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THE MARAMA BEAN

COMPOSITION & POTENTIAL

PhD Thesis by Mette Holse | 2012

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PhD Thesis · 2012

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Title
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Picture on second cover page: marama beans formed as Africa, by PhD Walter Chingwaru
Pictures on pages between publications by Margarida Dias Lima de Faria, IICT, Portugal

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Preface

This PhD project has been carried out in the research group Quality & Technology (Q&T) at the Department of Food Science, Faculty of Science, University of Copenhagen. The work has been sponsored partly by the EU funded project, MARAMA II, and partly by the Research School for Organic Agriculture and Food Systems (SOAR).

The project has been supervised by Associate Professor Åse Hansen, Professor Søren Balling Engelsen and Associate Professor Mikael Agerlin Petersen. I am grateful to Åse for inviting me to do this PhD and thereby introducing me to an exciting world of research benefitting developing countries. Thank you, Søren, for your valuable knowledge, help and inspiring discussions in regards to spectroscopy and Mikael, for useful discussions regarding aroma analysis.

I would also like to thank Professor Marena Manley from Stellenbosch University, South Africa for letting me visit her research group for four months during the fall 2009. It was a pleasure getting to know all the students and staff at the Department of Food Science and interesting and fruitful to be in a foreign research environment.

Warm thanks goes to my MARAMA II colleagues from around the world, for inspiring meetings, workshops and conferences held in Botswana, Namibia, South Africa and Denmark, which helped me see my marama bean research in a broader perspective.

I would like to thank all my Q&T colleagues for a great working environment. Special thanks goes to my officemate, Morten A. Rasmussen, who made my daily life as PhD student more fun and who on top of that contributed to valuable discussions regarding data analysis, to Flemming H. Larsen for practical help and valuable discussions regarding NMR spectroscopy, to Lisbeth T. Dahl

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I am grateful for the support from my friends and family during this process – not least you, Thomas, for being patient, supportive, understanding and believing in me during the process of finishing this PhD. Finally, thank you Emil for taking my mind elsewhere and putting life into perspective.

Mette Holse

Frederiksberg, January 2012

Abstract

The marama bean is an underutilised legume adapted to the adverse environmental conditions in semi-arid areas of Southern Africa. It thrives in poor-quality soils under harsh climatic conditions with long periods of drought and unpredictable rainfall, yet it produces highly nutritious beans.

Until now, the marama bean has only been found in the wild. It has been one of the world's many neglected plants despite its enormous potential. The research in this thesis work is part of an effort to unravel the secrets of the marama bean in an effort of lifting it out of obscurity. The research is also aimed at evaluating the potential of developing the marama bean into a crop, which may contribute to the food supply in some of the world's most challenging agricultural locations.

This thesis presents the most comprehensive study of the chemical composition of marama beans to date. It has been found that the chemical composition of marama beans on dry matter basis is 29-38% protein, 32-42% lipids and 19-27% dietary fibre, an ideal nutritional composition for combating malnutrition. Furthermore, marama beans proved to be an excellent source of several vitamins and minerals. For instance they are a good source of vitamin E and of important minerals such as calcium, iron and zinc that a large percentage of people living in Africa are deficient in. Additionally, the beans are rich in phytonutrients such as lignans that possess a wide range of health benefits. Finally, marama beans did not contain any of the potent allergens found in lupine and peanut and were found not to be cyanogenic.

Spectroscopic analyses of single beans revealed considerable chemical variations between marama beans harvested in the same geographical area (same batch). This bean-to-bean variation turned out to be as large as the overall variation between the batches; information that is highly important in a cultivation and breeding context.

The chemical attributes of marama beans make them suitable for production of a wide range of products. Marama bean products in the form of pressed oil and roasted beans were evaluated for their storage stability. Especially the oil proved to possess excellent storage stability, which is a great benefit for the potential commercial utilisation of the marama bean.

The many positive nutritional and functional properties of marama beans underline their large potential as a nutritious food, which may contribute to increasing the nutritional status and improving livelihoods of the people living in the areas where the beans grow.

Sammendrag

Maramabønnen er en bælgplante, der gror under vanskelige klimatiske forhold i ørkenområder i det sydlige Afrika. Her trives den i særdeles mager jord under barske klimatiske forhold med lange tørkeperioder og uforudsigelige mængder nedbør. Ikke desto mindre producerer maramaplanten særdeles næringsrige bønner.

Indtil videre har maramabønnen kun vokset vildt og har været en af verdens mange oversete planter på trods af sit enorme potentiale. Forskningen i denne afhandling bidrager til at kortlægge maramabønnens egenskaber i et forsøg på at synliggøre dens mange anvendelsesmuligheder. Samtidig vurderes potentialet for at gøre maramabønnen til en afgrøde, der kan bidrage til fødevareforsyningen i nogle af verdens mest udfordrende landbrugsområder.

I afhandlingen præsenteres det hidtil mest omfattende studie af den kemiske sammensætning af maramabønnen. Det er fundet, at bønnen på tørstofbasis indeholder 29-38% protein, 32-42% fedt og 19-27% kostfibre; en ernæringsmæssig sammensætning, der kan bidrage til bekæmpelse af underernæring. Maramabønnen er desuden rig på flere vigtige vitaminer og mineraler. Den indeholder f.eks. høje koncentrationer af E-vitamin samt mineralerne calcium, zink og jern, som en stor del af befolkningen i Afrika er i underskud af. Desuden er bønnerne rige på phytonæringsstoffer såsom lignaner, der har en bred vifte af sundhedsfremmende virkninger. Endelig indeholder maramabønnen ikke potente allergener kendt fra lupin og jordnød, ligesom den har vist sig ikke at være cyanogen.

Spektroskopiske analyser af enkelt-bønner har afsløret betydelige kemiske variationer imellem maramabønner høstet i samme geografiske område (samme batch). Denne bønne-til-bønne variation har vist sig at være lige så stor som den samlede variation imellem batches, hvilket er en yderst vigtig information i dyrknings- og forædlingsøjemed.

Maramabønnens kemiske egenskaber gør den velegnet til fremstilling af en lang række produkter. Lagringsstabiliteten af produkter fremstillet af maramabønner i form af presset olie og ristede bønner er blevet evalueret. Især olien viste sig at have en fremragende lagringsstabilitet, hvilket er en stor fordel i forbindelse med kommerciel udnyttelse af maramabønnen.

De mange positive ernæringsmæssige og funktionelle egenskaber fremhæver maramabønnens store potentiale som en næringsrig fødevare, der kan være med til at forbedre ernæringsstatus og levevilkår for befolkningen i de områder hvor bønnen gror.

List of publications

Paper I

Holse, M., Husted, S. & Hansen, Å. (2010) Chemical composition of marama bean (*Tylosema esculentum*) – A wild African bean with unexploited potential. *Journal of Food Composition and Analysis* 23, 648-657.

Paper II

Holse, M., Larsen, F.H., Hansen, Å. & Engelsen, S.B. (2011) Characterization of marama bean (*Tylosema esculentum*) by comparative spectroscopy: NMR, FT-Raman, FT-IR and NIR. *Food Research International* 44, 373-384.

Paper III

Holse, M., Petersen, M.A., Maruatona, G.N. & Hansen, Å. (2012) Headspace volatile composition and oxidative storage stability of pressed marama bean (*Tylosema esculentum*) oil. *Food Chemistry* 132, 1749-1758.

Paper IV

Holse, M., Skov, T. & Hansen, Å. (2011) Oxidative storage stability of roasted marama beans (*Tylosema esculentum*). *Food Research International*. *In press* (doi: 10.1016/j.foodres.2011.10.027).

Additional publications

Paper V

Jackson J.C., Duodu K.G., **Holse M.**, de Faria M.D.L., Jordaan D., Chingwaru W., Hansen A., Cencic A., Kandawa-Schultz M., Mpotokwane S.M., Chimwamurombe P., de Kock H.L. & Minnaar A. (2010) The Morama Bean (*Tylosema esculentum*): A Potential Crop for Southern Africa. In L.T. Steve (Ed) *Advances in Food and Nutrition Research, Volume 61*. Academic Press, Oxford, UK.

Paper VI

Alvarez-Jubete, L., **Holse, M.**, Hansen, Å., Arendt, E. & Gallagher, E. (2009) Impact of baking on vitamin E content of pseudocereals amaranth, quinoa, and buckwheat. *Cereal Chemistry* 86, 511-515.

Popular science publications in Danish

Holse, M. (2010) Vild ørkenbønne øger levestandarden for fattige afrikanere. www.ulandsnyt.dk.

Ørkenbønne skaber nyt håb i Afrika (2010) www.landbrugsavisen.dk.

Ørkenens bønner (2011) www.foodoflife.dk.

Rothenborg, M. (**18. September** 2011) Buskmændenes mirakelbønne giver næring til håb i Afrika. *Politiken*, Viden, p. 4.

Mirakelbønne kan hjælpe mod underernæring (2011) www.foodoflife.dk.

Holse, M. (2011) Miraklet marama – bønner der høres. *Dansk Kemi*, 92, no. 12, p. 8-12.

List of abbreviations

α-TE	α-tocopherol equivalents
CP	cross-polarisation
DF	dietary fibre
dm	dry matter
ELISA	enzyme-linked immunosorbent assay
FT	Fourier transform
GC	gas chromatography
HPLC	high-performance liquid chromatography
HR	high resolution
ICP	inductively coupled plasma
IR	infrared
MAS	magic angle spinning
MS	mass spectrometry
MSC	multiplicative scatter correction
NIR	near infrared
NMR	nuclear magnetic resonance
PC	principal component
PCA	principal component analysis
PV	peroxide value
S	sedimentation coefficient
SP	single-pulse

Contents

Chapter 1

Introduction

Background

The world's population is growing! In October 2011 the world's population passed seven billion and projections show a continued increase leading to the population surpassing nine billion people at 2050, and more than ten billion by the end of this century (UN, 2011b). The largest proportional increase in population takes place in sub-Saharan Africa[1], which today accounts for 13% of the world's population but is estimated to reach 20% by 2050 (The World Bank, 2011).

The growing population is leading to a constant and serious worry for a looming food crisis. This situation is especially a thread to the developing world where climate changes and overexploitation of land lead to soil erosion, loss of vital mineral nutrients, reduction of land for agricultural use, loss of biodiversity and changing rain patterns resulting in water scarcity. These changes lead to a drop in food production and consequently global food security problems – not least in sub-Saharan Africa (Collier *et al.*, 2008; Boon, 2009).

Ironically, the world is producing enough food to feed all (The World Bank, 2011). Yet, the number of undernourished people in the world is close to one billion, which is the equivalent to the population of North America and Europe combined – 98% of them live in developing countries. The most serious situation is found in sub-Saharan Africa where one third of the population lack adequate nutrition to meet their daily needs (FAO, 2010). The number of starving people in this region is continuously increasing even though one of the UN Millennium Development Goals aims at reducing the proportion of people

[1]The designation sub-Saharan Africa is commonly used to indicate all of Africa except northern Africa, with the Sudan included in sub-Saharan Africa (UN, 2011a)

in the world suffering from hunger by 50% before 2015 (compared to the numbers in 1990) (UN, 2010). In addition to a rapidly growing population, rising food insecurity and a changing environment, poverty and unemployment are major challenges in sub-Saharan Africa (FAO, 2010).

There may be many alternatives to solving the continuous food crisis in the world. Among the commonly proposed solutions are genetic improvements of seeds resulting in higher-yielding varieties, dams to irrigate vast areas and fertilizers to revitalize depleted soils. However, these solutions have led to a reduction in crop diversity by only focusing on few types of crops such as rice, wheat, maize and potato. Furthermore, the production depends on chemicals and technology to a degree that makes it economically prohibitive for the vast majority of poor farmers. Finally, these solutions have ignored the advantage of preserving diversity of food culture (Boon, 2009; WFP, 2010; FAO, 2011).

Another alternative would be to exploit and utilize all available food crops. This seems trivial, but more than 50,000 **edible plants** are known and of these, mankind has used only around 3000 species for food throughout history. Over the past centuries, the trend has been to focus on still fewer species resulting in only a few hundred species contributing significantly to food supplies today. Merely 15 crops provide 90% of the world's food energy intake and of these, three species – rice, maize and wheat – make up two-thirds and are the staple food for more than half of the world population (Vietmeyer, 1986; FAO, 1995; FAOSTAT, 2011).

> *"The path toward alleviating worldwide hunger and poverty will more likely be found by focusing on small-scale local initiatives than simply producing more food"*
>
> (The Worldwatch Institute, 2011)

In its annual "State of the World" report, the Worldwatch Institute describes how small-scale approaches to agriculture can help alleviate hunger and poverty and mitigate the effects of climate change in sub-Saharan Africa. One aspect of this, is an increased awareness of **underutilised plants** that thrive well in harsh environments (Vietmeyer, 1986). Examples of such plants are wild growing **legumes**. Legumes could have a special role, since they are usually protein-rich and because protein-energy malnutrition poses one of the most serious

nutritional problems in developing countries (Aphane *et al.*, 2003; Bhat & Karim, 2009). Only few selected legumes have been in focus in recent decades, namely peanuts and soybeans and merely around 20 of the 18,000-19,000 recognised legume species are used extensively today (National Research Council, 1979; Graham & Vance, 2003; Bhat & Karim, 2009).

One of the potentially important underutilised legumes is **the marama bean** (*Tylosema esculentum*) – a wild growing plant indigenous to the Kalahari Desert and neighbouring sandy regions in Southern Africa[2], producing oil- and protein-rich beans. A unique nutritional composition of marama beans[3] (Paper I and II) makes these ideal for production of various food products. Additionally, the marama bean has an edible tuber acting as a water reservoir and the stems of the plant may provide livestock feed and prevent soil erosion (Jackson *et al.*, 2010).

Commercial exploitation of the marama bean could potentially contribute to solving hunger and malnutrition challenges in Southern Africa, while increasing incomes and improving livelihoods. Despite the many attributes of the marama bean, only little research has targeted commercialisation of this unique indigenous natural resource in the countries where it grows naturally. Due to the lack of scientific and technological information, the marama bean is still an underutilised wild growing plant.

This PhD project has been part of the EU-financed project[4]:

> "MARAMA II – Development of Innovative and Healthful Marama Bean (*Tylosema esculentum*) Products Targeting Niche Markets"

This project used a market driven approach to develop prototypes of innovative high quality healthy marama bean food products. These are initially targeted to niche markets in Southern Africa but may also be sold worldwide in the future.

[2]Defined as Botswana, Lesotho, Namibia, South Africa and Swaziland (UN, 2011a)

[3]Throughout the thesis the common name marama bean will be used as opposed to the Latin name *Tylosema esculentum*. "The marama bean" will be used when referring to the entire plant, while "marama beans" is used when referring to the seeds.

[4]The members of the MARAMA II consortium (EU FP-6 grant: FP6-2004-INCODEV-3-MARAMA II-032059) was the Universities of Botswana, Copenhagen, Maribor (Slovenia), Pretoria (South Africa) and Namibia and the Instituto de Investigação Científica Tropical (IICT), Portugal and Market Matters Inc., USA and South Africa

The findings of MARAMA II will, therefore, contribute to the programme objectives of this call:

> *"Increasing the food security of populations in Southern Africa, diversifying rural livelihoods into income generating activities while increasing the quality of foods consumed"*
>
> (EU FP6, Bio-diverse, bio-safe and value added crops)

When starting to explore a wild underutilised plant species (such as the marama bean) and the commercial potential of new products from this, many aspects have to be considered. This requires research on many levels ranging from genetics and domestication over biochemistry, chemical composition and variation, product quality testing, market analysis, strategies for value chain development etc. Additionally, not only the sustainability of the plant source but also the local people's livelihoods have to be taken into consideration (Faria *et al.*, 2011). An overview of these aspects is found in Figure 1. The highlighted boxes show the categories to which the research of this thesis work has contributed (Papers I – IV).

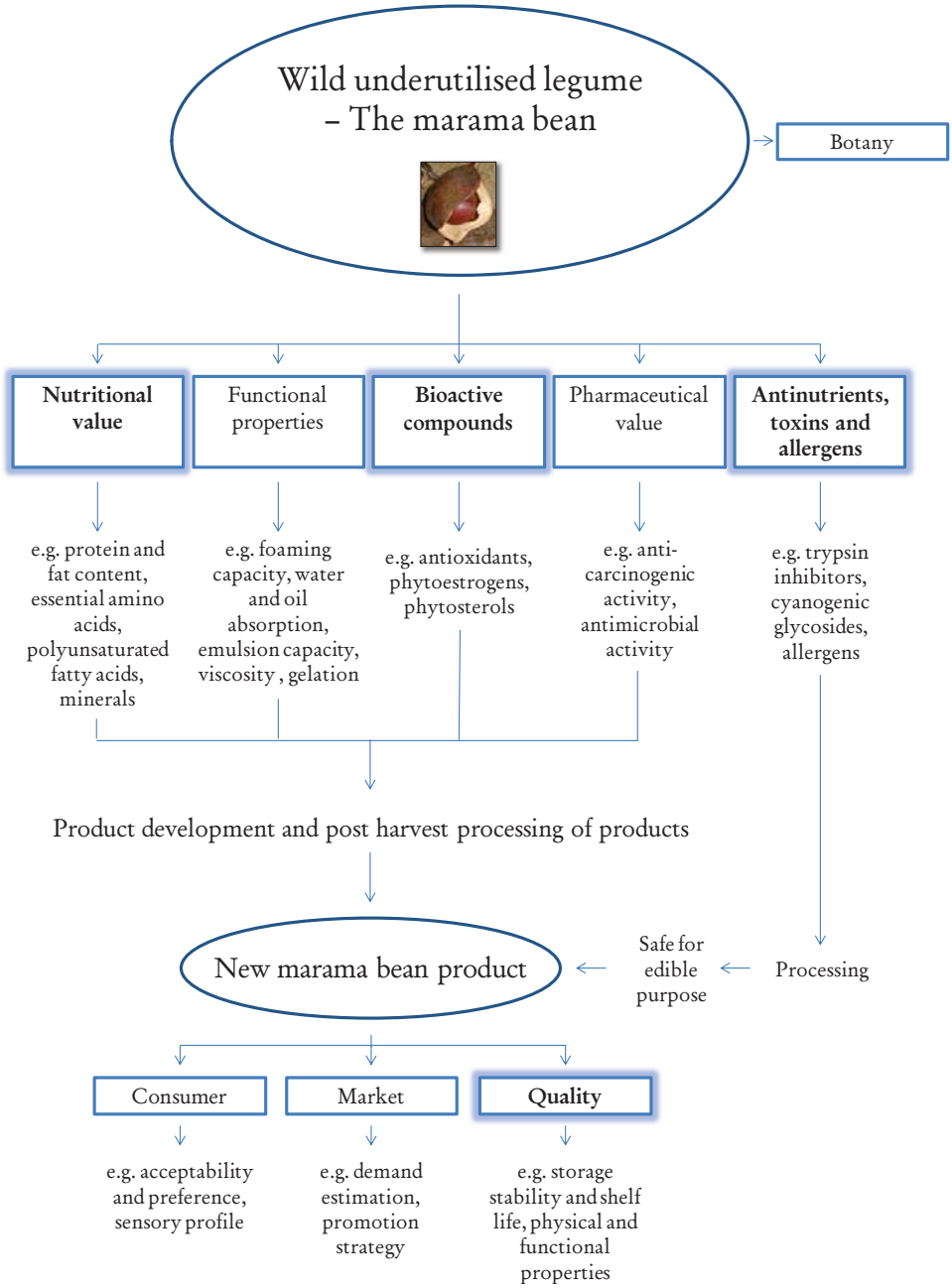

Figure 1 Schematic representation of important research steps in product development and commercialisation of indigenous underutilised plants, with the marama bean as an example. Idea for figure adapted from Bhat and Karim (2009). The highlighted boxes are the categories in which the research of this thesis work has contributed (Papers I – IV)

Aim of the thesis

The main focus of this thesis work was to analyse and evaluate the chemical composition of marama beans and to examine the storage stability of processed marama bean products. From this, to evaluate the potential of marama beans in relation to nutritional and functional aspects important in product development.

Thesis outline

The present thesis consists of a main part (*Chapter 1 – 8*) followed by four peer-reviewed scientific papers (I – IV). The main part introduces the reader to the marama bean and to the analytical methods applied. Additionally, the main experimental results from the papers are presented and discussed in a nutritional and food security perspective.

Chapter 2 gives a brief description of the botanical characteristics and traditional uses of the marama bean.

Chapter 3 provides an overview of the samples included in the thesis work, the experimental setup and the applied techniques; chemical (Paper I), spectroscopic (Paper II), chromatographic (Paper III and IV) and data analytical.

Chapter 4 gives a detailed description of the chemical composition and the variation thereof present in marama beans (Paper I and II). The composition of marama beans is compared to other more well known legumes and is discussed with emphasis on functionality in relation to product development.

Chapter 5 presents the nutritional qualities of the chemical composition of marama beans (Paper I and II).

Chapter 6 discusses the commercial potential of products made from marama beans and presents results on the storage stability of marama bean oil (Paper III) and roasted marama beans (Paper IV).

Chapter specific considerations and recommendations for future research are presented directly after each of the *Chapters 4 – 6*.

Chapter 7 is a brief conclusion of the results obtained in this thesis work.

Chapter 8 sums up the potentials and limitations of the marama bean and gives an outlook for the future of this wild growing fascinating nutritious bean with a view to both the developing countries and the Western world.

Chapter 2

The marama bean

Botanical classification

The genus *Tylosema* (previously *Bauhinia*) belongs to the plant family Fabaceae (Leguminosae) and subfamily Ceasalpinioideae. Within this genus five species, which are all endemic to Africa, have been characterised. Four of these were recognised by Coetzer and Ross (1976), nomenclature adjusted by Brummitt and Ross (1976) and later reviewed, most recently by Castro *et al.* (2005); *T. esculentum*, *T. fassoglense*, *T. argenteum* and *T. humifusum*. Castro *et al.* (2005) furthermore described a new species, *T. Angolense*.

Tylosema esculentum is the most well known and best described of the five species and is the species investigated in this thesis work. The generic name *Tylosema* refers to the torulose (i.e. a cylindrical or ellipsoid body) bean (Coetzer & Ross, 1976), while the word *esculentum* means edible and was given to the plant since it produces beans and tubers that are edible by people and tuberous stems that are consumed by browsing stock and game (Jackson *et al.*, 2010).

It goes by many names...

Tylosema esculentum – also known as marama bean • morama bean (Tswana) • marumana (Thonga) • gami (Khoi-khoi) • ombanui (Herero) • tsi, tsin (!Kung San) • gemsbokboontjie • moramaboontjie • elandboontjie • braaiboontjie (Afrikaans) • Camel's foot • tamani berry • gemsbuck bean (English) (National Research Council, 2006).

Beans from *Tylosema fassoglense* are also edible and consumed in the same way as marama beans (Dubois *et al.*, 1995). Therefore, few samples of this species have also been part of the sample material in Paper I and II.

Habitat

At present, the marama bean only grows in the wild (except in experimental cultivation programmes), in extreme environments with sizzling temperatures that may reach 50°C and erratic rainfall. In these regions some years will be characterised by long periods of drought whereas others will have rainfall exceeding 600 mm (Lawlor *et al.*, 2004; van der Maesen, 2006). Hence, the marama bean grows in regions where few conventional crops can survive.

The marama bean is endemic to the Kalahari Desert and the neighbouring semi-arid areas with nutritionally poor sandy soils. The plant occurs widespread and erratic in Botswana, Namibia and the northern part of South Africa (Northern Cape, North-West, Limpopo and Guateng Province) (Watt & Breyer-Brandwijk, 1962; Castro *et al.*, 2005; Nepolo *et al.*, 2009) as depicted in Figure 2. It is interesting that although the marama bean is restricted to Southern Africa, the four other species in *Tylosema* are distributed throughout eastern and central tropical Africa, from Sudan southwards, with *T. fassoglense* being the most widespread species (Castro *et al.*, 2005).

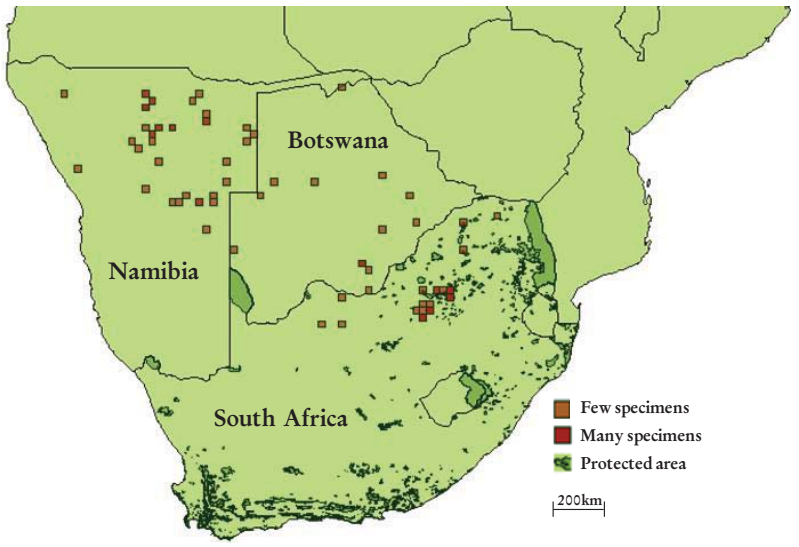

Figure 2 Geographical distribution of *Tylosema esculentum* in Namibia, Botswana and South Africa (SANBI, 2011)

The marama bean primarily thrives in deep sandy soils on the savannah. The soils are mostly well-drained, fine, calcareous sands, but may also consist of harder calcareous conglomerates and have a pH of 6 to 8. The plant is adapted to nutrient poor soils, where especially the prevalence of available phosphate and nitrogen is low and where only little organic matter exists (Dakora *et al.*, 1999; Lawlor *et al.*, 2004).

Morphology

The marama bean is a long-lived and perennial species that generates annually from a large underground tuber, which may contain up to 90% water and hereby acts as a water reserve (Travlos & Karamanos, 2006). Thus, the marama bean may survive during periods of drought, whereas it rots under persistent wet conditions (Lawlor *et al.*, 2004). The top of the tuber is found 45-85 cm below the soil surface (Bergström & Skarpe, 1981) and may reach a weight of 10 kg after a few years and tubers weighing up to 200-300 kg have been reported (Story, 1958; Kieth & Renew, 1975; Bousquet, 1981; Bergström & Skarpe, 1981).

Above ground the marama bean grows prostrate with numerous herbaceous stems that can be up to 3 m long and which creep over the soil surface (Coetzer & Ross, 1977; Castro *et al.*, 2005). The plant density is approximately 300-400 plants per hectare (Monaghan & Halloran, 1996). Yellow flowers develop midsummer and the fruits ripen in late autumn. The plant produces pods, which turn from being soft and reddish over light green to hard woody and dark brown in colour when ripe (Story, 1958; Wehmeyer *et al.*, 1969). The pods open at maturity and usually contain two but sometimes up to six edible seeds (**marama beans**) enclosed in a hard reddish to brownish-black hull. It may take two to four years before the plant produces beans (National Research Council, 2006).

The dimensions of a bean (length, width and thickness, respectively) are 19 x 17 x 13 mm on average (Jideani *et al.*, 2009; Mosele *et al.*, 2011b) and a single bean weighs 2.3 g on average (Paper I). In general, the structure of a legume seed comprises three main parts; hull (or seed coat), cotyledon and embryonic axis. The hull of marama beans is very thick, contributing to about 50% of the weight (Paper I), while the hull of other legume seeds solely accounts for 8-20% of the seed weight (Sathe & Venkatachalam, 2004). The cotyledon is primarily composed of parenchyma cells, which act as storage sites for most nutrients

(Salunkhe & Kadam, 1989). The marama tuber, plant, pods, beans, hull and cotyledon are shown in Figure 3.

Figure 3 Top left) marama tuber and right) flowering marama plant. Bottom left) semi-ripe (green) and ripe (brown) pods and ripe marama beans and right) cracked hull and cotyledon. Pictures provided by Margarida Dias Lima de Faria, IICT, Portugal and Mette Holse

Cultivation and domestication

The seed set of the marama bean is relatively low, which may be an adaption of the species to an environment in which rainfall is scarce (Hartley *et al.*, 2002). Production is approximately 100-300 kg per hectare (Lawlor *et al.*, 2004), which is quite low compared to other pulses that on average yield 925 kg per hectare and especially compared to peanuts and soybeans that yield approximately 1500 and 2200 kg per hectare, respectively (FAOSTAT, 2011). The low yield of the marama bean makes collection of beans from the wild an unsuitable way of reducing the food security problems in Southern Africa. Hence, if people in Southern Africa are to benefit from this important indigenous natural resource, sustainable commercialisation, cultivation and domestication approaches are of utmost importance (Faria *et al.*, 2011). Furthermore, such approaches are vital for the continued preservation of the marama bean in the wild, since it may become extinct due to overexploitation of its beans and stems as animal feed and

use of the land for cultivation of other crops (Keegan & Van Staden, 1981; National Research Council, 2006).

Over the years, **cultivation** of the marama bean as a crop for semi-arid and arid agriculture has been encouraged due to its ability to survive under unfavourable climatic and soil conditions as well as its nutritional and socio-economic potential (Kieth & Renew, 1975; National Research Council, 1979; Bousquet, 1981; Keegan & Van Staden, 1981; Vietmeyer, 1986; Wehmeyer, 1986; Ketshajwang *et al.*, 1998; Lawlor *et al.*, 2004; Nepolo *et al.*, 2009). Most recently, van Wyk (2011) examined 120 indigenous food plants from South Africa for their potential as new food and beverage products, and subjectively rated the potential of the marama bean as being high. Additionally, the indigenous populations themselves have shown interest in the development of the bean for their livelihoods (Chingwaru *et al.*, 2007).

As early as in 1924 small scale cultivation of the marama bean was initiated in various places in South Africa (Anonymous, 1924). Today, successful cultivation trials in Botswana (Botswana College of Agriculture) and Namibia (Sandveld Research Station) are ongoing (Lawlor *et al.*, 2004; Jackson *et al.*, 2010). Furthermore, a project called *Morama Engaged*[5] is currently being implemented in Botswana and Namibia, which focuses on the sustainable production of marama beans among other topics related to commercialisation of the marama bean.

Additionally, experimental cultivation has been attempted in various places. A study carried out in Texas, USA, at three different institutions examined the environmental tolerance and potential productivity of the marama bean, and concluded that it can be grown in arid regions of the United States. The plants exhibited vigorous perennial growth under conditions of ample water and a healthy seed crop was produced in about 4.5 years (Bousquet, 1981; Powell, 1987). Another trial carried out in Perth, Australia resulted in successful growth of the plants, however these failed to produce beans (Francis & Campbell, 2003). Moreover, experimental cultivation programmes in Northern Kenya (Hornetz, 1993), Pretoria in South Africa (Story, 1958) and Israel (van der Maesen, 2006) have been successful.

[5]Funded by the Swedish Government through the Swedish International Development Cooperation agency (SIDA)

Preliminary steps have been taken regarding domestication and selection of high yielding and early maturing genotypes of the marama bean (Lawlor *et al.*, 2004). The effects of different dormancy-breaking treatments of the **germination and emergence** (Lebutswe *et al.*, 2003; Travlos *et al.*, 2007a; Travlos *et al.*, 2007b) and the **reproductive biology** (Coetzer *et al.*, 1983; De Frey *et al.*, 1992; Hartley *et al.*, 2002) have been investigated. In addition, information on **genetic variation**, which is a prerequisite for the improvement of any plant species by breeding programs has been examined (Monaghan & Halloran, 1996; Nepolo *et al.*, 2009; Takundwa *et al.*, 2010a; Takundwa *et al.*, 2010b; Nepolo *et al.*, 2011). Furthermore, growth responses to **irrigation** (beneficial), **soil texture** (needs to be well-drained and sandy, not clay), **mineral fertilisation** (N and P not beneficial) and potassium in relation to leaflet movements and consequently the total **water economy of the plant** (extremely important) have been examined (Dakora *et al.*, 1999; Ramolemana *et al.*, 2003; Lawlor *et al.*, 2004; Travlos & Karamanos, 2006; Travlos *et al.*, 2008; Travlos & Karamanos, 2008).

Domestication of the marama bean has already been initiated in one of its native regions. The *Marama Domestication Project*[6], which is expected to run for 18 years, is an initiative to assess the possibility of domesticating the marama bean and to save it from extinction. Approximately 50 local farmers across Namibia have planted experimental marama bean fields. Through this, the project seeks to maximise production of the marama bean and utilise land that would not ordinarily be cultivated on account of deficiency in nutrients. One of the goals of the project is to develop early maturing cultivars of the marama bean. The project is particularly important for Namibia, which is one of the driest countries in sub-Saharan Africa with only 6% arable land.

Traditional uses

The marama bean is a traditional source of food for the indigenous populations in Southern Africa such as the San, Khoi, Herero and Tswana people (Wehmeyer *et al.*, 1969; National Research Council, 1979). Of these groups, the San, which are originally hunters and gatherers, and Herero, which are originally nomads, are best acquainted with marama beans (Faria *et al.*, 2011). Marama beans are gathered by hand from the wild and for some groups the beans are a staple food while others use them more rarely as a snack. Already in

[6]Funded jointly by the Kirkhouse Trust of the United Kingdom, the United Nations Development Program (UNDP) and the University of Namibia

1824, Burchell emphasised the importance and use of the beans by the indigenous population, especially of the Kalahari Desert. Today, marama beans are still praised as an important food with good taste and high nutrient value (Faria *et al.*, 2011). This is well illustrated by this quotation from a focus group interview conducted in Botswana:

> *"Eating marama makes you energetic, gain weight and feel good"*
>
> (Chingwaru *et al.*, 2007)

The importance of the marama bean for rural populations is further illustrated by their use of the beans as a supplement for babies and infants as well as for pregnant and breastfeeding women and for elderly (Jackson *et al.*, 2010). Several medical uses of marama beans have also been reported ranging from treatment of eye infections, eczema and diarrhoea to improved blood circulation (Chingwaru *et al.*, 2007; Jackson *et al.*, 2010).

Marama beans are not consumed raw since they are relatively tasteless and have an unpleasant slimy texture when chewed. They are primarily eaten as a snack after roasting in hot sand, which makes the slimy texture disappear and gives the bean a pleasant taste resembling that of roasted cashew nuts or peanuts (Verdoorn, 1959; Chingwaru *et al.*, 2007). The roasting process is carried out slowly in sand in a pot over open fire (Faria *et al.*, 2011). The sand provides an even distribution of heat and ensures that shattered beans do not make any damage (Story, 1958). The traditional method for preparation of roasted marama beans is shown in Figure 4.

The roasted beans may also be pounded into flour or ground into small pieces and boiled in water to produce a cocoa-like beverage or mixed with e.g. maize flour and then boiled in water and eaten as porridge, in this way increasing the nutritional value of the cereal porridge (Wehmeyer *et al.*, 1969; National Research Council, 2006; Chingwaru *et al.*, 2007). Additionally, marama beans are used as a source of oil for cooking and skincare and the unripe marama beans may be eaten boiled in the same way as other legumes (van der Maesen, 2006; Faria *et al.*, 2011).

Figure 4 Preparation of roasted marama beans. Top left) gathering ripe beans by hand and right) roasting in hot sand over open fire. Bottom left) cooling in sand on the ground and right) cracking by hand using a stone. Pictures provided by Margarida Dias Lima de Faria, IICT, Portugal

Usually the beans are only gathered in small quantities for household consumption, but uncooked as well as roasted marama beans are sold through small scale local informal market systems (van der Maesen, 2006; Faria *et al.*, 2011). Marama beans store well, especially under dry conditions, and remain edible for years due to their low water content (van der Maesen, 2006). Some local communities take advantage of this by saving the beans as a kind of "life saver" in times where other foods are not readily available or they cannot afford to buy food (Faria *et al.*, 2011). Hence, marama beans are an important contributor to food security.

In addition to the many uses of marama beans, the marama tuber may also be eaten either baked, boiled or roasted as a vegetable dish, and is reported to have a sweet pleasant flavour. Normally only young tubers of about 1 kg are eaten since larger tubers become fibrous and bitter (Burchell, 1824; Story, 1958).

However, the large tubers offer an important emergency source of water for humans and animals (van der Maesen, 2006; Faria *et al.*, 2011).

Wildlife such as gemsbok and other antelopes feed on the leaves and seeds of the marama plant (National Research Council, 2006). However, it has been shown that free-range beef cattle do not choose the marama plant as their preferential feed-stuff (Müseler & Rothauge, 2006).

Chapter 3

Experimental design and analytical platform

Sample set and experimental design

This thesis work includes two sample sets; one for investigating the chemical composition of marama beans and one for investigating the oxidative storage stability of different marama bean products.

In order to investigate the chemical composition of marama beans a sample set consisting of eighteen different marama bean batches was collected in different locations in Botswana, Namibia and South Africa during the growing seasons 1990 and 2005-2008 and was then analysed. A mixture of 10 g of dehulled beans from each batch were analysed by chemical analyses (Paper I)[7], while three single beans from each batch were analysed by spectroscopic methods (Paper II).

In order to investigate the oxidative storage stability and shelf life of marama bean products three different products were examined; marama bean oil (Paper III), roasted marama beans (Paper IV) and partly defatted marama bean flour (unpublished results). Preparation of the samples and products as well as the overall experimental setup are illustrated in Figure 5.

[7]Chemical measurements were carried out on sixteen of the batches. For two batches very little material was provided and this was only enough to evaluate the single bean composition

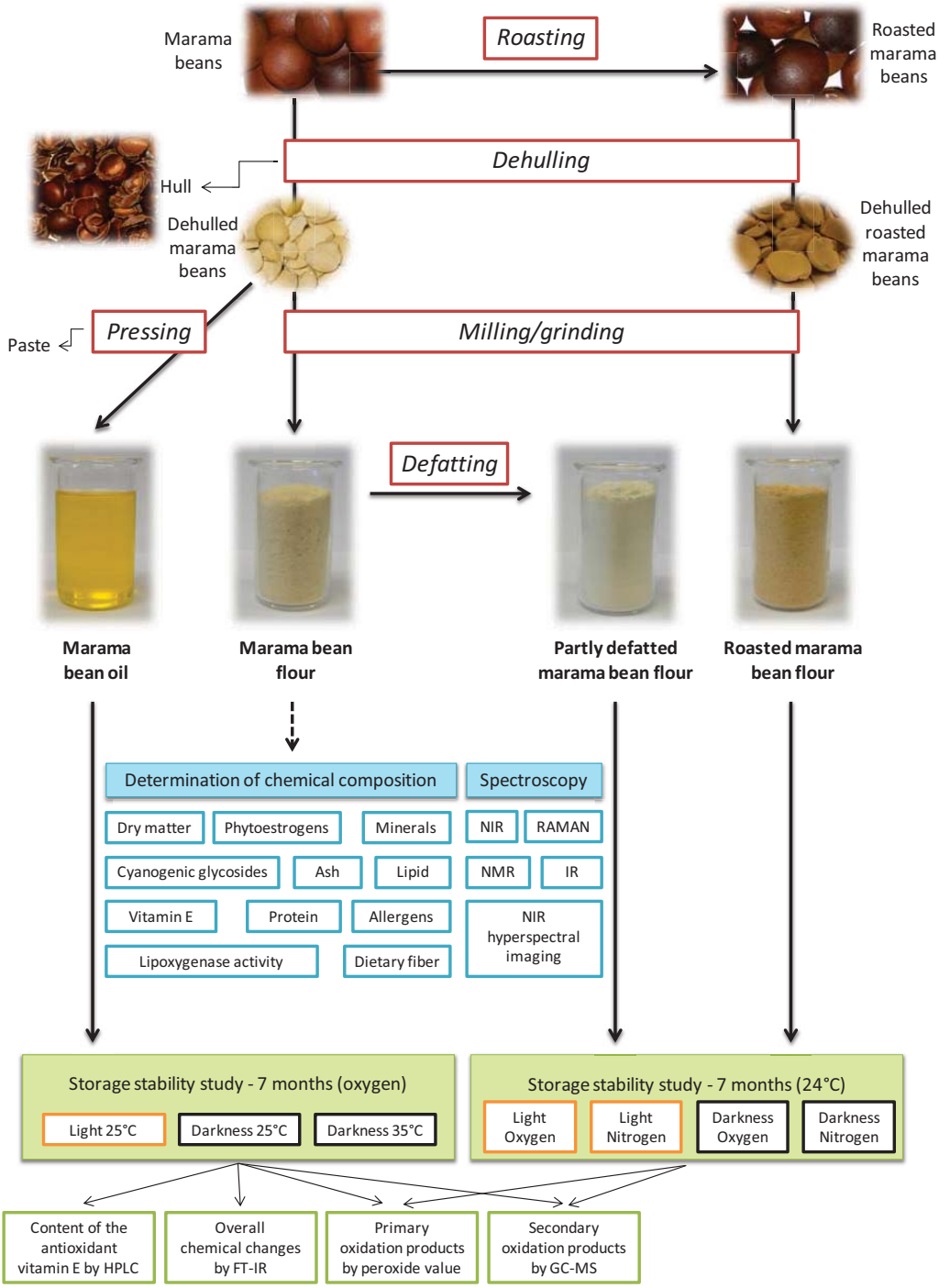

Figure 5 Sample preparation and experimental design for the present thesis work. The results from the partly defatted flour are not included in this thesis

Overview of applied methods

Before the start of the MARAMA II project, only limited information regarding the chemical composition of marama beans were available (Anonymous, 1924; Wehmeyer *et al.*, 1969; Bower *et al.*, 1988; Amarteifio & Moholo, 1998; Ketshajwang *et al.*, 1998; Francis & Campbell, 2003; Müseler & Schönfeldt, 2006). In the present thesis work, well known standard wet chemistry methods and advanced analytical methods (chromatography and spectroscopy) were applied to obtain detailed and comprehensive information of the chemical composition of marama beans.

Table 1 gives an overview of the many different types of analyses applied during this thesis work (Paper I – IV). The table is divided into several categories and sub-categories of analytical methods. The physical/chemical principle behind each method is briefly described along with the specific use(s) of the method in this thesis work. The chemical groups of minor and major nutrients are highlighted in different colours in order to provide an overview of which methods that may be applied for determination of specific types of nutrients.

