but not for the boiled samples.

Table 2 gives the amount of phytic acid in the kernels after treatment with phytase for different times. For *A. digitata* kernels, the phytic acid content after 2 minutes'

incubation was only somewhat lower than in the untreated kernels, but a slight decrease was observed after 15 minutes. This value was maintained up to 60 minutes. However, after 4 hours' incubation, the content had decreased to around 63% of the original value, and this decrease was significant. Similar results were obtained for *S. birrea* kernels, and after 4 hours' incubation the phytic acid content was only about 42% of the original value; this decrease was significant. The lowest phytic acid contents were seen after 60 minutes' incubation with a 5-fold higher enzyme concentration, when the value decreased significantly to 48% and 33% of the initial value in *A. digitata* and *S. birrea* kernels, respectively.

The results of the analysis of the supernatant of some of the incubated samples regarding their contents of iron, magnesium and zinc are given in Table 3. The results show that the amount of minerals in the supernatant was higher than in the samples not incubated, apart from zinc for the *A. digitata* sample. The value for zinc was unexpectedly high and could not be explained. The time for enzyme incubation did not seem to have a major influence on the mineral content, although there was a tendency for the longest incubation time to result in an increase in the concentration of magnesium in the supernatants from both kernels.

4.0 Discussion

This study is part of an on-going investigation of some wild fruits and kernels in Mozambique and the contents of compounds that are important from a nutritional point of view. Few data are available in the literature on phytic acid in the kernels studied here, and are summarized in Table 4. We have not found any data of phytic acid in these kernels from Mozambique. This means that our data will make a substantial contribution to the knowledge concerning phytic acid content in kernels from these two wild fruits. It is difficult to compare literature data on phytic acid as the results are sometimes expressed in terms of fresh weight, and sometimes based on dry matter content, while in other cases no information is given about fresh or dry weight. Furthermore, different analytical methods may have been used, which are sometimes not clearly described. The differences may also be explained to some extent by harvest year, location, climate and weather conditions.

Based on the analyses of phytic acid conducted using HPIC, our results for heated *A. digitata* kernels (3.6 – 4.4 g/100 g) generally agree with the results reported from other studies using other methods (Table 4). Our results are, however, lower than results from previous studies (Adubiaro et al., 2011; Mitchikpe et al., 2008) and higher than in others (Ezeagu, 2005; Nkafamiya et al., 2007; Proll et al., 1998;

Saulawa et al., 2014). In addition, there is one report showing markedly lower values of phytic acid (Osman, 2004). Our results regarding the amounts of phytic acid in heated *S. birrea* kernels (1.8 – 2.2 g/100 g) are around five times higher than that found in another study (Muhammad et al., 2011). It is interesting to note that the phytic acid content in peanuts, which are commonly consumed, has been reported to be around 2 g/100 g (Lazarte et al., 2015).

The addition of *L. plantarum* bacteria had a significant effect on the phytic acid content in the kernels which had been autoclaved before incubation. A slight reduction of the of phytic acid content in *A. digitata* seeds was reported after 72 hours fermentation (Nnam and Obiakor 2003) by the microflora on the seeds. *Lactobacillus* strains have been reported to produce extracellular phytase (Sreeramulu et al., 1996), which can reduce the phytic acid content (Reale et al., 2007).

During soaking or enzyme incubation, phytic acid is transferred to the surrounding liquid. The addition of phytase to the medium reduced the phytic acid content in the supernatants by 40-60% after 4 hours' treatment, but a five-fold higher enzyme concentration was more efficient. Thus future experiments should include different ratios of solids to liquid and different enzyme concentrations.

Results from a previous study showed that the phytic acid content in *A. digitata* kernel flour decreased by 65.57% after boiling for 1 hour, and changing the water at 20 minutes' intervals (Saulawa et al., 2014). This also suggests that the ratio of solid to liquid is an important factor for the reduction of phytic acid in the kernels. Furthermore, this indicates that boiling for prolonged period may decrease the phytic acid content. Changing the water may reduce the content of important minerals, as we observed that the amounts of iron, magnesium and zinc in the supernatant increased after enzyme incubation, and this would probably also be the case in boiling. As can be seen from Table 3, almost 50% of the original mineral content can be found in the supernatant after only a few minutes' enzyme incubation, apart from zinc in *A. digitata* (20%).