Table 1 Overview of methods applied in Paper I – IV. The physical/chemical principles as well as the actual obtained results for each method are presented. The paper(s) in which the method was applied is given

Type of method	Method	Physical/chemical principle	Results acquired by the method	Paper no.
Wet chemical analysis	Dry matter content	Gravimetric determination of dry matter content by evaporation of water. This is done by heating the sample in an oven (130°C) until a constant weight is obtained	**Dry matter** content of marama beans	I
	Ash content	Gravimetric determination of ash content by vaporisation of water and other volatile materials and burning of organic substances. This is done by heating the sample at high temperature (900°C) in a muffle furnace	**Ash** content (=total **mineral** content) of marama beans	I
	Kjeldahl	Determination of nitrogen content in a sample by decomposition of organic substances with strong acid followed by distillation with alkali to liberate ammonia for determination by titration. The amount of protein is then calculated from the nitrogen concentration by a conversion factor (the factor for soybean, 5.7, is used for marama beans)	**Protein** content of marama beans	I
	Soxhlet	Determination of the lipid content by solvent (ether) extraction	**Lipid** content of marama beans	I
	Total, insoluble and soluble dietary fibre analysis	Determination of the content of water soluble and insoluble dietary fibre (DF) by sequential enzymatic digestion by heat-stable α-amylase, protease and amyloglucosidase to break down starch and protein components. Subsequent filtering leaves the soluble DF in the filtrate solution (precipitated from solution by adding 95% alcohol), and the insoluble DF in the filter. The contents are determined gravimetrically	**Soluble, insoluble** and **total dietary fibre** content of marama beans	I

Table 1 continued (2/6)

Type of method	Method	Physical/chemical principle	Results acquired by the method	Paper no.
Wet chemical analysis	Enzyme-linked immunesorbent assay (ELISA)	Detection of specific proteins by use of antibodies. Antibodies are fixed to a solid surface (e.g. the surface of a well in a microtiter plate) and an antigen is added. Then an antibody-enzyme conjugate (same antibodies couples to an enzyme) is added. When a substrate (colourless) is added it reacts with the antibody-enzyme conjugate and a coloured product is produced from the enzyme and detected using a spectrophotometer by recording the absorbance at 450 nm	Evaluation of Immunoglobulin E-mediated (antibodies) allergenicity of marama beans by determination of proteins (antigens) with allergenic potential known from peanut and lupine	I
	Western blot	Western blot is a similar technique to ELISA used for detection of specific proteins by use of antibodies. Gel electrophoresis is used to separate native proteins, which are then transferred to a membrane, where they are detected by use of antibodies specific to the target protein	(Same as for ELISA)	I
	Cyanogenic glycoside analysis	Detection of cyanogenic glycosides in a sample by two different approaches. 1) Detection of the presence of cyanogenic glycosides by extraction of metabolites from the sample and subsequent addition of specific enzymes, which are able to release cyanide. 2) Detection of enzymes able to release cyanide from possibly present cyanogenic compounds. The cyanide content is determined spectrophotometrically by recording the absorbances at 580-750 nm	Evaluation of the potential content of cyanogenic glycosides and enzymes with ability to release hydrogen cyanide from these in marama beans	I
	Peroxide value (PV)	Determination of the peroxide value (expressed as meq O_2/kg lipid) based on a spectrophotometric determination of ferric ions (Fe^{3+}) derived from the oxidation of ferrous ions (Fe^{2+}) by hydroperoxides, in the presence of ammonium thiocyanate (NH_4SCN). The peroxide value is determined using a spectrophotometer by recording the absorbance at 500 nm	Measure of the extent to which primary lipid oxidation has occurred during storage of marama bean products	III & IV

Table 1 continued (3/6)

Type of method	Method	Physical/chemical principle	Results acquired by the method	Paper no.
Wet chemical analysis	Lipoxygenase isozyme activity test	Detection of the presence of lipoxygenase isozymes by spectrophotometric determination of a methylene blue decolourisation reaction. The mechanism of methylene blue bleaching by lipoxygenase involves the specific abstraction of hydrogen from hydroperoxide 13C-OOH isomers (formed from linoleic acid by the lipid peroxidation reaction if lipoxygenase isozymes are present) by methylene blue, which is then reduced to a colourless compound. The lipoxygenase activity is determined using a spectrophotometer by recording the absorbance at 660 nm	Detection of lipoxygenase isozymes in marama beans for the evaluation of potential enzymatic **lipid oxidation** during storage of marama products	III
Elemental analysis	Inductively coupled plasma-mass spectrometry (ICP-MS)	Quantification of minerals by ionisation of the atoms in a sample (by ICP – a plasma that contains sufficient concentrations of ions and electrons to make the gas electrically conductive), followed by detection by mass spectrometry (MS – see GC-MS)	Content of specific **minerals** (macro and trace elements) in marama beans	I
Chromatography	High-performance liquid chromatography (HPLC)	Chromatographic separation of individual components in a liquid mixture. The injected sample passes through a stationary phase (column) by use of a mobile phase. Hereby, the components in the sample are separated according to their chemical and physical interactions (polarity, solubility, size etc.) with the stationary phase and due to this elute from the column at different retention times. Together with a detector (florescence in Paper I and III, and coulometric electrode array detection in Paper I), the specific retention times often makes it possible to identify and quantify the eluting components	Content of eight **tocopherol and tocotrienols** in marama beans as well as in marama oil during storage Total **isoflavone** content in marama beans and roasted marama beans	I & III I

Table 1 continued (4/6)

Type of method	Method	Physical/chemical principle	Results acquired by the method	Paper no.
Chromatography	Gas chromatography–mass spectrometry (GC-MS)	Chromatographic separation of volatile organic compounds in a mixture followed by mass detection. A sample is vaporized when entering the column due to a temperature program. Here, the compounds are distributed between the stationary phase (column) and the mobile (gas) phase. The components held preferentially in the stationary phase are retained longer than those distributed selectively in the mobile phase. Due to this, components are separated and elute in the order of their increasing distribution coefficient with respect to the stationary phase. The separated components enter a mass detector, where ionization and fragmentation take place. The fragments/ions are separated according to their mass to charge ratio (m/z) and from this a unique fingerprint of each component is obtained – a mass spectrum, which is used for identification	Content of six specific **lignans** in marama beans and roasted marama beans	I
			Headspace profile of the marama products before and during storage → hereby examination of the **aroma** volatile composition and the changes of this due to **lipid oxidation**	III & IV
			Quantification of important volatile **lipid oxidation** products for determination of optimal storage conditions and shelf life of marama bean products	III & IV
Vibrational spectroscopy	Fourier transform-infrared (FT-IR)	Absorption measurement of the fundamental molecular vibrations of the molecules in a sample. The sample is exposed to infrared radiation (400-4000 cm^{-1}). When the radiant energy matches the energy of a specific molecular vibration in a molecule (e.g. C-H stretching), absorption of light occurs. In order for a molecule to be IR active there must be a change in dipole moment as a result of the vibration that occurs. IR spectroscopy measures molecular vibrations of the functional groups (e.g. bending and stretching) and gives strong signals for polar groups such as O-H, C-H and C-O	Determination of secondary structure of marama bean **proteins**	II
			Evaluation of the bean-to-bean and batch-to-batch variations in marama beans	II
			Overview of chemical changes due to **lipid oxidation** during storage of marama bean products	III

Table 1 continued (5/6)

Type of method	Method	Physical/chemical principle	Results acquired by the method	Paper no.
Vibrational spectroscopy	Near infrared (NIR)	Absorption measurement of the overtones and combination tones of the fundamental molecular vibrations of the molecules in a sample. The sample is exposed to non-destructive near infrared radiation (780-2500 nm). NIR spectroscopy gives strong signals for anharmonic molecular bonds such as C-H, O-H, N-H	Overview of bulk components in marama beans; water, lipid, carbohydrate and protein	II
			Evaluation of the bean-to-bean and batch-to-batch variations in marama beans	II
	FT-Raman	Inelastic scattering measurement of the fundamental molecular vibrations of the molecules in a sample. The sample is exposed to strong near infrared (or visible or near ultraviolet) radiation from a laser. The laser light interacts with molecular vibrations in the functional groups, resulting in the molecules being polarized by the incoming light, hereby placing the molecules into an excited state. When the molecule attempts to return to its base state it emits two types of light; one type is the complete return to the original base state (same wavelength as the original excited light) (Rayleigh scattering), while the other type occurs with the electron in its base state, with the vibrational state transitioning to an energy level resembling an excited state (Raman scattering). The shift in energy gives information about the vibrational modes in the system. Raman spectroscopy gives strong signals from non-polar groups with symmetrical stretches such as C-C, C=C, C≡N, S-S	Assignment of non-polar functional groups in marama bean lipids	II
			Evaluation of the degree of unsaturation of marama bean lipids	
			Determination of the absence of β-glucan and starch in marama bean carbohydrates and the predominance of pectins	
			Determination of the presence of aromatic amino acids in marama bean proteins	
			Evaluation of the bean-to-bean and batch-to-batch variations in marama beans	
	NIR hyperspectral imaging	Exploration of the spatial resolution of chemical constituents in a sample by use of a combination of NIR and an imaging system. The technique gives complete spectral information in the NIR wavelength region for each pixel in the image taken	Description of chemical differences and gradients within single marama beans (water, lipid, carbohydrate and protein)	(Not published)

Table 1 continued (6/6)

Type of method		Method	Physical/chemical principle	Results acquired by the method	Paper no.
Nuclear magnetic resonance (NMR) spectroscopy	Solution-state NMR	Proton high resolution-magic angle spinning (^{1}H HR-MAS) (+2D ^{1}H-^{1}H COSY, ^{1}H -^{1}H TOCSY and ^{1}H-^{13}C HSQC)	Detection and quantification of protons belonging to the mobile phase of a sample. Hereby, determination of chemical structures in the sample. General for NMR is that it relies on the phenomenon of nuclear magnetic resonance, hence only atomic nuclei with a magnetic moment (arising from the spin of the protons and neutrons) (such as ^{1}H, ^{13}C and ^{31}P) can be analyzed. The sample is placed in a strong static magnetic field where the nuclei are aligned and radiated at a frequency characteristic of the isotope. The nuclei absorb the electromagnetic radiation and are brought out of equilibrium. Different nuclei in a molecule absorb at slightly different resonant frequencies. The different shieldings of the nuclei results in different chemical shifts in the NMR spectrum. In MAS NMR the solid or rather semi-solid sample is rotated at the magic angle 54.74° (the diagonal in a cube) at which the dipole-dipole interactions cancel out and the otherwise broad solid state lines become narrow.	In general, description of the mobile part of marama beans. Hence, observation of hydrogens in lipids, small carbohydrates, amino acids and small peptides Assignment of protons in marama bean lipids Determination of the presence of raffinose among water soluble marama bean carbohydrates Exclusion of certain soluble carbohydrates in marama beans, e.g. mixed-linkage β-glucan and starch degradation products Determination of tyrosine as the only water soluble aromatic amino acid in marama bean suspension Evaluation of the bean-to-bean and batch-to-batch variations in marama beans	II
	Solid-state NMR	Carbon single-pulse magic angle spinning (^{13}C SP/MAS)	Detection and quantification of all carbon signals in a solid sample. See above.	In general, a more complete description of marama beans compared to solution state NMR Assignment of carbons in marama bean lipids Determination of degree of unsaturation and average fatty acid length of marama bean lipids (by linear combination of SP and CP/MAS spectra)	II
		Carbon cross-polarisation magic angle spinning (^{13}C CP/MAS)	Detection and quantification of carbon resonances originating from the immobile regions of the sample. See above.	Determination of degree of esterification in marama bean polysaccharides Observation and quantification of aromatic amino acids in marama bean protein	II

Data analysis

When measuring the chemical composition of marama beans each sample is characterised by several parameters (e.g. lipid, protein and vitamin E content). The nature of such data makes it ideal to use multivariate data handling methods instead of more classical univariate measures. Multivariate data analysis, also called chemometrics, makes it possible to simultaneously investigate the sample for common characteristics (e.g. interactions between chemical parameters) and to evaluate the differences and similarities between the samples analysed.

Several multivariate methods are available ranging from exploratory methods, over classification methods to calibration/regression methods. Within these methods it is possible to include pre-processing, validation and variable selection (just to name of few things) to ensure that proper data are analysed, that the analysis is valid and that the interpretation is as simple as possible, respectively.

In this thesis work, the exploratory approach has been used to characterise the marama beans from different countries, the bean-to-bean and batch-to-batch variation and the changes in chemical composition and aroma profile of marama bean products during storage. One of the often applied exploratory methods in chemometrics is principal component analysis (PCA) (Wold *et al.*, 1987).

In short, PCA is a descriptive method used for investigation of large data structures in order to study the relationships between or within the different samples and variables and to detect trends, groupings and outliers. PCA is a decomposition method that provides an approximation of a data matrix (X) in terms of the product of two data matrices; the score matrix (T) and the loading matrix (P). The idea is to describe common and systematic chemical information and collect this in fewer latent variables (principal components) than found in the original data. This is often possible due to samples being similar and variables correlating in a certain way. For marama beans a PCA model is for instance expected to reveal a greater similarity of samples from individual countries; a similarity obtained due to a specific pattern (correlation between the measured chemical compounds) in samples from the same country.

Written as an equation PCA can be described as

$$\mathbf{X = T P' + E}$$

Where E is the residual matrix capturing information not explained in the systematic part (scores and loadings). The product of scores and loadings is denoted a principal component (PC).

In a bit more detail, the first principal component describes the direction in the data that holds the largest variation between the samples. This could e.g. be that marama bean samples from South Africa are different from those harvested in Namibia and Botswana. The chemical compounds will each have a unique contribution to this difference as some might hold country specific information alone or when combined, while others contain information of something different (e.g. harvest year or rainfall). The size of these unique contributions (the loadings) can be considered as a pattern showing the importance of each chemical compound. The differences between the samples are summarized in the corresponding scores. The scores hold the amount of the found pattern e.g. differentiating between samples from South Africa and Namibia/Botswana. In the same way, the second principal component (second largest variation) can be calculated and interpreted. In PCA the direction of the second principal component is orthogonal to the first and successive components making the principal components independent.

Chapter 4

Chemical composition of marama beans

Overall composition

The marama bean belongs to the legume family, Fabaceae. Of all plants, only the family Poaceae (cereals and grasses) is superior to Fabaceae when it comes to importance in relation to human consumption (Vietmeyer, 1986). This is reflected in the global production of cereals being almost 2500 million tons (estimated gross production value of app. 570 billion $US) yearly, while the legume production accounts for only about 325 million tons (app. 125 billion $US) (FAOSTAT, 2011). However, when looking at the gross production values it appears that legumes have a substantially higher value per kg than cereals.

In Table 2 the average chemical composition of marama beans (Paper I) is compared to the chemical composition of some of the world's most important legumes and cereals. The legumes are divided into pulses (legume seeds with low lipid content) and legume oil crops (legume seeds with high lipid content). The carbohydrate content has been divided into digestible carbohydrate (sugars and starch) and dietary fibre (soluble and insoluble).

Table 2 Comparison of the proximate composition of marama beans with the most important legume oil crops, pulses and cereals according to world production. Values for the proximate composition are mean values, in some cases including numbers for several species and subspecies. Names separated by comma are different subspecies while names in parentheses are synonyms of common names. Major groups of pulses (as divided into groups by FAOSTAT) are in bold

Common name	Latin name	World annual production 2009 (million tons)[a]	Proximate composition (g/100g)[b]					
			Moisture	Ash	Lipid	Protein	Digestible carb.[c]	Dietary fibre
MARAMA BEANS								
Marama beans[d]	*Tylosema esculentum*	-	2	3	37	32	2	23
LEGUME OIL CROPS								
Soybeans	*Glycine max*	223	9	5	20	36	21	9
Peanuts (groundnuts)	*Arachis hypogaea*	36	7	2	49	26	7	9
PULSES								
Dry beans (Common beans) (*Phaseolus* spp. including several species now in the genera *Vigna*), e.g.								
Kidney beans, haricot beans, pinto beans, navy beans	*Phaseolus vulgaris* subspp.		12	4	1	22	41	20
Lima beans (butter beans)	*Phaseolus lunatus*	21	10	4	1	21	44	19
Azuki beans (adzuki beans)	*Vigna angularis*		13	3	1	20	50	13
Mung beans (golden grams, green grams)	*Vigna radiate*		9	3	1	24	47	16
Dry peas, e.g. garden peas, protein peas	*Pisum sativum* subspp.	10	11	3	1	25	34	26
Chickpeas (garbanzo beans, Bengal grams)	*Cicer arietinum*	10	12	2	6	19	44	17
Dry cowpeas, e.g. black-eyed peas	*Vigna unguiculata* subspp.	5	11	3	2	24	49	11
Dry broad beans, e.g. broad beans (faba beans), horse beans	*Vicia faba* subspp.	4	11	3	2	26	33	25
Lentils	*Lens culinaris*	4	10	3	1	26	29	31
Pigeon peas (cajan peas, Congo beans, toor dāl, arhar dāl)	*Cajanus Cajun*	3	11	3	1	22	48	15

Table 2 continued

Common name	Scientific classification	World annual production 2009 (million tons)[a]	Proximate composition (g/100g)[b]					
			Moisture	Ash	Lipid	Protein	Digestible carb.[c]	Dietary fibre
CEREALS								
Maize	*Zea mays* subspp.	819	10	1	5	9	67	7
Wheat	*Triticum* spp.	686	11	2	2	12	61	12
Rice	*Oryza sativa*	685	12	1	2	7	76	3
Barley	*Hordeum vulgare*	152	9	2	2	12	56	17
Sorghum	*Sorghum bicolour*	56	9	2	3	11	69	6
Millet	(Species in several genera)	27	9	3	4	11	64	9
Oats	*Avena sativa*	23	8	2	7	17	55	11
Rye	*Secale cereal*	18	11	2	2	10	61	15

[a]Production statistics obtained from FAOSTAT (2011).
[b]Nutrient data retrieved from the National Nutrient Database for Standard Reference (USDA, 2011). All data are for mature raw seeds.
[c]Digestible carbohydrate = carbohydrate by difference - total dietary fibre.
[d]Data on the average marama bean composition are from Paper I.

A PCA conducted on the proximate composition results (Table 2) on dry matter (dm) basis (Figure 6) reveals that marama beans are most similar to oil crop legumes such as peanuts and soybeans since they all have high lipid and protein contents. Marama beans are quite different from other pulses and even more so compared to cereals, primarily due to the high protein and lipid content of the beans and their low content of digestible carbohydrates. Therefore, soybeans and peanuts are the primary reference crops in the following discussion of the more detailed composition of marama beans.

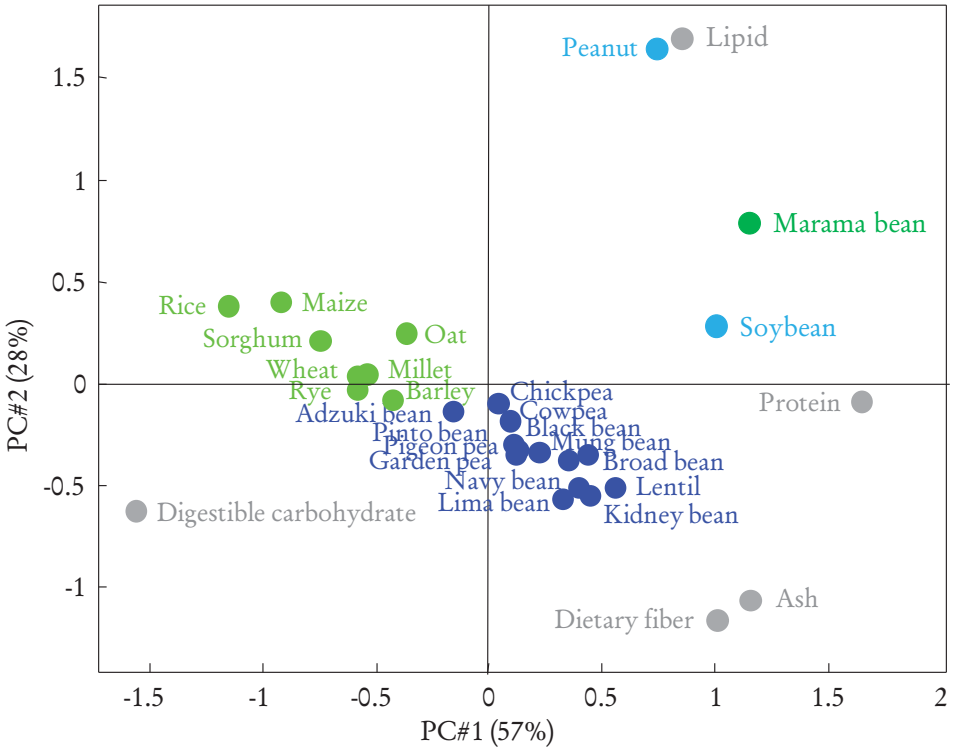

Figure 6 Bi-plot of PC1 vs. PC2 from a PCA model including contents (dry matter basis) of ash, lipid, protein, total dietary fibre and digestible carbohydrate (all shown in grey) for the legumes and cereals mentioned in Table 2. Samples are coloured according to the groups in Table 2; **marama bean**, **legume oil crops**, **pulses** and **cereals**

Chemical variations in marama beans

The proximate chemical composition of marama beans has been investigated and reported several times. Yet, compared to other more common legumes (e.g. peanuts and soybeans) marama beans are still rather unexplored. This is especially noticeable when looking at the sample sets analysed in existing studies. For the majority of studies only one batch is included in the sample set and only in a couple of studies more than two batches are explored.

Table 3 summarizes the proximate composition of marama beans and *T. fassoglense* beans reported so far (including data from Paper I). In all these studies, the samples analysed are mixtures of several marama beans. To highlight that such results are not from single marama beans, they will be denoted as *bulk*

Table 3 Proximate composition (g/100 g) of the marama bean reported as mean ± standard deviation (studies with only one batch investigated) or range (studies with more than one batch analysed). A literature survey

Examined batches[a]	Country of harvest	Harvest year	Moisture	Lipid	Protein	Carbo-hydrate[b]	Dietary fibre	Ash	Reference
1	South Africa	–	4.0	41.6	32.8	17.2	1.3[d]	3.1	Anonymous (1924)[f]
1	Botswana	–	5.2	36.1	31.6	23.2	1[d]	2.9	Wehmeyer *et al.* (1969)[f]
1	Botswana	1978–80 (mixed)	3.9±1.0[h]	42.2±1.6	31.8±1.1	18.9±2.2	–	3.2±0.1	Bower *et al.* (1988)[f]
2 (*T. fassoglense)*	Burundi and Zaire	–	–	24–30	–	–	–	–	Dubois *et al.* (1995)[f]
1	Botswana	–	–	48.2	–	–	–	–	Ketshajwang *et al.* (1998)[f]
1	Botswana	–	–	33.5±0.04[i]	34.1±0.12	24.1±0.02	4.4±0.13[d]	3.7±0.14	Amarteifio and Moholo (1998)[e]
1	–	–	–	41.7	38.4	–	–	–	Francis and Campbell (2003)[f]
6 (roasted beans)	Botswana and Namibia	2001–03	2.0–4.1	31.5–40.2	34.0–41.3	9.4–14.5[c]	3.5–4.3[d]	3.08–3.51	Müseler and Schönfeldt (2006)[f]
1	Botswana	–	–	38.4	–	–	–	–	Mitei *et al.* (2008)[f]
1	Botswana	–	6.5±0.5[h]	35.2±6.2	31.6±0.8	24.2±6.2	–	2.6±0.2	Jideani *et al.* (2009)[f]
14 (*T. esculentum*) 2 (*T. fassoglense)*	Botswana, Namibia and South Africa	1990 and 2005–08	1.3–6.6[g]	32.0–41.9	28.8–38.4	–	18.7–26.8	2.5–3.7	Paper I[e]
1	Botswana	2006	–	38.1±0.0[h]	34.3±0.5	24.8±0.1	–	2.7±0.0	Kayitesi *et al.* (2012)[e]
2 (*T. esculentum*) 1 (*T. fassoglense)*	Botswana and South Africa	2007–08	5.1–7.1[g]	30.6–37.3	29.2–36.2	14.9–18.3	7.9–8.4[d]	3.2–3.4	Amonsou *et al.* (2011)[f]
1	Botswana	2008	5.3±0.2[h]	40.0±0.7	32.3±0.8	19.4	–	3.0±0.0	Mosele *et al.* (2011b)[f]

[a] *T. esculentum* if not otherwise stated [b] Obtained by difference [c] Total non-structural carbohydrates [d] Crude fibre [e] Results on dry matter basis
[f] Results as is [g] Duplicate [h] Triplicate [i] Four replicates

samples while results stemming from one single marama bean (as will be used later) will be denoted as a *single bean sample*. Where the bulk sample may consist of beans from one or several *batches* with different characteristics (such as harvest year, location, season, maturity and genotype) the single bean sample will have a unique harvest year, location, season, maturity and genotype.

The studies using just one bulk sample cannot be explored with respect to variations between beans and between harvest years and places. Other studies (including Paper I) conducted on several bulk samples from different harvest years and/or places make it possible to investigate the *batch-to-batch variation*. Since none of the studies listed in Table 3 are conducted on single beans it is not possible to evaluate the *bean-to-bean variation* within a specific batch.

In studies with just one bulk sample only the *analytical method variation* can be investigated (e.g. deviation between replicate samples of the same homogeneous bulk sample). An overview of which type of sample specific variation that can be considered for different sample sets (together with the reported proximate composition) is shown in Figure 7.

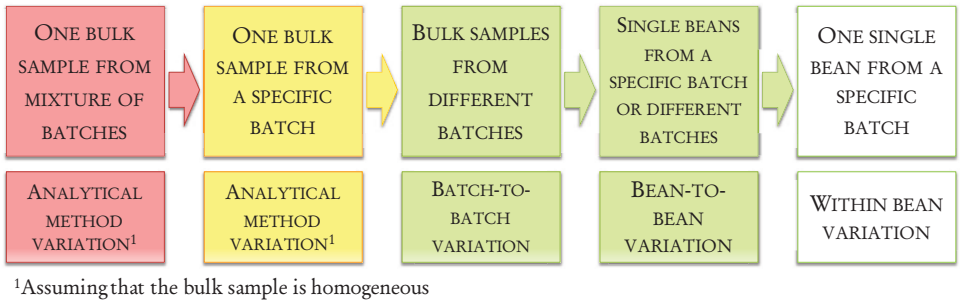

Figure 7 Type of sample and corresponding type of variation. One batch will have a unique harvest year, location, season, maturity and/or genotype

Until recently, most marama bean studies belonged to the red or yellow boxes. Only a few more recent studies include batch-to-batch (Dubois *et al.*, 1995; Müseler & Schönfeldt, 2006; Paper I; Amonsou *et al.*, 2011) and bean-to-bean (Paper II) variations.

Batch-to-batch variation

Table 3 reveals large batch-to-batch variation in the chemical composition between the marama bean batches from different harvest areas and years. This is

seen by the large ranges provided for studies where several bulk samples have been analysed. This variation, entailing geographical and season related differences, is crucial and should be further investigated. In Paper I (Figure 2), this batch-to-batch variation is illustrated in a bi-plot from PCA on chemical data from 16 marama bean batches, which is also given in a modified version below in Figure 8A.

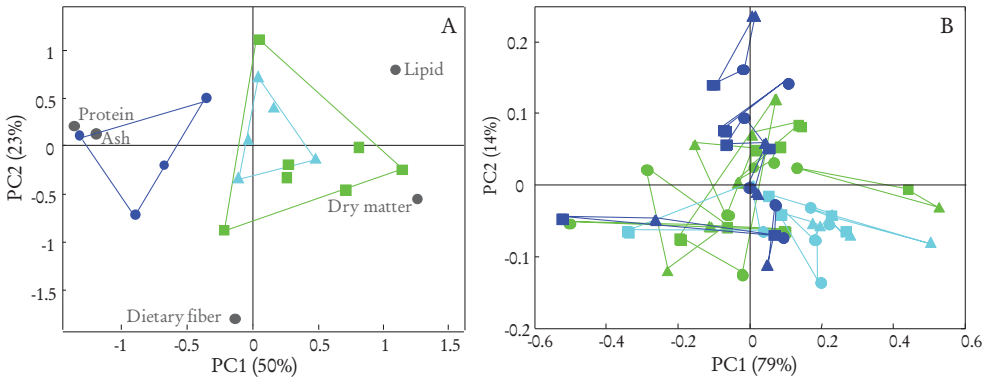

Figure 8 A) Bi-plot from PCA on average chemical data (duplicates) from 16 marama bean samples (bulk samples). Border points are connected by lines. B) Score plot from PCA on all NIR spectra (780–2498 nm) from the duplicate measurements of 3 single marama bean samples from each of 18 batches. Square, triangle and circle indicate sample a, b and c within each of the batches (connected with lines). Both figure A and B are coloured according to country; **Botswana**, **Namibia** and **South Africa**

Figure 8A shows that determination of the proximate chemical composition makes it possible to differentiate between batches of marama beans harvested in South Africa as opposed to the samples harvested in Namibia and Botswana, which do not seem to differ considerably from each other. There is a tendency towards a higher content of ash, protein and water, and a lower content of lipid in samples from South Africa compared to the samples from Namibia and Botswana. These differences were mainly ascribed to varying climate and soil conditions of the harvest places (Paper I).

Bean-to-bean variation

In order to be able to investigate the bean-to-bean variation, spectroscopic analysis of single beans from several batches were carried out (Paper II). This was possible, since spectroscopy, as opposed to wet chemical analyses, provides a method of analyzing even small amounts of an intact marama bean sample. In

Paper I it was discussed that a large bean-to-bean variation could be present and this was confirmed by the spectroscopic studies presented in Paper II where three representative single beans, from each of the batches examined in Paper I, were analysed.

None of the applied spectroscopic techniques (NIR, IR, Raman and NMR) gave data with representative information for the respective country of origin or harvest year. This was proposed to be due to the large bean-to-bean variation. In Figure 8B the bean-to-bean variation is illustrated in a score plot from PCA on NIR spectra from all single beans analysed (Paper II, Figure 2). Figure 8B shows that the beans from the same batch can be very different and overshadow the otherwise clear geographical differences from the bulk sample study (Paper I). Because of the large bean-to-bean variation it was not possible to find any quantitative relationship between the spectra (single bean samples) and the different chemical components (bulk samples), e.g. to predict the protein content from NIR spectra.

Besides the results obtained from Paper II, the variation between single marama beans has not previously been examined. However, Monaghan and Halloran (1996) examined the genetic diversity by examining leaves collected from 16 different marama plants from three different populations in Botswana (app. 1 ha in area, 6-800 km apart). They found a considerable amount of genetic variation in the marama plants and concluded that most of the genetic variation (85%) occurred within (plant-to-plant) rather than between (batch-to-batch) populations. More recent studies have initiated a focused effort of understanding the genetic variation and distribution of the marama bean. These studies have included more than 300 marama plants from 10 to 20 populations for examination of the genetic diversity of the marama bean. All studies confirmed the relatively high level of genetic diversity in the marama bean and that the primary variation is within rather than between populations (Nepolo *et al.*, 2009; Takundwa *et al.*, 2010a; Takundwa *et al.*, 2010b; Nepolo *et al.*, 2011).

Within bean variation

To date no studies have been conducted to examine the *within bean variations* (the white box in Figure 7), that is the spatial resolution of chemical constituents in marama beans. This type of variation can be examined by use of several different analytical techniques. One way would be to apply different kinds of microscopy, however, these techniques require cumbersome and destructive slicing and staining of the chemical constituents of interest. Some microscopic

analyses have been carried out (Amonsou *et al.*, 2011; Mosele *et al.*, 2011b), but these have only analysed selected chemical features in selected areas of a single marama bean. Hence, not providing information on the entire chemical variation within a marama bean.

A promising approach to gain spatial chemical information in a sample is hyperspectral NIR imaging (Amigo, 2010), which is a combination of NIR spectroscopy and an imaging system. This approach provides spectral information in the NIR wavelength region for each pixel. The generated hyperspectral image or "hypercube" makes it possible to describe chemical differences and gradients in the marama bean and can therefore be used for exploration, classification and to some degree quantification. NIR hyperspectral imaging has previously been applied for examination of e.g. single cereal kernels (Smail *et al.*, 2006; Gorretta *et al.*, 2006; Weinstock *et al.*, 2006; Berman *et al.*, 2007; Mahesh *et al.*, 2008; Nansen *et al.*, 2008; Williams *et al.*, 2009).

In a preliminary study, NIR hyperspectral imaging was applied to marama beans (own work, unpublished). Results from the NIR hyperspectral image analysis of a slice from a single marama bean is shown in Figure 9. The score plot and score image reveal that PC2 describes a chemical gradient that changes from the core to the back of the bean (gradient 1) while PC3 contains information that separates the tips of the bean from the back (gradient 2). The loading plot for PC2 suggests that the separation between the inner and outer parts of the bean is due to a gradual change in the lipid concentration, among other things. The highest lipid content is found in the core of the marama bean. Likewise, the gradient that changes from the tip of the bean to the back of the bean is due to a gradual change in the carbohydrate concentration (loadings for PC3). The highest carbohydrate content is present in the tips of the bean.

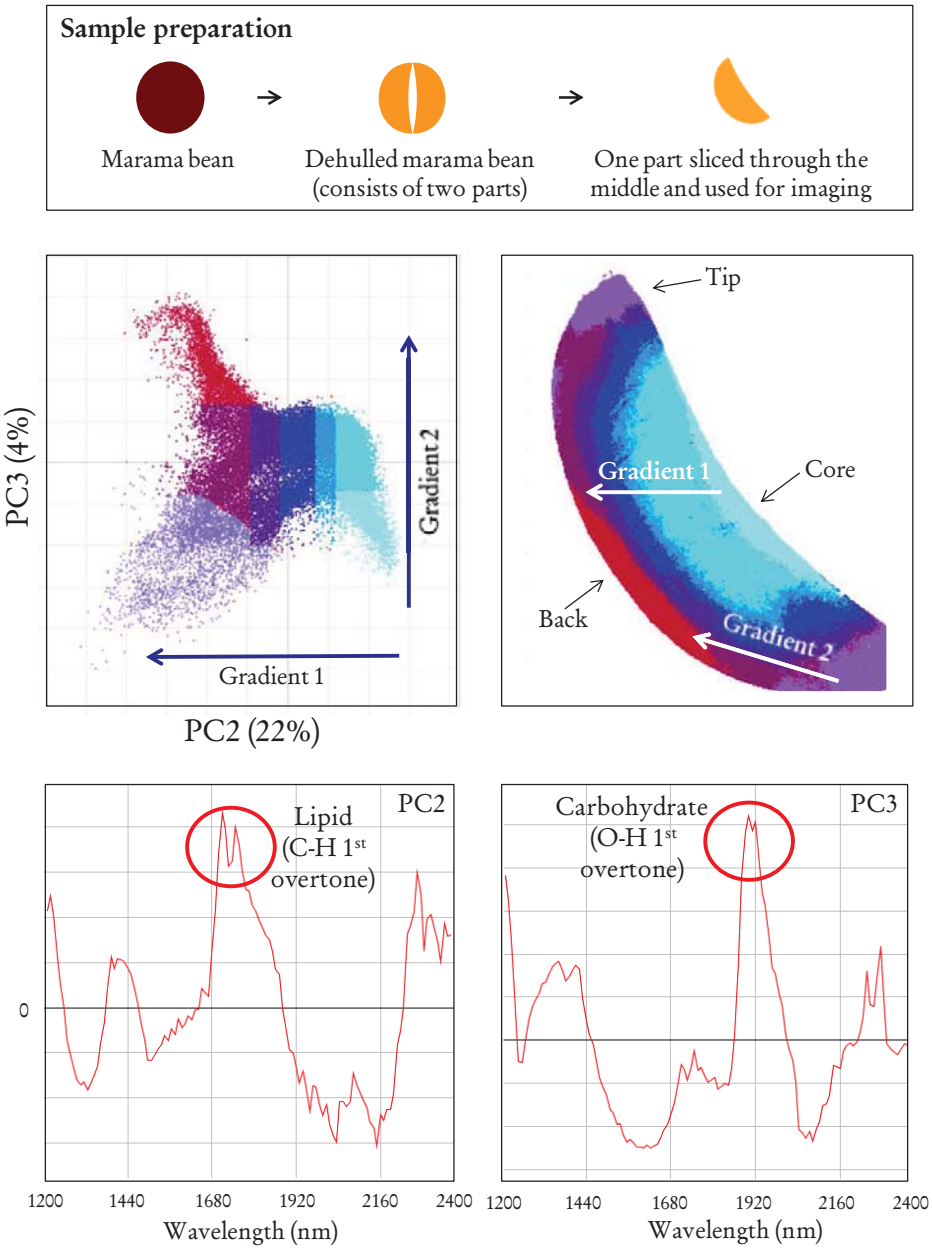

Figure 9 Top) sample preparation for NIR hyperspectral image analysis. Middle) PCA score plot (PC2 vs. PC3) and corresponding score image of the sliced marama bean. The colours indicate chemical gradients in the marama bean. Bottom) corresponding loading plots with two of the major peaks assigned

Composition of the protein, lipid and carbohydrate fractions

The chemical composition of marama beans influences their functional properties and nutritional quality and this knowledge must be utilised when developing new food products containing marama beans. This section reviews the chemical composition and structure of protein, lipid and carbohydrate fractions of marama beans focusing on physiochemical and functional properties. These properties are discussed in relation to the characteristics of other legumes already used in food products (e.g. soybeans). This is followed by a more nutritional discussion of the chemical composition of marama beans in *Chapter 5*, where also the micronutrient composition of marama beans is presented and discussed.

Protein composition 

The protein composition and structure of marama beans affect the physiochemical and functional properties such as the seed hardness, protein digestibility, protein solubility, water and oil absorption, emulsion and foaming capacity, viscosity and gelation (Maruatona *et al.*, 2010; Amonsou *et al.*, 2011). It is thus important to obtain detailed knowledge about the protein composition when the aim is to develop new protein rich products or ingredients from marama beans.

Total protein content

The high protein content of marama beans ranging from 28.8 to 38.4% dm (Paper I, Table 2) is higher than that of nearly all legumes and equalling that of soybeans (Table 2). This gives marama beans a great potential both as a nutritive food by itself and as a protein-rich food ingredient for supplementation in other food products or for improving the functionality of more complex food systems.

Microstructure of the bean

The microstructure of marama beans is important for processing and industrial applications. The physical location and protein body structure of the storage protein in marama beans were recently investigated by Amonsou *et al.* (2011). They found that the proteins were clustered as spherical protein bodies surrounded by lipid bodies. This was confirmed by a high resolution imaging study by Mosele *et al.* (2011b) who characterised the complete microstructure of

marama beans. The microstructure was investigated by different microscopy techniques and one of the results is shown in Figure 10. This illustrates how the protein bodies (green) are surrounded by lipid bodies (black) within each parenchyma cell.

The shape and physical location of protein bodies in marama beans are similar to those known from other legumes including soybeans and peanuts (Lott & Buttrose, 1978). The findings and similarities with soybean, suggest that extraction of marama protein may be done in the same way as for soybeans (Amonsou *et al.*, 2011). In addition to the protein bodies within the parenchyma cells, the cell walls also contain some protein, which was apparent from microscopy of marama bean tissue stained with Aniline Blue Black (Mosele *et al.*, 2011b).

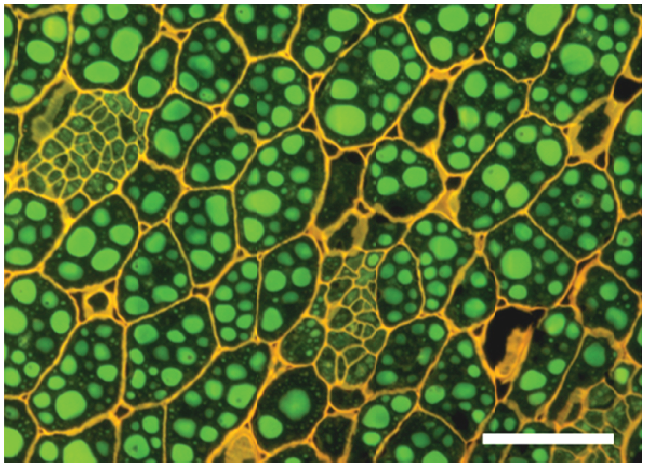

Figure 10 Parenchyma cells in a marama bean (coriphosphine O-staining), showing protein bodies and **lipid bodies** within the cells and pectin in middle lamella and cell corners. Bar = 100 μm (Mosele *et al.*, 2011b)

Two types of inclusions within marama protein bodies were identified, namely spherical globoids and druse crystals (cluster of small crystals in an arrangement called a druse or rosette), which function as storage sites for minerals (Amonsou *et al.*, 2011). Spherical globoids are the most common inclusions in many legumes including peanuts and from these legumes the spherical globoids are known to be rich sources of phosphate (deposited as insoluble phytate), potassium, calcium and magnesium. Likewise, druse crystals are known to be rich in calcium (Lott & Buttrose, 1978).

Storage proteins

The storage proteins, which are normally found in high concentrations in legumes, function as reserves of minerals and amino acids. The minerals and amino acids may be mobilised and utilised for maintenance and growth of the seed (Berg *et al.*, 2002). The storage proteins of marama beans may be classified according to their solubility, and are composed mainly of globulins (dilute salt soluble) (53%) and albumins (water soluble) (23%) (Bower *et al.*, 1988) as is the case for most other legume species (Gueguen, 1983). Marama bean protein furthermore contains aqueous alcohol soluble prolamins (16%) and alkali and acid soluble gluteins (7.7% and 0.5%, respectively) (Bower *et al.*, 1988). This protein composition resembles that of e.g. dry broad beans and peas more than that of common beans, soybeans and lupines (Gueguen, 1983).

In this work, the protein composition was investigated by ^{1}H HR MAS NMR (Paper II). However, this method is only able to analyse the mobile phase of the sample, and therefore it was only possible to investigate the signals from approximately 23% of the proteins (albumins). Nevertheless, ^{1}H HR MAS NMR revealed that tyrosine is predominant and the only aromatic amino acid in this fraction of the marama bean proteins (Paper II, Figure 8).

Globulins in common legume species are composed of two major proteins characterised by their sedimentation coefficients (S); vicilin (7S) and legumin (11S), and the ratio between these differ between species (Sathe & Venkatachalam, 2004; Marcello, 2006). Amonsou *et al.* (2012) found that vicilin-like subunits are absent in marama protein and concluded that this is most unusual, if not unique for a legume seed. They furthermore found that the only major proteins in marama beans are basic legumin-like (11S) storage proteins and two additional proteins of high molecular weight (63 and 148 kDa). The legumin-like proteins of marama beans seem to lack disulphide bonds and are present in higher amounts than in soybean protein. Storage protein composition significantly influences the functionality of proteins and these findings suggest that the marama bean proteins may have increased heat stability compared to soybean proteins due to strong hydrophobic interactions associated with the basic legumin-like proteins. In general, the protein profile of marama beans is different to those of soybeans, peanuts and other protein-rich oilseeds such as canola and sunflower indicating that the functionality of marama protein is different (Amonsou *et al.*, 2012).

Amino acids

The amino acid composition of marama beans is largely dominated by glutamic (app. 15 g/100 g) and aspartic acid (app. 10 g/100 g) as well as tyrosine (app. 11 g/100 g) (Bower *et al.*, 1988; Amonsou *et al.*, 2012). Actually the tyrosine content is substantially higher than that of most other legumes including soybeans and peanuts (USDA, 2011), while the content of tyrosine in lentils is extremely high and about three times that of marama beans (Iqbal *et al.*, 2006).

The strong presence of aromatic amino acids in marama bean protein (histidine, phenylalanine, tryptophan and tyrosine, app. 20% of the total amino acids) was evident from Raman spectra as well as ^{13}C CP/MAS and ^{1}H HR MAS NMR spectra of marama bean flour (Paper II, Figures 5, 4 and 8, respectively).

The high content of aromatic amino acids together with the aliphatic amino acids (alanine, isoleucine, leucine, proline and valine, app. 24% of the total amino acids) may increase the hydrophobicity and stability of marama protein in comparison to other legumes that have lower contents of these amino acids, e.g. soybeans. The high tyrosine content may contribute to the structural stability of marama protein in a similar way as in gluten, since tyrosine is involved in polypeptide cross-linking (Tilley *et al.*, 2001; Takasaki *et al.*, 2005). A cross-link between two tyrosine residues (dityrosine) in a polypeptide is depicted in Figure 11.

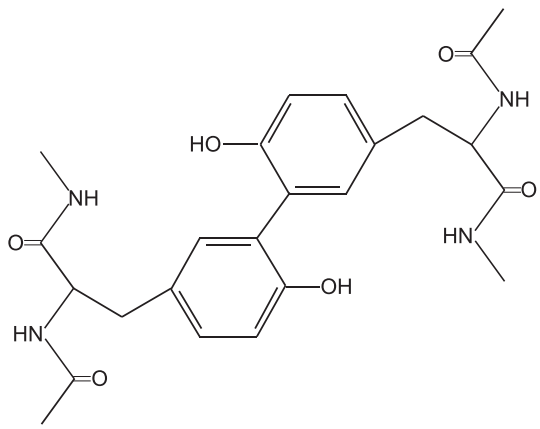

Figure 11 Polypeptide cross-linking by two tyrosine residues in marama bean protein

Secondary structure

The secondary structure of proteins affects the nutritional properties in relation to protein digestibility and influences the functionality of the protein, which is

important in relation to processing of functional ingredients (Carbonaro *et al.*, 2008). The secondary protein structure has been investigated by use of IR spectroscopy (Paper II, Figure 9). In particular the spectral region containing the amid I band (1660 cm^{-1}), arising from the stretching vibrations of C=O in the peptide bonds (Jackson & Mantsch, 1995) was examined. The frequency of the carboxyl group depends on the secondary structure of the protein and by calculation of the second derivatives of the spectra it is possible to assign the spectral components of the amide I band (Susi *et al.*, 1967; Krimm & Bandekar, 1986; Dong *et al.*, 1990). Furthermore, it is possible to investigate the relative intensity of the spectral component associated with a given secondary structural element and hereby gain knowledge about the relative content of the different secondary structures.

The secondary structure of marama protein is dominated by α-helixes and β-sheets, and to much lesser extent β-turns, while no random coil conformations or molecular aggregates were identified. Hence, marama bean protein is highly structured (Paper II). The high level of β-sheets compare well to the secondary protein structure of other legumes such as common beans and lentils, which have been investigated using the same technique by Carbonaro *et al.* (2008). However, these legumes also contain molecular aggregates in addition to α-helixes and β-turns. Likewise, peanut protein has high levels of β-sheet secondary structure and lower levels of α-helixes, β-turns and random coils (Marcone *et al.*, 1998). Soybean protein consists mainly of β-sheets and β-turns and has a higher level of α-helixes than peanut protein, but a substantially lower contribution from random coils (Dev *et al.*, 1988; Zhao *et al.*, 2008). In conclusion, it seems that the secondary protein structure of marama beans resembles that of soybeans the most.

Lipid composition

The composition of marama bean lipids impacts both the nutritional, sensory and functional properties of the bean.

Total lipid content

The total lipid content of marama beans is exceptionally high ranging from 32.0 to 41.9% dm (Paper I, Table 2). The lipid content by far exceeds that of other pulses (Table 2) and compares with that of oil seeds used for production of

commercial vegetable oils such as sunflower seeds (22–36%) and rapeseeds (22–49%) (Belitz *et al.*, 2004) and closely approaches that of peanuts (Table 2).

Storage lipids

In most seeds, storage lipids are in the form of triglycerides (Murphy, 1990). These are made up of a variety of fatty acids esterified to the hydroxyl groups of a glycerol backbone. The physical and chemical properties of lipids depend primarily on their triglyceride profile.

The microstructural studies carried out by Mosele *et al.* (2011b) showed that the lipid in marama beans exists mostly as neutral lipids in droplet form, surrounding the protein bodies in the parenchyma cells (Figure 10). This finding is in good agreement with the fact that 83% of the lipid is made up of triglycerides and free fatty acids (Mitei *et al.*, 2008). The positional distribution of fatty acyl chains on the glycerol backbone of triglycerols in marama bean oil (Mitei *et al.*, 2008) is given in Figure 12.

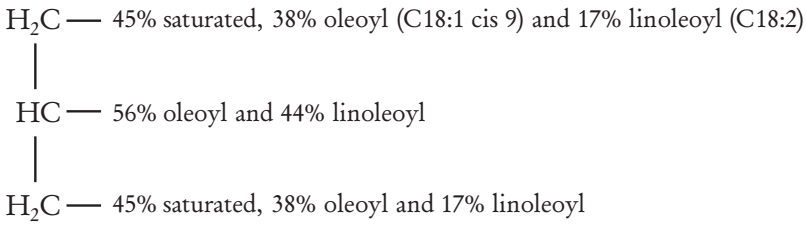

Figure 12 Positional distribution of fatty acyl chains on the glycerol backbone of triglycerols in marama bean oil

Besides the triglycerides, marama oil contains mono- and diglycerols (almost 10% in total) and lower levels of glycolipids (1.6%), phospholipids (1.3%), sterols (3.7%) as well as hydrocarbons (1.4%) (Mitei *et al.*, 2008).

A content of less than 5% free fatty acids has been determined in marama bean oil (Bower *et al.*, 1988) suggesting that the activity of lipases is negligible in the beans. According to Codex Alimentarius (2001), ordinary virgin olive oil is allowed a free acidity of 3.3 g/100 g oil, indicating that marama bean oil is of an acceptable quality in this regard.

Fatty acid composition

The fatty acid composition is dominated by unsaturated fatty acids (app. 75%), the principal being oleic acid (C18:1, n-9) (43%) followed by linoleic (C18:2, n-6)

(22%) and α- and γ-linolenic acid (C18:3, n-3/n-6) (2.2%) as well as lower concentrations of erucic (C22:1, n-9) (2.1%), arachidonic (C20:4, n-6) (2.0%), palmitoleic (C16:1, n-7) (1.1%) and gadoleic acid (C20:1, n-9) (0.7%). Saturated fatty acids of marama beans comprise palmitic acid (C16:0) (14%) and lower concentrations of stearic (C18:0), arachidic (C20:0), behenic (C22:0), myristic (C14:0) and margaric acid (C17:0) (Engelter & Wehmeyer, 1970; Bower *et al.*, 1988; Ketshajwang *et al.*, 1998; Francis & Campbell, 2003; Mitei *et al.*, 2008). The fatty acid composition is compared to that of other oil crops in Figure 13, which shows that it largely resembles the lipid profile of peanuts.

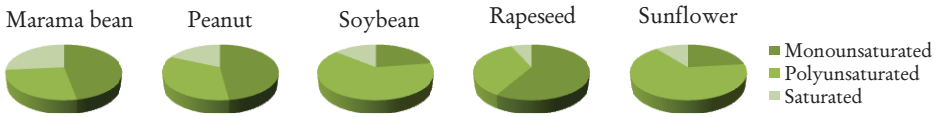

Figure 13 Average fatty acid composition of marama bean oil (Paper II) and other oil crops (Saxholt *et al.*, 2008) showing the percentage of saturated, monounsaturated and polyunsaturated fatty acids

Raman spectroscopy resulted in exceptionally well-resolved and intense spectra, providing good quantitative and overall information on the lipid composition of marama beans (Paper II, Figure 5). The degree of unsaturation was furthermore evaluated by several different ratios between peaks in the Raman spectrum (Ozaki *et al.*, 1992; Li-Chan, 1996). This enabled the detection of even minor differences in the degree of unsaturation.

The combined use of three different NMR techniques (Table 1) made it possible to assign all protons and carbons in the marama bean lipids hereby providing an overall picture of the functional groups of the fatty acids (Paper II, Figures 3 and 4). However, individual fatty acids could not be assigned in such a complex matrix. A linear combination of the spectra from ^{13}C SP/MAS and CP/MAS NMR resulted in a spectrum only comprising the resonances from lipids (Paper II, Figure 4). From this, it was possible to calculate a range of overall descriptors of the lipids. For instance the average fatty acid chain length was determined to be 18.1 carbons, which is in good agreement with the wet-chemical analysis (17.7 carbons). The degree of unsaturation was also investigated as the ratio of unsaturated to carbonyl and saturated carbons, which was found to be 1:8.5. This was also in fair agreement with the expected ratio of 1:7.3 from the lipid

composition analysed by wet chemistry. The spectra furthermore confirmed that more than half of the unsaturated fatty acids are monounsaturated.

Carbohydrate composition 

Knowledge about the carbohydrate composition of marama beans is important in relation to the potential use of the carbohydrate fractions in different food applications. Especially polysaccharides are important in the food industry due to their role as thickeners, stabilisers, texturisers and gelling agents (Willats *et al.*, 2006). However, very little research has been carried out on the carbohydrate composition of marama beans.

Total carbohydrate content

The total content of carbohydrates has only been determined indirectly as the difference between 100% and the content of proteins, lipids and ash. These contents vary between 9 and 25% (Table 3). In comparison, the total carbohydrate content of other pulses accounts for about 60% while the oil crops peanut and soybean contain 16% and 30% carbohydrate, respectively (Table 2). Hence, as previously stated, marama beans resemble legume oil crops more than pulses. However, determining the total carbohydrate content by the "by difference" approach possesses a number of problems, since the determined value will also include a number of non-carbohydrate components such as organic acids, tannins, waxes and some Maillard products. Additionally, all the analytical errors from the different proximate analyses will be added (Southgate, 1991; Englyst & Hudson, 1996). Instead, it is more useful to identify the different types of carbohydrates in marama beans and in this way gain a better understanding of the potential physiological properties and health benefits of these.

Dietary fibre

Dietary fibre (DF) is defined as carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine, and includes polysaccharides, oligosaccharides, lignin and associated plant substances (AACC, 2001). DF may be divided into soluble and insoluble DF. The soluble DF, such as pectin, may be degraded by anaerobic bacteria in the colon, while insoluble DF, such as cellulose, may only be fermented to a limited extent in the colon (Anderson *et al.*, 2009).

Marama beans have a DF content varying between 18.7% and 26.8% dm of which only about 4% is water soluble (Paper I, Table 3). In comparison with the total DF content of peanuts and soybeans, which is 9 and 10% dm, respectively (Table 2), it appears that marama beans have a considerably higher level of DF than other legume oil seeds. The DF content even approaches some of the pulses with the highest contents of DF (dry peas and broad beans) (Table 2).

Insoluble polysaccharides

The application of ^{13}C SP/MAS NMR made it possible to examine both the soluble and insoluble polysaccharides in marama beans (Paper II, Figure 4). The spectra revealed a major contribution from ester or acid groups, indicating that a large part of the polysaccharides are present as pectins/galacturonic acids. Raman spectroscopy showed the strong presence of α-anomeric (non-starchy) carbohydrates (Paper II, Figure 7) and in combination with the NMR spectra indicated that a large part of the carbohydrates are homogalacturonans. The chemical structure of a homogalacturonan is shown in Figure 14, illustrating how galacturonan units are linked by (1→4)-α-linkages.

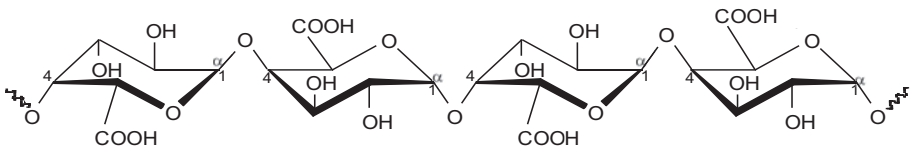

Figure 14 Homogalacturonan structure. Modified from Brejnholt (2009)

These results correspond well with the results obtained recently by Mosele *et al.* (2011b). They investigated marama beans by histochemical methods and electron microscopy and found that the majority of the carbohydrates in marama beans are insoluble polysaccharides (pectin and cellulose), which are present in the cell walls. The pectin is visible in the cell walls (orange) in Figure 10. They furthermore concluded that lignin is absent in marama beans.

The starch content of marama beans has been determined by Mosele *et al.* (2011a) who found it to be negligible (0.2% dm). This was also confirmed by spectroscopic studies where contributions from starch could not be detected neither in ^{1}H HR-MAS NMR nor Raman spectra (Paper II, Figures 6 and 7). The negligible starch content distinguish marama beans from most other legumes, in which starch is usually the most abundant carbohydrate (22-45%) (Hoover & Sosulski, 1991). Yet, legume oil crops usually have extremely low

starch contents, for instance soybeans have a starch content of 0.2-1%. Actually, starch is present in soybeans during maturation (6-9%) but is degraded before the seeds reach maturity. It is suggested that the starch is consumed for the production of soluble oligosaccharides (Saldivar *et al.*, 2011), which might also be the case for marama beans.

Soluble carbohydrates

The minor fraction of soluble carbohydrates in marama beans has been analysed by solution state NMR (Paper II, Figure 6). The obtained spectrum was rather simple in the anomeric region and only three resonances were tentatively assigned, suggesting a high content of raffinose (O-α-d-galactopyranosyl-(1→6)-O-α-d-glucopyranosyl-(1→2)-β-d-fructofuranoside), among the water soluble carbohydrates. The structure of raffinose is shown in Figure 15.

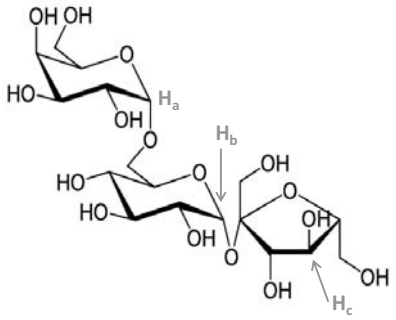

Figure 15 Raffinose. The protons assigned by ^{1}H HR-MAS NMR are given. H_a originates from the H_1 in the α-glucose unit, H_b originates from H_1 in the α-galactose unit and H_c is from the H_3 in the fructofuranose unit (Paper II, Table 3)

A comprehensive assignment of the resonances from the pyranosidic carbohydrate protons was hampered by a large number of overlapping resonances. Yet, even though no specific carbohydrate composition could be obtained by spectroscopic measurements, NMR and Raman spectra (Paper II, Figures 6 and 7) revealed that marama beans do not contain measurable amounts of abundant water soluble carbohydrates such as β-glucans and glucose normally found in cereals.

Chemical analysis carried out by Mosele *et al.* (2011a) confirmed a very low content of soluble sugars (<1% dm) in marama beans, which is common for legumes. They found sucrose to be the dominant sugar followed by raffinose and myoinositol and smaller amounts of maltose, arabinose and ribose, hence

confirming the content of raffinose and absence of glucose identified by NMR spectroscopy.

Considerations and recommendations for future research

Although the large variations in chemical composition between single beans advocate for the use of bulk samples to ensure a good measure of an average sample taken at a certain place or time, this will also hide some very relevant information; the bean-to-bean variation. Being able to characterise and understand the bean-to-bean variation will most certainly help the domestication process, since beans with specific traits may be selected and used for breeding. The large bean-to-bean variation also highlights the importance of proper sampling when evaluating single beans.

The beans used in Paper I and II as well as the beans used to prepare the products analysed in Paper III and IV were all collected in Southern Africa and sent to Denmark for analyses. Information on harvest sites and years were provided, however more specific information was lacking. In future studies such information would be useful, e.g. specific harvest site (e.g. GPS coordinates), specific harvest date, which plant in the specific population, which stem on the plant, which pod on the stem, how many beans in the pod and the genetics of the plant. Moreover, it would be useful to include information on weather conditions throughout the growing and harvest seasons, e.g. rainfall (mm) and sun (hours) and soil conditions, e.g. soil type and mineral content. These data could yield important information in relation to selection of specific plants in breeding programs.

In order to gain more knowledge on the physiochemical and functional potentials of marama beans in relation to product development, more research is needed on the protein and carbohydrate composition of the beans. The protein, lipid and carbohydrate results (Paper II) illustrate how NMR in combination with the vibrational spectroscopic techniques (NIR, IR and Raman) can be applied to a chemically intact food matrix and hereby provide qualitative and semi-quantitative information about the chemical composition. These techniques could also be applied to protein, lipid and carbohydrate fractions of marama beans. This would provide a more comprehensive characterisation of these chemical classes since spectral areas with many overlapping peaks found in spectra of the intact marama bean would be less packed and hence easier to identify. One example is the region at about 50-80 ppm in the ^{13}C CP/MAS

NMR spectrum where the aliphatic side chains in amino acids and non-anomeric carbons in the carbohydrates significantly overlap (Paper II, Figure 4).

Spectroscopy could also be applied in relation to product development. As an example IR could be used to monitor conformational changes of the marama proteins occurring during processing such as roasting where the heat most likely will alter the (secondary) protein structure.

Chapter 5

Nutritional perspectives of marama beans

Background

Undernourishment[8] is a major challenge – especially in the developing world. The overall number of undernourished people in the world is approaching one billion (FAO, 2010) and more than a quarter of these live in Sub-Saharan Africa (The World Bank, 2011).

Especially children are at risk and globally about 171 million, or more than one in four, children under five years are stunted (low height-for-age). This is especially a problem in developing countries, not least in Africa where 38% of the children under five years are stunted (de Onis *et al.*, 2012). This is a consequence of chronic undernutrition beginning already before birth if the mother is undernourished (UNICEF, 2009). Undernourishment of children is detrimental in many ways since it undermines both their growth, development and cognitive abilities and make them more susceptible to infectious diseases. As adults they may also face a higher risk of chronic illnesses such as heart disease and diabetes (UNICEF, 2009). Moreover, it is estimated that inadequate nutrition contributes to more than one third of all deaths of children under the age of five (UNICEF, 2009) partly because it is an underlying cause associated with illnesses such as diarrhoea, pneumonia, malaria and measles (Caulfield *et al.*, 2004).

[8]Undernutrition is defined as the outcome of insufficient food intake, inadequate care and infectious diseases. It includes being underweight for one's age, too short for one's age (stunting), dangerously thin for one's height (wasting). It is generally applied to protein and energy deficiency, but it also relates to micronutrient deficiencies (UNICEF, 2009)

Malnutrition is generally caused by poor absorption of food, caused e.g. by diseases, and by the lack of access to adequate nutritious foods, which in turn has many reasons ranging from poverty over climate changes to the operation of economical and political systems in the world (UNICEF, 2009).

Protein-energy malnutrition

Protein-energy malnutrition is one of the most serious problems in the developing countries today (Aphane *et al.*, 2003; Bhat & Karim, 2009), with the shortage of proteins being the most important factor.

Generally there are two main sources of protein; animals and plants. In developing countries the major source of protein is cereals (60%) followed by meat (9%) and pulses (7%), while in developed countries cereals and meat both provide just below 30% and milk and other dairy products provide 17% of the consumed protein (Friedman, 1996). This distribution is primarily due to the high costs of animal protein. Since plant protein in general is less favourable than animal protein in a nutritional perspective, the developing countries are in need of other high-quality protein foods. The protein quality depends on the amino acid composition, digestion, the effect of processing of the food and the amount of energy in the food (or diet), since low energy intake reduces the efficiency of utilisation of the proteins (Friedman, 1996).

Especially in developing countries cultivation and use of legumes may be the fastest and most effective way of producing enough food protein to meet everyone's needs (Bhat & Karim, 2009). The unique chemical composition of marama beans (*Chapter 4*), with high protein and lipid contents, makes these an ideal food when dealing with protein-energy malnutrition.

Protein

All essential amino acids are present in marama beans and the percentage of essential amino acids is higher compared to most other legumes (Figure 16). In this regard, marama protein is generally superior to most other legumes. Moreover, marama beans are rich in lysine while they contain quite low amounts of methionine and cystein (Bower *et al.*, 1988; Amonsou *et al.*, 2012). This is typical for legumes and in this way marama beans – as other legumes – complement cereals with reverse amino acid composition (Hymowitz *et al.*, 1972).

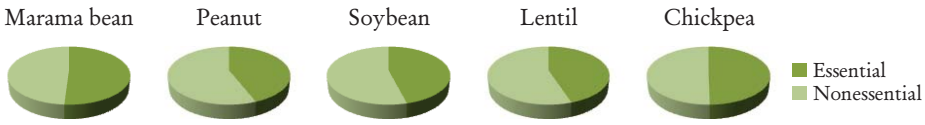

Figure 16 Amino acid composition of marama beans (Bower *et al.*, 1988) and other oil crops (Saxholt *et al.*, 2008) and pulses (Iqbal *et al.*, 2006) showing the percentage of essential and nonessential amino acids

Lipid

The lipid of marama beans is present mainly as unsaturated fatty acids with approximately 48% monounsaturated and 27% polyunsaturated fatty acids (*Chapter 4*, Figure 13) and contains both of the two essential fatty acids, linoleic and α-linolenic acid (Figure 17).

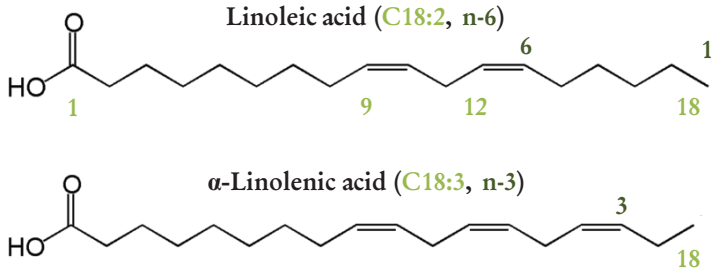

Figure 17 The two essential fatty acids, linoleic and α-linolenic acid, both present in marama beans

Marama beans are a good source of linoleic acid with an average content of 22 g/100g oil (Paper II) comparable to that of canola oil, which is also 22 g/100g (Saxholt *et al.*, 2008). However, it is not nearly as rich in linoleic acid as e.g. safflower (75 g/100g), soybean (53 g/100g) and peanut oil (32 g/100g) (Saxholt *et al.*, 2008), which are known to be rich sources of this essential fatty acid.

The average content of α- and γ-linolenic acid in marama bean oil is 2.2 g/100g in total (Paper II), hence the content of the essential α-linolenic acid must be quite low. However, it has not been determined on its own. The content is in the range of that of peanut oil (0.2 g/100g) and is far from reaching that of flaxseed oil (12.7 g/100g) (Saxholt *et al.*, 2008), which is known as an excellent source of α-linolenic acid.

Carbohydrate

Marama beans are quite unique because of their high dietary fibre and low starch content. Dietary fibres promote beneficial physiological effects including blood cholesterol lowering, blood glucose attenuation, blood pressure lowering and improvement of the immune function. Therefore, high dietary fibre content as seen in marama beans may provide many health benefits such as a reduced risk for developing coronary heart disease, stroke, hypertension, diabetes, obesity and certain gastrointestinal disorders (AACC, 2001; Anderson *et al.*, 2009).

Micronutrient malnutrition

Food security is not only about the access to adequate amounts of protein and calories. Around 30% of people in developing countries are affected by vitamin and mineral deficiencies (The World Bank, 2011). In the following, a brief overview of the micronutrients found in marama beans is presented along with corresponding nutritional considerations for some of the important constituents.

Vitamins

Wehmeyer *et al.* (1969) determined the content of a range of vitamins in marama beans including B vitamins, vitamin C and β-carotene. More recently, Müseler and Schönfeldt (2006) re-investigated the vitamin content and found that marama beans contain considerable amounts of vitamin A (0.27 mg/100g dm), B_3 (nicotinic acid) (9.21 mg/100g dm), B_6 (1.71 mg/100g dm), B_9 (folic acid) (0.14 mg/100g dm), B_{12} (0.004 mg/100g dm) and E (6.27 mg/100g dm) and minor contents of the vitamins B_1 (thiamin) (0.38 mg/100g dm), B_2 (riboflavin) (0.06 mg/100g dm) and K (0.22 mg/100g dm). In the same study, Müseler and Schönfeldt (2006) calculated the nutrient density of marama beans and in that way estimated the relative nutritional quality of different micronutrients in relation to the recommended daily allowances for different age groups (NAS, 1999; NAS, 2000; NAS, 2001). They concluded that marama beans are an excellent source of the **vitamins A, B_3, B_6, B_{12}, E** and **folic acid**. This list includes some of the most important vitamins in relation to health consequences of poor people in developing countries. For instance, **vitamin A** deficiency affects more than 200 million people worldwide and is the major cause of preventable visual impairment and blindness, reduced immunity and improper

growth of children (Aphane *et al.*, 2003; UNICEF & The Micronutrient Initiative, 2004; UNICEF, 2009).

The content and composition of **vitamin E** was examined in detail in Paper I. These analyses showed that the total content of vitamin E isomers in marama beans is 114 μg/g, which is almost double the amount that was determined by Müseler and Schönfeldt (2006). Marama beans have a considerably higher content of vitamin E (44 μg α-TE/g, α-tocopherol equivalent) compared to various cereal grains, legumes, vegetables and fruits (1-23 μg α-TE/g) (Bramley *et al.*, 2000). However, peanuts (152 μg α-TE/g) and soybeans (171 μg α-TE/g) have substantially higher vitamin E contents than marama beans (Bramley *et al.*, 2000).

The high vitamin E content has numerous health benefits. For instance vitamin E acts as a powerful antioxidant by neutralizing free radicals in the body that cause tissue and cellular damage and it contributes to the regulation of cellular signalling and gene expression (Azzi & Stocker, 2000; Brigelius-Flohé, 2006).

The vitamin E in marama beans is dominated (76%) by γ-tocopherol (59-234 μg/g) (Figure 18), while only lower concentrations of α- (14-48 μg/g) and β-tocopherol (1.1-3.3 μg/g) are present. Only traces of δ-tocopherol as well as the two tocotrienols β- and γ-tocotrienols were seen and only in some samples (Paper I, Table 4).

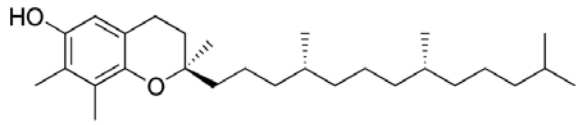

Figure 18 One of the eight vitamin E isomers, γ-tocopherol, which is present in the highest concentration in marama beans

The high content of γ-tocopherol is of particular biological relevance since it possess potential anti-carcinogenic and anti-inflammatory activities (Saldeen & Saldeen, 2005; Brigelius-Flohé, 2006).

Minerals

The **ash content** of marama beans varies between 2.5 and 3.7% dm (Paper I, Table 2). The level of the **macro-elements** (potassium, phosphorus, magnesium and calcium) is similar to that of peanuts and approaching that of soybeans (USDA, 2011). The beans furthermore contain several **trace-minerals** (zinc, iron

and copper) in amounts that match the content of soybeans and peanuts (Paper I, Table 5).

The relatively high level of minerals in marama beans is astonishing when considering the poor soils in which they grow. Most likely, the macro-elements mainly originate from the globoid protein storage sites (see *Chapter 4*). Additionally, druse crystals, which are present in marama bean proteins, are known to be rich in calcium and the high calcium in marama beans is probably due to this. In comparison, soybeans, which do not contain druse crystals have a substantially lower content of calcium (Amonsou *et al.*, 2011).

Müseler and Schönfeldt (2006) reported on the relative nutritional quality of different minerals in relation to the recommended daily allowances (NAS, 1999; NAS, 2000; NAS, 2001), and determined that marama beans are an excellent source of the minerals **calcium, iron, zinc** and **iodine**. **Iron** deficiency is the most widespread nutritional disorder in the world affecting about two billion people, of which the majority are women and children in developing countries. It is the principal cause of anaemia, which for infants has health consequences such as premature birth, low birth weight, infections and ultimately a higher risk of death. Later in childhood, physical and cognitive development are impaired resulting in learning difficulties. Moreover, anaemia contributes to more than 20% of all deaths of pregnant women. **Zinc** has a critical role in the physical growth of humans and normal functioning of the gastrointestinal tract and immune system and an inadequate intake of **iodine** influences the mental health of especially children (Aphane *et al.*, 2003; Hotz & Brown, 2004; UNICEF & The Micronutrient Initiative, 2004; UNICEF, 2009).

Health benefits

In addition to the many nutritional advantages of marama beans, some of their potential health benefits have also been examined. A study conducted by Chingwaru *et al.* (2011) demonstrated high **antimicrobial** activity of marama bean extracts (especially methanol and ethanol extracts) against both bacteria and yeast. In another study, Chingwaru *et al.* (2012) demonstrated that extracts (especially ethanol extracts) of marama beans were strong **antiviral** agents against rotavirus infection, which is a major cause of diarrhoea among infants, young children and immunocompromised people. This fits well with the fact that marama beans are used as traditional medicine against diarrhoea (Chingwaru *et al.*, 2007; Jackson *et al.*, 2010).

The microbicidal action against bacteria, yeast and rotavirus are proposed to be ascribed to the high amount of phytonutrients in marama beans such as gallic acid, phytosterols and lignans as well as certain fatty acids (such as palmitic and oleic acid), peptides (specifically protease inhibitors) and amino acids (Chingwaru *et al.*, 2011; Chingwaru *et al.*, 2012). These phytonutrients are presented and discussed in more detail in the following.

Phytonutrients

Phytonutrients (also known as phytochemicals) are an enormous group of biologically active chemical compounds, which occur naturally in plants and may affect human health positively (Rosa *et al.*, 2010).

Phenolic compounds

Phenolic compounds are one group of compounds within the phytonutrients, and may be divided into several classes, such as hydroxybenzoic acids, hydroxycinnamic acids, anthocyanins, proanthocyanidins, flavonols, flavones, flavanols, flavanones, isoflavones, stilbenes and lignans (Manach *et al.*, 2005).

The total content of phenolic substances of marama beans was reported by Chingwaru *et al.* (2011) to be around 100 mg/100g dm, which is considerably lower than the total phenolic content of soybeans, which varied between 270-470 mg/100g among 20 cultivars (Malencic *et al.*, 2007). In marama beans, flavonoids constitute the major class of phenolics (46 mg/100g), with myricetin and quercetin being the dominant sources. For the phenolic acids, cinnamic acid derivatives (primarily caffeic acid) constitute 38 mg/100g, while benzoic acid derivatives (mainly gallic, vanillic and protocatechuich acids) constitute 21 mg/100g of the total phenolic substances. In general, phenolic compounds are known to possess various health protective effects against degenerative diseases such as cancer and cardiovascular diseases (Manach *et al.*, 2005).

Phytoestrogens

The content of lignans and isoflavones in marama beans were examined in Paper I (Table 6). These compounds belong to a group of plant-derived compounds with structural and functional similarities to estrogens and are therefore termed phytoestrogens. Marama beans were found to be an excellent source of lignans with an average total content of approximately 1100 µg/100g. The content of the lignans secoisolariciresinol (Figure 19) and lariciresinol was higher than that of soybean, while the level of pinoresinol was lower (Mazur, 1998; Penalvo *et al.*, 2004).

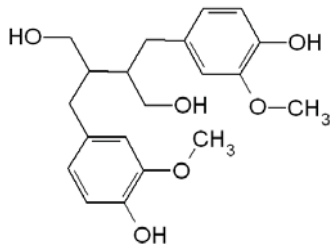

Figure 19 Chemical structure of the lignan, secoisolariciresinol, present in marama beans

Lignans may be converted by intestinal bacteria into enterolignans, which possess a range of health promoting effects in humans such as anti-estrogenic and antioxidant action and may reduce the risk of certain types of cancers as well as cardiovascular diseases (Adlercreutz, 2007).

No isoflavones were found in marama beans, which is contrary to soybeans that are rich in isoflavones (Mazur *et al.*, 1998).

Phytosterols

Mitei *et al.* (2009) determined the composition of phytosterols in marama bean oil and found 4-desmethylsterols to be the most abundant (77%), consisting primarily of β-sitosterol, stigmasterol and campesterol. Marama oil also contained significant amounts of 4,4-dimethylsterols and 4-monomethylsterols (16% in total). Phytosterols may have important bioactive properties such as cancer prevention and lowering of plasma total cholesterol (Woyengo *et al.*, 2009).

Health risks

Plants may contain a range of compounds that are directly or indirectly responsible for health related risks. These include a variety of antinutritional factors, which are substances that may adversely affect health and growth by preventing the absorption of the present nutrients, as well as toxic constituents and allergens.

Antinutritional factors

Marama beans, like many other plant seeds, contain proteins such as protease inhibitors (Elfant *et al.*, 1985; Starcher *et al.*, 1985; Starcher *et al.*, 1986; Bower *et al.*, 1988; Nadaraja *et al.*, 2009). One kind of protease inhibitor found in marama

beans is a relatively strong trypsin inhibitor, constituting about 20% of the total marama protein (Bower *et al.*, 1988). The presence of trypsin inhibitor is typical in legumes where it usually makes up about 5-10% of the total protein (Nadaraja *et al.*, 2009). The trypsin inhibitor activity may be destroyed by heat (Bower *et al.*, 1988; Maruatona *et al.*, 2010), for instance during processing into roasted marama beans. Another protease inhibitor identified by Nadaraja *et al.* (2009) is an elastase inhibitor representing about 14% of the total acid-extractable marama protein, which is significantly higher than in other known beans or nuts.

Allergens

The marama beans have been analysed for their potential allergenicity (Paper I). The analyses revealed no presence of proteins cross-reacting with the allergenic proteins from lupine and peanut, which are some of the most prevalent allergenic foods in the western world (WHO, 2003). Hence, there is no reason to suspect that people suffering from lupine or peanut allergy will suffer from allergic reactions when consuming products containing marama beans. This may provide large potentials in relation to product development of marama bean products, since marama beans might be used as substitute for peanut or lupine in different food products. However, assuming that people will not have allergic reactions to marama beans is not possible from just one study, as there might be other potential allergens not tested. Nevertheless, respondents from a focus group interview conducted in Botswana reported that they did not encounter any side effects of moderate consumption of marama beans (Chingwaru *et al.*, 2007).

Toxic constituents

Upon tissue disruption in some plants, e.g. legumes, cyanogenic glycosides may be enzymatically hydrolysed to the respiratory poison hydrogen cyanide (Poulton, 1990). Marama beans did not contain cyanogenic glycosides nor the enzymes that break these down to hydrogen cyanide (Paper I), which is in accordance with results from other studies (Anonymous, 1924; Mazimba *et al.*, 2011).

Considerations and recommendations for future research

More research on the effects of different types of processing on the nutritional quality of marama beans such as the digestibility of the protein and carbohydrate fractions should be carried out. It has been shown that heating

increases the in vitro protein digestibility of marama bean flour, however this will concurrently reduce some of the functional properties such as protein solubility and emulsifying capacity (Maruatona *et al.*, 2010). Hence, these pros and cons of heating during processing of marama bean products need to be taken into account during product development.

The bioavailability of the micronutrients in marama beans should be examined. For instance, legumes usually contain phytate, which is a main inhibitor of iron and zink absorption (Sandberg, 2002). Some polyphenols may also complex-bind iron hereby making it unavailable for absorption (Sandberg, 2002). If it is confirmed that e.g. the content of phytate is a problem in relation to mineral uptake from marama beans, the food processing steps should be optimised in such a way that phytate would be degraded making the minerals bioavailable. This may be done by techniques that increase the activity of native phytases such as soaking, germination and fermentation, by fermentation with phytase-producing microorganisms or by addition of phytase (Sandberg, 2002).

There is plenty of room for more studies on the presence of phytonutrients in marama beans and the bioavailability of these, which may reveal new health benefits of the bean. For instance soybeans have been extensively studied in order to identify the bioactive components responsible for their many health benefits. From these examinations, phytonutrients such as isoflavones, saponins, phytosterols, lignans, phytic acid and oligosaccharides have all been found to exert biological activities and contribute to the overall health effects observed with soy consumption (Kang *et al.*, 2010). In relation to this, it is also crucial to do more analyses on antinutritional factors (such as tannins, saponins, phytic acid and glycosinolates – some compounds may act as both phytonutrients and antinutrients), toxic constituents (such as quinolizidine alkaloids, triterpenes, cardiac glycosides) (Wink, 2004) and allergens (known from other sources than peanut and lupine, e.g. soybean, lentils, walnut, hazelnut and sesame) (Breiteneder & Radauer, 2004) to assure that there are no potential hazards in marama beans before commercialisation proceeds.

Chapter 6

Marama bean products

Commercialisation

A rich diversity of plants is found in Africa and about one fourth of the total number of higher plants in the world is present in Sub-Saharan Africa. Roughly 3000 traditional medicinal plants are regularly used in Southern Africa and less than 2% of these have been commercialised. Hence, many of these underutilised indigenous plants may be valuable resources for product development (van Wyk, 2008). An example is the African indigenous plant *Aspalathus linearis* that has been turned from a wild harvested crop into a successful commercial product worldwide; rooibos tea (Reinten & Coetzee, 2002). This plant was traditionally used as a herbal tea of the Khoi-San people. Commercialisation started in 1904 and today rooibos tea is popular worldwide as a healthy and tasty tea and as an ingredient in cosmetics because of its anti-mutagenic and antioxidant effects. Exports to Germany alone exceeds local consumption and has greatly improved the financial situation of many poor communities (van Wyk, 2008).

In order to turn the marama bean into a large scale agricultural crop, it is crucial to develop value added products that can be sold domestically in Southern Africa and later potentially also in global niche markets. The first steps towards commercialisation of marama bean products have been taken by the MARAMA II project, which has created and analysed prototype marama bean products.

Marama beans are perfect for production of value added protein-rich ingredients similar to those commercially available from soybeans, e.g. **defatted marama bean flour** with potential as a functional food ingredient (Maruatona *et al.*, 2010). The applicability of legume flours in foods depends on their nutritional value as well as their sensory and functional properties. Jideani *et al.* (2009) assessed the functional properties of raw and roasted marama bean flour. They

found that the flour (roasted as well as raw) possesses good functional properties, such as emulsifying and stability properties, and can be used as a protein supplement in processed foods such as weaning, baked and soup products. For instance, **roasted marama bean flour** used in composite sorghum porridges improves both taste (adds a roasted nut aroma and flavour), nutritional quality (sorghum, a staple in many African countries, has a low protein content and is deficient in the essential amino acid lysine) and texture (makes the porridge less viscous) (Maruatona *et al.*, 2010; Kayitesi *et al.*, 2010; Kayitesi *et al.*, 2012).

The high lipid content of marama beans makes them a potential source for production of e.g. **pressed marama bean oil**. An increase is seen in the interest for pressed oil in favour of extracted oil as many aroma components disappear during the extraction procedure. A further advantage of pressed marama bean oil is that it may easily be produced in villages in Southern Africa by use of simple pressing technology. Processing of marama bean products may be based on existing technology used to manufacture products from e.g. peanuts and soybeans. Ideally, all parts of the bean may be used, for instance the defatted press rest from production of marama bean oil might be readily available as a source of protein for fortification of other food products or for potential extraction of useful carbohydrates (Mosele *et al.*, 2011a).

Local respondents in socioeconomic and anthropological surveys conducted by Faria *et al.* (2011) supported the development of different marama bean products including butter, oil, milk and fortified flours. Studies have also shown that marama beans may be used for value added products such as yoghurt and ice-cream, marama cookies and other snack products, meat analogues and canned marama beans in tomato sauce (Jackson *et al.*, 2010). Some of these prototype products are shown in Figure 20.

Figure 20 Prototype marama bean products. Left) butter and cookies, middle) milk and right) oil. Pictures provided by University of Botswana and NFTRC (National Food Technology Research Centre), Botswana (sponsored by MARAMA II and SIDA (AKT 2010-064))

Research on the health benefits of marama beans have also shown that they may have potential for use in pharmacology and in functional foods. Additionally, products made from other parts of the marama bean, e.g. the tuber and seed hull may also be used commercially due to nutritional and health benefits (Mazimba *et al.*, 2011; Chingwaru *et al.*, 2011; Chingwaru *et al.*, 2012).

Analyses of the market conditions for marama bean products have been carried out by Faria *et al.* (2011). These studies focused on economical, social and cultural conditions in Southern Africa and concluded that "*assuring the sustainability of local people's livelihoods while creating a larger market is only achievable through community organisations supported by a broad marketing strategy and using cultivated marama beans*".

Storage stability

After harvesting of marama beans, during dehulling and processing of marama bean products and distribution and storage of these, deteriorative processes are unavoidable. These may lead to changes in functional and sensory quality of the product as well as a decrease in nutritional quality and food safety. Oxidative reactions of lipids and proteins are major causes of chemical deterioration in food. Therefore, it is vital to gain knowledge on the storage stability of marama bean products.

Lipid oxidation

As previously described, lipid of marama beans is highly unsaturated with oleic and linoleic acid accounting for approximately 70% of the total fatty acids (Mitei *et al.*, 2008). These two fatty acids are highly prone to oxidation (Belitz *et al.*, 2004), making lipid oxidation in marama bean products during storage a crucial quality control parameter. Oxidation of unsaturated lipids may proceed by three different pathways; autoxidation, photosensitised oxidation and enzymatic oxidation.

Lipoxygenase enzymes are present in many plants similar to the marama bean such as peanut and soybean (St.Angelo *et al.*, 1979; Suda *et al.*, 1995). Lipoxygenase may catalyze the oxidation of unsaturated fatty acids to their corresponding hydroperoxides (primary oxidation products), which may again be broken down to volatile secondary oxidation products (Robinson *et al.*, 1995). In order to elucidate whether enzymatic lipid oxidation would be a potential problem in marama bean products during storage, the activity of the

lipoxygenase isozymes was examined in raw marama beans (Paper III, Figure 1). These results suggest that the lipoxygenase isozymes are either absent or naturally inhibited in marama beans and therefore enzymatic lipid oxidation should not be a potential problem during storage of marama bean products.

Autoxidation is a free radical-induced chain reaction where unsaturated fatty acids react with triplet oxygen to form hydroperoxides. These labile compounds further decompose to produce a complex mixture of volatile compounds (secondary oxidation products), which are mainly carbonyl compounds such as aldehydes, ketones, carboxylic acids, esters and amides, as well as hydrocarbons and furans (Frankel, 1987; Choe & Min, 2006). The reaction involves three stages; initiation, propagation and termination. The development in the content of fatty acids, antioxidants, primary and secondary oxidation products during the three stages of lipid oxidation is illustrated in Figure 21.

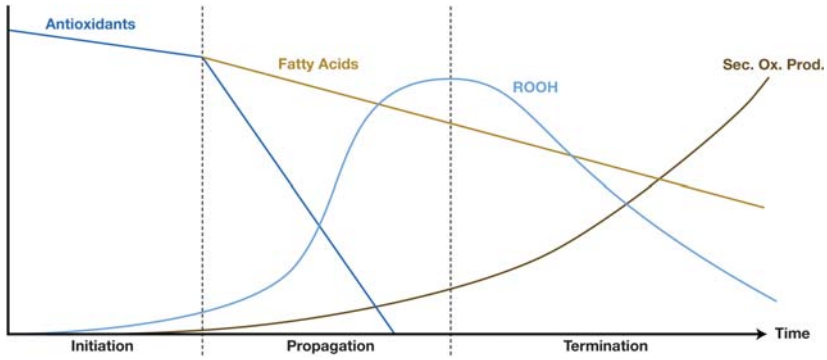

Figure 21 The development in fatty acids, antioxidants, primary and secondary oxidation during the three stages of lipid oxidation

Photosensitised oxidation of unsaturated fatty acids is triggered by exposure to light in the presence of oxygen and a photosensitizer. Natural pigments found in foods, e.g. riboflavin, which is also found in marama beans (Wehmeyer *et al.*, 1969), have the ability to act as photosensitizers by absorbing energy from light and transferring it to triplet oxygen to form the excited singlet oxygen (Frankel, 1980; Min & Boff, 2002).

Hydroperoxides formed during lipid oxidation are generally flavour- and odourless. However when broken down to volatile secondary oxidation products an increase in flavour deterioration in e.g. food is seen (Frankel, 1982; Frankel, 1987; Choe & Min, 2006). The impact of the different volatile

oxidation products on the quality of the marama products depends on their odour threshold values. This value is a measure of the odour intensity and is defined as the minimal concentration of the compound that is detectable by the human nose. If exceeding the odour threshold value, the specific volatile compound will most likely deteriorate the quality of the food product.

Factors affecting the oxidative stability of marama bean products

The oxidative stability of marama bean products is influenced by various internal factors such as fatty acid composition, presence and activity of pro- and antioxidants, moisture content and nature of the surface of the product. In addition, various extrinsic factors such as oxygen concentration, light, temperature and relative humidity all affect the rate of lipid oxidation (Choe & Min, 2006). The sensitivity towards the external factors depends considerably on the specific products and their internal factors. Getting to know the impact of these extrinsic factors on the marama bean products is vital for selection of the right packaging in order to retain a good quality during storage.

The oxidative stability of marama bean products stored in different packaging materials and under different storage conditions was investigated by examination of the primary and secondary oxidation products. These were measured as the peroxide value (PV) and content of volatiles, respectively. IR spectroscopy was also applied in order gain an overview of the chemical changes taking place during the storage period. Additionally, the content of the antioxidant vitamin E was measured through the storage period.

Pressed marama bean oil

Marama bean oil was studied during seven months of storage under different light and temperature conditions (Paper III). Figure 22 shows the development of primary and a selected secondary oxidation product (hexanal) as well as vitamin E during the storage period (Paper III, Figures 3, 4 and 6). Compared with the theoretical development of oxidation products and antioxidants in Figure 21, a close resemblance is seen.

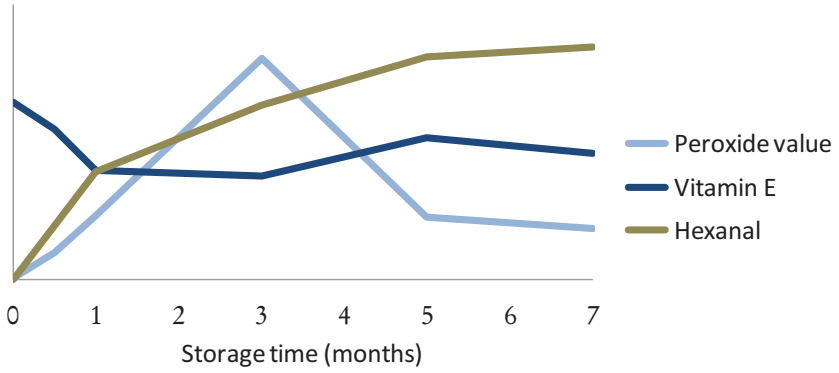

Figure 22 Development in the content of primary (peroxide value) and secondary (illustrated as the content of hexanal) lipid oxidation products as well as the content of the antioxidant **vitamin E** during storage of marama bean oil in light at 25°C for seven months (the tree curves are scaled to fit in the same figure)

The pressed marama oil had a PV of 0.3 meq/kg before the storage experiment was initiated. Since freshly refined lipids should have a PV below 1 meq/kg (Rossell, 1994), this shows a good stability of the marama oil during processing. Interestingly and in relation to this, the marama oil proved to have good natural antioxidant properties, since a stable nature of the vitamin E isomers was seen during storage. Additionally, a rise in the content of vitamin E was seen during the later months of storage in light. This rise was ascribed to regeneration of the α-tocopheroxyl radical, which is formed during antioxidant action of α-tocopherol. Pazos *et al.* (2007) demonstrated that some natural phenolic compounds were able to regenerate α-tocopherol via reduction of α-tocopheroxyl radical, and since marama beans contain phenolics (Jackson *et al.*, 2010; Mazimba *et al.*, 2011) this seems like a reasonable explanation.