In this study, we observed a weak relation between the content of phytic acid and minerals in the supernatant of the enzyme-treated samples, although the incubation time had no significant effect on the mineral content. One explanation of this could be that part of the enzyme was bound to the kernel matrix, reducing the effect.

4.1 Conclusions

New data on the content of phytic acid in kernels from two wild fruits have been obtained. The results show that the presence of phytic acid in the kernels can be reduced by various processing techniques. The addition of bacteria had significant

effect on the phytic acid content in the autoclaved kernels. Enzyme treatment reduced the phytic acid content by 20-30% after 15 minutes, and a higher enzyme concentration was found to be more efficient. Almost 50% of the estimated original content of minerals was found in the supernatant after a few minutes' enzyme incubation. This suggests that the water used for boiling should be included in the dishes if the kernels are to be used as a dietary supplement.

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Figure legend

Figure 1: Chromatogram from the analysis of *A. digitata* kernels after phytase treatment. The retention time for the peak corresponding to inositol hexaphosphate is around 5 minutes.

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Table 1. Phytic acid in *A. digitata* and *S. birrea* kernels pre-treated by boiling or autoclaving for 15 minures before incubation with *L. plantarum* for 48 hours at 37°C, (n=2).

Sample	Addition of *L. plantarum*	Phytic acid (g/100 g dry matter)	
		A. digitata	*S. birrea*
Boiled	No	4.4 ± 0.6	2.2 ± 0.1
Boiled	Yes	4.2 ± 0.0	1.9 ± 0.2
Autoclaved	No	3.6 ± 0.1	1.8 ± 0.0
Autoclaved	Yes	3.3 ± 0.0	1.6 ± 0.0

Table 2. Phytic acid content in *A. digitata* and *S. birrea* kernels incubated at 55°C with phytase for different times and then boiled for 5 minutes, (n=2).

Phytase	Incubation time	*A. digitata*	*S. birrea*
		Phytic acid	
(mg/g sample)	(min)	(g/100 g dry matter)	
0	0	4.0 ± 0.1	2.4 ± 0.2
10	2	3.9 ± 0.2	1.9 ± 0.3
10	15	2.9 ± 0.7	2.0 ± 0.1
10	30	3.2 ± 0.4	2.1 ± 0.3
10	60	3.3 ± 0.0	2.3 ± 0.2
10	240	2.5 ± 0.2	1.0 ± 0.1
50	60[1]	1.9 ± 0.1	0.8 ± 0.2

[1]50mg/ml of phytase

Table 3. Contents of iron, magnesium and zinc in the supernatant from *A. digitata* and *S. birrea* kernels incubated at 55°C with or without phytase for different times, (n=2).

Phytase (mg/g sample)	Incubation time (min)/Sample	Fe	Mg	Zn
		(mg/100 g dry matter)		
	A. digitata	*4.0-6.0*[*]	*626 - 706*[*]	*5.2-5.7*[*]
0	0	0.9 ± 0.09	109 ± 5.51	4.5 ± 0.04
10	2	4.4 ± 0.14	329 ± 5.65	1.3 ± 0.00
10	15	3.0 ± 0.28	404 ± 23.9	1.1 ± 0.08
10	30	2.6 ± 0.16	373 ± 21.9	0.9 ± 0.00
10	60	2.1 ± 0.12	359 ± 17.1	1.2 ± 0.01
10	240	2.1± 0.08	409 ± 24.6	1.4 ± 0.00
	S. birrea	*4.0*[*]	*346 - 436*[*]	*4.5*[*]
0	0	0.2 ± 0.03	49 ± 0.03	0.7 ± 0.00
10	2	2.3 ± 0.10	299 ± 20.2	2.6 ± 0.00
10	15	2.4 ± 0.11	289 ± 17.3	2.8 ± 0.02
10	30	2.4 ± 0.09	311 ± 20.0	2.7 ± 0.01
10	60	2.1 ± 0.09	310 ± 16.2	2.2 ± 0.02
10	240	2.1 ± 0.11	344 ± 21.8	2.4 ± 0.02

[*]Results from a previous study on mineral content in the kernels (Magaia et al., 2013).