Other compounds found in marama beans may also contribute to the antioxidative activity and hereby stability of the oil. For instance it is known that marama beans contain vitamin C, β-carotene (Wehmeyer *et al.*, 1969; Müseler & Schönfeldt, 2006), phytosterols (Mitei *et al.*, 2009) and phenolic compounds such as flavonoids (Mazimba *et al.*, 2011; Chingwaru *et al.*, 2011), which all possess antioxidant activity. Additionally, marama beans contain lignans (Paper II) that may be converted by intestinal bacteria into enterolignans, also possessing antioxidant activity (Adlercreutz, 2007).

The study in Paper III showed that light had a larger effect on the lipid oxidation than temperature, yet both parameters should preferably be low in order to obtain the best storage stability of marama bean oil.

Roasted marama beans

Roasted marama beans were studied during seven months of storage in the presence or absence of oxygen and with or without exposure to light (Paper IV). Again light was found to have the largest oxidative effect, however the oxygen level in the packaging material should also be kept as low as possible to obtain the best storage stability of the roasted marama beans.

Recommendations for storage of marama bean products

In general, marama bean products should be stored in darkness in a packaging material with low oxygen availability and at the lowest possible temperature.

A range of the volatile oxidation products were quantified in marama bean oil (Paper III, Table 2) and roasted marama beans (Paper IV, Table 2). These concentrations were compared to the detection threshold values of the corresponding volatiles and used to determine whether the product quality would be compromised and hereby the shelf life of the products was determined. Table 4 summarizes the recommended storage conditions along with the minimum shelf life at these conditions for the two marama products.

Table 4 Summary of the recommended storage conditions, the minimum shelf life and the limiting factor (secondary oxidation product) at these conditions for each of the marama bean products

	 **Pressed marama bean oil**	 **Roasted marama beans**
Recommended storage condition	Glass bottle Darkness (or dark bottle) 25°C	Plastic bag Low amount of oxygen Darkness (or bag that does not transmit light) 25°C
Minimum shelf life at recommended storage condition	7 months	5 months
Limiting factor	Hexanal	Hexanal

Considerations and recommendations for future research

In a future study investigating the influence of external factors on the storage stability of marama bean products, it would be interesting to vary other storage parameters such as the relative humidity and the type of packaging material (e.g. the oxygen permeability) as well as a wider range of storage temperatures. It would also be interesting to investigate the effect of adding additional natural singlet oxygen quenchers such as β-carotene and tocopherols to the products in order to see how these would influence the storage stability.

An additional analysis for evaluation of the lipid oxidation and the general storage stability of marama bean products could be a sensory evaluation, which could be correlated to the chemical determinations. Furthermore, GC-olfactometry could be used to confirm when the products develop detectable

amounts of unwanted off-flavours due to lipid oxidation. This would also add valuable knowledge to confirming or updating the odour detection threshold values.

With the high protein content of marama beans, a study focusing on the oxidation of proteins in marama bean products would lead to a more comprehensive overall oxidative knowledge.

Finally, it would be interesting to look into the loss of nutrients during longer storage periods, since this could potentially reduce the product quality.

Chapter 7

Conclusions

The aim of this thesis work was to examine the chemical composition of marama beans and to determine the storage stability and shelf life of marama bean products. This should ultimately lead to an examination of the potential of the marama bean as a contributor to increasing food security and alleviating malnutrition in Southern Africa.

This thesis work presents the most comprehensive study of the chemical composition of marama beans to date. From this study it was found that the chemical composition of marama beans on dry matter basis was 29-38% protein, 32-42% lipids, 19-27% dietary fibre and 2.5-3.7% ash. The chemical analyses revealed differences in the composition between different batches of marama beans. Beans from South Africa seemed to have higher moisture, ash and protein content than the beans from Namibia and Botswana, which did not differ considerably from each other. Spectroscopic analysis of single bean samples furthermore revealed considerable chemical variations between marama beans harvested in the same geographical area, and this bean-to-bean variation within each batch turned out to be as large as the overall variation between the batches.

Marama beans proved to be a good source of several different vitamins, minerals and phytonutrients, hereby providing many nutritional and health benefits. The beans were especially a good source of vitamins A, B_3, B_6, B_{12}, E and folic acid and of important minerals such as calcium, iron, zinc and iodine. The beans contained phytonutrients such as flavonoids, phenolic acids, phytosterols and lignans. Finally, marama beans did not seem to possess any health risks, since they did not contain any of the potent allergens found in lupine and peanut and were found not to be cyanogenic.

NMR in combination with the vibrational spectroscopic techniques; NIR, IR and Raman, gave qualitative and semi-quantitative information about the

chemical composition of the chemically intact marama bean. The spectroscopic results confirmed many of the findings from the wet chemical analysis and provided information on the secondary protein structure and the carbohydrate composition of marama beans.

The storage stability of marama bean oil and roasted marama beans was evaluated and especially the oil was found to be stable due to its natural antioxidant properties. The fact that marama bean products may be stored over a longer period of time, improves the potential for commercial use of the products.

The many positive nutritional and functional properties of marama beans underline their large potential as a nutritious food, which may also be developed into a variety of commercial products. In this way, the marama bean may contribute to increasing the nutritional status and improving livelihoods of the people living in the areas in which it grows.

Chapter 8

Perspectives

The unique chemical composition of marama beans makes this underutilized nutritive legume an interesting subject for food processing applications and hereby a prospective crop plant. To sum up some of the many nutritional and health benefits of marama beans, Figure 23 gives a brief overview of some of these potentials. Yet, there are a number of limitations to be overcome before this is realistic. The perhaps most important feature in relation to the marama bean gaining success is cultivation and domestication. The plant needs to be developed into a crop and further developed into desirable high yielding and early maturing cultivars. These efforts should especially be focused in the areas where the marama bean is already known and appreciated (around the Kalahari Desert in Southern Africa), but could later on be expanded to other desert areas around the world – areas that seem to be ever expanding due to climate changes. Some efforts to do so have already been made, but much more is needed if the full potential of the marama bean is to be reached.

How can developing countries benefit from marama beans?

The exceptionally high lipid and protein content of marama beans make them an ideal food for undernourished people, and at the same time the beans provide many important micronutrients. The high protein content of marama beans make them ideal for production of protein-rich ingredients, which can be used to fortify staple cereal-based foods, while the high oil content makes marama beans an obvious source of pressed oil, which may be used for cooking or in salads. Yet, there may also be a number of products that could be targeted specifically at vulnerable groups of undernourished people, for instance small children. One possibility would be to produce a ready-to-use marama bean-based product in the same way as the peanut-based paste Plumpy'Nut®, developed by

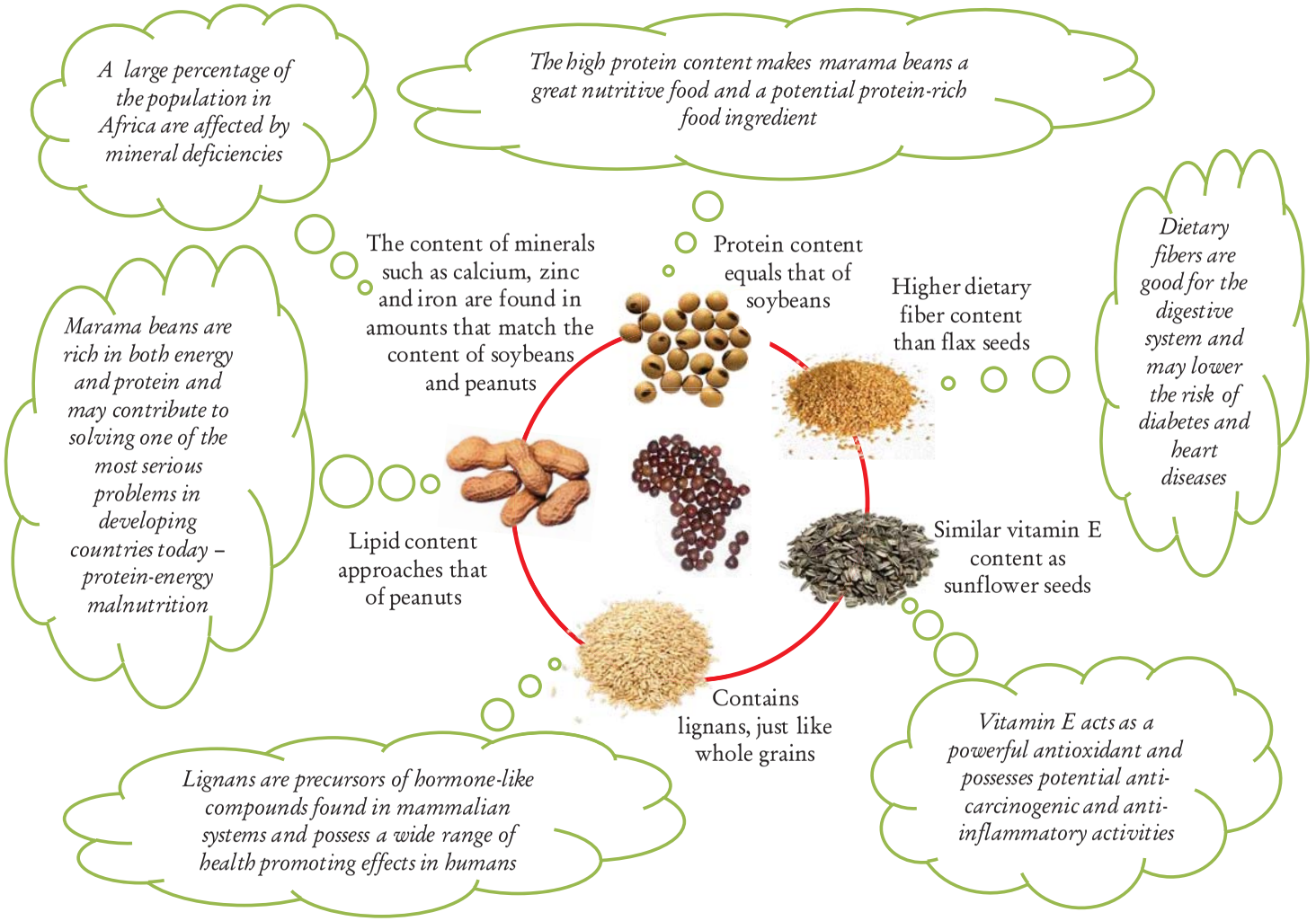

Figure 23 Summary of some of the nutritional and health benefits of marama beans (Paper I)

Nutriset in 1996. This revolutionised the treatment of severe acute malnutrition and similar products are still widely used today (Enserink, 2008). Marama bean paste would most definitely not be able to compete with peanut based products already on the market, but could be a niche product targeted at children suffering from the very serious peanut allergy. In addition to reducing malnutrition and replenishing lost nutrients and energy, another advantage of using marama beans over peanuts would be that this product could be produced locally in the dry regions of Southern Africa using modest technologies. Hence, the production of such a product would also have economical advantages.

Can marama beans also be promoted in the Western world?

The marama bean should primarily be grown and used in its natural habitats, the drought-prone sandy areas of Southern Africa, and in these areas be aimed at alleviating malnutrition. However, if and when this mission succeeds, the potential of also using marama beans in niche products targeted at markets worldwide would be an excellent idea.

Several aspects of the marama bean may be interesting in regards to the Western market. As mentioned marama beans seem to be safe for people suffering from lupine or peanut allergy. Hence, these people could consume products containing marama beans instead. For instance low fat protein rich marama flour or marama protein extracts could be used as an ingredient in food products instead of soybean or lupine protein. Today these are incorporated in a wide variety of processed food products such as baked goods, cereals, pasta, meat and dairy products to impart nutritive value and functional properties (Moure *et al.*, 2006).

Another potential aspect of the marama bean is that it grows in the wild and hence is organic, and even when domestication is initiated it may be grown by the principles for organic crop production. Hence, marama bean products may be marketed as local organic specialties with an exotic story conveyed on the packaging material of for example a snack product like roasted marama beans. Such a product could both be sold in specialty stores and for instance be used as a snack on flights to and from Europe and Southern Africa as an authentic African alternative to bags of peanuts or other well known snacks. Another potential niche market could be as an exclusive food used in the kitchens of top-restaurants where people go specifically to try new and interesting foods.

The marama bean alone will not save the world – but I sincerely hope that in the near future, it will help improve the lives of thousands of people living in the poor arid areas in Southern Africa!

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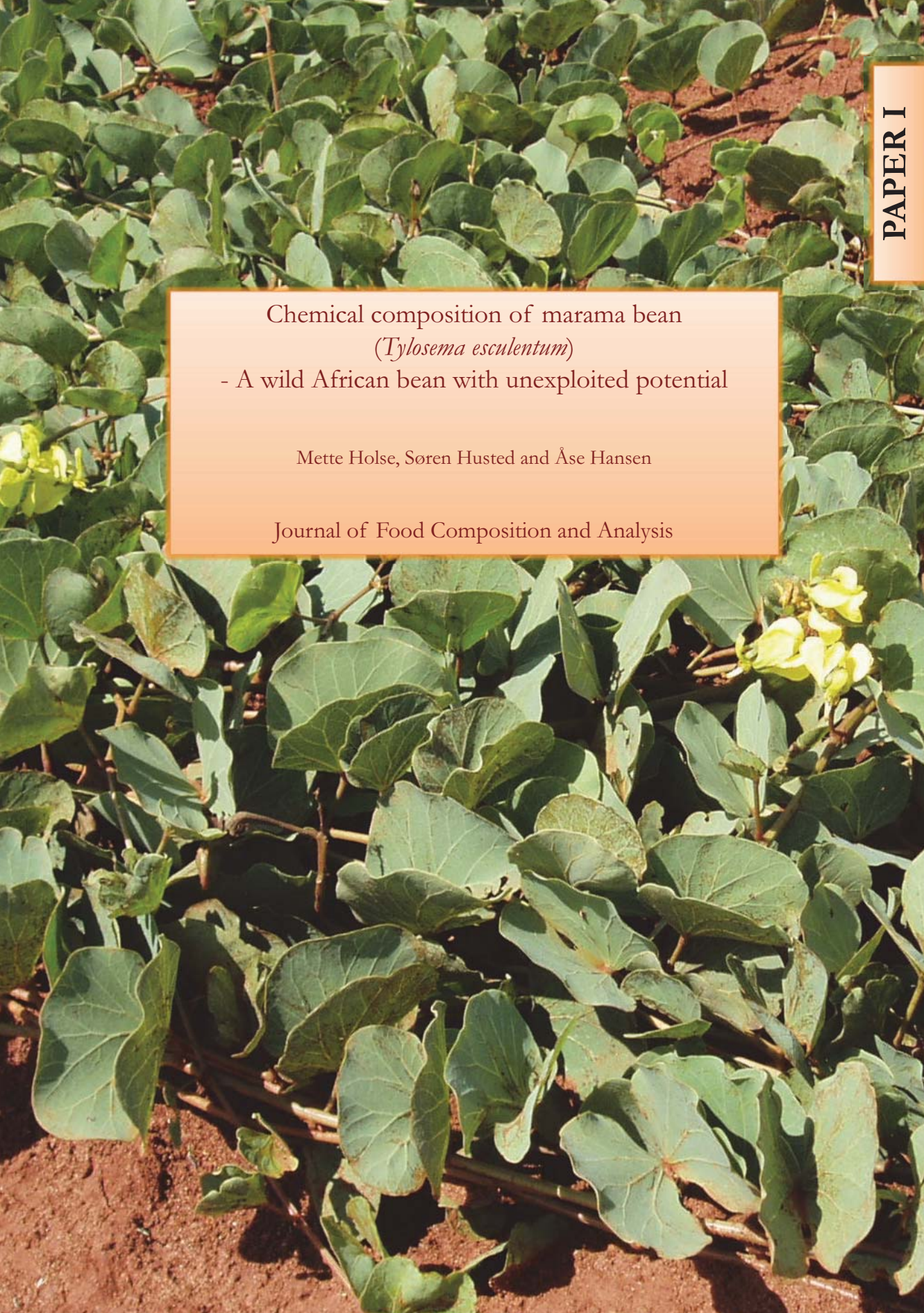

PAPER I

Chemical composition of marama bean (*Tylosema esculentum*) - A wild African bean with unexploited potential

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ABSTRACT

Marama bean is an underutilized legume which grows wild in Southern Africa and forms part of the diet for the indigenous population. The seeds are rich in lipid and protein, and have the potential to improve nutrition and increase food security for people living in these rural areas. Sixteen samples of marama beans from Botswana, Namibia and South Africa harvested in 1990–2008 were examined for chemical composition. The nutrient content on dry matter basis was: protein 29–38%, lipids 32–42%, dietary fiber 19–27% and ash 2.5–3.7%. In general, beans from South Africa had higher content of protein and ash compared to beans from Botswana and Namibia. The vitamin E isomers α-, β- and γ-tocopherols were found at levels of 14–48, 1.1–3.3 and 59–234 μg/g, respectively. In general, beans from Namibia contained the highest amount of vitamin E. The marama bean had a mineral content similar to that of peanut and approaching that of soybean, and is a good source of the important trace-elements Zn and Fe. The three lignans secoisolariciresinol, lariciresinol and pinoresinol were present in the marama bean. Additionally, the marama bean did not seem to contain any of the potent allergens found in lupine and peanut, and it was not cyanogenic.

1. Introduction

The marama bean (*Tylosema esculentum*) is a long-lived, perennial, tuberous legume which occurs naturally in extreme environments with high temperatures (typical daily maximum of 37 °C in the growing season), low rainfall (100–900 mm) and long periods of drought (van der Maesen, 2006). The plant is indigenous to the Kalahari Desert and neighboring areas with poor semi-arid soils in Botswana, Namibia and the northern part of South Africa, but also occurs in Angola, Zambia and Mozambique. Furthermore, experimental cultivation in Kenya, South Africa, Australia, Israel and the United States (Texas) has been successful (Bousquet, 1981; van der Maesen, 2006).

The genus *Tylosema* belongs to the plant family Fabaceae. Five species within the genus, all native to Africa, have been characterized. Four of these were recognized by Coetzer and Ross (1977) in 1977 and later reviewed, most recently by Castro et al. (2005); *T. esculentum* (Burch.) Schreiber, *T. fassoglense* (Schweinf.) Torre & Hillc., *T. argenteum* (Chiov.) Brenan, and *T. humifusum* (Pic.-Serm. & Roti. Mich.) Brenan. Castro et al. (2005) furthermore described a new species, *T. angolense* Silveira & Castro. *T. esculentum*, also called marama/morama bean, is the most well-known and -documented species. However, *T. fassoglense* is also widely distributed throughout the East African countries from Ethiopia to the northern region of South Africa and westwards to Angola and northern Namibia. Beans of *T. fassoglense* are consumed in the same way as marama beans and their chemical composition has been examined by Dubois et al. (1995).

Abbreviations: dm, dry matter; TDF, total dietary fiber; IDF, insoluble dietary fiber; SDF, soluble dietary fiber; SD, standard deviation.

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The marama plant is a creeper with stems up to 3 m long arising from a large tuber with forked tendrils which facilitate climbing opposite the leaves (Castro et al., 2005). It produces very hard pods, which usually contain two, but sometimes as many as six, large (2–3 g) dark brown edible seeds (Wehmeyer et al., 1969). The seed is a neglected and underutilized traditional food, which forms part of the diet for the indigenous population in Southern Africa. Until recently, the seeds were gathered by hand from the wild and mostly eaten as a snack after roasting in hot sand, but they can also be ground after roasting and made into porridge, or boiled and eaten as other beans before they are fully ripe. After roasting, they have a delicious nutty flavor, resembling roasted cashew nut (Victor, n.d.). The beans are never eaten raw due to their slimy texture and unpleasant taste (Wehmeyer et al., 1969; Wehmeyer, 1986). The raw beans keep well and remain edible for years due to their hard outer shell and low water content.

The seeds have a high lipid and protein level, giving them an enormous socio-economic value. Besides the large potential of the roasted seeds, the marama bean also has potential as a source of oil and various other healthy food products such as marama milk and defatted marama flour as an ingredient (van der Maesen, 2006). Hence, this neglected legume has the potential to improve nutrition, increase food security and diversify livelihoods for the people living in these rural areas (Rachie, 1979; van der Maesen, 2006).

In spite of its significant potential, the marama bean has not yet been studied very extensively. However, a number of studies have evaluated the domestication of the plant and its potential as a food crop (Francis and Campbell, 2003; Monaghan and Halloran, 1996; Powell, 1987) as well as the biology of the legume (Coetzer et al., 1983; De Frey et al., 1992; Hartley et al., 2002; Mitchell et al., 2005; Travlos et al., 2007a,b). There are also a number of studies describing the main chemical composition of the beans, especially their high lipid and protein content.

The lipid content has been reported in several studies and was reported to range between 35 and 48% dry matter (dm) (Amarteifio and Moholo, 1998; Bower et al., 1988; Francis and Campbell, 2003; Keegan and Van Staden, 1981; Ketshajwang et al., 1998; Wehmeyer et al., 1969). The high amount of lipids in marama beans is comparable to the content found in seeds used for production of commercial vegetable oils such as sunflower seed (22–36%) and rapeseed (22–49%) and closely approaches that of peanuts (45–55%) (Belitz et al., 2004; Salunkhe and Kadam, 1989). The amount of lipids is twice that of soybeans (17–20% dm) (Belitz et al., 2004; Street and Öpik, 1975), which is the legume that compares best with the high protein content of the marama beans. The fat is present mainly (app. 75%) as unsaturated fatty acids, the principal fatty acid being oleic acid (47–49%). The beans furthermore contain linoleic (19–26%) and palmitic acid (12–14%) as well as stearic, arachidic, linolenic, arachidonic, erucic, behenic, myristic, palmitoleic and gadoleic acid in lower concentrations (Bower et al., 1988; Engelter and Wehmeyer, 1970; Francis and Campbell, 2003; Ketshajwang et al., 1998; Mitei et al., 2008). The fatty acid composition resembles that of olive oil. In addition, an in depth study of the physiochemical parameters and the fatty acids and triacylglycerol profiles of marama beans has been carried out by Mitei et al. (2008).

Amarteifio and Moholo (1998), Bower et al. (1988) and Keegan and Van Staden (1981) all investigated the protein content of marama beans and found that it ranges from 30 to 39% dm. The protein content is comparable to or higher than most other legume seeds such as dry peas, chick peas, lentils, kidney beans, cowpea and lupine with contents between 20 and 40% dm (Gueguen, 1983; Nassar et al., 2008) and equals that of soybeans (33–46% dm) (Belitz et al., 2004; Gueguen, 1983; Hymowitz et al., 1972). The protein composition of marama beans is largely dominated by glutamic and aspartic acid as well as tyrosine (Bousquet, 1981; Bower et al., 1988; Dubois et al., 1995). All the essential amino acids are present in the beans; methionine, however, is present in rather low amounts.

The dietary fiber content of the beans has hardly been investigated, and has only been obtained indirectly as the difference between 100% and the content of protein, lipid and minerals (dm basis). Wehmeyer et al. (1969), Amarteifio and Moholo (1998) and Bower et al. (1988) reported carbohydrate contents of 24, 25 and 20% dm, respectively.

Marama bean is furthermore a good source of macro-elements such as potassium (7760–8490 μg/g), phosphate (3970–4840 μg/g), magnesium (2580 μg/g) and calcium (1360–1520 μg/g), sodium (41–890 μg/g), as well as the trace elements zinc (38 μg/g), iron (33–49 μg/g) and copper (10 μg/g) (Amarteifio and Moholo, 1998; Wehmeyer et al., 1969). However, the mineral content has not been examined thoroughly. Likewise, not many previous studies have looked at the vitamin content of the marama bean. Wehmeyer et al. (1969) published results on the content of B vitamins (thiamine, riboflavin, nicotinic acid), vitamin C and β-carotene and found that marama bean is a good source of B and C vitamins, but a poor source of β-carotene. The content of vitamin E has not been investigated previously. This is an interesting area to examine since vitamin E is present in abundant amounts in other seeds, nuts and their respective oils (Bramley et al., 2000) and since vitamin E has numerous health benefits. The content of other constituents with potential health benefits such as phytoestrogens, phytosterols or phytate has not been examined either. Another area where almost no attention has been paid is the presence of toxic constituents or antinutritional factors in the seeds, such as allergens or cyanogenic glycosides.

The available studies on the chemical composition are all limited by the low number of samples analyzed, which means that these studies are not fully representative for the variation that might be expected in the chemical composition of uncultivated seeds. Therefore, the aim of this study was to obtain a more representative and comprehensive description of the chemical composition of the marama bean. Hence, its chemical composition was investigated by analysis of a wide selection of marama beans from different harvest years and geographical locations. This provided the fundamental basis for understanding the variation in the beans.

Samples from Botswana, Namibia and South Africa were analyzed for the content of dry matter, ash, lipid and protein, as well as dietary fibers. In addition, examination of vitamin E isomers was carried out along with an investigation of the mineral composition of the beans and the presence of phytoestrogens. The beans were screened for the presence of proteins cross-reacting with allergenic proteins from lupine and peanut and for the existence of cyanogenic glycosides.

2. Materials and methods

2.1. Samples

Sixteen different marama bean batches were examined, 14 samples of the species *Tylosema esculentum* and 2 samples of the species *T. fassoglense*. The batches were collected from wild growing plants from different locations in Botswana, Namibia and South Africa during the growing seasons 1990 and 2005–2008. Each sample represents the harvest from a certain location in the specific year, and the batches are listed in Table 1 along with ID number, origin, harvest year and species. The beans were stored in plastic bags at 4 °C and 40% relative humidity until analyses were performed.

Table 1
List of batches of marama bean samples used in this study by ID number, origin, harvest year and species.

ID number[a]	Origin	Harvest year	Species
BO9001	Botswana	1990	*T. esculentum*
BO0501	Botswana	2005	*T. esculentum*
BO0601	Botswana	2006	*T. esculentum*
BO0602	Botswana	2006	*T. esculentum*
BO0603	Botswana	2006	*T. esculentum*
BO0701	Botswana	2007	*T. esculentum*
BO0803	Botswana	2008	*T. esculentum*
NA0701	Namibia, Okakarara	2007	*T. esculentum*
NA0702	Namibia, Gobabis – Sandveld station	2007	*T. esculentum*
NA0703	Namibia, Gobabis – Sandveld station	2007	*T. esculentum*
NA0801	Namibia, Gobabis – Sandveld station	2008	*T. esculentum*
NA0802	Namibia, Okakarara	2008	*T. esculentum*
SA0701	South Africa, Entabeni	2007	*T. fassoglense*
SA0702	South Africa, Babeni	2007	*T. fassoglense*
SA0703	South Africa, University of Pretoria, Experimental farm	2007	*T. esculentum*
SA0705	South Africa, Rooidraaitrust	2007	*T. esculentum*

[a] First two letters: country of origin; next two numbers: harvest year; last two numbers: batch no. from this year.

A sample of soybean with known chemical content (dm: 89.5, ash: 4.6, protein: 40.0, fat: 16.5, soluble dietary fiber: 7.3, insoluble dietary fiber: 10.0, total dietary fiber: 17.3 all as % dm) was used as a control sample for each set of analytical samples in the proximate analyses. This was done in order to control the accuracy of the analysis and to assure no deviations in any particular run.

2.1.1. Visual examination of the beans

A picture of each sample was obtained by scanning 20 whole beans in a scanner (HP ScanJet 6200C, Hewlett Packard, US).

2.1.2. De-hulling of the beans

The beans were de-hulled with a nut de-huller. The hulls and cotyledons of 20 beans were weighed and the weight-percentage of the de-hulled bean compared to the whole bean weight was calculated as well as the average weight of one cotyledon.

2.1.3. Milling of bean samples

10 g of the cotyledons were milled in a laboratory mill (IKA A10, Labortechnik, Staufen, Germany) for 10 s. The milling procedure has been optimized in order to obtain an appropriate particle size ($\leq$250 μm) and to avoid extended milling which can lead to prolonged sample aeration and heating. The milled bean samples were kept in light and air tight plastic containers at 4 °C and 40% relative humidity until analysis.

2.1.4. Reduced sample size

Because of a limited amount of sample material, the standard methods for measuring dry matter, ash, lipid and protein content were optimized in order to use the smallest amount of sample possible, without compromising the standard deviations of the analyses.

2.2. Determination of chemical composition

2.2.1. Dry matter content

The dry matter content of the milled samples was determined in triplicate according to a modified version of ICC-Standard No. 110/1 (ICC, 1991). Instead of using 2 g of sample, only 0.5 g of the sample was applied.

2.2.2. Ash content

Ash content was determined in triplicate according to a modified version of ICC-Standard No. 104/1 (ICC, 1991). 0.5 g sample was applied instead of 3 g as applied in the original standard method.

2.2.3. Protein content

The crude protein content was determined in duplicate according to a modified version of AOAC Official Method 2001.11 (AOAC, 2007) using Kjeltec Tecator equipment: 2020 Digestor, 2001 Scrubber Unit, 1026 Distilling Unit (Foss, Hillerød, Denmark) and ABU 900 Autoburette (Radiometer A/S, Brønshøj, Denmark). Only 12 mL of concentrated H_2SO_4 was applied for sample digestion, instead of 15 mL as otherwise recommended for samples with a fat content of more than 10%. The nitrogen-protein conversion factor for soybeans (5.7) was used in determination of the protein content.

2.2.4. Lipid content

The total fat content was determined in duplicate by a modified version of AACC Method 30-25 (AACC, 2000) where samples were defatted with ether in a Soxhlet extractor (Foss, Hillerød, Denmark). 1 g sample was applied instead of 2 g as applied in the original standard method.

2.2.5. Dietary fiber content

The content of total (TDF), soluble (SDF) and insoluble dietary fibers (IDF) was determined in duplicate according to AOAC Method 991.43 (AOAC, 2007) and AACC Method 32-07 (AACC, 2000). The analysis was done using the Megazyme K-TDFR Assay Kit (Megazyme International Ireland Ltd., Wicklow, Ireland). Since the fat content is larger than 10% the samples where defatted prior to analysis. The fiber content was determined in all samples except for SA0701 and SA0702 because of lacking sample material.

2.3. Minor chemical components

2.3.1. Vitamin E (tocopherols and tocotrienols) content

The content of tocopherols and tocotrienols was determined in duplicate according to a modification of the method described by Nielsen and Hansen (2008). Using this procedure 1 g of milled sample was extracted with 30 mL hexane by placing the extract in a centrifuge (Sorvall RT 6000D, Buch & Holm A/S) at 6000 rpm for 10 min at 20 °C (in the original method the extraction was done in an ultrasonic bath). The supernatant was transferred to a 30 mL brown bottle and was evaporated to dryness under nitrogen gas. Hereafter, the dry pellet was dissolved in 3 mL hexane. Prior to injection onto the HPLC column, the extract was filtered through a 0.2 μm filter. The quantitative HPLC separation was performed using a Varian HPLC (Star 9012, Varian, Inc. Scientific Instruments, Walnut Creek, CA, US), equipped with a fluorescence detector (Varian, 9070, Varian, Inc. Scientific Instruments, Walnut Creek,

CA, US). The detector was set to an excitation wavelength of 290 nm and an emission wavelength of 330 nm. The column used was a LiChrosorb Si 60 (125 mm × 4 mm i.d., particle size 5 μm, VWR International ApS, Rødovre, Denmark) normal-phase column, protected with a steel guard column (50 mm × 3 mm i.d., particle size 5 μm) filled with ChromGuard S (Bie & Berntsen, Copenhagen, Denmark). The temperature of the column oven was 25 °C. The mobile phase contained 94.6% hexane, 3.6% ethyl acetate and 1.8% acetic acid and the flow rate of the mobile phase was set at 1.0 mL/min.

A calibration curve for all eight isomers (α, β, γ and δ-tocopherol standards >95% purity (VWR International ApS, Rødovre, Denmark) and α, β, γ and δ-tocotrienol standards >95% purity (Davos Life Science Pte Ltd., The Helios, Singapore)) was prepared over a concentration range of 3.0–75.0 μg/mL and the tocopherols and tocotrienols were then identified by retention time and quantified by use of linear regression. In addition, the total amount of vitamin E was expressed in total equivalents based on biological activity (α-TE), which is an expression based on the activity relative to the naturally occurring, most active form of the vitamin, α-tocopherol. α-TE in food is calculated by adding up all amounts of vitamin E after multiplying the amount of α-T by 1.0, β-T by 0.5, γ-T by 0.1, δ-T by 0.03, α-T3 by 0.3, β-T3 by 0.05, and γ-T3 by 0.01 (Eitenmiller and Lee, 2004). Barley grain containing all eight isomers (α-T: 5.4, β-T: 0.5, γ-T: 1.7, δ-T: 0.4, α-T3: 22.0, β-T3: 3.0, γ-T3: 15.5, δ-T3: 2.3 all in μg/g dm, and 12.7 mg of α-TE) was used as a control sample to assured the accuracy of the analysis.

All samples were analyzed for vitamin E content except for the samples SA0701 and SA0702 because of small quantity of sample material.

2.3.2. Mineral content

The mineral content was determined in duplicate in three marama bean samples (BO0603, NA0701 and SA0703) using an inductively coupled plasma mass spectrometer (ICP-MS) (Agilent 7500c, Agilent Technologies, Manchester, UK) equipped with an octopole reaction system (ORS) according to the method described by Husted et al. (2004). For each batch of marama beans, seven blank samples were included in order to determine the limit of quantification (LOQ). Samples of a certified reference material (Apple leaf, standard reference material 1515; NIST: National Institute of Standards and Technology, Gaithersburg, MD, USA) were also digested to estimate the accuracy of the analysis. Samples were diluted to the same acid concentration (1.75%, v/v HNO_3) as standards and quantification was performed by external calibration (P/N 4400 ICP-MS, Multi-elemental calibration standard, CPI-International, Amsterdam, Holland). Dilutions were performed in a Teflon-coated class 100 laminar flow bench (KR-170s Biowizard, Kojair Tech Oy, Vilppula, Finland). Analytical results were accepted when they were above the LOQ (10 sigma) and when an accuracy >90% was obtained, relative to the NIST 1515 plant tissue used as certified reference material.

2.3.3. Phytoestrogen content

The content of various phytoestrogens was investigated in a sample (BO0603) of roasted and unroasted marama beans. The roasted beans were prepared by roasting in an oven for approximately 50 min at 145–150 °C and thereafter de-hulling. The content of six dietary plant lignans (pinoresinol, lariciresinol, medioresinol, syringaresinol, secoisolariciresinol and matairesinol) was analyzed using a modified gas chromatography/mass spectrometry (GC–MS) method, where the Sep-Pak was replaced by ether extraction, according to Penalvo et al. (2005). The total amount of isoflavones was analyzed with high performance liquid chromatography (HPLC) using coulometric electrode array detection (CEAD) as descried by Penalvo et al. (2004b). All phytoestrogens were determined in duplicate. Accuracy of the analysis was determined using two types of control samples, millet and rye bran, with low and high lignan content, respectively.

2.3.4. Allergens

The allergenicity of a marama bean sample (BO0603) was investigated in duplicate by use of the cholera toxin mouse model as described by Foss et al. (2006). This method is used for evaluating IgE-mediated food allergy. By use of sera from mice fed peanut or lupine the cross-reaction between proteins from lupine, peanut and marama bean were assessed. Using this procedure, 3 g of milled marama bean was suspended in 20.0 mL 0.1 M NH_4HCO_3, pH 7.80, and gently stirred for 24 h at 4 °C. Supernatant was obtained by centrifugation at 1800 × *g* for 10 min. The marama bean extract was analyzed by two protein analysis; enzyme-linked immunosorbent assay (ELISA) and Western blotting.

2.4. Cyanogenic glycosides

Two marama bean samples (BO0603 and BO0803) were tested for their potential content of cyanogenic glycosides by their ability to release hydrogen cyanide (Morant et al., 2008). Two different approaches were applied; first, a freeze/thaw-method was carried out to see if the bean itself had enzymes (β-glucosidases releasing glucose is the first step, the next step can happen spontaneously or by the enzyme hydroxyl nitrile lyase that releases cyanide) that would release cyanide from possibly present cyanogenic compounds. In the second approach, the bean was boiled in order to extract metabolites and hereafter specific enzymes, which are able to release cyanide, were added. Two enzymes were applied: linamarase, which cleaves the cyanogenic glycosides linamarin and lotaustralin, and emulsin, which cleaves e.g. dhurrin. These enzymes have different pH optima and hence the buffers tricin and MES (2-(N-morpholino)ethanesulfonic acid) were used, respectively. Furthermore, background values were obtained by extracting metabolites from a sample but not adding enzymes. The cyanide content was determined spectrophotometrically. The method is modified from Halkier and Møller (1989) and Jørgensen et al. (2005).

2.5. Statistical analysis

One-way analysis of variance (ANOVA) was used to test levels between countries. For significant response variables the levels were subsequently compared using two-sided *t*-tests. All analyses used $p < 0.05$ as significance level. Principal component analysis, PCA (PLS toolbox v.4.1 Eigenvector Research, Inc., Manson, WA, USA) was used to investigate the variance structure in the chemical data. All other data analyses were performed using the Statistics toolbox for MatLab (MatLab v.7.6.0 R2008a, Mathworks, USA).

3. Results and discussion

3.1. The marama bean

Sixteen different marama bean samples from three different countries and various harvest years have been examined. The appearance and size of the beans vary considerably (Fig. 1). Especially the *T. fassoglense* samples (e.g. SA0702) differ from the *T. esculentum* in being flatter, and in having a softer shell and a lighter brown color.

The de-hulled bean makes up 43–57% (on average 50%) of the total weight of the bean, and the average weight of the de-hulled bean is 2.3 g (varying between 1.8 and 3.1 g). There are no

Fig. 1. Pictures of two marama bean (*T. esculentum*) samples from Botswana and Namibia, as well as one *T. fassoglense* sample from South Africa.

significant differences in the weight according to origin of the beans.

3.2. Chemical composition

3.2.1. Dry matter and ash content

The dry matter content of the marama beans range from 93.4 to 98.7% (Table 2). Hence, it can be concluded that the water content is very low and variations may arise from external factors such as soil composition, climate and harvest time. However, no significant differences in dry matter were found between beans from the three different countries of origin

The ash content of the beans varies between 2.5 and 3.7% dm (Table 2). The South Africa beans have significantly higher ash content than beans from both Namibia and Botswana, which do not differ significantly. The ash content is a measure of the total mineral content in the beans. The mineral composition is discussed further below (Table 5). Only few previous studies report the dry matter and ash contents of the beans. These studies report a water content of approximately 4% (Bower et al., 1988) and an ash content of 3.5% (Amarteifio and Moholo, 1998; Bower et al., 1988), so our results are in accordance with the literature.

3.2.2. Protein content

The marama bean is an excellent source of protein as the content is between 28.8 and 38.4% dm, with a significantly higher amount in the beans from South Africa compared to beans from Botswana. The amount of protein in beans from Namibia is between the contents from the two other countries (Table 2). The content determined in this study is in agreement with the level ranging from 30 to 39% found in other investigations (Amarteifio and Moholo, 1998; Bower et al., 1988; Keegan and Van Staden, 1981). Since the beans have a protein level equaling that of soybeans, the beans will have a great potential both as a nutritive food itself and as a protein-rich food ingredient for supplementation in other food products.

Table 2
Dry matter (dm) content (percent) and content (percentage on dry matter basis, % dm) of ash, protein and lipid in marama bean samples from different harvest years and areas.

Sample	Dry matter[a] (%)	Ash[a] (% dm)	Protein[b] (% dm)	Lipid[b] (% dm)
BO9001	95.8 ± 0.2	2.8 ± 0.1	30.5–30.8	38.9–39.1
BO0501	98.3 ± 0.1	2.5 ± 0.0	30.9–30.9	40.0–40.0
BO0601	98.4 ± 0.1	2.5 ± 0.1	28.7–28.8	41.8–42.1
BO0602	98.7 ± 0.1	2.5 ± 0.1	31.2–31.2	38.2–38.3
BO0603	96.3 ± 0.2	2.9 ± 0.0	31.9–32.0	40.3–41.4
BO0701	97.0 ± 0.1	2.9 ± 0.0	31.7–31.9	31.7–32.2
BO0803	94.7 ± 0.2	2.8 ± 0.0	32.0–32.4	38.7–39.3
NA0701	98.2 ± 0.1	3.1 ± 0.1	29.3–29.6	39.2–39.4
NA0702	95.6 ± 0.0	2.9 ± 0.0	32.7–32.8	38.1–38.7
NA0703	96.5 ± 0.0	3.0 ± 0.1	31.4–31.5	38.0–38.7
NA0801	97.3 ± 0.0	2.9 ± 0.0	35.6–35.7	37.2–37.7
NA0802	97.2 ± 0.2	2.9 ± 0.1	35.0–35.1	38.3–38.8
SA0701	96.9 ± 0.1	3.7 ± 0.1	34.6–35.0	35.5–35.6
SA0702	93.4 ± 0.2	3.3 ± 0.1	38.3–38.4	33.9–34.3
SA0703	94.5 ± 0.1	3.2 ± 0.0	35.4–35.9	34.8–35.0
SA0705	95.8 ± 0.2	3.6 ± 0.1	34.3–35.0	41.5–41.8
Soybean control	89.4 ± 0.1	4.6 ± 0.1	40.1 ± 0.3	16.5 ± 0.3
One-way ANOVA *p*	0.10	<0.0001	0.004	0.47
Botswana average	97.0 ± 1.5	2.7 ± 0.2 a	31.1 ± 1.2 a	38.7 ± 3.2
Namibia average	96.9 ± 1.0	3.0 ± 0.1 a	32.9 ± 2.6 a,b	38.4 ± 0.7
South Africa average	95.2 ± 1.5	3.5 ± 0.2 b	35.9 ± 1.8 b	36.5 ± 3.5
Total average	96.5 ± 1.5	3.0 ± 0.4	32.8 ± 2.6	38.1 ± 2.7
Average SD on analysis	0.1	0.1	0.3	0.4

[a] Values represent the average of three independent replicates ± the standard deviation (SD).
[b] Values represent the range between the two independent replicates.
For significant effects, pair-wise comparisons are made. Different letters indicate statistically significant differences between the averages ($p < 0.05$).
The average SD on the analysis method gives a measure of the precision of the method itself.

Table 3
Content (percentage on dry matter basis, % dm) of soluble and insoluble dietary fibers in marama bean samples from different harvest years and areas[a].

Sample	Soluble dietary fiber (% dm)	Insoluble dietary fiber (% dm)	Total dietary fiber (% dm)
BO9001	0.3–1.1	23.9–24.5	24.2–25.7
BO0501	0.3–1.6	20.9–22.6	21.2–24.2
BO0601	1.6–1.9	22.0–23.3	23.6–25.2
BO0602	0.3–0.9	22.2–25.2	23.1–25.5
BO0603	0.8–1.1	20.6–27.4	21.4–28.5
BO0701	0.6–1.1	23.3–25.2	24.4–25.8
BO0803	0.3–0.6	18.0–18.6	18.5–18.8
NA0701	0.5–0.6	22.7–22.9	23.2–23.6
NA0702	0.9–0.9	20.1–24.7	21.0–25.6
NA0703	1.1–1.7	19.6–20.3	21.3–21.4
NA0801	0.3–0.8	22.4–25.7	23.2–26.0
NA0802	0.8–1.1	17.7–20.2	18.6–21.3
SA0703	0.3–1.2	25.5–26.6	25.8–27.8
SA0705	1.0–1.3	17.7–25.4	19.0–26.4
Soybean control	7.2 ± 0.3	9.9 ± 0.4	17.1 ± 0.4
One-way ANOVA p	0.98	0.50	0.47
Botswana average	0.9 ± 0.4	22.7 ± 2.2	23.6 ± 2.3
Namibia average	0.9 ± 0.3	21.6 ± 2.1	22.5 ± 1.9
South Africa average	0.9 ± 0.3	23.8 ± 3.2	24.8 ± 2.9
Total average	0.9 ± 0.4	22.5 ± 2.2	23.4 ± 2.2
Average SD on analysis	0.4	2.4	2.4

[a] Values represent the range between the two independent replicates.
The average SD on the analysis method gives a measure of the precision of the method itself.

3.2.3. Lipid content

The marama beans are a rich source of lipids with a content of 32.0–41.9% dm (Table 2). In spite of this variation there are no statistically significant differences between the content in the beans from the three countries. But, it indicates that the observed differences are due to the variation that can be expected in uncultivated beans regardless of origin. The values obtained in this study are in general agreement with results from previous studies, where the content has been reported to be between 35 and 44% dm (Amarteifio and Moholo, 1998; Bower et al., 1988; Francis and Campbell, 2003; Keegan and Van Staden, 1981). However, Ketshajwang et al. (1998) found a lipid content of 48% (w/w) which is substantially higher than other reported results.

The high lipid content is a great advantage of the beans, especially in regards to producing healthy products that may improve the nutritional status of undernourished people in Southern Africa.

3.2.4. Dietary fiber content

The dietary fiber analysis resulted in improved determinations when de-fatting the beans before analysis. The content of TDF is quite high and varies between 18.7 and 26.8% dm (Table 3) of which the majority is insoluble and only about 4% of the dietary fibers are soluble. The content does not vary significantly between countries.

The content of dietary fiber has not previously been determined in marama beans. The content of carbohydrates reported in other investigations (19–24% dm) is based on indirect values, calculated as the difference between 100% and the content of protein, lipid and minerals (dm basis) (Amarteifio and Moholo, 1998; Bower et al., 1988; Wehmeyer et al., 1969). Hence, the level of dietary fibers obtained in this study is in accordance with the previously reported level of carbohydrates, indicating that fibers make up the majority of the carbohydrate part of the bean.

Summarizing the content of the major chemical components determined in this study (lipid, protein, total dietary fiber and minerals (dm basis)) on average makes up more than 97% of the beans, which means that the content of starch must be very low. This is in contrast to other legumes, in which starch is usually the most abundant carbohydrate (22–45%) (Hoover and Sosulski, 1991). Comparing with the TDF content of peanut and soybean, which is 9 and 10% dm, respectively (U.S. Department of Agriculture, 2007), it appears that the marama bean has a considerably higher level of dietary fiber than other legume seeds investigated. This means that the marama bean is quite unique because of its high dietary fiber and low starch content.

3.2.5. Multivariate data analysis

To further investigate the data from the proximate analysis a PCA was performed on the autoscaled data (mean centered + scaling each variable to unit variance). A bi-plot (scores and loadings) is given in Fig. 2. The loadings show how the chemical parameters are correlated while the scores illustrate the variation within the sample set.

From the figure it appears that PC1 encompassing 50% of the variation in data contains information about the proximate composition of the beans. There is a tendency towards a higher content of both ash, protein and water (less dm), and a lower content of lipid in samples from South Africa as opposed to samples from Namibia and Botswana, which do not seem to differ considerably from each other. However, these differences were not all statistically significant (Table 2).

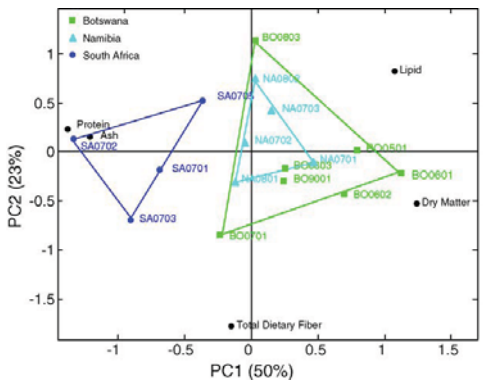

Fig. 2. Bi-plot from PCA on chemical data from 16 marama bean samples. Samples are colored according to country and border points are connected by lines.

Table 4
Concentrations (micrograms per gram on a dry matter basis, μg/g dm) of tocopherol isomers (α-, β- and γ-) and total vitamin E equivalents (milligrams of α-TE) in marama bean samples from different harvest years and areas[a].

Sample	α-Tocopherol (μg/g dm)	β-Tocopherol (μg/g dm)	γ-Tocopherol (μg/g dm)	α-TE (mg α-TE)
BO9001	24.7–24.9	1.1–1.1	94.1–95.4	34.8
BO0501	13.8–14.1	–	72.3–78.1	21.4
BO0601	23.4–24.4	–	88.8–89.2	32.8
BO0602	24.5–24.6	–	70.6–71.3	31.7
BO0603	25.7–28.0	2.1–2.9	77.2–85.4	36.2
BO0701	30.3–31.5	1.3–1.5	56.9–62.0	37.5
BO0803	55.2–58.5	1.7–1.9	90.0–96.4	67.1
NA0701	31.8–32.3	3.3–3.3	156.3–161.6	49.6
NA0702	32.3–33.5	1.7–1.7	122.8–128.1	46.3
NA0703	26.4–30.4	1.6–1.8	93.4–108.3	39.3
NA0801	27.3–28.6	–	229.6–239.0	51.4
NA0802	46.3–49.7	–	141.1–152.7	62.7
SA0703	36.1–37.9	–	74.3–77.0	44.6
SA0705	36.6–37.5	0.6–0.6	158.3–158.8	53.2
Soybean control	5.3 ± 0.2	0.6 ± 0.1	1.7 ± 0.1	12.8
One-way ANOVA *p*	0.58	0.29	0.019	0.20
Botswana average	28.8 ± 13.4	1.7 ± 0.6	80.6 ± 12.9 a	37.4 ± 14.1
Namibia average	33.9 ± 8.2	2.2 ± 0.9	153.3 ± 50.4 b	49.9 ± 8.5
South Africa average	37.0 ± 0.0	0.6 ± 0.0	117.1 ± 58.6 a,b	48.9 ± 6.1
Total average	31.8 ± 10.7	1.8 ± 0.8	111.7 ± 48.1	43.5 ± 12.6
Average SD on analysis	1.4	0.2	4.9	–

[a] Values represent the range between the two independent replicates.
For significant effects, pair-wise comparisons are made. Different letters indicate statistically significant differences between the averages ($p < 0.05$) for each isomer of tocopherol.
The average SD on the analysis method gives a measure of the precision of the method itself.

One of the reasons why these tendencies towards regional differences are not statistically significant is most likely due to the large variation within beans from each of the three countries and especially due to a heterogenic distribution of beans harvested in the same national location at distinct harvest times. This has been confirmed several times in single-bean in-house pre-studies.

PC 2 describes 23% of the variation and gives mainly information on the content of total dietary fiber. However, it is not possible to distinguish between beans from different countries due to their fiber content as was also seen in Table 3. Finally, it has not been possible to draw any conclusions on the influence of harvest years.

Looking further into the geographical placement of the harvest sites, the sites in Namibia and Botswana appear to be situated relatively close to each other in the Northern part of the Kalahari Desert, while the South African beans are harvested south of the Kalahari Desert. Both climate and soil conditions differ in the two ends of the desert. This may explain the similarity in chemical composition of the beans from Namibia and Botswana and their difference to the South African beans. The regional differences might be interesting to take into consideration when initializing cultivation of marama beans, e.g. if beans with a high protein content are desired South Africa might be the best place to cultivate these.

The two *T. fassoglense* samples (SA0701 and SA0702) do not seem to differ considerably from the *T. esculentum* samples from South Africa (SA0703 and SA0705). This indicates that even though the visual appearance (Fig. 1) of the two species differs quite markedly, the proximate chemical composition is similar. This is confirmed when comparing the results from this study to the findings by Dubois et al. (1995) who investigated the chemical composition of *T. fassoglense*.

It has not been possible to draw any conclusions on the influence of harvest years on the chemical components, even though the weather conditions in Southern Africa varied greatly from extremely dry seasons (e.g. 2007) to very rainy seasons (e.g. 2008).

3.3. Minor chemical components

3.3.1. Vitamin E

The content of the eight vitamin E isomers have been determined, but only α-, β- and γ-tocopherols are present in measurable quantities in marama beans (Table 4). α- and γ-tocopherols are present in all beans, while β-tocopherol is present in the majority of the beans. The content of vitamin E isomers differs largely between samples and is dominated by γ-tocopherol with 59–234 μg/g followed by α- and β-tocopherols with 14–48 μg/g and 1.1–3.3 μg/g, respectively. Furthermore, traces of δ-tocopherol as well as β- and γ-tocotrienols are present in some samples. The remaining two tocotrienols (α- and δ-) are not present in the beans

The content of γ-tocopherol accounts for approximately 76% of the total vitamin E content of the beans, and the content varies significantly between beans from Namibia and Botswana, and in a considerably higher amount in the beans from Namibia. The content of γ-tocopherol in beans from South Africa is between the levels from the two other countries. For α- and β-tocopherols there are no significant differences between countries. The high content of vitamin E in marama beans is most likely related to their high fat content and abundance of unsaturated fatty acids. However, no correlation is found between the content of lipids and the content of tocopherols in total or the different vitamin E isomers.

Vitamin E has numerous health benefits. For instance vitamin E acts as a powerful antioxidant by neutralizing free radicals in the body that cause tissue and cellular damage and it contributes to the regulation of cellular signaling and gene expression (Azzi and Stocker, 2000; Brigelius-Flohé, 2006). The high content of γ-tocopherol is of particular biological relevance as it has shown potential anti-carcinogenic and anti-inflammatory activities (Brigelius-Flohé, 2006; Saldeen and Saldeen, 2005).

When comparing the average α-TE of marama beans, 44 mg α-TE, with that of various cereal grains, legumes, vegetables and fruits (1–23 mg α-TE) (Bramley et al., 2000), it appears that marama beans have a significantly higher vitamin E content. The vitamin E content of marama beans seems to fall somewhere

Table 5
Concentrations (micrograms per gram, μg/g) of minerals in marama bean samples from the three different countries[a].

Mineral	NA0701	BO0603	SA0703
K	9423–9522	7502–7650	12,957–13,379
P	4050–4576	3307–3383	5488–5594
Mg	3580–3593	2330–2647	3712–3783
S	1849–1978	2188–2220	2335–2399
Ca	937–1462	2038–2176	1313–1361
Zn	31–39	33–33	38–39
Mn	22–29	15–16	14–15
Na	29–29	2–3	6–6
Al	18–19	81–83	4–6
B	18–19	19–21	21–21
Fe	12–14	13–14	35–40
Cu	6–7	7–7	15–16

[a] Values represent the range between the two independent replicates.

between that of sesame seeds (23 mg α-TE) and sunflower seeds (59 mg α-TE). However, peanuts (122 mg α-TE) and soybeans (560 mg α-TE), which have been used as references in the previous sections, have considerably higher vitamin E content than marama beans (Bramley et al., 2000; Nishiba et al., 2007).

3.3.2. Minerals

The mineral content of the three examined marama bean samples is given in Table 5. The beans have a level of macro-elements (K, P, Mg and Ca) similar to that of peanuts and approaching that of soybeans (U.S. Department of Agriculture, 2007). The beans furthermore contain several trace-minerals (Zn, Fe, Cu and Mn) in amounts that match the content of soybeans and peanuts. In general the values are in accordance with the previously published results. However, the sodium content found in this study is considerably lower than previously reported (Amarteifio and Moholo, 1998; Wehmeyer et al., 1969).

The relatively high mineral levels in the marama beans are quite surprising when taking into account the poor soils in which they grow. The bean sample from South Africa (SA0703) seems to have higher amounts of K, P, Fe and Cu compared to the beans from Namibia (NA0701) and Botswana (BO0603), which corresponds well to the higher ash content in beans from South Africa (Table 2). The sample from Botswana has the highest content of Ca and Al, where the sample from Namibia has the highest content of Mn and Na. However, more samples are needed to be able to draw statistically significant conclusions on the differences between countries.

In regards to the nutritional quality, the high contents of zinc and iron are extremely important. Large percentages of the population in Africa are deficient or at risk of inadequate intake of zinc and iron (Hotz and Brown, 2004; UNICEF and Micronutrient Initiative, 2004). Therefore, the marama beans might be a good supplement to a diet otherwise low in these trace elements. However, the bioavailability of the minerals will be limited if they are bound by phytic acid, as the case is in most legume seeds.

3.3.3. Phytoestrogens

Samples of roasted and unroasted marama beans were investigated for content of phytoestrogens, which are naturally occurring plant-derived phytochemicals. These compounds are precursors of hormone-like compounds found in mammalian systems and posses a wide range of health promoting effects in humans (Branca and Lorenzetti, 2005; Kwiatkowska, 2007).

The three lignans; secoisolariciresinol, lariciresinol and pinoresinol, were found in the raw marama bean; lariciresinol showed the highest level, followed by secoisolariciresinol. Secoisolariciresinol and lariciresinol were also found in the roasted beans, while pinoresinol disappeared during roasting (Table 6). There are no

Table 6
Concentration (micrograms per 100 gram, μg/100 g) of lignans in a marama bean sample (BO0603)[a]. The concentration is measured in both roasted and unroasted samples.

Sample	Secoisolariciresinol	Lariciresinol	Pinoresinol
Unroasted marama bean	305–362	675–825	21–23
Roasted marama bean	394–406	614–620	–
Millet control	25.6 ± 2.6	148.7 ± 12.8	325.2 ± 17.3
Rye bran control	80.7 ± 2.0	921 ± 14.2	798.3 ± 5.7
One-way ANOVA *p*	0.16	0.22	

[a] Values represent the range between the two independent replicates.

significant differences between the amount of secoisolariciresinol and lariciresinol in the roasted and unroasted beans. The lignans matairesinol, syringaresinol and medioresinol are not present in the beans.

The content of secoisolariciresinol in marama beans is equal to or higher than that of soybean (13–273 μg/100 g) and peanut (333 μg/100 g) (Mazur et al., 1998). The level of lariciresinol is lower in soybeans (287 μg/100 g) and the level of pinoresinol is higher in soybeans (446 μg/100 g) (Penalvo et al., 2004a) than in marama beans. Contrary to soybeans which are rich in isoflavones (Mazur et al., 1998), no isoflavones were found in the marama bean samples. Summing up, it seems as if marama bean is an excellent source of lignans but lacks isoflavones. The level of secoisolariciresinol and lariciresinol is retained during roasting, which is an important characteristic since the beans usually are eaten roasted.

3.3.4. Allergens

Since allergy is one of the fastest growing health problems in the western world (WHO, 2003), an evaluation of new foods with respect to their allergenic potential is important. Even though the marama bean has been consumed in Southern Africa for centuries, it should be tested prior to an introduction to the western market, where it is not a common constituent of the diet. Lupine and peanut are potent allergens and are some of the most prevalent allergenic foods in the western world. Humans suffering from allergic reactions to these legumes may also present an allergic reaction to other legumes with proteins that are able to cross-react with proteins of lupine or peanut. Consequently, the IgE antibodies that have been used to test marama beans are antibodies that react with the major allergens in lupine and peanut.

The analyses revealed no presence of proteins cross-reacting with the allergenic proteins from lupine and peanut. Hence, there is no reason to suspect that people suffering from lupine or peanut allergy will suffer from allergic reactions when consuming products containing marama beans. However, we cannot rule out the possibility that people will have an allergic reaction to marama beans as there might be other potential allergens not tested.

3.3.5. Cyanogenic glycosides

Marama bean samples were investigated for their potential cyanogenesis, which is the ability of some plants, including grain legumes and nuts, to release cyanide during degradation of cyanogenic glycosides. Upon tissue disruption, the cyanogenic glycosides may be enzymatically hydrolyzed to the respiratory poison hydrogen cyanide (Breiteneder and Radauer, 2004; Poulton, 1990). Hence, it is important to ensure that this is not a problem in marama beans and products thereof.

The analyses have shown that marama beans do not seem to contain either cyanogenic glycosides or the enzymes that break these down to hydrogen cyanide. This was revealed since cyanide was not released from the beans by their own enzymes during the freeze/thaw-method, nor during the boiling method where specific enzymes were added to the beans. However, not all enzymes

capable of breaking down cyanogenic glycosides have been tested, hence it may only be concluded that the cyanogenic glycosides which are degraded by the two tested β-glucosidases (linamarase and emulsion) are not present in the beans. The fact that marama beans (*T. esculentum*) do not seem to be cyanogenic is in accordance with results from Dubois et al. (1995), who found that *T. fassoglense* seeds did not contain any detectable amounts of cyanogenic glycosides.

3.4. Overall discussion

The studies on the chemical composition of marama beans published so far all used only one or very few marama bean samples. The results vary considerably, which may be due to differences in extraction and analysis methods, but certainly also might be due to the fact that no attention has been paid to the large differences within marama beans from different growth areas, harvest years and even due to differences in beans harvested at the same time and place. Gaining knowledge about this variation is interesting for several reasons. First, this will make it possible to evaluate and utilize available research on the beans in a more objective manner; secondly, it is useful to be able to interpret novel results; and finally, this will improve how to set up the sample plan in future scientific studies of marama beans. Furthermore, it is important to know the regional differences to be able to select beans with certain characteristics for future cultivation. Moreover, in the production of marama-bean-based products, more knowledge is needed about the bean-to-bean variation to be able to optimize production and to obtain standardized products.

The excellent proximate composition of marama beans, their high content of minor components such as vitamin E, important minerals and lignans, and the absence of allergens and cyanogenic glycosides as potential health risks, make it clear that marama bean can be an alternative to soybean and peanut as a food source or ingredient. This will improve the lives of indigenous people in Southern Africa, especially people living in areas where the potential of this uncultivated plant is yet to be exploited. Unfortunately, the beans only grow in limited quantities today, but work on cultivation of the beans is being carried out.

4. Conclusion

This analysis of marama bean samples from different harvest years and areas has given a more representative and comprehensive description of the chemical composition compared to previously published work. The marama bean has a great potential as a food product or as an ingredient in other food products, because of its quite unique composition with high protein (29–38%), fat (32–42%) and dietary fiber (19–27%) contents and very low starch content.

The chemical analyses have revealed differences in the proximate composition between beans harvested in Namibia, Botswana and South Africa. Beans from South Africa seem to have higher moisture, ash and protein content than the beans from Namibia and Botswana, which do not differ considerably from each other. However, there are large variations within beans from each of the three countries, and a heterogenic distribution of beans harvested in the same national location at distinct harvest times.

Marama bean proved to be a good source of vitamin E. The isomers α- and γ-tocopherol were present in all beans, while β-tocopherol was present in the majority of the beans, with contents of 14–48, 59–234 and 1.1–3.3 μg/g, respectively. In general, beans from Namibia contained the highest amount of vitamin E. Marama bean was furthermore found to have mineral contents comparable to that of peanut and approaching that of soybean and with a high content of the important trace-elements Zn and Fe. Additionally, the three lignans secoisolariciresinol, lariciresinol and pinoresinol were present in the beans, while isoflavones were not found.

Finally, marama bean did not exhibit allergic cross-reactions with peanut and lupine, nor did it contain cyanogenic glycosides and the enzymes that break these down to hydrogen cyanide.

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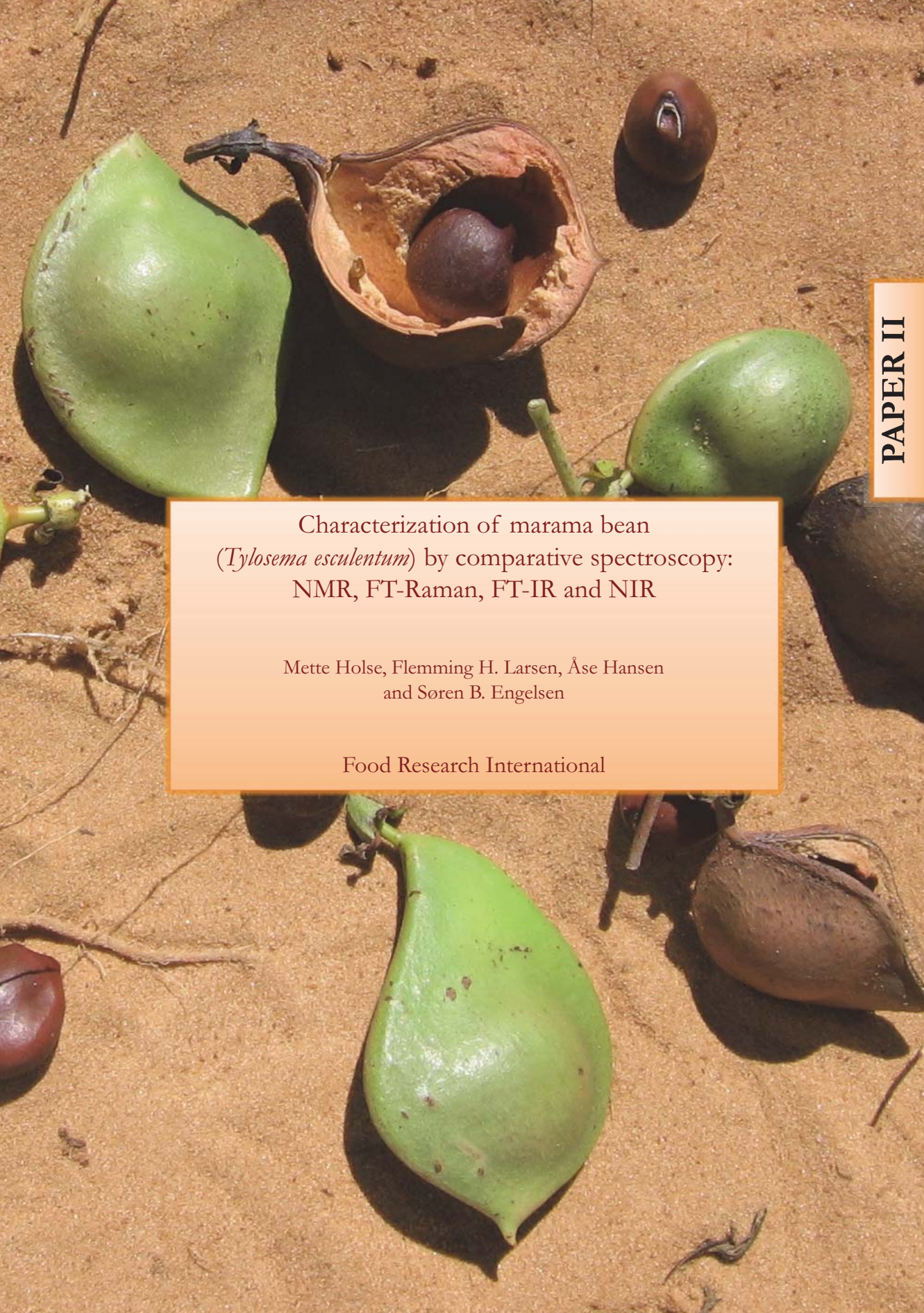

PAPER II

Characterization of marama bean (*Tylosema esculentum*) by comparative spectroscopy: NMR, FT-Raman, FT-IR and NIR

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ABSTRACT

The marama bean from Southern Africa has proven to be a source for production of various healthy food products. In order to exploit its commercial potential, it is important to know its chemical composition in more detail. In this study, marama beans from different geographical sites and harvest years were analyzed by use of infrared, near infrared, Raman, and ^{1}H as well as ^{13}C nuclear magnetic resonance spectroscopy. These techniques can measure single beans in a rapid and non-destructive manner. By comparative application, the qualitative composition of the marama bean was explored in detail, revealing large amounts of protein, dietary fiber and unsaturated fat. The carbohydrate fraction was largely present as pectins and a minor fraction of smaller water soluble carbohydrates were tentatively assigned to raffinose. It is characteristic that the beans do not contain starch or β-glucans and that the water soluble part of the proteins/peptides have a high content of the aromatic amino acid tyrosine.

1. Introduction

The marama (also called morama) plant (*Tylosema esculentum*) is a wild, long-lived, perennial, tuberous legume which grows in extreme environments with high temperatures, low rainfall and long periods of drought. The plant grows naturally in the Kalahari Desert and neighboring areas with poor semi-arid soils in Botswana, Namibia and the northern part of South Africa. Another *Tylosema* species *T. fassoglense* (Schweinf.) is widely distributed throughout Sub-Saharan Africa, from Ethiopia through Angola to Namibia and the northern part of South Africa (Dubois et al., 1995). The edible and nutritious seeds from these plants are underutilized as food, but constitute a part of the traditional diet for the San people and other indigenous groups in Southern Africa (van der Maesen, 2006). The marama beans are gathered from the wild and are mostly eaten as a snack after roasting in hot sand (van der Maesen, 2006).

The seeds have a high content of lipid (approx. 38% dry matter basis, % db) and protein (approx. 33% db) (Holse, Husted, & Hansen, 2010) which give them an important socio-economic value. Besides the large nutritional value of the roasted seeds, the marama bean also has potential as a source for production of oil and various other healthy food products such as marama milk and defatted marama flour (van der Maesen, 2006). Hence, this neglected legume has the potential to both improve nutrition, increase food availability in dry regions and diversify livelihoods for the people living in these rural areas (van der Maesen, 2006; Rachie et al., 1979).

A number of studies describing the main chemical composition of the marama bean have been carried out (Amarteifio & Moholo, 1998; Bower, Hertel, Storey, & Oh, 1988; Dubois et al., 1995; Francis & Campbell, 2003; Hertel, Bower, & Storey, 1987; Ketshajwang, Holmback, & Yeboah, 1998; Engelter & Wehmeyer, 1970). These studies describe its high content of lipids and proteins as well as the composition of fatty acids and amino acids. Recently, a comprehensive characterization of the chemical composition of marama bean was made on samples from different harvest years and countries (Holse et al., 2010). This and most other studies of the marama bean have been carried out by use of chemical and chromatographic analysis (Amarteifio & Moholo, 1998; Bower et al., 1988; Ketshajwang et al., 1998; Hertel et al., 1987; Francis & Campbell, 2003; Dubois et al., 1995; Engelter & Wehmeyer, 1970; Holse et al., 2010). Until now, only the marama oil has been analyzed by spectroscopic methods (NMR and MS) (Mitei, Ngila, Yeboah, Wessjohann, & Schmidt, 2008) and no previous studies have applied spectroscopic techniques to analyze intact marama bean tissue. The advantage of using spectroscopic techniques is the exploratory character of the measurements that enables simultaneous detection of several different and even non-anticipated compounds. Hence, spectroscopy will provide an excellent overview of the marama bean composition representing the four major chemical groups in food: water, lipids, carbohydrates and proteins. Spectroscopy in combination with chemometrics has abundantly been applied to cereals

Abbreviations: ATR, attenuated total reflectance; CP/MAS, cross-polarization magic-angle spinning; db, dry matter basis; FT, Fourier transform; HR-MAS, high-resolution magic-angle spinning; IR, infrared (reflectance); MSC, multiplicative scatter correction; NIR, near infrared (reflectance); NMR, nuclear magnetic resonance; PC, principal component; PCA, principal component analysis; SP/MAS, single-pulse magic-angle spinning; TSP-d4, sodium 3-(trimethylsilyl)propionate-d4.

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and vegetables and its fundamental importance has recently been demonstrated in a spectroscopic application for barley seeds (Munck et al., 2010).

In this study, the marama bean was analyzed using ^{1}H HR-MAS NMR (proton high-resolution magic-angle spinning nuclear magnetic resonance) and solid-state ^{13}C CP and SP/MAS NMR (carbon cross-polarization and single-pulse MAS NMR) spectroscopy as well as the vibrational spectroscopic techniques NIR (near infrared) spectroscopy, FT-IR (Fourier transform infrared) spectroscopy and FT-Raman (Fourier transform Raman) spectroscopy. The subsequent spectral analyses aim to detect specific features enabling characterization of the chemical composition of the marama bean as well as variations in the composition of the beans as a function of harvest time and geographical origin. These spectroscopic investigations of intact marama tissue were made to pave the way for subsequent analysis of the content of micro and macro components in the marama bean.

2. Materials and methods

2.1. The samples

The marama bean sample set investigated in this study consisted of three replicate marama beans (a, b and c) from 18 different batches resulting in a total of 54 samples. Fifteen of the batches were of the species *T. esculentum*, while three were *T. fassoglense*. The batches were collected from wild growing plants at different locations in Botswana, Namibia and South Africa during the growing seasons 1990 and 2005–2008. Each batch represents the harvest from a certain location in the specific year. The beans were stored in plastic bags at 4 °C and 40% relative humidity until analysis to minimize changes over time. The batches are listed in Table 1.

The hard outer hull of the bean was removed by use of a nutcracker. Hereafter, each bean was ground in a mortar to a small particle size (≤250 μm). The ground marama beans were stored separately in sealed glass containers at the same conditions as the whole beans until the spectroscopic analyses were carried out. No further treatments of the ground marama beans were carried out before analysis.

In addition to the bean tissue, marama oil (from marama beans harvested in Botswana in 2006, prepared at University of Botswana in 2008 by use of a mechanical press) and linoleic acid (>99%, SIGMA, India) were examined and used as standard reference material.

2.2. NIR spectroscopy

The measurements were carried out in reflectance mode on a NIR spectrometer (System 6500, FOSS NIRsystems, Silver Springs, USA) equipped with a spinning module. The instrument uses a split detector system with a silicon (Si) detector from 400–1098 nm and a lead sulphide (PbS) detector from 1100–2498 nm. Absorption values (log (1/R)) were obtained every 2 nm giving a total of 1050 variables. The ground marama bean was analyzed in a half sized cup (internal diameter, 1.2 cm; depth, 0.3 cm) through a quartz window. Each spectrum represents the average of 16 scans ratioed against a background (32 scans) measured on an internal white ceramic disc. Duplicate measurements were made. Scatter effects in the raw spectra were removed by application of MSC (Multiplicative Scatter Correction) (Geladi, Macdougall, & Martens, 1985; Rinnan, van den Berg, & Engelsen, 2009).

2.3. FT-IR spectroscopy

The absorbance measurements were performed on an Arid-Zone MB100 FT-IR instrument (ABB Bomen, Quebec, Canada) using an Attenuated Total Reflectance (ATR) device with a triple-bounce diamond crystal. IR spectra were recorded in the range from 4400–750 cm^{-1} using a spectral resolution of 4 cm^{-1}. The ground marama bean was positioned on the crystal surface and squeezed towards the diamond crystal by use of a concave needle compressor. Each spectrum represents the average of 32 scans ratioed against the background (64 scans) collected with the empty crystal and stored as absorbance spectra. Between the samples, the crystal was thoroughly cleaned with ion exchanged water. Each sample was measured in duplicate.

Table 1
Applied marama bean samples.

ID number[a]	Origin	Harvest year	Species
BO9001	Botswana	1990	*T. esculentum*
BO0501	Botswana	2005	*T. esculentum*
BO0601	Botswana	2006	*T. esculentum*
BO0602	Botswana	2006	*T. esculentum*
BO0603	Botswana	2006	*T. esculentum*
BO0701	Botswana	2007	*T. esculentum*
BO0803	Botswana	2008	*T. esculentum*
NA0701	Namibia, Okakarara	2007	*T. esculentum*
NA0702	Namibia, Gobabis–Sandveld station	2007	*T. esculentum*
NA0703	Namibia, Gobabis–Sandveld station	2007	*T. esculentum*
NA0801	Namibia, Gobabis–Sandveld station	2008	*T. esculentum*
NA0802	Namibia, Okakarara	2008	*T. esculentum*
SA0701	South Africa, Entabeni	2007	*T. fassoglense*
SA0702	South Africa, Babeni	2007	*T. fassoglense*
SA0703	South Africa, University of Pretoria, Experimental farm	2007	*T. esculentum*
SA0705	South Africa, Rooidraaitrust	2007	*T. esculentum*
SA0706*	South Africa, Steenbokpan	2007	*T. esculentum*
SA0707*	South Africa, R33 30km from Lephalala and R518 halfway between Marken and Makamole	2007	*T. fassoglense*

*No chemical reference data.
[a] First two letters: country of origin; next two numbers: harvest year; last two numbers: batch no.

2.4. FT-Raman spectroscopy

The measurements were carried out on a Perkin Elmer System 2000 FT-NIR Raman interferometer (Perkin Elmer Instruments, Waltham, Massachusetts, USA) equipped with an Indium–Gallium–Arsenide (InGaAs) detector. Raman signals were collected as 180° backscatter by illuminating the ground marama bean with a Nd:YAG laser emitting 500 mW at 1064 nm. Samples were packed in a small ring cup (internal diameter, 0.5 cm and depth, 0.5 cm). Spectra were collected in the range from 3600–200 cm^{-1} using a spectral resolution of 8 cm^{-1}. Each spectrum represents the direct intensity average of 64 scans and each sample was measured in duplicate.

2.5. ^{1}H HR-MAS NMR spectroscopy

The proton NMR spectra were acquired using a Bruker Avance 400 (9.4 T) spectrometer (Bruker Biospin Gmbh, Rheinstetten, Germany), operating at a Larmor frequency of 400.13 MHz for protons, using a High-Resolution Magic-Angle Spinning (HR-MAS) probe equipped with a 4 mm (outer diameter, o.d.) rotor. Samples were prepared from 0.5–1.0 mg ground marama bean suspended in 50 μl of D_2O (containing 5.8 mM sodium 3-(trimethylsilyl)propionate-d4 (TSP-d4)). All spectra were referenced to the TSP-d4 signal (0.0 ppm). All experiments were performed at 75°C using a pulse sequence (zgcppr) by which the water resonance is pre-saturated before a composite 90-degree pulse is applied (Bax, 1985). All spectra were acquired using a recycle delay of 4 s, 64 scans, a spin-rate of 7 kHz and a dwell time of 60.4 μs for acquisition of 32 k data points resulting in a total acquisition time of 1.979 s.

In addition, a series of 2D experiments were recorded (^{1}H–^{1}H COSY (Ancian, Bourgeois, Dauphin, & Shaw, 1997), ^{1}H–^{1}H TOCSY (Bax & Davis, 1985), and ^{1}H–^{13}C HSQC (Bodenhausen & Ruben, 1980)) in order to perform spectral assignments.

2.6. Solid-state ^{13}C MAS NMR spectroscopy

The ^{13}C single-pulse SP/MAS and cross-polarization CP/MAS NMR spectra were recorded on a Bruker Avance 400 (9.4 T) spectrometer (Bruker Biospin Gmbh, Rheinstetten, Germany), operating at Larmor frequencies of 400.13 and 100.62 MHz for ^{1}H and ^{13}C, respectively. The experiments were carried out using a double-tuned (CP/MAS) probe equipped with a 4 mm (o.d.) rotor. ^{1}H and ^{13}C rf-field strengths of 80 kHz were utilized during both TPPM–^{1}H-decoupling (Bennett, Rienstra, Auger, Lakshmi, & Griffin, 1995), single pulses and cross-polarization. The variable amplitude CP scheme (Peersen, Wu, Kustanovich, & Smith, 1993) was employed to enhance the CP performance during fast spinning. Both spectra were acquired at room temperature using a spin-rate of 9 kHz and an acquisition time of 47.6 ms. For the CP/MAS spectrum (contact time = 1.5 ms) a recycle delay of 4 s and 8000 scans were used whereas a recycle delay of 128 s and 900 scans were employed for the SP/MAS spectrum. Prior to Fourier transformation the free induction decays (FID) were apodized by Lorentzian line broadening of 50 Hz. Both spectra were referenced (externally) to the carbonyl resonance in α-glycine at 176.5 ppm.

2.7. Data processing

NIR, IR and Raman spectra were analyzed using MatLab (v.7.7 R2008b, Mathworks, US). The NMR spectra were processed and analyzed using the software package Topspin (v.1.3, Bruker Biospin, Rheinstetten, Germany) and the assignments of 2D NMR spectra were performed using the Sparky program version 3.114 (Goddard & Kneller, 2010). In addition, the PLS toolbox (v.4.1, Eigenvector Research, Inc., Manson, WA, US) running in MatLab was used for the multivariate data analysis (principal component analysis, PCA (Wold, Esbensen, & Geladi, 1987)).

3. Results and discussion

3.1. Characterizing bean-to-bean and batch-to-batch variation — NIR as an example

In the previous chemical composition study of the same sample set, the overall chemical composition of the marama bean was determined to be: protein 29–38% db, fat 32–42% db, dietary fiber 19–27% db, ash 2.5–3.7% db and a total dry matter content in the range from 93 to 99% (Holse et al., 2010). The fat fraction is present mainly as unsaturated fatty acids (approx. 74%) (Mitei et al., 2008) and the dietary fibers are mainly water insoluble (approx. 96%) (Holse et al., 2010). The protein and carbohydrate composition has not yet been analyzed in detail.

NIR spectroscopy measures overtones and combinations of the fundamental molecular vibrations and gives strong signals from anharmonic molecular bonds i.e. bonds that contain at least one hydrogen atom. NIR is thus an excellent probe for the four bulk components in foods: water (O–H), fat (C–H), protein (N–H) and carbohydrate (C–H and O–H).

In Fig. 1 (top), a representative FT-IR spectrum (4400–750 cm^{-1}) of marama bean is shown and the region containing the fundamental C–H, N–H and O–H stretching vibrations is highlighted. It is primarily these vibrations that are repeated as combinations and overtones in the NIR spectra (780–2498 nm) of the marama bean shown in the bottom of Fig. 1. All NIR spectra (18 batches × 3 beans × 2 replicates = 108 spectra) are shown in Fig. 1 and the 1st overtone region is highlighted.

Peaks arising from C–H stretches are visible as combination bands at around 2300–2350 nm and as 1st, 2nd and 3rd overtones in the regions 1730–1760, 1195–1225 and 910–940 nm, respectively. The C–H stretches result primarily from the CH_2 groups in fats but with a considerable contribution from C–H bonds in carbohydrates and proteins. The peak at 2170 nm may be a combination band originating

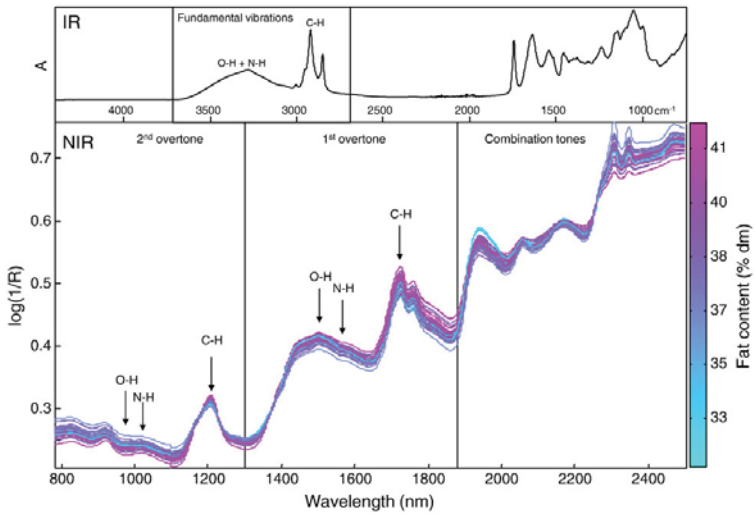

Fig. 1. Top: IR spectrum (4400–750 cm^{-1}) of marama bean. The fundamental C–H, N–H and O–H stretching vibrations region is highlighted. Bottom: MSC corrected NIR spectra (780–2498 nm) of duplicate measurements of 54 marama bean samples, the 1st overtone region is highlighted. The spectra are colored according to the fat content (% db) as indicated on the color bar.

from amide (Bruun, Holm, Hansen, & Jacobsen, 2006) and/or olefinic HC=CH groups from the high amount of protein and unsaturated fatty acids in the beans. The remaining peaks in the region from approx. 2000–2200 nm are N–H combination bands from protein which have 1st and 2nd overtones at approx. 1600 nm and just above 1000 nm, respectively. The peak at 1940 nm is an O–H stretch/O–H deformation combination band primarily from the carbohydrates. From the NIR spectra it can be concluded that marama beans are characterized by a low water content (low absorption at 970, 1450 and 1940 nm) and major contents of the three other bulk components of food: protein, fat and carbohydrate. Mainly bulk components can be investigated by NIR whereas changes in minor components (e.g. a particular amino acid) are difficult to detect. Visually, the spectra display a similar magnitude and curvature, however by coloring the spectra according to fat content (% db) in the batches, differences can be observed.

In order to investigate the main variation amongst the samples principal component analysis (PCA) was performed on the entire spectral data set resulting in the score plot in Fig. 2.

From Fig. 2 it can be observed that the instrumental error of the NIR analysis is very small since the two replicates of each bean are positioned almost on top of each other. It was not possible from the scores to see groupings according to country of origin or harvest year (this was also confirmed using supervised classification methods such as partial least squares discriminant analysis (PLS-DA) (Barker & Rayens, 2003) and extended canonical variates analysis (ECVA) (Nørgaard, Bro, Westad, & Engelsen, 2006)). This can be attributed to a large bean-to-bean variation in each batch that is similar in magnitude to the overall batch-to-batch variation. Accordingly, it was not possible to distinguish between the three *T. fassoglense* samples (SA0701, SA0702 and SA0707) and the *T. esculentum* samples from South Africa (SA0703, SA0705 and SA0706) which is confirmed by the similarity in chemical composition between the two species (Dubois et al., 1995; Holse et al., 2010). The results are also to some degree contradictory to the fact that the beans from South Africa have a higher content of protein and ash compared to beans harvested in Botswana and Namibia (Holse et al., 2010). However, the study by Holse et al. (2010) was performed on bulk samples consisting of flour from many beans from the same batch, which makes a direct comparison of the two studies difficult. Because of the large bean-to-bean variation it was not possible to find any quantitative relationship (results not shown) between the spectra and the different chemical components.

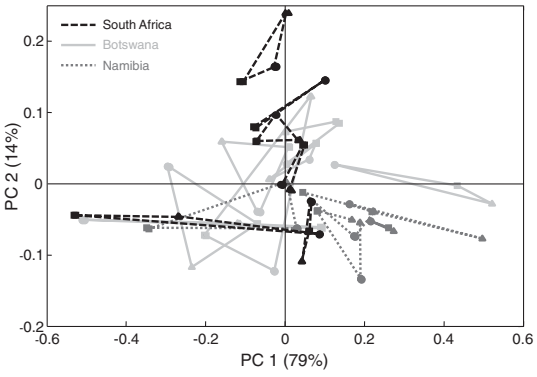

Fig. 2. Score plot from PCA on all MSC preprocessed NIR spectra (780–2498 nm) from the duplicate measurements of the 54 marama bean samples. Square, triangle and circle indicate sample a, b and c within each of the 18 batches (connected with lines). South Africa (black dashed lines), Botswana (light grey solid line) and Namibia (dark grey dotted line).

3.2. Describing chemical features in the marama bean

Despite a large within batch variation, the spectra for all applied spectroscopic methods contain relevant and descriptive chemical information that can be used in a complementary manner to wet-chemistry analyses (Holse et al., 2010). The spectroscopic analysis of the marama bean is presented in the following and provides valuable information on the qualitative chemistry of both major and minor chemical components.

A similar PCA procedure as above was carried out for the IR, Raman and NMR spectral data sets. Consistently, the spectral techniques revealed large variations between beans from the same batch, which is most likely due to the fact that the marama plant is a wild growing plant. Knowledge about this variation is valuable when sampling in future studies. This study will exploit the large bean-to-bean variation by investigating and discussing spectra of the two beans, regardless of harvest place and year, that span the largest variation as described by the first principal component of the PCA model.

In the subsequent sections, the fat, carbohydrate and protein composition of the marama bean will be investigated by combining the information obtained from the different spectroscopic techniques. Throughout the paper the focus will be on two types of chemical features; 1) bulk features that have already been evaluated by wet-chemical methods and 2) finer features such as carbohydrate composition and secondary protein structure. The investigated features are described for single beans, which has not been done in previous marama beans studies.

3.3. Fat composition

The fat of marama beans is present mainly as unsaturated fatty acids and the principal fatty acid is oleic acid (approx. 43%). The beans furthermore contain linoleic acid (approx. 22%) and palmitic acid (approx. 13%) as well as lower concentrations of a range of other fatty acids. In Table 2 a summary of results from the literature is given, describing the fatty acid composition of marama beans.

The top of Fig. 3 shows a ^{1}H HR-MAS NMR spectrum (0.6–8.0 ppm) of an aqueous suspension of marama bean acquired at 75°C. The ^{1}H HR-MAS NMR spectrum only shows the protons belonging to the mobile phase of the suspended marama bean. Therefore only hydrogens in lipids, small carbohydrates, amino acids or small peptides are observed. The spectrum is divided into three regions. In the R1 region (6.0–8.0 ppm) resonances from aromatic protons primarily from the amino acids are observed. The R2 region (3.0–6.0 ppm) contains resonances from anomeric and pyranosidic protons in carbohydrates, protons in unsaturated lipids and glycerol backbones as well as α/β protons in proteins or amino acids. The R3 region (0.6–3.0 ppm) contains resonances from aliphatic protons primarily from lipids but also from amino acids. This spectral region is enlarged in the bottom of Fig. 3, with the chemical structure of linoleic acid presented above the spectra. The different lipid protons are assigned by use of lower case letters and the chemical shift assignments are given in Table 3. Assignments of the resonances listed in Table 3 were obtained from 2D COSY, TOCSY and ^{13}C-HSQC spectra (not presented here).

The ^{1}H HR-MAS NMR spectrum gives a good overview of the importance of fat in the marama bean composition. A comparison between the two selected spectra reveals that they only differ to a small degree in fat composition. When scrutinizing the spectra it is revealed that the small peaks at around 2.2 ppm (f) and 2.8 ppm (d) are more pronounced in the sample from South Africa. Differences in the intensity of peak d indicate that the sample from South Africa contains more poly-unsaturated fatty acids than the sample from Namibia.