Table 4. Literature data on the phytic acid content of the seeds of *A. digitata* and kernels of *S. birrea.*

Phytic acid (%)	**Based on**	**Reference**
A. digitata		
6.66 and 7.13	-[1]	Adubiaro et al., 2011
4.903*	DW[2]	Mitchikpe et al., 2008
1.75 and 0.62	-	Saulawa et al., 2014
1.40*	-	Proll et al., 1998
1.20	-	Ezeagu, 2005
0.20	-	Nkafamyia et al., 2007
0.0730*	FW[3]	Osman, 2004
0.18 and 0.16[4]	-	Nnam & Obiakor, 2003
S. birrea		
0.423*	DW	Muhammad et al., 2011

*= recalculated

[1]- = not given; [2]DW= dry weight. [3]FW = fresh weight; [4] = units not stated.

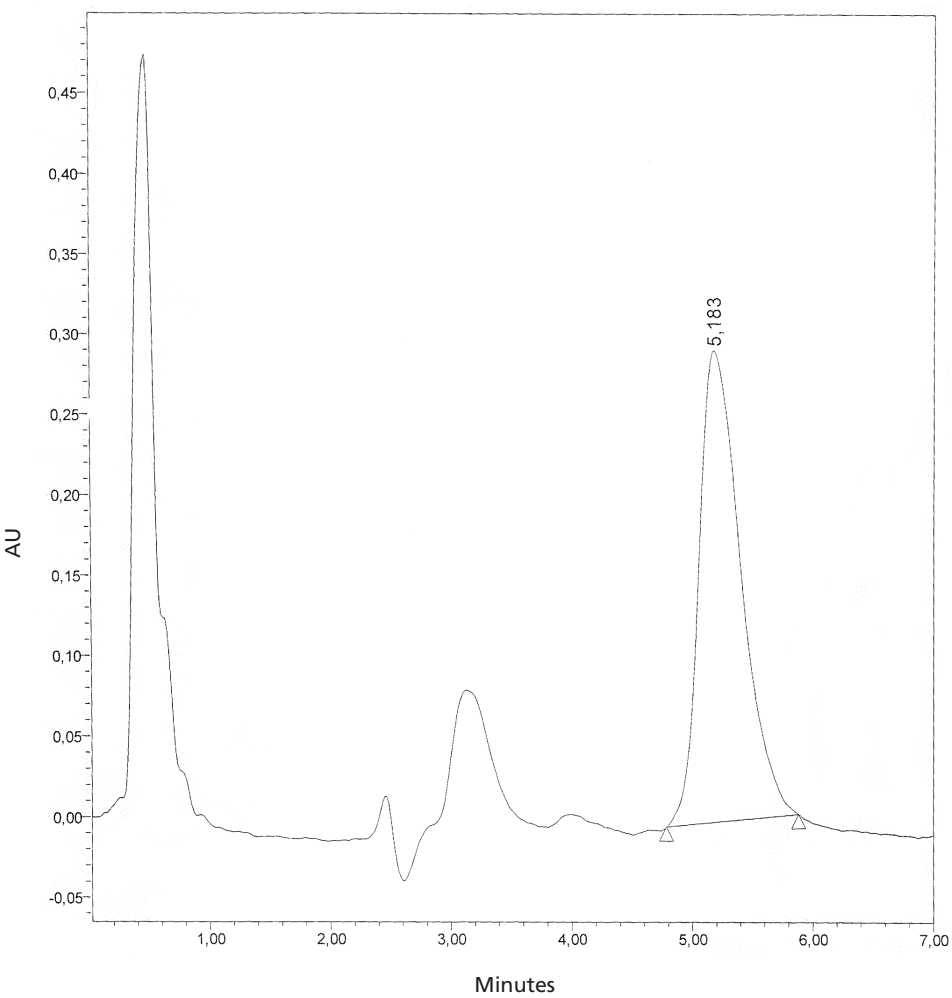

Figure 1: Chromatogram from the analysis of *A. digitata* kernels after phytase treatment. The retention time for the peak corresponding to inositol hexaphosphate is around 5 minutes.