While ^{1}H HR-MAS NMR spectra provide a good description of the mobile part of the marama bean suspension, a more complete

Table 2
Fatty acid composition (% of total fatty acids) of marama bean. Summary of results from the literature. The major fatty acids in marama bean is in bold. The last column gives the average fatty acid composition (normalized to 100%).

Fatty acid		Engelter and Wehmeyer (1970)	Bousquet et al. (1981)	Bower et al. (1988)	Ketshajwang et al. (1998)	Francis and Campbell (2003)	Mitei et al. (2008)	Average
Myristic	(14:0)	Trace		1.3 ± 0.3	0.13			1.7
Palmitic	**(16:0)**	**14.1 ± 1.3**	**16.9**	**13.8 ± 5.0**	**12.09**	**12.8**	**12.93 ± 0.06**	**13**
Margaric	(17:0)				0.09			0.1
Stearic	(18:0)	6.5 ± 0.3	10.0	9.7 ± 7.0	6.75	7.3	8.82 ± 0.12	7.7
Arachidic	(20:0)	3.3 ± 0.4	3.4	2.8 ± 1.3	2.76		3.31 ± 0.03	2.9
Behenic acid	(22:0)						1.03 ± 0.02	1.0
Palmitoleic	(16:1)	0.7 ± 0.1	1.8	1.7 ± 0.3	0.38			1.1
Oleic	**(18:1)**	**47.9 ± 0.9**	**34.8**	**48.5 ± 8.0**	**47.61 (n − 9) + 1.67 (n − 7)**	**49.0**	**47.27 ± 0.43**	**43**
Gadoleic	(20:1)	1.0 ± 0.2			0.57		0.61 ± 0.00	0.7
Erucic	(22:1)	1.8 ± 0.2					2.63 ± 0.01	2.1
Linoleic	**(18:2)**	**24.6 ± 0.4**	**26.3**	**19.2 ± 9.5**	**26.43**	**23.5**	**23.40 ± 0.42**	**22**
(no common name)	(16:2)	Trace						
α- and γ-Linolenic	(18:3)		2.3	2.0 ± 1.5		2.7		2.2
Arachidonic	(20:4)		2.1					2.0
Others	–			1.2 ± 1.0	1.54			1.3

description of the intact marama bean can be obtained from solid-state ^{13}C MAS NMR spectroscopy. By applying the ^{13}C single-pulse SP/MAS experiment all carbon signals are detected quantitatively correct, whereas the resonances originating from the immobile regions are enhanced using the cross-polarization CP/MAS experiment due to transfer of polarization from the nearby protons. The solid-state ^{13}C NMR spectra of the marama bean are presented in Fig. 4A and B, respectively. From Fig. 4A it is readily observed that the narrow resonances from the highly mobile carbons present in the lipids are by far the most intense in the SP/MAS spectrum whereas their presence is insignificant in the CP/MAS spectrum (Fig. 4B), in which the intensity of carbons located in the immobile regions of the sample are enhanced.

By a linear combination of the SP/MAS and CP/MAS spectra (0.75A–0.05B) a difference spectrum can be obtained (Fig. 4C), leaving only the resonances from the lipids. The horizontal baseline and the exclusive presence of lipid resonances in this spectrum indicates that the polysaccharides and protein fractions can be evaluated quantitatively correct based on the CP/MAS spectrum (Fig. 4B). Based on the spectrum in Fig. 4C and the assignments in Table 3, the ratio between the lipid-chain carbons (CH_2-methylene) and the CH_3-carbons can be determined to 16.1 meaning that the

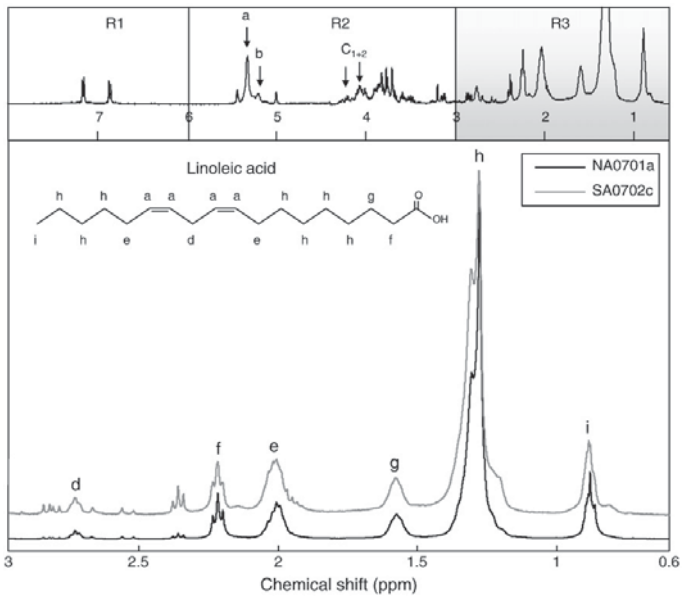

Fig. 3. Top: ^{1}H HR-MAS NMR spectrum (0.6–8.0 ppm) of an aqueous suspension of marama bean. The spectral regions R1, R2, and R3 are indicated on the figure. Bottom: Enlargement of the R3 region (0.6–3.0 ppm). The chemical structure of linoleic acid is given in the figure and the corresponding protons are assigned in the spectrum. The assignments are furthermore given in Table 3. The shown spectra are the two most different samples based on scores in the first principal component of a PCA model on only the marked spectral region. The spectra have been normalized to the fat peak at 1.3 ppm.

Table 3
Assignments of ^{1}H and ^{13}C chemical shifts observed in the NMR spectra of marama bean from 2D COSY, TOCSY and ^{13}C-HSQC spectra at 75 °C. Chemical shifts are referenced to internal TSP-d4. Note that the ^{13}C chemical shifts are 2 ppm higher than those observed in the ^{13}C CP/MAS spectra acquired at 25 °C (Fan, 1996; Biological Magnetic Resonance Data Bank, 2010). For the fatty acids the assigned H and C are highlighted in bold.

Compound	δ_{1H} (ppm)	δ_{13C} (ppm)	Assignment	Assignment on Figures	Seen in figure no.
Glyceryl	5.199	71.85	–CH	b	Figs. 3, 4A and 6
	4.264	64.62	$–CH_2$	c_1	
	4.082	64.62	$–CH_2$	c_2	
Fatty acid(s)	5.314	132.4	C–C–C–**CH**=**CH**–C–C–C– or C–C–**CH**–C=C–C–C=**CH**–C–C-C	a_1	
	5.314	130.7	C–C–C–**CH**=C–C–**CH**=C–C–C–C	a_2	
	2.754	28.34	C=C–**CH_2**–C=C	d	Figs. 3 and 4A
	2.036	29.93	C=C–**CH_2**-saturated carbon	e	
	2.245	36.52	O=C–**CH_2**	f	
	1.591	27.51	O=C–**CH_2**–**CH_2**	g	
	1.316	32.34	–(**CH_2**)$_n$–	h_1	
	1.316	25.36	–**CH_2**–CH_3	h_2	
	1.316	34.65	–**CH_2**–CH_2–CH_3	h_3	
	0.889	16.59	**CH_3**	i	
		172.4	O–**CH**=O (observed in ^{13}C SP/MAS spectrum)	j	Fig. 4A
Raffinose(?)	5.426	95.09	C_1H_1 in glucose	A	Fig. 6
	3.570	73.97	C_2H_2 in glucose	B	
	3.764	75.52	C_3H_3 in glucose	C	
	3.935		H_4 in glucose	D	
	4.998		H_1 in galactose	E	
	3.856		H_2 in galactose	F	
	4.208		H_3 in fructose	G	
	4.060		H_4 in fructose	H	
	3.894		H_5 in fructose	I	
	3.817		H_6 in fructose	J	
Choline	3.193	56.72	$–CH_3$	"Choline"	
Tyrosine (Y)	7.151	133.5	$–C_\delta H_\delta$	Star	Fig. 8
	6.853	118.2	$–C_\varepsilon H_\varepsilon$	Triangle	
Amino acids		156.9	C_ζ in either Arg or Tyr (observed in ^{13}C CP/MAS spectrum)	II	Fig. 4B
Protein/polysaccharides		173.4	Carbonyl groups in the protein amide bonds AND esters and acid groups in the polysaccharides (~1:1 ratio) (observed in ^{13}C CP/MAS spectrum)	I	

average chain length of the fatty acids is 18.1 carbons. The average fatty acid composition given in Table 2, gives an average chain length of 17.7 in good agreement with the NMR results. The ratio of unsaturated to carbonyl and saturated carbons calculated from Fig. 4C is 1:8.5, which is in fair agreement with the expected ratio of 1:7.3 from the lipid composition mentioned above. In addition, the ratio

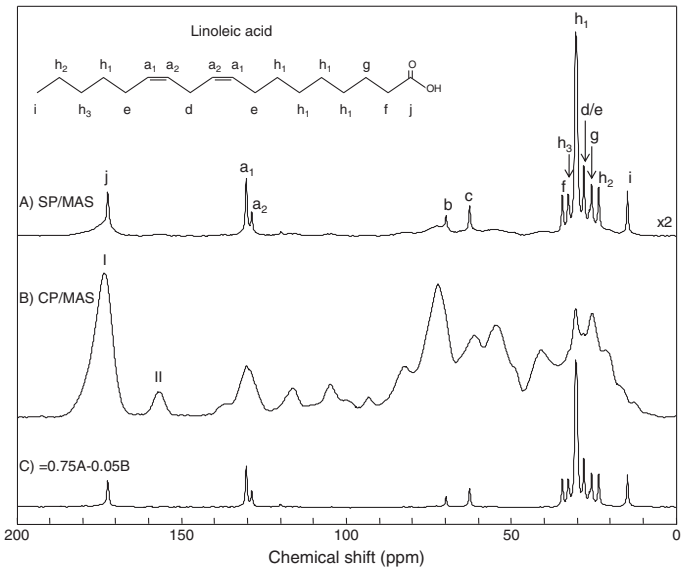

Fig. 4. A) ^{13}C CP/MAS NMR and B) ^{13}C SP/MAS NMR spectra (0–200 ppm) of marama bean (BO0603). A) is vertically scaled by a factor of 2. Chemical shifts are assigned in the figure (lipid carbons: lower case letters, I: carbonyl carbon in either esters, carboxylic acids or amide bonds, and II: C_ζ in either Arg or Tyr) and the corresponding assignments are given in Table 3. C) linear combination of the SP/MAS and the CP/MAS spectra (0.75A–0.05B).

between the integrals of the resonances of the two unsaturated carbons (a_1 and a_2) is 2.4:1 close to the expected ratio of 2.3:1. When combined, the three different NMR techniques provide a complete picture of the average of the functional groups of the fatty acids in marama beans. However, no individual fatty acids can be assigned from the NMR spectra of such a complex matrix.

Fig. 5 shows the Raman spectrum (top) and an enlargement of the carbonyl and fingerprint region (bottom). Spectra of linoleic acid and marama bean oil are superimposed on the two marama bean spectra.

In addition to the information gained from the different NMR experiments, the unusually sharp and intense FT-Raman spectrum of the marama bean provides good quantitative information on the lipid composition (Engelsen, 1997). Especially non-polar functional groups (such as C=C) have intensive bands in Raman spectra in contrast to IR which gives strong signals for polar groups (e.g. O–H and C–H). The marama bean Raman spectra (solid lines) display a rather week carbonyl band at 1745 cm^{-1} due to the C=O stretching vibrations from the ester bonds in the triglycerides. Obviously the carbonyl peak is absent in the pure linoleic acid spectrum (grey dashed line). The peak at 1655 cm^{-1} is the cis C=C stretching vibrations from the unsaturated fatty acids, which dominate the fatty acid composition of the bean (about 75% of the total lipid content). This peak is very sharp, especially for the marama oil (dotted black line) and the linoleic acid. However, when comparing the two selected samples it appears that they differ in their degree of unsaturation even though they are harvested in the same country during the same harvest year.

The large broad peak at 1442 cm^{-1} arises from CH_2 bending vibrations. The intensity ratio of the two bands I_{1655}/I_{1442} have been shown to correlate linearly with the iodine value and is thus a good indicator for estimating the unsaturation level of lipid containing foods (Ozaki, Cho, Ikegaya, Muraishi, & Kawauchi, 1992). The ratio shows as expected that linoleic acid (C18:2) is more unsaturated than the marama oil, approx. by a factor 2 (Table 2). The degree of unsaturation may also be evaluated by the ratio of the area of the C=C stretching band to the area of the C=O stretching band instead of the CH_2-band (Li-Chan, 1996). The two bands at 1265 and 1301 cm^{-1} are due to =CH in-plane deformations and $(CH_2)_n$ wagging and twisting, respectively. The relative intensity of these bands also changes according to the degree of unsaturation of the lipids (Li-Chan, 1996). Comparison of the two extreme marama bean samples reveal only minor differences in the degree of unsaturation with the SA0703b sample having a slightly higher degree of unsaturation than the SA0706b sample.

3.4. Carbohydrate composition

The composition of the dietary fibers that make up 19–27% of the dry weight of the marama bean (Holse et al., 2010) has not yet been investigated. The aim of the present study was to provide an overall description of the carbohydrate fraction of the marama bean using vibrational and NMR spectroscopy.

NMR is an excellent method for exploring the carbohydrate composition in aqueous solution but only a minor fraction of the carbohydrates in marama bean is soluble. This is why only approx. 4% of the total dietary fibers (Holse et al., 2010) will be detectable in the ^{1}H HR-MAS NMR spectrum. In order to study the non-soluble polysaccharides quantitatively correct it is necessary to perform ^{13}C SP/MAS NMR.

In the top of Fig. 6, the ^{1}H HR-MAS NMR spectrum is shown and the region containing the signals from carbohydrate protons (R2: 3.0–6.0 ppm) is highlighted. In the bottom of Fig. 6, the carbohydrate region is enlarged and assignments are given. It is a rather simple spectrum in the anomeric region and only two resonances are tentatively assigned to raffinose (Table 3). Raffinose (O-α-d-galactopyranosyl-(1→6)-O-α-d-glucopyranosyl-(1→2)-β-d-fructofuranoside) is found in a large variety of seeds, beans and vegetables. The peak at 5.426 ppm (A) originates from the H_1 in the α-glucose unit, the peak at 4.998 ppm (E) originates from H_1 in the α-galactose unit and the peak

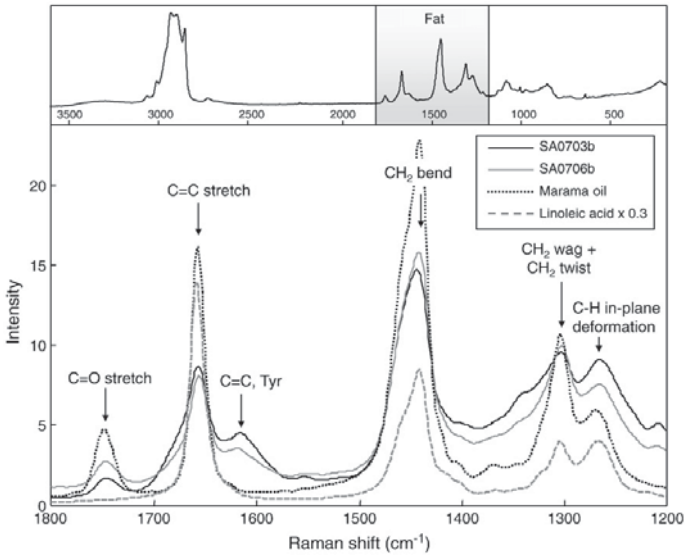

Fig. 5. Top: Raman spectrum (3600–200 cm^{-1}) of marama bean, the lipid region is highlighted. Bottom: Raman spectrum (1800–1200 cm^{-1}) of marama bean sample SA0703b (black solid line) and SA0706b (grey solid line), marama oil (black dotted line) and linoleic acid (grey dashed line, vertically scaled by a factor of 0.3), all acquired at 64 scans, 8 cm^{-1}, 500 mW. Assignments are given on the figure. The shown spectra are the two most different samples based on scores in the first principal component of a PCA model on only the marked spectral region (MSC preprocessing).

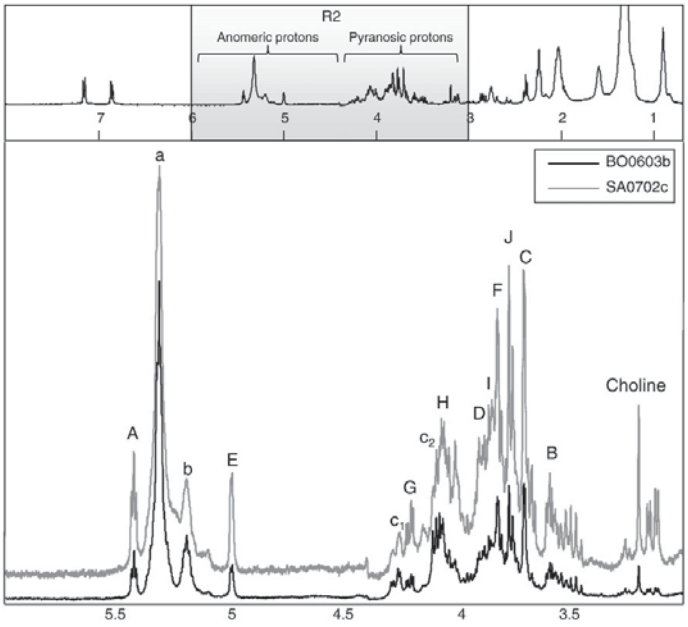

Fig. 6. Top: ^{1}H HR-MAS NMR spectrum (0.6–8.0 ppm) of marama bean. The R2 region is highlighted and the regions of the anomer protons and the pyranose ring protons in carbohydrates are shown. Bottom: Enlargement of the carbohydrate region (3.0–6.0 ppm). The chemical shifts of the carbohydrate protons are assigned in the spectrum (capital letters) along with protons from choline and lipid (lower case letters). The assignments are furthermore given in Table 3. The shown spectra are the two most different samples based on scores in the first principal component of a PCA model on only the marked spectral region. The spectra have been normalized to the fat peak at 1.3 ppm.

at 4.208 ppm (G) is from the H_3 in the fructofuranose unit. In the region where resonances from the pyranosic carbohydrate protons are found (3–4.5 ppm) a comprehensive assignment is hampered by the many overlapping resonances.

When comparing the two spectra from the samples from Botswana and South Africa it appears that the sample from South Africa has a higher soluble carbohydrate/fat ratio since the spectra are normalized according to the methylene peak at 1.3 ppm. Additionally, there are small but noteworthy differences in the pattern of pyranosidic carbohydrate protons.

Inspection of the CP/MAS spectrum (Fig. 4B) reveals a range of broad resonances due to overlapping resonances from a complex mix of soluble as well as insoluble polysaccharides and proteins. Some functional groups may be identified. First of all, the resonance at 173.4 ppm (marked I in Fig. 4B) originates from carbonyl groups either in the amide bonds of the proteins or from esters or acid groups in the polysaccharides. The resonance at 156.9 ppm (marked II) originates from C_ζ in the amino acids Arg or Tyr. The ratio of the integrals of the two resonances is 92:8. From previous studies Arg and Tyr are known to constitute 17–20% of the amino acids in marama bean (Bower et al., 1988; Dubois et al., 1995). Hence, if peak I only originated from protein, the integral of peak I should be 5–6 times bigger than that of peak II. However, it is observed that peak I is around 11.5 times larger than peak II and therefore it would appear that peak I contains a major contribution from ester and acid groups in the insoluble polysaccharides.

Due to extensively overlapping resonances in the spectral region containing the anomeric carbons in polysaccharides (~90–110 ppm) it is difficult to obtain an accurate integral of this region, but it contains approximately one third (33%) of the carbonyl resonances (peak I). This would require that the ratio between ester or acids and the anomeric carbons is around 3:2 indicating that a large part of the polysaccharides is present as pectins/galacturonic acids.

In addition to the assignments made above, the NMR spectra can be used to exclude the presence of certain carbohydrates in marama beans. It is for example characteristic that the marama bean does not contain measurable amounts of abundant plant carbohydrate compounds such as β-glucans (no anomeric signal in the β-anomeric region 4.45–4.85 ppm), glucose (no anomeric signals at 4.64 (β) and 5.23 (α) ppm) and starch (no anomeric α-(1→4) starch proton at 5.36 ppm) (Seefeldt, Larsen, Viereck, Wollenweber, & Engelsen, 2008). The absence of starch which was confirmed in the wet-chemistry study by Holse et al. (2010) is in contrast to other legumes where starch is the most abundant carbohydrate (22–45%) (Hoover & Sosulski, 1991). Combined this composition makes the marama bean a unique legume with a high dietary fiber content and no starch.

Fig. 7 displays the fingerprint region of the marama bean Raman spectrum which contains signals from carbohydrates, the glycerol backbone of fats and aromatic compounds. In order to search for information from other sources than lipid, a marama oil spectrum is shown for comparison. The characteristic anomeric specific band at 854 cm^{-1} originates from alpha-anomeric carbohydrates and is consistent with the predominance of pectins as reported by NMR. Beta-anomeric forms should have the corresponding peak near 890 cm^{-1} (Thygesen, Løkke, Micklander, & Engelsen, 2003), which is overlapped with signals from the fats and presumably absent. When comparing the two selected spectra, it appears that they differ in the content of alpha-anomeric carbohydrates despite being from the same harvest year and place and that they are similar with respect to regions containing peaks arising from fat.

3.5. Protein composition

The average amino acid composition of marama bean proteins is given in Table 4 and it is noteworthy that marama beans are especially

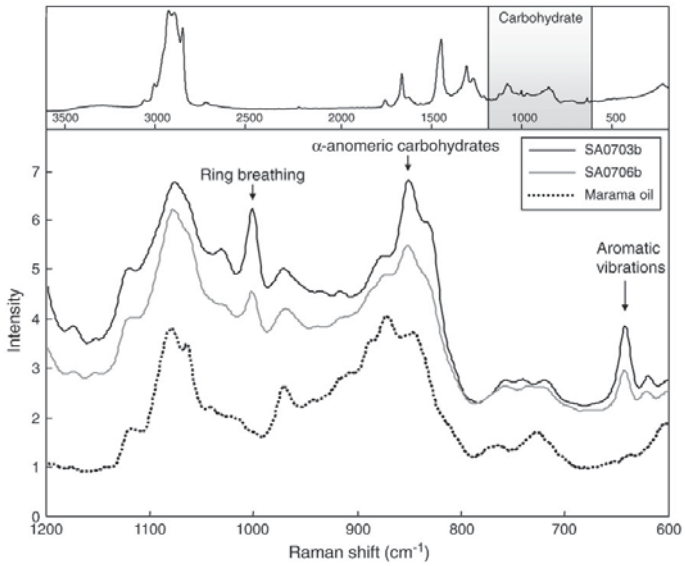

Fig. 7. Top: Raman spectrum (200–3600 cm^{-1}) of marama bean, the carbohydrate region is highlighted. Bottom: Raman spectrum (600–1200 cm^{-1}) of marama bean sample SA0703b (black solid line), SA0706b (grey solid line) and marama oil (black dotted line) acquired at 64 scans, 8 cm^{-1}, 500 mW. Assignments are given on the figure. The shown spectra are the two most different samples based on scores in the first principal component of a PCA model on only the marked spectral region (MSC preprocessing).

high in glutamic acid, tyrosine and aspartic acid and low in cysteine and methionine (Bower et al., 1988; Dubois et al., 1995).

The Raman spectrum of marama bean (Fig. 7) reveals protein characteristic peaks at 1003 cm^{-1}, 643 cm^{-1} and 621 cm^{-1} due to ring breathing and other aromatic vibrations from the aromatic amino acids (De Gelder, De Gussem, Vandenabeele, & Moens, 2007; Li-Chan, 1996). In addition, the peak at 1613 cm^{-1} (Fig. 5) originates from C=C stretches in aromatic amino acids. This is in good agreement with the fact that these make up approx. 21% of the amino acids in marama beans (tyrosine, phenylalanine, histidine and tryptophan, Table 4). The two presented spectra differ markedly in intensity of the aromatic vibrations despite being from the same harvest year and place which highlights the large bean variation in the sample set.

Table 4
Amino acid composition (% of total amino acids, normalized to 100%) of marama bean. Summary of results from the literature. Amino acids present in the highest amounts in marama beans are highlighted in bold.

Amino acid	Bower et al. (1988)	Dubois et al. (1995)[a]
Essential		
Arginine	6.3	7.8
Cysteine	0.8	1.0
Histidine	2.4	2.8
Isoleucine	4.0	3.3
Leucine	5.9	5.8
Lysine	5.5	5.5
Methionine	0.8	0.3
Phenylalanine	4.8	4.2
Threonine	3.0	3.3
Tyrosine	**11.6**	**11.9**
Tryptophan	1.7	ND
Valine	4.4	3.9
Nonessential		
Alanine	3.1	3.1
Aspartic acid	**10.8**	**10.5**
Glutamic acid	**15.6**	**17.7**
Glycine	5.7	6.0
Proline	6.9	7.6
Serine	5.3	5.4
Ammonia	1.3	ND

[a] T. *fassoglense*.

Information about amino acid and protein protons is found throughout the entire ^{1}H HR-MAS NMR spectrum. Bower et al. (1988) found that globulins are the most abundant (53%) proteins in marama beans, followed by albumins (23.3%), prolamins (15.5%), alkali soluble gluteins (7.7%) and acid soluble gluteins (0.5%). Of these classes albumins is the only water soluble class of the proteins and hence it is only expected to detect signals from around ¼ of the proteins. In the aromatic region (R1), shown in Fig. 8, only two doublets arising from tyrosine at 7.151 ppm and 6.853 ppm are found indicating that tyrosine is the only and dominant source of aromatic signals. The reason why we do not see trace amounts of the other aromatic amino acids present in marama beans is because they are bound in a form that is not soluble and therefore does not show up in the spectrum of the mobile phase.

The two selected spectra show large differences in the intensity of the tyrosine peaks. Since the spectra are normalized to the methylene peak (1.3 ppm) the tyrosine content in the mobile phase is much lower for the South African bean sample.

In the R2 region of the ^{1}H HR-MAS NMR spectrum α and β protons from amino acids may be seen, however, due to the dominance of the carbohydrate peaks it is not possible to assign any protons from amino acids unambiguously.

Resonances from amino acids are also observed in the CP/MAS spectrum (Fig. 4B), where a mix of resonances from the aromatic amino acids is detected in the region from 110–165 ppm. Additionally, the aliphatic side chains in amino acids give rise to signals in the region 5–50 ppm. C_α and C_β can even overlap with the carbohydrate region, as was also seen for the α and β protons in the ^{1}H HR-MAS NMR spectrum. The overlapping peaks cannot be assigned specifically.

In order to gain information on the secondary structure of the proteins in the marama bean, the FT-IR spectral region containing the amid I band (1660 cm^{-1}), arising from the stretching vibrations of

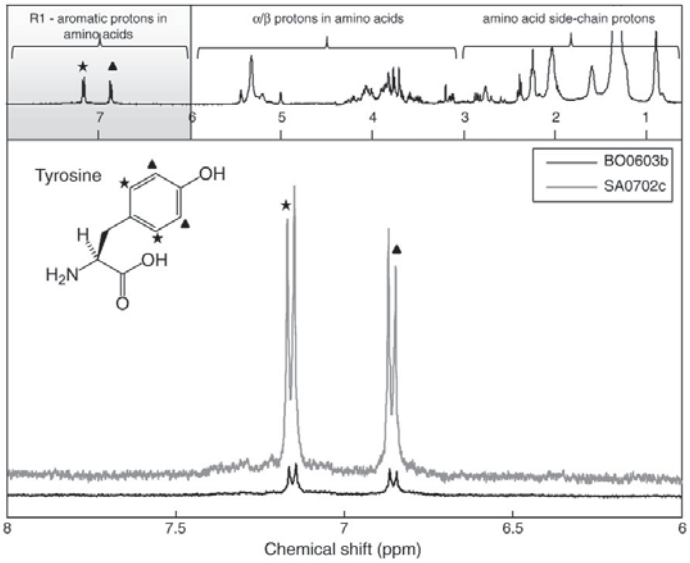

Fig. 8. Top: ^{1}H HR-MAS NMR spectrum (0.6–8.0 ppm) of marama bean. The region containing aromatic protons from amino acids (R1) is highlighted, furthermore, the regions where α/β protons in amino acids and amino acid side-chain protons is found is indicated. Bottom: The aromatic region (R2: 6.0–8.0 ppm) of the ^{1}H HR-MAS NMR spectrum of marama bean. Assignments are given as stars and triangles on the figure and in Table 3. The shown spectra are the two most different samples based on scores in the first principal component of a PCA model on only the marked spectral region. The spectra have been normalized to the fat peak at 1.3 ppm.

C=O in the peptide bonds is examined. The vibration energies of the carboxyl group depend on the different conformations of the protein, such as β-sheet and α-helix structures, β- and α-turns, and inter- or intra-molecular aggregates. Calculating the second derivatives of the spectra (Rinnan et al., 2009) makes it possible to assign the spectral components of the amide I band, as shown in Fig. 9. It appears that the secondary structure of the marama proteins is mainly α-helixes and β-sheets, and to a much lesser extent β-turns (Susi, Timashef, &

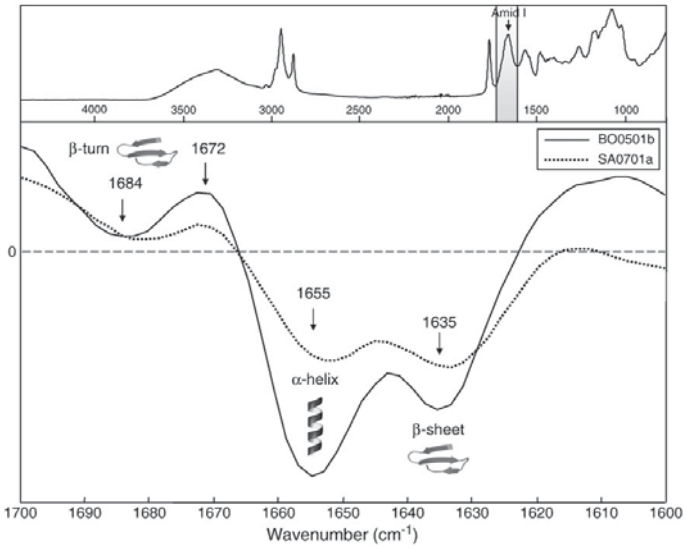

Fig. 9. Top: IR spectrum (4400–750 cm^{-1}) of marama bean, the Amid I band is indicated on the figure. Bottom: Smoothed second derivative of the IR spectrum (1700–1600 cm^{-1}) – a valley in the second derivative represents a peak in the raw spectrum. Assignments of the secondary protein structure are given on the figure. The shown spectra are the two most different samples based on scores in the first principal component of a PCA model on only the marked spectral region (MSC preprocessing).

Stevens, 1967; Dong, Huang, & Caughey, 1990). Random coil conformations are usually associated with the IR band between 1640 and 1648 cm^{-1} (Krimm & Bandekar, 1986). Hence, the marama bean protein is highly structured. The two samples show clear differences in the secondary protein structure. The SA0701a has an almost similar content of α-helixes and β-sheets, while the BO0501b sample appears to be more structured (sharper peaks) and to contain larger quantities of α-helixes than β-sheets.

4. Conclusions

This study has shown how the chemical composition of a chemically intact food matrix such as the marama bean can be studied qualitatively and semi-quantitatively by the combined use of vibrational spectroscopy (NIR, IR and Raman) and NMR spectroscopy.

Combined use of Raman, ^{13}C SP/MAS and ^{1}H HR-MAS NMR made it possible to assign all protons and carbons in the marama bean lipids. By use of the spectrum resulting from a linear combination of ^{13}C SP/MAS and CP/MAS spectra it was possible to calculate the degree of unsaturation and the average fatty acid chain length in agreement with the wet-chemical analysis. The marama tissue resulted in exceptionally well-resolved and intense Raman spectra and even minor differences in the degree of unsaturation could be evaluated.

The carbohydrate composition on the other hand was much more difficult to elucidate. Results from solid-state NMR suggested a high degree of esterification in the polysaccharides and by ^{1}H HR-MAS NMR the major presence of raffinose among the water soluble carbohydrates was suggested. However, no specific carbohydrate composition was obtained but perhaps more importantly the absence of bulk carbohydrates such as β-glucans and starch was demonstrated. This result was confirmed by Raman spectroscopy. Furthermore, Raman spectroscopy showed the presence of α-anomeric carbohydrates. In combination with the NMR results this indicates that a large part of the pectins are α-linked.

From the NMR spectra, tyrosine was identified as the only aromatic amino acid in the mobile phase and the Raman spectra supported the strong presence of phenylic aromatic amino acids. In addition, the CP/MAS NMR spectra revealed the presences of other aromatic amino acids than only tyrosine. IR was useful in determining the secondary structure of the proteins of the marama bean, which has not previously been done. This showed that the protein structure was highly structured, dominated by α-helixes and β-sheets, and that relative variations in the secondary structures exist.

The spectral information was furthermore used to characterize the compositional variations in marama beans harvested in the same geographical area at the same time. However, the bean-to-bean variation within each batch turned out to be as large as the overall variation between the batches.

All in all, this spectroscopic study has given a very comprehensive chemical description of the marama bean and introduced how these tools can extract detailed chemical information from single beans in a fast and holistic manner. This study has paved the way for subsequent quantitative analysis of micro and macro components in the marama bean and suggests that the important sampling issue should be taken into account.

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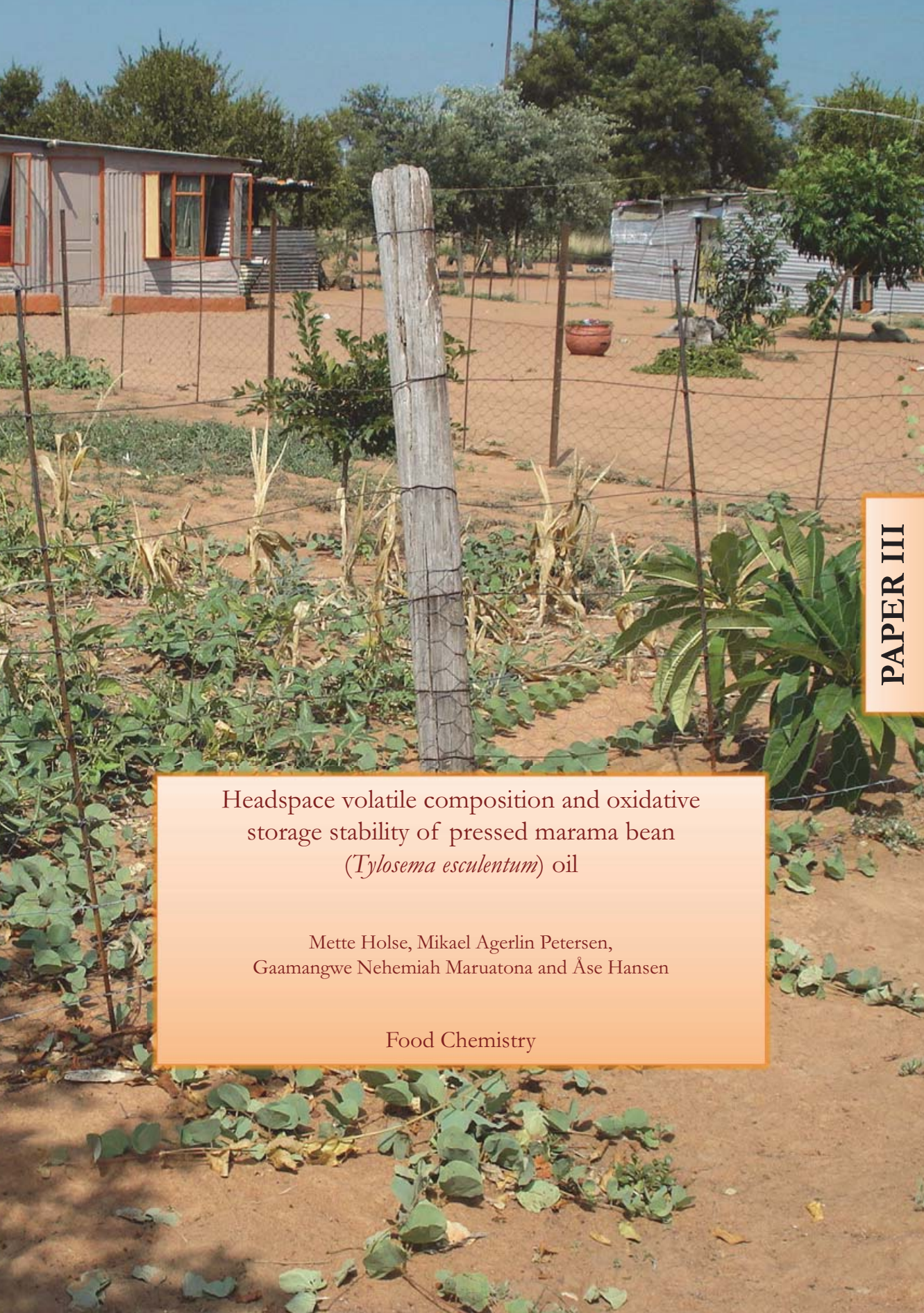

PAPER III

Headspace volatile composition and oxidative storage stability of pressed marama bean (*Tylosema esculentum*) oil

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ABSTRACT

Marama bean (*Tylosema esculentum*) is an underutilised legume indigenous to the Kalahari Desert region of southern Africa. The bean has high lipid content and hence is a potential source for production of edible oil. The headspace volatile composition of freshly pressed marama bean oil was explored and the oil was further studied during 7 months of storage under different light and temperature conditions. The oxidative stability of the oil was examined by measuring peroxide value, vitamin E content and FT-IR spectra. Additionally, the headspace volatile composition of the oil was investigated during storage by use of dynamic headspace GC–MS. The results showed that marama oil is highly stable and has good natural antioxidant properties; enzymatic lipid oxidation does not take place in marama oil. Light has a greater effect on the lipid oxidation than has temperature, and hence marama oil should preferably be stored in darkness and rather at 25 °C than 35 °C. Under these conditions, the marama oil has a shelf life of at least 7 months.

1. Introduction

The marama (also called morama) bean (*Tylosema esculentum*) is a wild, perennial, tuberous legume, indigenous to the Kalahari Desert and neighbouring areas, with poor arid soils, in Botswana, Namibia and the northern part of South Africa (Bousquet, 1981). The plant produces very hard pods with large (2–3 g) dark brown edible seeds. The seed is part of the traditional food eaten by some of the indigenous populations in southern Africa, but is otherwise underutilised (van der Maesen, 2006).

The marama bean is a good source of food energy with a lipid content of 32–42% on a dry matter basis (% db) (Holse, Husted, & Hansen, 2010). The high content of lipids in marama beans is comparable to the content found in seeds used for production of commercial vegetable oils, such as sunflower seeds (22–36%) and rapeseeds (22–49%) and closely approaches that of peanuts (36–54%) (Salunkhe & Kadam, 1989). Furthermore, the content of lipids is twice that of soybeans (17–20% db) (Street & Öpik, 1975), which is the legume that compares best with the high protein content of the marama beans (29–38% db) (Holse et al., 2010). This makes the marama bean a potential source for production of pressed oil. An increase of interest in pressed oil (rather than extracted oil) has arisen as many aroma components disappear during the extraction procedure. A further advantage of pressed marama oil is that it may easily be produced in villages in southern Africa by use of simple technology.

An important issue, when producing a new food product, is the stability of the product during storage. Here a range of reactions will take place, deteriorating the quality of the product (functional and sensory changes, as well as decreasing nutritional quality and food safety), e.g. microbial spoilage, lipid and protein oxidation.

The lipid of marama beans is highly unsaturated (app. 75%), the principal unsaturated fatty acid being oleic acid (43%). The beans, furthermore, contain the unsaturated fatty acids linoleic (22%) and linolenic acid (2.2%) as well as erucic (2.1%), arachidonic (2.0%), palmitoleic (1.1%) and gadoleic (0.7%) in lower concentrations (Bower, Hertel, Storey, & Oh, 1988; Engelter & Wehmeyer, 1970; Francis & Campbell, 2003; Ketshajwang, Holmback, & Yeboah, 1998; Mitei, Ngila, Yeboah, Wessjohann, & Schmidt, 2008). With respect to oxidation of foods the most important fatty acids are oleic, linoleic and linolenic (Belitz, Grosch, & Schieberle, 2004). Hence, the lipid composition of the marama bean makes it susceptible to oxidative deterioration. The level of lipid oxidation

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is therefore a critical quality control parameter of marama bean products during storage.

Oxidation of unsaturated lipids may proceed by three different pathways (autoxidation, photosensitized oxidation and enzymatic oxidation), which are triggered by free radicals, light plus a photosensitizer and lipoxygenase enzymes, respectively. Lipoxygenase enzymes are present in many plants similar to the marama bean, such as peanut and soybean (Belitz et al., 2004). Through all three pathways, unsaturated fatty acids are turned into hydroperoxides (primary oxidation products). These labile compounds further decompose to produce a complex mixture of volatile compounds (secondary oxidation products), which are mostly low molecular weight aldehydes, ketones, alcohols and short-chain hydrocarbons. Hydroperoxides are generally flavourless and odourless; however, their decomposition products have a great impact on flavour and odour. Hence, the quality of edible oils does not deteriorate until the volatile secondary oxidation products are formed (Choe & Min, 2006; Frankel, 1980, 1987).

The rate of oxidation of oils is influenced by various internal factors, such as fatty acid composition and the presence and activity of pro- and antioxidants. Marama oil contains tocopherols which are natural antioxidants (Mitei, Ngila, Yeboah, Wessjohann, & Schmidt, 2009). Holse et al. (2010) found that marama beans have a relatively high content of vitamin E (44 mg α-TE), higher than various cereal grains, legumes, vegetables and fruits (1–23 mg α-TE). In addition to the internal factors that may affect the rate of lipid oxidation, various extrinsic factors, such as oxygen concentration, light, temperature and relative humidity all influence lipid oxidation. Until dehulling of the bean, the seed is protected by the hull, preventing oxidative deterioration. During dehulling and pressing into oil, the seeds are affected by mechanical injury and exposed to light and oxygen, which initiates lipid oxidation.

The aim of this study was to examine the headspace volatile composition of pressed marama oil and to evaluate the oxidative stability of the oil during storage under different light and temperature conditions.

The oil was evaluated through examination of primary oxidation products, by determination of peroxide value (PV) and examination of the secondary oxidation products, by use of dynamic headspace gas chromatography mass spectrometry (DHS-GC–MS). Furthermore, the content of the antioxidant, vitamin E, was determined using high-performance liquid chromatography (HPLC). In addition, Fourier transform infrared spectroscopy (FT-IR) was applied for evaluation of the overall changes taking place during storage. Furthermore, the activity of lipoxygenase isozymes in raw marama beans was examined, in order to know whether enzymatic lipid oxidation would be a potential problem in marama oil during storage.

2. Materials and methods

2.1. Materials

Cold-pressed marama oil was produced from marama beans harvested in Botswana in 2006. The oil was prepared at the University of Botswana, in 2008, by use of a mechanical press and stored in brown bottles at 5 °C until analysed at the University of Copenhagen, carried out within 1 week.

2.2. Analysis of volatiles

2.2.1. General

DHS-GC–MS was used to measure the headspace volatile profile of the marama oil before and during storage and thereby to examine the volatile composition and the changes of this due to lipid oxidation. DHS-GC–MS is a technique that involves the collection, separation, detection and identification of volatile organic compounds, i.e. compounds that exists in the vapour phase and is a widespread technique used in flavour analyses of food (Wampler, 2002).

2.2.2. Dynamic headspace GC–MS

Ten gram of marama oil were added, directly, to a 150 ml purge flask. The sample was equilibrated to 30 ± 1 °C in a circulating water bath and then purged with nitrogen (150 ml/min) for 60 min, while being stirred by a magnetic stirrer at 200 rpm. The volatile compounds were collected on a Tenax-TA trap (250 mg, mesh size 60/80, Buchem BV, Apeldoorn, The Netherlands), which was then sealed with caps and kept at 5 °C prior to GC–MS analysis. Duplicate measurements were carried out.

The trapped volatiles were desorbed using an automatic thermal desorption unit (ATD 400, PerkinElmer, Norwalk, USA). Primary desorption was carried out by heating the trap to 250 °C with a flow (60 ml/min) of carrier gas (He) for 15.0 min. The stripped volatiles were re-trapped in a Tenax-TA cold trap (30 mg, 5 °C), which was subsequently heated at 300 °C for 4 min (secondary desorption, outlet split 1:10). This allowed for rapid transfer of volatiles to the GC–MS through a heated (225 °C) transfer line.

Separation of volatile compounds was carried out on a gas chromatography system (G1800A GCD System, Hewlett–Packard, Palo Alto, CA, USA) consisting of a gas chromatograph, a mass selective detector, and a data system. The GC was equipped with a polar DB-Wax capillary column (J&W Scientific, 30 m long × 0.25 mm internal diameter, 0.25 μm film thickness). The column flow rate was 1.0 ml/min, using helium as carrier gas. The column temperature was kept at 45 °C for 10 min, increased at 6 °C/min to 240 °C, and finally kept isothermal for 10 min.

The GC was equipped with a mass spectrometric detector (Agilent 5973 Mass Selective Detector) operating in the electron ionisation (EI) mode at 70 eV. Mass-to-charge ratios between 15 and 300 were scanned. The MS transfer line was maintained at a temperature of 280 °C.

2.2.3. Analysis of GC–MS data

The GC–MS data were analysed using the software MSD ChemStation G1701EA (Version E.01.00.237, Agilent Technologies Inc., Palo Alto, CA, USA). Volatile compounds were identified by matching their mass spectra with those of a commercial database (Wiley275.L, G1035A, Agilent Technologies, Inc.) and areas of the peaks in the total ion chromatogram (TIC) were obtained by integration. Additionally, Kováts retention indices (Kováts, 1958) of all identified volatiles were calculated as the retention time of the volatile normalised to the retention times of adjacently eluting *n*-alkanes.

In order to quantify the identified volatiles, multiple headspace extraction (MHE) was carried out. MHE is a stepwise headspace extraction for the quantitative analysis of volatiles in solid or complex liquid samples (Kolb & Pospisil, 1977). Duplicates of the oil sample, stored for 7 months in light at 25 °C, were prepared as previously described and used for the MHE experiment. Each sample was continuously purged with nitrogen (30 °C, nitrogen flow 150 ml/min) and seven consecutive dynamic headspace samplings (60 min duration) were made (after 0, 1, 2, 3, 4, 5 and 23 h). Analysis of the Tenax TA traps was performed as described previously.

The total amount of volatile analyte present in the sample was calculated from the estimated sum of all partial peak areas (A_i), obtained in the series of chromatograms, where i is the number of the extraction (Kolb & Pospisil, 1977):

$$\sum_{i=1}^{n} A_i = \frac{A_1}{1 - e^{-q'}}$$

The exponent q' describes the exponential decline of the peak areas during the stepwise MHE procedure, and is obtained from the linear regression analysis:

$$lnA_i = -q'(i-1) + lnA_1$$

where the q' value is equal to the slope of the linear regression line and ln A_1 is given by the y-intercept.

Finally, for conversion of the estimated sum of all partial peak areas into absolute amounts, five pure standard compounds were analysed; 10 μl of each compound (hexanal, octanal, pentan-1-ol, hexan-1-ol and 2-pentylfuran) were mixed with 10 ml heptane (1000 ppm). A standard series, with 500, 100 and 10 ppm, was made; 2 μl of each were injected directly into a Tenax-TA trap (in triplicate), and the Tenax-TA traps were analysed as described previously.

2.3. Packaging and storage conditions

The effects of the external factors, light and temperature, on the oxidative stability of pressed marama bean oil were investigated during 7 months of storage at 25 °C in light and darkness and at 35 °C in darkness. For each storage condition, two samples of each 40 g of marama oil were filled into 50 ml transparent bottles, and sealed with a lid. Samples that were to be stored in light at 25 °C were placed in a room without direct sunlight and with the electric light (Philips Master TL-D Super 80, 36W/830) turned on constantly. The samples that were to be stored in darkness at 25 °C were kept in the same room inside two black plastic bags. A temperature logger (Testo 174 mini temperature data logger) was used to monitor the temperature during the entire storage period. The oil samples that were to be stored in darkness at 35 °C were wrapped in aluminium foil and left in an oven (with an internal thermometer) set to 35 °C. These storage conditions were chosen in order to make the storage realistic in relation to what would be possible in an African village. Hence, the temperature range 25–35 °C was chosen, instead of e.g. 5 °C, which would require a refrigerator.

The oil samples were analysed at the time points 0, ½, 1, 3, 5 and 7 months. Two identical bottles were analysed at each day of analysis and, from each of these, two replicates were prepared for each analysis. This resulted in four replicates for each storage treatment on each day of analysis. In both the sample preparation step and the analytical step, the samples were randomized to minimise the introduction of systematic effects in data. The codes L25, L35 and D35 are used, with the numbers indicating the storage temperature and L = light and D = darkness. These codes are used in the results and discussion section.

2.4. Lipoxygenase isozyme activity

The presence of the lipoxygenase isozymes L-1 and L-2 was examined by the spectrophotometric method described by Suda, Hajika, Nishiba, Furuta, and Igita (1995). Clean whole marama beans were dehulled using a DF sample cracker (WMC Sheet Metal Works, Tzaneen, South Africa), whereafter they were ground with a chilled mortar and pestle to fine flour (80–100 mesh). Soybean flour (used as a standard) was prepared in a similar way but the beans were dehulled manually. For each sample, 0.5 g of flour was homogenised with 49 ml of ice-cooled deionised water at 9500 rpm with an Ultra-turrax T25 homogeniser (IKA-Labortechnik, Germany) and allowed to stand for 1 h at 4 °C. The homogenates were centrifuged in 15 ml centrifuge tubes (1000 rpm, 10 min, 4 °C) and the supernatant obtained was used as the extract for detection of lipoxygenase isozymes.

In screening for L-1 activity, a mixture was prepared containing 1.0 ml of 0.2 M sodium borate buffer (pH 9.0), 0.2 ml of 100 μM methylene blue, 0.2 ml of 10 mM sodium linoleate substrate, 0.2 ml of distiled water and 0.6 ml of soybean or marama bean flour extract. The absorbance at 660 nm was measured with a Lambda EZ150 UV/Vis Spectrophotometer (Perkin–Elmer Corporation, USA) at intervals of 10 s for 3 min at 23 °C. In screening for L-2 activity, the reaction mixture contained 0.8 ml of 0.2 M sodium phosphate buffer (pH 6.0), 0.2 ml of 100 μM methylene blue, 0.2 ml of 0.2 M dithiothreitol in 0.2 M sodium phosphate buffer (pH 6.0), 0.2 ml of acetone, 0.2 ml of 10 mM sodium linoleate substrate and 0.4 ml of soybean or marama bean extract. The absorbance at 660 nm was measured at intervals of 1 min for 10 min at 23 °C.

According to Toyosaki (1996), the methylene blue decolourising reactions involve hydroperoxides, especially 13C-OOH isomers (i.e. hydroperoxides with 13 carbons), that are formed during lipid peroxidation. The mechanism of methylene blue bleaching by lipoxygenase involves the specific abstraction of hydrogen from the hydroperoxide 13C-OOH isomer by methylene blue (MB), which is then reduced to MB-H, which is colourless.

2.5. Peroxide value

Measurements of PV and preparation of a standard curve were performed according to The International Dairy Federation method 74A:1991 (Shantha & Decker, 1994). The PV of the oil was calculated as milliequivalents of peroxides/kg lipid (meq/kg). All measurements were carried out in duplicate.

2.6. Tocopherol and tocotrienol contents by HPLC

The content of tocopherols and tocotrienols were determined, in duplicate, according to a modification of the method described by Nielsen and Hansen (2008). Using this procedure, 1 g of marama oil was transferred to a 30 ml brown bottle and dissolved in 3 ml of hexane. Prior to injection onto the HPLC column, the extract was filtered through a 0.2 μm filter.

The quantitative HPLC separation was performed using a Varian HPLC (Star 9012, Varian, Inc. Scientific Instruments, Walnut Creek, CA, US), equipped with a fluorescence detector (Varian, 9070, Varian, Inc. Scientific Instruments, Walnut Creek, CA, US). The detector was set to an excitation wavelength of 290 nm and an emission wavelength of 330 nm. The column used was a LiChrosorb Si 60 (125 × 4 mm i.d., particle size 5 μm, VWR International ApS, Rødovre, Denmark) normal-phase column, protected with a steel guard column (50 × 3 mm i.d., particle size 5 μm) filled with ChromGuard S (Bie & Berntsen, Copenhagen, Denmark). The temperature of the column oven was 25 °C. The mobile phase contained 94.6% of hexane, 3.6% of ethyl acetate and 1.8% of acetic acid and the flow rate of the mobile phase was set at 1.0 ml/min.

A calibration curve for all eight isomers (α, β, γ and δ-tocopherol standards >95% purity (VWR International ApS, Rødovre, Denmark) and α, β, γ and δ-tocotrienol standards >95% purity (Davos Life Science Pte., Ltd., The Helios, Singapore)) was prepared over a concentration range of 3.0–75.0 μg/ml and the tocopherols and tocotrienols were then identified by retention time and quantified by use of linear regression.

2.7. FT-IR spectroscopy

The Fourier transform infrared absorbance measurements were performed on an Arid-Zone MB100 FT-IR instrument (ABB Bomen, Quebec, Canada), using an attenuated total reflectance (ATR)

device with a triple-bounce diamond-crystal. IR spectra were recorded in the interval from 4400 to 550 cm^{-1}, using a spectral resolution of 4 cm^{-1}. The marama oil was positioned on the crystal surface with a pipette. Each spectrum represents the average of 32 scans ratioed against the background (64 scans) collected with the empty crystal and stored as absorbance spectra. Between the samples, the crystal was thoroughly cleaned with deionised water. Each sample was measured in duplicate.

2.8. Data analysis

During storage, sample 7L25B changed colour from bright yellow to colourless and was removed from the sample set. For the replicate samples, from months 0 and ½, a high uncertainty of the GC–MS data was observed and these data were removed from the subsequent data analysis.

For the GC–MS data, principal component analysis (PCA) was used as a descriptive method during the first step of the data exploration. PCA allows the main variability aspects of the dataset to be visualised. The main goals of this procedure are to find relationships between the different parameters (objects and variables) and to detect possible clusters within objects and/or variables. In addition, PCA was used to remove outliers in data. PCA was performed using LatentiX (v.2.00, Latent5, Copenhagen, Denmark).

For the IR data, a calibration model, partial least squares regression (PLS), was made, linking spectra to storage time. This was also performed in LatentiX.

3. Results and discussion

3.1. Headspace volatile profile of pressed marama oil

Through the DHS-GC–MS analysis 25 different volatile compounds were identified in the headspace of pressed marama oil samples. These volatiles were all present throughout the storage period, but in varying quantities. In Table 1 the volatiles are listed according to their chemical classes, along with their odour characteristics reported in the literature. The oil contained a high number of typical lipid oxidation products, e.g. short chain (up to C_8) alcohols, aldehydes and ketones. The alcohols have sweet spirituous odours while many of the aldehydes and ketones have characteristic sharp and unpleasant odours. In addition to this, the oil contained a range of volatile esters of short chain fatty acids, which are important volatile compounds with sweet fruity odours, as well as low molecular weight carboxylic acids with more unpleasant pungent odours. Finally, the oil contained the terpene limonene, which has a fresh citrus odour and the furan 2-pentylfuran which has a fruity and green yet pungent odour. This is the first investigation of volatile components in marama oil; thus data cannot be compared with other analyses of pressed or extracted marama oil.

3.2. Lipoxygenase activity in marama beans

In order to elucidate whether enzymatic lipid oxidation would be a potential problem in marama oil during storage, the activities of the lipoxygenase isozymes, L-1 and L-2, were examined in raw marama beans. As seen in Fig. 1, marama bean extract did not bleach methylene blue at pH 9.0 as the absorbance of the methylene blue did not decrease with time. This suggests the absence of lipoxygenase isozyme L-1 in raw marama beans. Similar results were obtained for the L-2 isozyme at pH 6.0 (Fig. 1), also signifying the absence of L-2 isozyme in marama beans. Soybean extract, which was used as reference material, exhibited bleaching activity toward methylene blue, as indicated by the decrease in absorbance with time (Fig. 1), confirming the presence of L-1 and L-2 isozymes in soybeans, as is also known from the literature (Suda et al., 1995).

These results suggest that the lipoxygenase isozymes are either absent or naturally inhibited in some way in marama beans and therefore enzymatic lipid oxidation should not be a potential problem during storage of marama bean oil.

Many phenolic compounds have some form of inhibitory activity toward lipoxygenase enzymes. Since marama bean cotyledons have a total phenolic content of 2.8 mg of catechin equivalents/100 mg db (Jackson et al., 2010), these compounds may possibly be responsible for inhibiting lipoxygenase in marama beans. More specifically, gallic acid polymers have been reported to be effective in retarding lipoxygenase oxidation of linoleate (Nawar, 1996). The cotyledons of marama beans contain 23 mg of gallic acid/100 g db (Jackson et al., 2010). However, it is not known whether the gallic acid in marama beans is in monomeric or polymeric form. Additionally, Skrzypczak-Jankun, Zhou, and Jankun (2003) demonstrated that the flavonoid quercetin, which is also found in marama beans (24 mg/100 g db) (Jackson et al., 2010), complexed with soybean lipoxygenase, thereby inhibiting it. Yet another phenolic compound found in marama beans, caffeic acid (39 mg/100 g db) (Jackson et al., 2010), inhibited rat leucocyte 5-lipoxygenase at 200 μm (de la Puerta, Gutierrez, & Hoult, 1999). Furthermore, although no amount was given, St. Angelo, Kuck, and Ory (1979) reported that peanut tannins (catechol-like) gave 67% inhibition of soybean lipoxygenase at pH 8.4, possibly through cross-linking with the enzyme.

Marama beans also contain 2.6 g/100 g of erucic acid (Mitei et al., 2008), a fatty acid identified by St. Angelo et al. (1979) and St. Angelo and Ory (1984) as a lipoxygenase inhibitor as it completely inhibits soybean and peanut lipoxygenase at a minimum concentration of 7.3 μmol/100 g. Of the oilseeds that were studied (soybeans, rapeseed and peanuts), lipoxygenase activity was not detected in rapeseed only and this was attributed to the presence of erucic acid and tannins in rapeseed (St. Angelo et al., 1984).

3.3. Storage stability of pressed marama oil

3.3.1. General

Since the fatty acid composition of marama oil is dominated by the unsaturated fatty acids oleic, linoleic and linolenic, it is expected that the oil will be very susceptible to oxidation.

3.3.2. Overview of chemical changes during storage – FT-IR spectroscopy

FT-IR is a powerful instrumental tool for both quantitative and qualitative analysis of edible oils, due to the functional group information (e.g. strong signals for polar groups such as O–H, C–H and C–O) contained within an IR spectrum (Dobson, 2001). Therefore, FT-IR has been used to study oil oxidation by sequentially recording the spectra of samples taken over time from oils under oxidative stress (Guillen & Cabo, 1999, 2000; Vandevoort, Ismail, Sedman, & Emo, 1994).

In this study, FT-IR spectra of the marama oil samples were recorded during the 7 months of storage. Initially, a PCA model was made from the IR data to explore the spectral changes over storage time. Scatter score plots (e.g. score 1 vs. score 2) were coloured according to storage time and some of the plots showed a tendency to time-dependent changes in the samples (for brevity these plots are not shown). This was further investigated using a multivariate regression model (PLS), in order to elucidate potential chemical changes going systematically up or down during the storage period. Fig. 2A shows the model performance given as an actual vs. predicted plot while Fig. 2B shows the corresponding regression coefficients.

Table 1
Volatile compounds identified by DHS-GC–MS in the headspace of marama oil throughout the storage period. Volatiles are grouped according to chemical classes. The relative contents of the volatiles compared to the total content at month 7 are indicated in percent (calculated from the average peak areas (n = 12, all storage conditions) from the total ion chromatograms (TIC) divided by the total area). Kováts retention indices and published odour characteristics are given.

Chemical class	Volatile compound	Relative content of total (%) – month 7	Retention index	Published odour characteristics[a,b]
Alcohols	2-Ethylhexan-1-ol	<1	1147	Mild, oily, sweet, slightly floral
	Octan-1-ol	<1	1147	Fresh, orange-rose, fatty
	Heptan-1-ol	<1	1216	Fragrant, woody, heavy, oily, faint, aromatic, fatty
	3-Methylbutan-1-ol	1	1251	Fruity, banana, sweet, fragrant, powerful
	Butan-1-ol	1	1452	Fusel-like sweet and pleasant
	Hexan-1-ol	10	1535	Herbaceous, woody, fragrant, mild, sweet, green fruity
	Pentan-1-ol	7	1553	Fusel-like sweet and pleasant
Aldehydes	Oct-2-enal	<1	878	Almond, fatty, fruity, green, nutty
	Pentanal	15	1070	Powerful, acrid, pungent
	Octanal	<1	1178	Fatty, fruity, orange peel, pungent, soapy
	Hexanal	20	1281	Fatty, green, grassy, powerful, penetrating and fruity
	Nonanal	1	1313	Citrus, fatty, floral, green
	Heptanal	<1	1489	Fatty, green, heavy, oily, planty green
	(E)-Hept-2-enal	1	1596	Fatty, fruity, green, melting plastic
Ketones	Propan-2-one	9	899	Characteristic aromatic odour
	Octan-2-one	<1	1187	Floral, fruity, musty, soapy
	Heptan-2-one	1	1272	Blue cheese, fruity, musty, soapy
	3-Hydroxybutan-2-one	<1	1277	Bland, woody, yogurt
Hydrocarbons	1-Methyl-4-(1-methylethenyl)-cyclohexene (limonene)	<1	1035	Pleasant, lemon-like, fruity
Acids	Hexanoic acid	3	894	Sickening, sweaty, rancid, sour, sharp, pungent, cheesy, fatty, unpleasant
	Ethanoic acid	3	1865	Strong, pungent, characteristic vinegar
	Pentanoic acid	3	1881	Unpleasant, sweet, cheese-like
Esters	Ethyl acetate	21	1182	Pleasant ethereal-fruity, brandy-like
	Methyl hexanoate	<1	1224	Ether-like, reminiscent of pineapple
Furans	2-Pentylfuran	2	921	Fruity, green, pungent, sweet

[a] Burdock (2005).
[b] www.odour.org.uk.

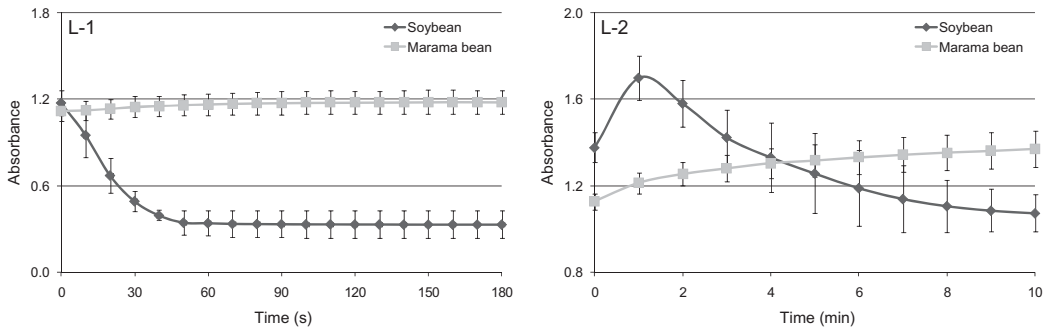

Fig. 1. Examination of the presence of lipoxygenase isozymes (L-1 and L-2) in soybean and marama bean. Curves show methylene blue bleaching by soybean or marama bean extracts determined by absorbance measurements at 660 nm.

The regression coefficients reveal that, e.g. the –C–H-bonds in the lipids (2928 cm^{-1}), decrease during storage while the –C–O-bonds (1165 cm^{-1}) increase. This suggests that lipids are oxidised over time and that certain oxidation products are formed. However, multivariate regression, linking primary and secondary oxidation products to the FT-IR data, resulted in poor models. This proposes that the chemical changes captured in the spectral data are not due to individual primary or secondary lipid oxidation products but rather a combination of different oxidation effects plus other chemical changes taking place over time, (i.e. a combination of lipid oxidation and changes in the content of phenolic compounds, tocopherols and tocotrienols, pigments). Thus, the information in the spectra can be seen as a measure of increasing oxidation taking place over time. From the model in Fig. 2, it will be possible to estimate the approximate storage time (i.e. overall oxidation status) for a new marama oil sample with high certainty (RMSECV = 0.29).

3.3.3. Primary oxidation products – peroxide value

The peroxide value (PV) was used to establish the primary oxidation state of the marama oil. PV was monitored during storage and the development of PV in the oil as a function of storage time is presented in Fig. 3. The pressed marama oil had a low PV of 0.26 ± 0.2 meq/kg before the storage experiment was initiated. This

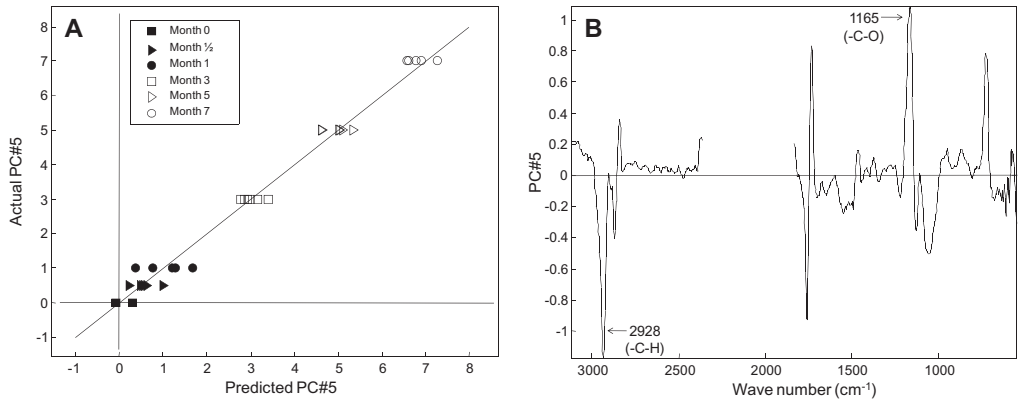

Fig. 2. PLS model of IR spectra (scatter corrected and smoothed) linked to storage time, using five PLS components. (A) Model performance (actual vs. predicted: $r^2 = 0.99$, RMSECV = 0.29 weeks) and (B) regression coefficients, with assignment of two of the most important peaks.

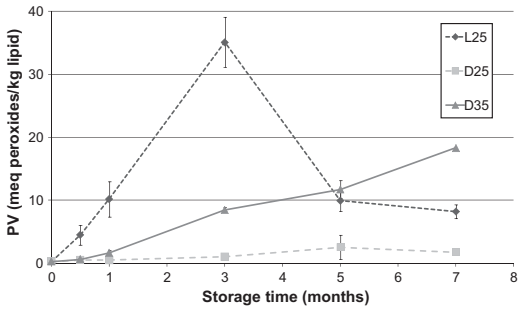

Fig. 3. Effect of storage on peroxide values (mean of two replicates; error bars represent standard deviation) of marama oil stored under different temperature and light conditions for 7 months. L25, oil stored in light at 25 °C; L35, oil stored in light at 35 °C; D35, oil stored in darkness at 25 °C. Notice that small error bars are hidden behind the data points.

compares well with the slightly higher PV found in extracted marama bean oil of 4.01 ± 0.05 meq/kg (Mitei et al., 2008) and shows a good stability of the marama oil during processing, since it is stated in the literature that freshly refined lipid should have PV below 1 meq/kg (Rossell, 1994).

As may be seen from Fig. 3, lipid hydroperoxides are formed during storage, confirming that the oils are being oxidised, regardless of the storage conditions. Fig. 3 clearly shows that samples stored in light at 25 °C are subjected to the fastest and highest degree of lipid oxidation, i.e. compared to the samples stored in darkness even at higher temperatures (35 °C). This difference is evident already after half a month of storage. Hence, light has a larger influence on the lipid oxidation taking place in the marama oil than has temperature. This is in agreement with studies of the stability of other oils, such as olive oil (Pristouri, Badeka, & Kontominas, 2010). The samples stored in light at 25 °C show an increase in hydroperoxides until month three (35.1 ± 4.0 meq/kg), where after the hydroperoxides start to decrease and are broken down to secondary oxidation products. Comparing the peroxide value at three months with the maximum peroxide value given for virgin olive oil with an acceptable quality (PV = 20 meq/kg) (Codex Alimentarius, 2001), it seems that marama oil stored in light at 25 °C may be expected to be of unacceptable quality after 3 months of storage.

In darkness, the increase in PV is solely due to autoxidation. Of the samples stored in darkness, those kept at 35 °C become more oxidised than the ones stored at 25 °C. Hence, the rate of formation of primary oxidation products increases with temperature. The samples stored at 35 °C reached a level of 18.3 ± 7.2 meq/kg while the samples stored at 25 °C only reached 1.74 ± 0.4 meq/kg after 7 months of storage. The PV values show that storage in darkness for up to 7 months does not seem to compromise the quality of the marama oil. However, the PV of the samples stored at 35 °C closely approaches the Codex Alimentarius (2001) quality limit for olive oil of 20 meq/kg.

3.3.4. Secondary oxidation products – dynamic headspace GC–MS

Secondary oxidation products are volatiles formed through decomposition of the primary oxidation products. These volatiles may give the marama oil undesirable rancid flavours and can be used as a measure of the oxidative status of the product. In this study, the volatiles formed during lipid oxidation were determined by DHS-GC–MS.

To visualise the development of the volatile compounds identified throughout the storage period (Table 1), eight of the components are plotted as peak area vs. months of storage in Fig. 4. Fig. 4 shows that, for all of the volatile compounds given as representative examples, the samples stored in light have been oxidised the most and hence have the largest content of the respective volatile compound. For the samples stored in darkness, those stored at 35 °C have developed larger amounts of volatiles than the samples stored at 25 °C. In general, light has a larger effect on lipid oxidation than temperature, since samples stored in light at 25 °C are oxidised more than samples stored in darkness at 35 °C, confirming the trend seen from the peroxide value data.

While some volatile compounds seem to have a continued increase in concentration until month 7 (hexanal and pentanoic acid), others seem to level off towards the end of the storage experiment (pentanal, heptan-2-one and 2-pentylfuran), and others even start to decline in concentration after about 3 to 5 months of storage (butan-1-ol, (E)-hept-2-enal and pentan-1-ol).

In order to get an overview of the data, a PCA model was made, including all samples and all the identified volatiles. The score and loading plots are shown in Figs. 5A and B, respectively. PC1 describes the storage time and, furthermore, distinguish between the storage conditions. The main observation is that samples stored in light generally have higher concentrations of the volatile

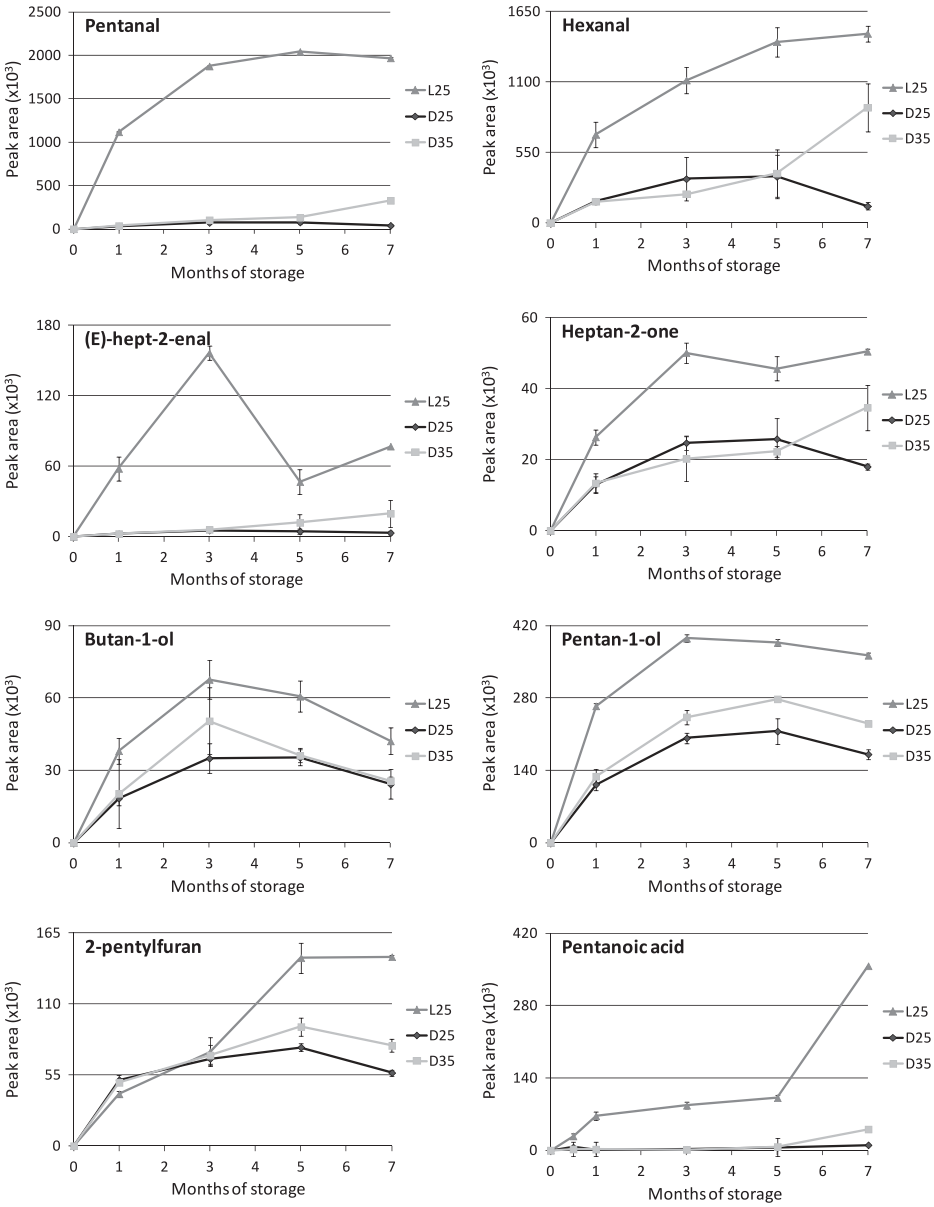

Fig. 4. Effect of storage on the levels of eight selected volatiles identified in marama oil stored under different temperature and light conditions for 7 months. Data are presented as mean peak area (four replicates) with error bars representing the standard deviation. The peak areas for month 0 are set to zero. L25, oil stored in light at 25 °C; L35, oil stored in light at 35 °C; D35, oil stored in darkness at 25 °C.

compounds than have samples stored in darkness. The samples stored in darkness at two different temperatures may not be separated by PC1 or PC2. This confirms that light has a larger influence than has temperature on lipid oxidation, as previously discussed.

A PCA model, made solely on the samples stored in darkness, indicates a difference between the samples stored at 25 °C and 35 °C with the samples stored at the highest temperature reaching the highest concentrations of the oxidation products after 7 months of storage (results not shown). This confirms the findings illustrated in Fig. 4.

Besides looking at the development of the amount of each compound over time, it is important to combine this with the actual

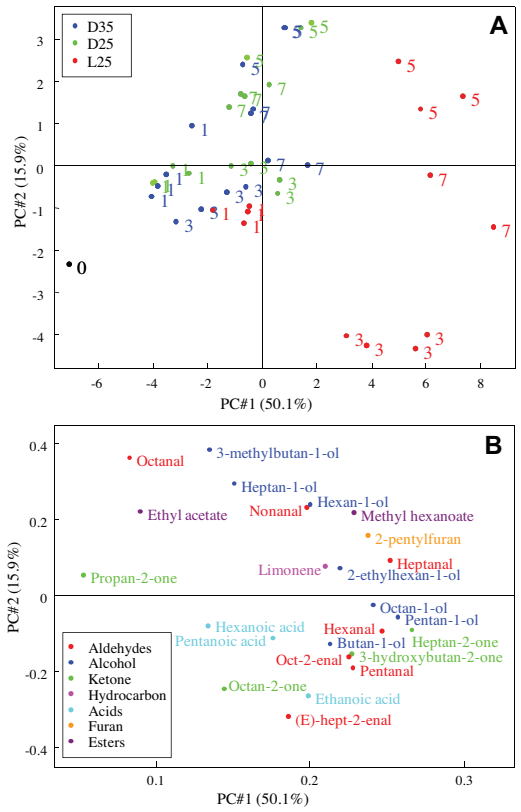

Fig. 5. (A) Score plot, PC1 vs. PC2, from a PCA model including all the marama oil samples stored under different temperature and light conditions for 7 months and all identified volatiles in these samples; coloured according to storage conditions. (B) Loading plot of PC1 vs. PC2 coloured according to chemical classes.

concentrations of the compounds and their threshold values. Five selected volatile compounds were quantified and used as examples. Hexanal is a dominant oxidation product of linoleic acid and other *n*-6 fatty acids, which are found in large quantities in marama beans. Hence, hexanal can be used as an effective indicator of lipid oxidation (Choe & Min, 2006; Shahidi & Pegg, 1994). Another aldehyde, octanal, which is one of the major oxidation products formed from oleic acid, was also analysed, along with two alcohols (pentan-1-ol and 1-hexan-1-ol). Finally, the furan, 2-pentylfuran, which is well known as a significant contributor to off-flavour in soybean oil (Chang, Smouse, Krishnam, Mookherj, & Reddy, 1966), was quantified.

Data from MHE analysis of stored marama oil, as well as standard curves for the five pure volatile compounds, were used to calculate the concentrations in the samples and made it possible to compare the actual concentrations at certain storage times with the odour threshold values in oil of each reference compound (Table 2). From Table 2, it appears that only hexanal reached a concentration higher than its threshold value. Octanal, hexan-1-ol, pentan-1-ol and 2-pentylfuran were not formed in concentrations even approaching the detection threshold values in oil. Hence, the significant increases of these compounds over time, seen in Fig. 4, are not large enough to influence the sensory properties of the marama oil. However, it has to be taken into consideration that synergistic effects between sub-threshold odour compounds may affect the overall odour sensation (Ito & Kubota, 2005), but sensory analysis is needed in order to confirm such effects.

Samples stored for 1 month in light at 25 °C reached hexanal concentrations of 287–413 ppb. This exceeds the odour detection threshold, which is reported to be in the interval 75–300 ppb for hexanal in oil. During the consecutive months of storage, the hexanal concentration further increased and reached 787 ppb at 7 months. Thus, the sensory quality of these oils will most certainly be affected. Samples stored in darkness at 35 °C, reached hexanal concentrations of 374–552 ppb at 7 months of storage and thereby also exceeded the odour threshold value. However, oils stored for 7 month in darkness at 25 °C only contained 52–75 ppb of hexanal, which is just below the detectable threshold value. Hence, this would be the preferred storage condition for pressed marama oil. In this condition it should be possible to store the oil for at least 7 months without obtaining detectable amounts of unwanted odours.

3.3.5. Antioxidants – vitamin E content by HPLC

The concentrations of all eight tocopherol and tocotrienol isomers were determined in order to evaluate their influence on the oxidative stability of the marama oil. Only α- and γ-tocopherols were present in detectable amounts in the oil, with concentrations of 113 μg/g db and 339 μg/g db, respectively, before the storage experiment was initiated. The contents of α- and γ-tocopherols have previously been determined in raw marama bean samples

Table 2
Concentration range of the quantified volatile compounds in the oil samples, their threshold values and odour. The samples that have passed their threshold value are indicated.

Volatile compounds	Concentration range during month 1–7 for all storage conditions (ppb)	Odour detection threshold in oil (ppb)	Samples with concentrations above the threshold value	Published odour characteristics[g]
Hexanal	52–787	75–300[a–d]	Month 1–5: light at 25 °C Month 7: light at 25 °C and darkness at 35 °C	Fatty, green, grassy, powerful, penetrating and fruity
Octanal	2–9	56–900[a,b,d]	Month 1–7: none	Fatty, fruity, orange peel, pungent, soapy
Pentan-1-ol	27–122	470–3000[b,c]	Month 1–7: none	Fusel-like sweet and pleasant
Hexan-1-ol	16–63	400[e]	Month 1–7: none	Herbaceous, woody, fragrant, mild, sweet, green fruity
2-Pentylfuran	10–47	250–6000[f]	Month 1–7: none	Fruity, green, pungent, sweet

[a] Fazzalari (1978).
[b] Morales, Luna, and Aparicio (2005).
[c] Aparicio and Luna (2002).
[d] Reiners and Grosch (1998).
[e] Aparicio and Morales (1998).
[f] Min and Boff (2002).
[g] Burdock (2005).

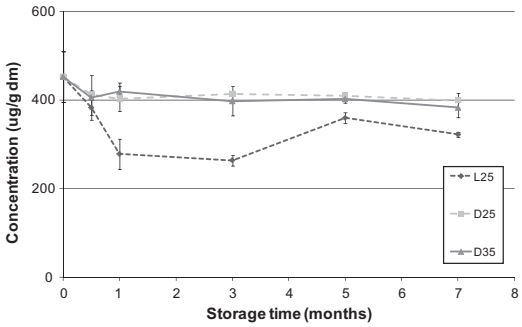

Fig. 6. Effect of storage on α- and γ-tocopherol contents (means of four replicates; error bars represent standard deviation) of marama oil samples stored under different temperature and light conditions for 7 months. L25, oil stored in light at 25 °C; L35, oil stored in light at 35 °C; D35, oil stored in darkness at 25 °C.

and were found to be 14–48 μg/g db and 59–234 μg/g db, respectively (Holse et al., 2010). This fits well with the fact that lipids account for about 1/3 of the dry matter of the marama bean (Holse et al., 2010).

During the 7 months of storage, the α- and γ-tocopherols acted as antioxidants, reducing their levels to final concentrations of 52–87 μg/g db and 270–312 μg/g db, respectively, depending on the storage conditions. The loss equals 23–54% and 8–20%, respectively, of the initial concentrations. In Fig. 6, the total vitamin E content (α- and γ-tocopherols) is given for the marama oil samples during the 7 months of storage.

For the samples stored in darkness (at either 25 °C or 35 °C) the content of vitamin E decreased during the first ½–1 month of storage (approximately 10% loss) and thereafter stabilized for the rest of the storage period. The stable nature of the vitamin E isomers in marama oil is very interesting, since it was expected that the amount of antioxidants would decrease throughout the storage period.

The sample stored in light at 25 °C showed a considerably greater decrease in vitamin E content during the first three months (approximately 40% loss), which is most likely due to a more pronounced lipid oxidation taking place and hence more antioxidant being used. After three months, the vitamin E content began to rise until month 5, where after it stabilized. Hence, after 7 months of storage, 70% of the initial amount of tocopherols and tocotrienols in the marama oil was present. The sudden rise in the content of vitamin E might be due to regeneration of the α-tocopheroxyl radical, a radical formed during antioxidant action of α-tocopherol. Pazos, Andersen, Medina, and Skibsted (2007) demonstrated that some natural phenolic compounds were able to regenerate α-tocopherol via reduction of the α-tocopheroxyl radical and, since marama beans contain phenolics (Jackson et al., 2010), this seems to be a reasonable explanation.

4. Conclusions

The volatile profile of pressed marama oil was found to be dominated by short chain alcohols and aldehydes. Through the study, it was found that the marama oil was very stable and had good natural antioxidant properties. Investigations of the raw marama beans revealed that enzymatic lipid oxidation would not be an issue during storage of marama bean products. The study, furthermore, showed that light had a greater effect on the stability of pressed marama bean oil than temperature. Therefore, the oil should preferably be stored in darkness and rather at 25 °C than 35 °C, in order to keep the non-enzymatic lipid oxidation at the lowest possible level. If these storage conditions are used, the oil can be stored for at least 7 months without showing undesirable odours.

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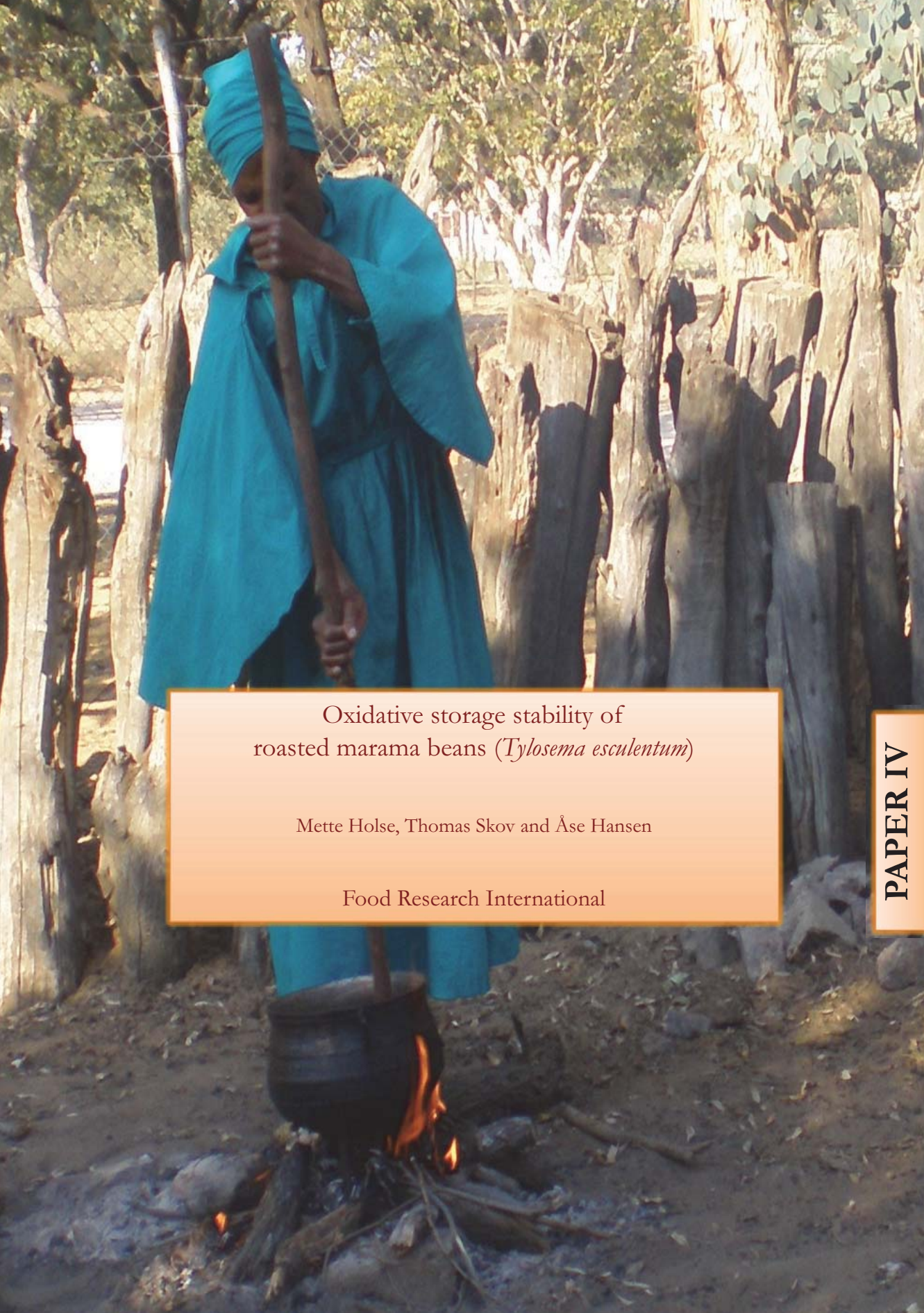

Oxidative storage stability of roasted marama beans (*Tylosema esculentum*)

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ABSTRACT

The marama bean is an underutilized traditional source of food for the indigenous population in Southern Africa such as the Herero, Tswana and Khoisan people and is mostly eaten as a snack after roasting in hot sand. The beans have a high content of protein and oil (mainly unsaturated lipid). This composition gives the marama bean a significant potential for production of various nutritious food products such as roasted beans. The aim of this study was to investigate the influence of different storage conditions on the oxidative stability of roasted marama beans. This was evaluated by packing the roasted marama beans in the presence or absence of oxygen and storing them with or without exposure to light for seven months. During the storage period, the changes in flavor related oxidation products (i.e. secondary oxidation products) were investigated. It was found that roasted marama beans should preferably be stored in darkness in bags containing a low amount of oxygen. Under these conditions the beans could be stored for at least five months without obtaining undesirable odors caused by lipid oxidation. Hexanal was found to be the limiting storage factor as it was the first volatile exceeding its odor detection threshold.

1. Introduction

The marama (or morama) bean (*Tylosema esculentum*) is a perennial, tuberous legume which grows wild in the Kalahari Desert region of Southern Africa (Bousquet, 1981). The plant produces very hard pods with edible seeds, which are part of the traditional food eaten by various rural communities in Southern Africa (National Research Council, 2006), but is otherwise underutilized (van der Maesen, 2006). The seeds are surrounded by a solid hull and must be dehulled before consumption.

The dehulled seeds are extremely nutritious with high contents of lipids and proteins, 32–42% dry matter basis (% db) and 29–38% db, respectively (Holse, Husted, & Hansen, 2010). The high lipid content approaches that of peanuts (36–54%) (Salunkhe & Kadam, 1989) while the protein content is comparable to that of soybeans (33–46% db) (Gueguen, 1983; Hymowitz, Collins, Walker, & Panczner, 1972; Salunkhe & Kadam, 1989). Traditionally, marama beans are eaten as a snack after roasting in hot sand, but may also be used for production of various products such as marama oil and marama milk. Of particular significance is the high protein content, which makes the marama bean ideal for production of value-added protein-rich ingredients similar to those commercially available from soybeans. One example is roasted marama bean flour, which may be used in e.g. composite sorghum porridges in order to improve both taste and nutritional quality (Kayitesi, Duodu, Minnaar, & de Kock, 2010; Maruatona, Duodu, & Minnaar, 2010).

Before setting up a commercial production of marama products such as roasted marama beans, it is important to know the stability of the product during storage. Within this it is essential to identify potential oxidation products that contribute to the flavor (i.e. flavor compounds in form of secondary lipid oxidation products). The rate of lipid oxidation in the marama bean products may be influenced by various internal factors such as fatty acid composition, presence and activity of pro- and antioxidants, moisture content and nature of the surface of the product as well as external factors such as light, oxygen concentration, temperature and relative humidity. In the intact marama bean the solid hull is preventing oxidative deterioration of the seed. During dehulling and processing into various products the seeds are affected by mechanical injury and exposed to light and oxygen, which initiates lipid oxidation.

Marama bean lipids are highly unsaturated (app. 75%) with the major unsaturated fatty acids being oleic acid (approx. 40%) and linoleic acid (approx. 20%) (Mitei, Ngila, Yeboah, Wessjohann, & Schmidt, 2008). Hence, the lipid composition of marama beans makes them highly susceptible to oxidative deterioration. However, Holse et al. (2010) found that marama beans have a relatively high content of the natural antioxidant vitamin E (44 mg α-TE), compared to various cereal grains, legumes, vegetables and fruits (namely 1–23 mg α-TE).

Oxidation of the unsaturated lipids in marama beans may proceed through different mechanisms resulting in the formation of hydroperoxides (primary oxidation products) which may decompose into volatiles (secondary oxidation products), hereby affecting the quality of the product. Autoxidation is a spontaneous oxidation of unsaturated

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fatty acids in the presence of oxygen, while photosensitized oxidation of unsaturated fatty acids is triggered by exposure to light in the presence of oxygen and a photosensitizer (Frankel, 1980; Min & Boff, 2002). Another potential oxidation pathway is through the presence of lipoxygenase enzymes found in many plants similar to the marama bean. However, it has recently been shown that these enzymes are either absent or naturally inhibited in marama beans (Holse, Petersen, Maruatona, & Hansen, 2011).

In the present study, we focused on the oxidative storage stability (i.e. appearance of undesirable flavor compounds) of roasted marama beans. The storage stability was examined under different packaging conditions where packaging gas (21% oxygen/<1% oxygen) and light exposure (light/dark) were varied during seven months of storage at room temperature. The oxidative stability of the roasted beans was evaluated by examination of secondary oxidation products by investigation of the formation and breakdown of volatile compounds using dynamic headspace gas chromatography-mass spectrometry (GC-MS). Additionally, volatile compounds formed during the roasting process were also monitored during the storage period. Within the GC-MS analysis, multiple headspace extraction (MHE) was used in order to quantify some of the important volatiles, which were then compared to reported odor detection thresholds. From the results of this storage experiment a recommended storage condition and shelf life was given for the roasted marama beans.

Fig. 1. Packed roasted marama beans.

2. Materials and methods

2.1. Sample material

Roasted marama beans were prepared from marama beans harvested in Namibia in 2008. The beans were roasted in their hull in an oven at 145–150 °C for 50 min. After cooling, the beans were dehulled by use of a nutcracker.

Before each day of analysis the roasted dehulled beans were removed from the packaging material and milled in a laboratory mill (IKA A10, Labortechnik, Staufen, Germany) for 10 s to a particle size of ≤250 μm. The milled samples were kept in light and air tight containers at 5 °C until analysis.

2.2. Packaging and storage conditions

The roasted marama beans were packed in transparent plastic laminate (PA/PE 20/70) with an oxygen transmission rate (OTR) of 32 ml/m^2/24 h/atm (23 °C, 75% RH) and a water vapor transmission rate (WVTR) of 1.0 g/m^2/24 h (23 °C, 85% RH). Each bag was filled with 40 g of roasted marama beans and then either sealed under atmospheric conditions (21% oxygen) or flushed with nitrogen (<1% oxygen). The bags were packed using a Multivac Packaging Machine (A 300/42, Multivac, Wolfertschwenden, Germany). Two replicate bags were prepared for each storage condition and for each month of analysis. The samples were denoted in the following way:

- LO (Light/Oxygen): samples stored in light in atmospheric air
- LN (Light/Nitrogen): samples stored in light in nitrogen
- DO (Darkness/Oxygen): samples stored in darkness in atmospheric air
- DN (Darkness/Nitrogen): samples stored in darkness in nitrogen

These codes are used in the Results and discussion section. Fig. 1 shows the packed roasted beans for a sample that was packed in atmospheric air and stored in light for five months.

The samples stored in light were placed in a room without direct sunlight and with the electric light (Philips Master TL-D Super 80, 36 W/830, Copenhagen, Denmark) turned on constantly. The samples that were to be stored in darkness were kept in the same room inside two black plastic bags. The samples were stored at room temperature and the average temperature during the entire storage period was 24.1 ± 1.8 °C, which was monitored by a temperature logger (Testo 174 mini temperature data logger, Testo Inc., Sparta, NJ, USA). The samples were analyzed after approximately 0, 1, 3, 5 and 7 months of storage (or exactly 0, 32, 101, 168 and 225 days, respectively). In the following, the time points will be given as the approximate number of months. The two replicates of each storage condition were analyzed on each day of analysis and from each of these, two sub-replicates were prepared for all the analyses. This resulted in four replicates for each storage treatment on each day of analysis. In the sample preparation and the analysis steps, the handling of the samples was randomized.

2.3. Gas composition

Before the bags were opened for analysis, the gas composition of the headspace above the packed product was determined by use of a CheckMate 9900 (PBI Dansensor A/S, Ringsted, Denmark). The gas composition was used to identify leaking bags.

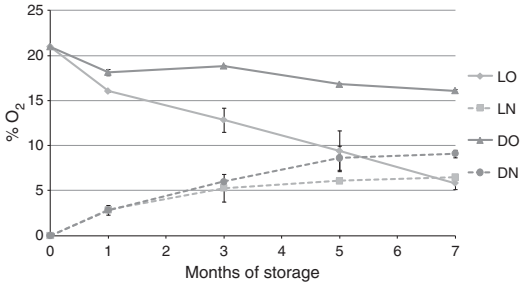

Fig. 2. Effect of storage on oxygen levels in the headspace in roasted marama beans packed in the presence or absence of oxygen and stored under different light conditions (light or dark) for seven months. Data are shown as mean values of the two replicates with error bars indicating ± std. LO: samples stored in light in atmospheric air; LN: samples stored in light in nitrogen; DO samples stored in darkness in atmospheric air; DN samples stored in darkness in nitrogen.

Table 1
Volatile compounds identified by GC-MS in the headspace of roasted marama beans packed in the presence or absence of oxygen and stored under different light conditions (light or dark) for seven months (collection of data from all samples). Volatiles are grouped according to chemical classes and reported odor characteristics are given.

Volatile compound	Odor characteristics[a]
Aldehyde	
Butanal	Pungent
2-methylbutanal	Powerful, choking
Pentanal	Powerful, acrid, pungent
Hexanal	Fatty, green, grassy, powerful, penetrating and fruity
(E)-2-hexenal	Sweet, fragrant, almond, fruity green, leafy, apple, plum
Heptanal	Fatty, green, heavy, oily, planty green
Octanal	Fatty, fruity, orange peel, pungent, soapy
(E)-2-octenal	Almond, fatty, fruity, green, nutty
Nonanal	Strong, fatty
Decylaldehyde	Penetrating, sweet, waxy, floral, citrus, fatty
(E)-2-decenal	Orange, slightly fatty, floral green
Benzaldehyde	Pleasant, almond-like
Ketone	
2-butanone	Sweet, apricot-like
3-hydroxy-2-butanone	Bland, woody, yogurt
2-heptanone	Blue cheese, fruity, musty, soapy
3-methyl-2-heptanone	–
2-octanone	Floral, fruity, musty, soapy
3-octen-2-one	Fruity, lemon
1-octen-3-one	Mushroom
Dihydro-5-methyl-2(3*H*)-furanone	Sweet, herbaceous
Dihydro-2(3H)-furanone	–
Hydrocarbon	
Pentane	–
Hexane	–
Heptane	–
Octane	–
3-methylnonane	–
2-hexene	–
1-heptene	–
1-octene	–
Undecane	–
Dodecane	–
Alcohol	
1-butanol	Fusel-like sweet and pleasant
2-butanol	Alcoholic
2-methyl-1-propanol	Penetrating, wine-like
1-pentanol	Fusel-like sweet and pleasant
2-pentanol	Mild green, fusel-oil
Heptanol	Woody, heavy, oily, faint, aromatic, fatty
3-heptanol	Powerful, herbaceous
1-hexanol	Herbaceous, woody, fragrant, mild, sweet, green fruity
2-methyl-3-buten-2-ol	–
(E)-2-hexen-1-ol	Powerful, leafy, green, wine-like, fruity
1-octen-3-ol	Powerful, sweet, earthy
(E)-2-undecen-1-ol	Mild, fatty-waxy
Acid	
Acetic acid	Strong, pungent, characteristic vinegar
2-methylpropanoic acid	Sweet, balsamic, fruity
Butanoic acid	Persistent, penetrating, rancid, butter-like odor
Pentanoic acid	Unpleasant, sweety, cheese-like
Hexanoic acid	Sickening, sweaty, rancid, sour, sharp, pungent, cheesy, fatty
Ester	
Formic acid, pentyl ester	Fruit-like
Formic acid, hexyl ester	Fruity, apple-like, unripe-plum
Formic acid, octyl ester	Fruity, rose-orange note
Acetic acid, methyl ester	Pleasant, fruity
Acetic acid, ethyl ester	Pleasant ethereal-fruity, brandy-like
Propanoic acid, 2-hydroxyethyl ester	–
Pentanoic acid, methyl ester	Pungent, green-fruity
Hexanoic acid, 2-propenyl ester	Fruity
Furan	
2-butylfuran	Non-characteristic, weak
2-pentylfuran	Fruity, green, pungent, sweet
Pyridine	
Pyridine	Characteristic, penetrating, burnt
3-ethyl-2,6-dimethylpyridine	–
Pyrazine	
2,3-dimethylpyrazine	Musty, nut skins, cocoa powdery, roasted, potato and coffee nuances
2,5-dimethylpyrazine	Nutty, peanut, musty, earthy, powdery, slightly roasted
2,6-dimethylpyrazine	Cocoa, roasted nuts, roast beef, coffee
Ethylpyrazine	Nutty, musty, fermented, coffee, roasted, cocoa, meaty nuances
2-ethyl-5-methylpyrazine	–
2-ethyl-6-methylpyrazine	Roasted potato
3-ethyl-2,5-dimethylpyrazine	Potato, cocoa, roasted, nutty
2-methyl-6-vinylpyrazine	Coffee
2-cyano-3,5-dimethylpyrazine	–

[a] (Burdock, 2005), www.thegoodscentscompany.com, www.odour.org.uk.

2.4. Dynamic headspace GC-MS

Duplicate samples of 10 g of roasted marama bean flour were added directly to a 280 ml purge flask. The samples were equilibrated to 30 ± 1 °C in a circulating water bath and then purged with nitrogen (75 ml/min) for 60 min, while being stirred by a magnet stirrer at 200 rpm. The volatile compounds were collected in a Tenax-TA trap (250 mg, mesh size 60/80, Buchem BV, Apeldoorn, The Netherlands), which was then sealed with caps and kept at 5 °C until GC-MS analysis. Duplicate measurements were carried out. The GC-MS setup and conditions were carried out as described by Holse et al. (2011).

The GC-MS data were analyzed using the software MSD ChemStation G1701EA (Version E.01.00.237, Agilent Technologies Inc., Palo Alto, CA, USA). Volatile compounds were identified by matching their mass spectra with those of a commercial database (Wiley275.L, G1035A, Agilent Technologies, Inc., Palo Alto, CA, USA) and areas of the peaks in the total ion chromatogram (TIC) were obtained by integration.

2.4.1. Multiple headspace extraction (MHE) for quantification of important volatiles

MHE was carried out in order to quantify the identified volatiles (secondary oxidation products) in the roasted marama beans (Kolb & Pospisil, 1977). A sample of roasted marama beans that had been stored for seven months in atmospheric air and in light was used for the MHE experiment, since this sample was expected to have undergone the highest degree of oxidation (most secondary oxidation products present). Duplicates were prepared and each sample was continuously purged with nitrogen (30 °C, nitrogen flow 75 ml/min) and seven dynamic headspace samplings (60 min duration) were made (after 0, 1, 2, 3, 4, 5 and 23 h). Additionally, five pure aroma compounds were quantified. 10 μl of each of the five compounds (hexanal, octanal, 1-butanol, 1-hexanol, 2-pentyl-furan) was mixed with 10 ml heptane (1000 ppm). A standard series with 500, 100 and 10 ppm was made. 2 μl of each was injected directly into a Tenax-TA trap (in triplicate). GC-MS analysis of the Tenax TA traps was performed as described by Holse et al. (2011).

The total amount of volatile analyte present in the sample was calculated from the estimated sum of all partial peak areas (A_i), obtained

in the series of chromatograms, where *i* is the number of extractions (Kolb & Pospisil, 1977):

$$\sum_{i=1}^{n} A_i = \frac{A_1}{1-e^{-q'}}$$

The exponent q′ describes the exponential decline of the peak areas during the stepwise MHE procedure, and is obtained from the linear regression analysis:

$$lnA_i = -q'(i-1) + lnA_1$$

where the q′ value is equal to the slope of the linear regression line and ln A_1 is given by the y-intercept.

2.5. Multivariate data analysis

For the GC-MS data, principal component analysis (PCA) was used as a descriptive method during the first step of the data exploration. PCA was performed using LatentiX (v. 2.00, Latent5, Copenhagen, Denmark).

3. Results and discussion

3.1. Monitoring of oxygen level in packaging material

The oxygen level in the bags is given in Fig. 2 for each of the four storage treatments. For the bags containing atmospheric air, the roasted beans exposed to light used more oxygen than the beans stored in darkness. This was observed throughout the entire seven months of storage. Hence, a higher degree of lipid oxidation was observed in the beans stored in light. For the roasted beans that were kept in bags flushed with nitrogen an increase in oxygen was seen during the storage period, and the concentration was >1% already after one month of storage. This was due to the oxygen permeability of the applied storage bags. As observed for the beans stored in atmospheric air, there was a higher amount of oxygen in the bags flushed with nitrogen and stored in darkness than the ones stored in light.

3.2. Secondary oxidation products — dynamic headspace GC-MS

Through the DHS-GC-MS analysis 69 different volatile compounds were identified in the headspace of the roasted marama beans

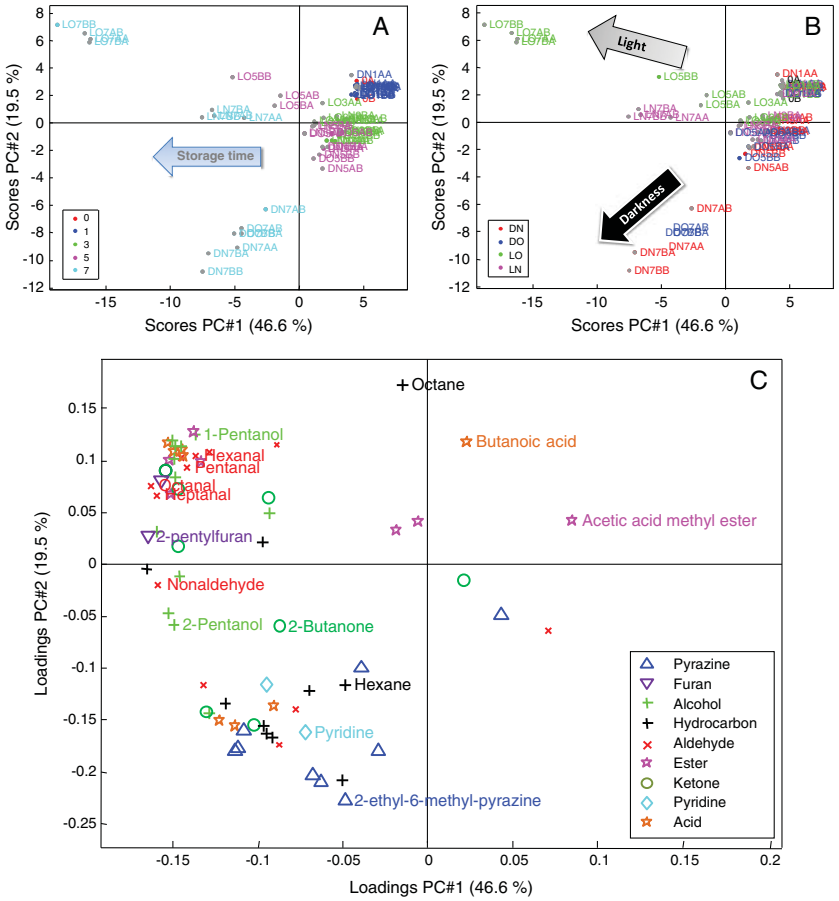

Fig. 3. Score plot of PC1 vs. PC2 from a PCA model including all the roasted marama bean samples packed in the presence or absence of oxygen and stored under different light conditions (light or dark) for seven months and all identified volatiles in these samples. A) colored according to months of storage and B) colored according to storage conditions. C) Loading plot of PC1 vs. PC2, colored according to chemical groups (selected labels are shown in order to give a better overview).

(Table 1). The majority of these compounds were typical secondary oxidation products, such as low molecular weight aldehydes, ketones, alcohols and short-chain hydrocarbons; many of these affecting the flavor of the product. Since there is no lipoxygenase activity in marama beans these volatiles must primarily arise from autoxidation and photosensitized lipid oxidation. The presence of pyrazines, pyridines and furans were attributed to Maillard reactions tacking place during the roasting process (Cerny, 2008; van Boekel, 2003). Most of the nine pyrazines identified in roasted marama beans are also found in roasted soybeans or roasted peanuts (Jung, Bock, Back, Lee, & Kim, 1997; Kato et al., 1981; Walradt, Pittet, Kinlin, Muralidhara, & Sanderson, 1971). From these food products, it is well known that pyrazines are partly responsible for the characteristic and desirable nut-flavor and that 2,5-dimethyl-pyrazine is the component which is most responsible for the pleasant nutty odor (Jung et al., 1997). The total pyrazine content increases with increasing roasting temperature (Abegaz, Kerr, & Koehler, 2004; Fadel, Mageed, & Lotfy, 2008; Jung et al., 1997) and continues to increase during storage. Likewise, pyridines are e.g. found in roasted coffee (Leino, Kaitaranta, & Kallio, 1992), but contributes to a distinctive, unpleasant fish-like odor.

A score and loading plot of a PCA model summarizing the results in Table 1 are shown in Fig. 3.

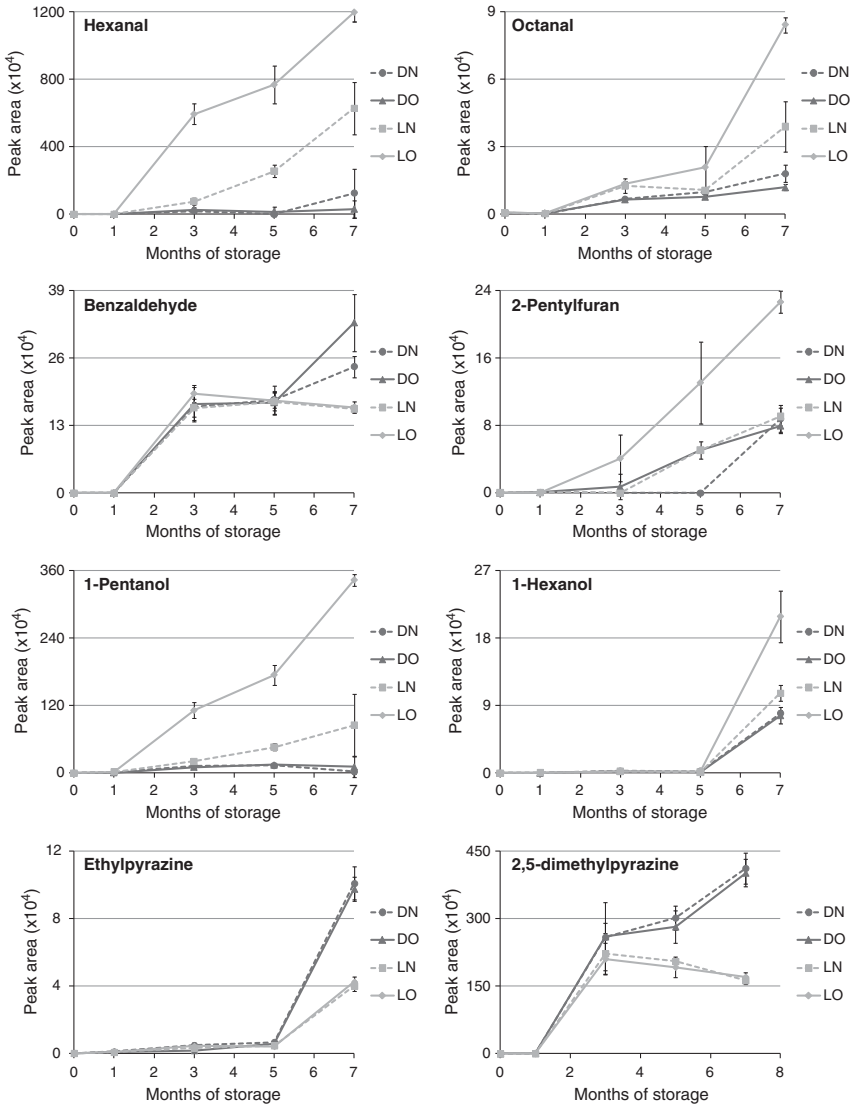

Fig. 4. Effect of storage on the level (given as peak area) of eight selected volatiles identified in roasted marama beans packed in the presence or absence of oxygen and stored under different light conditions (light or dark) for seven months. Data is presented as mean peak area (four replicates) with error bars indicating ± std. For several of the compounds, the increase is so small during the first months that it is not possible to see the actual increase on the figure. LO: samples stored in light in atmospheric air; LN: samples stored in light in nitrogen; DO samples stored in darkness in atmospheric air; DN samples stored in darkness in nitrogen.

In the score plot (Fig. 3A) the first principal component mainly describes the changes that occur over time. Focusing on the storage conditions (Fig. 3B) it appears that the samples stored in light and in darkness behave in different ways indicated by the two arrows. For the samples stored in light, it is noticeable that the largest difference between samples packaged in atmospheric air or nitrogen is seen at seven months and that this difference is larger than for samples stored in darkness. This goes hand in hand with the by far largest oxygen consumption in the samples stored in light and atmospheric air (Fig. 2). In a study on the storage stability of roasted peanuts, light exposure gave the greatest systematic variation in the formation of radicals as opposed to oxygen availability. Contrary, the development of hexanal was mainly affected by the oxygen availability (Jensen, Danielsen, Bertelsen, Skibsted, & Andersen, 2005).

From the corresponding loading plot (Fig. 3C) it appears that most of the volatile compounds are found in largest concentrations at longer storage times. This was also expected, since the production of secondary oxidation products will be most pronounced at longer storage times. This is in accordance with a study on the storage stability of roasted peanuts were they also found a larger production of secondary oxidation products at later storage times (El-Kayati, Fadel, Abdel Mageed, & Farghal, 1998). The plot furthermore reveals that pyrazines and pyridines are present in the highest amounts when samples are stored in darkness compared to light, while samples stored in light contain the largest concentrations of most of the identified esters and furans.

In order to visualize the development of the volatile compounds during the seven months of storage in more detail, eight selected volatiles (to show different chemical classes and oxidation developments) are plotted as peak area vs. months of storage (Fig. 4).

For the majority of the oxidation products (such as hexanal, octanal, 2-pentylfuran, 1-pentanol and 1-hexanol) the oxidation was most pronounced in the beans packaged in atmospheric air and stored in light as opposed to storage in atmospheric air and darkness or in packages flushed with nitrogen (whether in light or darkness). In general, the lowest levels of secondary oxidation products were formed in the samples stored in darkness. This fits well with higher levels of oxygen in the packaging for the roasted marama beans stored in darkness than in light. For many of the identified volatile compounds the largest increase in concentration is seen between five and seven months of storage.

On the contrary, some volatiles such as benzaldehyde and even more evidently the nut-flavor providing ethylpyrazine and 2,5-dimethylpyrazine are found in higher concentrations in the roasted beans stored in darkness. The lower concentrations of pyrazines in samples stored in light might to some extent be explained by depletion of several of the precursors for pyrazine formation in the presence of light. Whether this is the only explanation has not been investigated in this study.

3.3. Quantification of important flavor compounds — multiple headspace extraction

The actual concentrations of five selected volatiles were compared to their odor threshold values (Table 2). These volatiles are all well-known and undesirable oxidation products, which may be used as a measure of the oxidative status of the roasted beans (Choe & Min, 2006; Shahidi & Pegg, 1994). The actual concentrations are compared to reported odor detection thresholds of volatiles from oil samples. This was chosen (as opposed to threshold values found in water or air) since the roasted beans have a high lipid content. Volatile concentrations exceeding these threshold values give an indication, that the quality of the roasted beans is affected negatively.

For hexanal (a dominant oxidation product of linoleic acid and other ω-6 fatty acids, which are found in large quantities in marama beans), samples stored for seven months reach concentrations up to about 15700 ppb, which is far beyond the detectable odor threshold value for hexanal in oil (75–300 ppb). Already after three months of storage, the samples packed in the presence of oxygen and stored in either light or dark conditions reached values between 323 and 8431 ppb, making the sensory quality of these roasted beans questionable. For octanal (one of the major oxidation products formed from oleic acid) the roasted marama beans must be stored in darkness to ensure a proper quality after seven months of storage.

From the results in Table 2, it is evident that packaging the roasted marama beans without oxygen and storing them in darkness is the most optimal storage condition. If this is applied, the roasted beans may be stored for five months without obtaining unwanted off-flavors in levels above the detection thresholds. If any of the other three storage conditions (LO, LN, DO) are applied a good quality flavor can only be assured for the first month of storage.

Monitoring the development of hexanal is a key indicator of the progress in the undesirable lipid oxidation and can be used to ensure a proper quality of the roasted beans. Likewise, hexanal was found to be the limiting volatile compound in a storage stability study of marama bean oil (Holse et al., 2011). Additionally, a storage stability

Table 2
Concentration range of the quantified volatile compounds identified in the roasted marama beans packed in the presence or absence of oxygen and stored under different light conditions (light or dark) for seven months. The corresponding volatile threshold values in oil and the odor characteristics of the volatiles are also given. The samples that have passed the threshold value are indicated in bold letters.

Volatile compounds	Concentration range during month 0–7 for all storage conditions (ppb)	Odor detection threshold in oil (ppb)	Samples with concentrations above the threshold value	Odor characteristics[a]
Hexanal	0–15874	75–300[b–e]	Month 0–1: none **Month 3: LO, LN, DO** **Month 5: LO, LN, DO** **Month 7: All**	Fatty, green, grassy, powerful, penetrating and fruity
Octanal	0–240	56–900[b,c,e]	Month 0–5: none **Month 7: LO, LN**	Fatty, fruity, orange peel, pungent, soapy
1-butanol	0–255	500[a]	Month 0–7: none	Fusel-like sweet and pleasant
1-hexanol	0–55	400[f]	Month 0–7: none	Herbaceous, woody, fragrant, mild, sweet, green fruity
2-pentylfuran	0–114	250–6000[g]	Month 0–7: none	Fruity, green, pungent, sweet

[a] (Burdock, 2005).
[b] (Fazzalari, 1978).
[c] (Morales, Luna, & Aparicio, 2005).
[d] (Aparicio & Luna, 2002).
[e] (Reiners & Grosch, 1998).
[f] (Aparicio & Morales, 1998).
[g] (Min & Boff, 2002).

study on roasted peanuts confirmed that hexanal may be used as an indicator for oxidative changes (Jensen et al., 2005).

4. Conclusions

The results presented in this study, demonstrate the importance of controlling the storage conditions to ensure the quality of roasted marama beans. During storage, several secondary oxidation products are produced. Some are produced in concentrations exceeding their odor threshold values and thus, most likely deteriorating the quality of the roasted beans. For optimal storage, the beans should preferably be stored in darkness or alternatively in bags that do not transmit light. However, it was also shown that darkness alone cannot prevent oxidative deterioration in roasted marama beans when stored over a long time period. The amount of oxygen in the package also plays a major role and the bags should preferably contain the lowest possible amount of oxygen in order to prolong the shelf life. Storage in low oxygen concentration and in darkness resulted in a shelf life of five months without obtaining undesirable flavors (especially caused by hexanal) in the roasted beans.

Acknowledgments

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U-landsnyt.dk
DANMARKS UAFHÆNGIGE GLOBALE NYHEDSTJENESTE

Udgivet på *U-landsnyt.dk* (http://www.u-landsnyt.dk)

Vild ørkenbønne øger levestandarden for fattige afrikanere

10:16 | 06.08.10

Den 22.-26. august samles forskere i Sydafrika til verdenskongres om fødevarevidenskab og teknologi. Blandt deltagerne og talerne er forskere fra KU LIFE, der har opdaget, at en hidtil ukendt afrikansk bønne – Maramabønnen - som vokser vildt i ørkenområder med ekstreme vejrforhold har et enormt potentiale af næringsstoffer.

Af Mette Holse, ph.d.-studerende, Kvalitet og Teknologi, Institut for Fødevarevidenskab, LIFE, Københavns Universitet

I det barske ørkenmiljø, der ellers gør det meget vanskelligt at dyrke afgrøder, har den hidtil ukendte "Maramabønne" vist sig at være en glimrende kilde til både protein (ca. 38 %), fedt (ca. 33 %) og kostfibre (ca. 23 %). Den næringsrige sammensætning er ekstremt vigtig for befolkningen i disse områder, hvor fejlernæring er et udbredt problem. Mere end 20 % af den voksne befolkning er hiv-positive, og er derfor meget afhængige af at spise tilstrækkelige mængder af kalorier for at overleve.

En vifte af sunde indholdsstoffer
I vores forskning på KU-LIFE har vi opdaget, at maramabønnen er rig på flere vigtige vitaminer og mineraler. F.eks. indeholder bønnen høje koncentrationer af E-vitamin, som bl.a. virker som en kraftig antioxidant i kroppen, samt sporstofferne zink og jern, som en stor procentdel af befolkningen i Afrika lider af underskud af. Desuden indeholder bønnerne lignaner, der er forløbere til hormonlignende stoffer, som besidder en bred vifte af sundhedsfremmende virkninger.

Maramabønner kan hjælpe danske allergikere
Allergi er en af de hurtigst voksende sygdomme i den vestlige verden, og det er derfor yderst vigtigt at kende det allergiske potentiale af nye produkter. Folk der lider af allergi overfor visse bælgplanter kan risikere at reagere på andre bælgplanter, såsom maramabønnen, da proteinerne kan krydsreagere.
Peanut og lupin er nogle af de mest almindelige fødevarer, der er skyld i allergiske reaktioner i den vestlige verden. Derfor har vi testet, om bønnen indeholder proteiner, der kan krydsreagere med de allergene proteiner, som findes i peanut og lupin – og det gør den ikke! Det betyder, at vi kan anvende bønnen som erstatning for disse produkter, som en stor del af verdens befolkning er stærkt allergiske overfor.

Buskmændenes mirakel-middel
Maramabønnen vokser vildt i Kalahariørkenen i Namibia, Botswana og den nordlige del af Sydafrika. Her har Buskmændene (San-folket) igennem århundreder samlet og anvendt bønnerne. De er især blevet givet til syge, børn og gravide, som har ekstra brug for næring, men anvendes også som en delikatesse, da de har en lækker nøddeagtig smag, når de bliver ristet. Desuden er de blevet anvendt til udvindelse af olie, der kan bruges som creme eller i madlavning.

Nye maramaprodukter skal skabe sundhed og arbejde i Afrika
Den overordnede målsætning i det EU-finansierede projekt, som denne forskning er en del af, er at opnå viden om maramabønnen, som kan føre til produktion af nye sunde maramaprodukter. Produkterne skal kunne produceres ved hjælp af simpelt udstyr, der kan betjenes af de lokale, og produkterne skal derefter kunne sælges. Dette vil ikke blot forbedre de indfødtes ernæringsstatus, men også øge fødevaretilgængeligheden og muligheden for jobs for befolkningen i de fattige tørre landdistrikter i det sydlige Afrika.

Potentielle produkter er bl.a. koldpresset maramaolie, som kan bruges på samme måde som olivenolie, maramamel med et lavt fedtindhold, der kan anvendes til proteinberigelse af andre fødevarer eller ristede maramabønner, som kan spises som en snack ligesom peanuts.

Næste store skridt bliver at sætte gang i dyrkningen af maramabønnen i de egne, hvor den allerede i dag vokser vildt, så der kan høstes nok bønner til at sætte en kommerciel produktion af maramaprodukter i gang – og hvem ved, måske kan bønnen engang dyrkes i andre tørre egne verden over.

Finansiering
Projektet er finansieret af EU og er et spændende samarbejde mellem alt fra sociologer og marketingsfolk til naturvidenskabelige forskere fra både Europa of Afrika.

Læs mere om Marama-projektet på KULIFE: www.marama.life.ku.dk/ [2]
Læs om World Congress of Food Science and Technology http://www.iufost2010.org.za/ [3]

Ørkenbønne skaber nyt håb i Afrika

Landbrugsavisen.dk
Torsdag 12. august 2010 10:44

En næsten ukendt plante kan få stor betydning for sundheden i Afrikas ørkenområder. Det er danske forskere, der har fået øje på maramabønnens egenskaber.

I det barske afrikanske ørkenmiljø, hvor det meget vanskeligt at dyrke noget, har den hidtil upåagtede plante 'maramabønnen' vist sig at være en glimrende kilde til både protein, fedt og kostfibre.

Det er forskere fra Det Biovidenskabelige Fakultet på Københavns Universitet (KU Life), der har opdaget den hidtil ukendte afrikansk bønne, som vokser vildt i ørkenområder med ekstreme vejrforhold. Det oplyser U-landsnyt.dk.

Næringsrig
På grund af bønnens meget næringsrige sammensætning kan opdagelsen vise sig at blive ekstremt værdifuld for befolkningen i disse områder, hvor fejlernæring er udbredt. Mere end 20 procent af den voksne befolkning er hiv-positive og er derfor meget afhængig af at spise tilstrækkelige mængder af kalorier for at overleve.

"I vores forskning har vi opdaget, at maramabønnen er rig på flere vigtige vitaminer og mineraler. For eksempel indeholder bønnen høje koncentrationer af E-vitamin samt sporstofferne zink og jern, som en stor procentdel af befolkningen i Afrika ikke får nok af. Desuden indeholder bønnerne lignaner, der er forløbere til hormonlignende stoffer, som besidder en bred vifte af sundhedsfremmende virkninger," forklarer Mette Holse, ph.d.-studerende, Kvalitet og Teknologi, Institut for Fødevarevidenskab, KU Life.

22.-26. august skal hun sammen med andre forskere fra KU Life deltage i en verdenskongres om fødevarevidenskab og teknologi i Sydafrika, hvor maramabønnen bliver et af de mest interessante emner.

Målet er nu at sætte gang i en kommerciel produktion af maramabønner og

maramaprodukter. Måske kan bønnen også dyrkes på mere kølige breddegrader.

Buskmænd spiser den
Helt ukendt er maramabønnen nu ikke. Buskmændene i Kalahariørkenen i Namibia, Botswana og den nordlige del af Sydafrika har igennem århundreder samlet og anvendt bønnerne. De er især blevet givet til syge, børn og gravide, men anvendes også som en delikatesse, da de har en lækker nøddeagtig smag, når de bliver ristet.

Desuden er de blevet anvendt til at udvinde olie, der kan bruges som creme eller i madlavning.*Ritzau*

Ørkenens bønner

Alle fotos i artiklen: Jørgen Nielsen

Afrikas jord gemmer på mange hemmeligheder. Én af dem er genstand for et spændende forskningsprojekt på Institut for Fødevarevidenskab. San-folket i Kalahariørkenen har i århundreder kendt maramabønnens unikke egenskaber, og nu kan de komme resten af verden til gode.

Mette Holse er ph.d.- studerende på Institut for Fødevarevidenskab og fortæller om den nøjsomme plante, der lever vildt i de ekstremt tørre egne i Namibia, Botswana og Sydafrika:

"Den har en stor, op til 150 kg tung knold dybt nede i jorden, som samler den smule vand, der falder engang imellem. Herfra skyder lange grene henover jordoverfladen, og på trods af at planten gror et sted, hvor der næsten ikke er næring i jorden, har bønnerne alligevel et stort indhold af fedt, protein og kostfibre. De lokale plukker bønnerne og giver dem til især syge, børn og gravide. Fejlernæring og sygdom er udbredt i det sydlige Afrika, og derfor er der perspektiver i at sætte gang i en organiseret dyrkning af bønnen".

Godt nyt for allergikere

Der er indlysende fordele i at dyrke maramabønner i verdens tørre egne. Men hvad er potentialet i forhold til vores del af verden, hvor der ikke ligefrem er mangel på fedt og protein i maden?

"Her på stedet har vi fokuseret på de kemiske egenskaber, og fundet at maramabønner har et højt indhold af antioxidanten vitamin E. Til gengæld er der ikke nogle af de indholdsstoffer, der bl.a. betyder at mange reagerer allergisk på peanuts og andre bælgplanter. Man vil kunne bruge maramabønner i stedet for peanuts i mange produkter. Marama–proteinet kan oprenses og forædles til brug i andre fødevarer, og der kan presses olie til både madlavning og kosmetik. Der har været afprøvet mange produkter i løbet af projektet, og der har været lavet smagstests", siger Mette Holse, og fortsætter:

"Vores forskning er betalt af EU, og vi er syv forskellige partnere, bl. a. flere afrikanske universitetsinstitutter, der hver især har undersøgt forskellige aspekter af Marama–bønnens anvendelse. Det lige fra sociologer, der har lavet interviews med de lokale indbyggere, til marketingsfolk, der har undersøgt, hvilke produkter, der kan sælges på hvilke markeder".

Måske er Kalaharis hemmelighed på vej til at blive en del af et udbud af naturlige, sunde fødevarer på verdensmarkedet. På den måde hjælper forskningen verdens befolkning til bedre ernæring og gavner ulandenes økonomi – så hvem ved, en dag kan 'ørkenens bønner' om mere mad måske blive hørt.

Uddannelse indenfor fødevarevidenskab
Læs mere om LIFEs uddannelse i Fødevarevidenskab.

MARAMA-projektet er finansieret af EU's 6. rammeprogram.

HVAD FORSKER DU I?

Buskmændenes mirakelbønne

Mette Holse fra Københavns Universitet har fundet ud af, at den afrikanske maramabønne både kan forbedre ernæringen for de lokale, skabe job og erstatte allergifremkaldende fødevarer.

Hvad forsker du i?

»Jeg forsker i den kemiske sammensætning af den afrikanske maramabønne og i kvaliteten og holdbarheden af produkter fremstillet af den. For eksempel maramaolie, der kan bruges i madlavning og salater. Ristede maramabønner, der kan spises som snacks. Og maramamel med lavt fedtindhold, der kan anvendes som proteinberigelse af andre fødevarer«.

»Maramaplanten vokser vildt i ørkenområder omkring Kalahariørkenen i Namibia, Sydafrika og Botswana. Her kan kun få planter vokse på grund af tørke, men maramaplanten har en enorm knold, der ligger dybt i jorden, og som samler den smule regnvand, der falder. I århundreder har grupper som San-folket – buskmændene – samlet, gemt og anvendt bønnerne fra planten til syge, børn og gravide, som har brug for ekstra næring. Bønnen betragtes også som en delikatesse, da den har en lækker nøddeagtig smag, når den bliver ristet«.

Hvorfor har du valgt det emne?

»Af flere årsager. Ph.d.-forskning kan let blive fagnørdet, så det er fascinerende at arbejde hen imod noget så konkret som at forbedre levevilkårene for fattige i den tredje verden. Vi skal jo udvikle næringsrige produkter, der kan produceres ved hjælp af simpelt udstyr, og som derfor skaber job i de fattige tørre landdistrikter, samtidig med at de lokale får bedre ernæring. Det var også vigtigt for mig, at projektet gav mig mulighed for at samarbejde med andre forskere – jeg er en del af et større EU-finansieret projekt med partnere i seks lande og med meget forskellige faglige baggrunde. Og så har turene til Botswana, Namibia og Sydafrika været præcis så spændende og lærerige, som jeg havde håbet på«.

Hvordan har du grebet det an?

»Jeg har arbejdet med maramabønnen de seneste tre år. I begyndelsen brugte jeg mange timer på at undersøge bønnerne i laboratoriet ved hjælp af forskellige kemiske og ikkedestruktive spektroskopiske målemetoder. Det vil sige teknikker, der beskæftiger sig med stoffers vekselvirkning med elektromagnetiske bølger afhængigt af bølgelængden eller frekvensen, og som kan give en masse kemisk viden, uden at produktet, der undersøges, ødelægges. Det sker ellers ved traditionelle kemiske metoder. Senere handlede det om databehandling og artikelskrivning, og nu skal jeg færdiggøre selve ph.d.-afhandlingen – og forsvare den«.

Hvad har du så fundet ud af?

»Jeg har fundet ud af, at maramabønnen har en overraskende næringsrig sammensætning, på trods af at den gror i eks-

MICHAEL ROTHENBORG

giver næring til håb i Afrika

BØNNEFORSKER. **Ph.d.-studerende Mette Holse, Institut for Fødevarevidenskab på KU-Life, håber, at hendes projekt kan give konkrete resultater i form af bedre levevilkår for befolkningen i det sydlige Afrika.** Privatfoto

tremt tørre egne, hvor jorden er meget næringsfattig. Planten har vist sig at være en glimrende kilde til både protein (ca. 38 procent), fedt (ca. 33 procent) og kostfibre (ca. 23 procent). Denne næringsrige sammensætning er ekstremt vigtig for befolkningen i disse områder, hvor fejlernæring er et udbredt problem. Mere end 20 procent af den voksne befolkning er hiv-positive og er derfor meget afhængige af at spise tilstrækkelige mængder af kalorier for at overleve«.

»Desuden er maramabønnen rig på flere vigtige vitaminer og mineraler. For eksempel indeholder den høje koncentrationer af E-vitamin, som blandt andet virker som en kraftig antioxidant i kroppen, samt sporstofferne zink og jern, som en stor procentdel af befolkningen i Afrika har underskud af. Bønnerne indeholder også lignaner, som har en bred vifte af sundhedsfremmende virkninger«.

»Jeg har også fundet ud af, at maramabønnen ikke indeholder proteiner, der kan krydsreagere med de allergene proteiner, som findes i jordnød og lupin – nogle af de fødevarer, der er skyld i flest allergiske reaktioner i den vestlige verden. Det betyder, at maramabønnen vil kunne anvendes som erstatning for jordnød og lupin, som en betydelig del af verdens befolkning er allergisk over for«.

Hvad kan din forskning bruges til?

»I Afrika er der allerede holdt flere workshopper, hvor resultaterne fra hele projektet om maramabønnens gode egenskaber og anvendelsesmuligheder er blevet demonstreret for lokalbefolkningen, og mindre produktioner af maramaprodukterne er ved at blive etableret«.

»Forhåbentlig vil mine resultater være med til at vise det kæmpe potentiale, jeg mener, at maramabønnen har, og på den måde bane vejen for, at mine og de andre forskeres resultater ikke blot bliver til interessante forskningsresultater, men at bønnen også kan udbredes, opdyrkes og blive til produkter, som kan være med til at forbedre levevilkårene for befolkningen i det sydlige Afrika«.

michael.rothenborg@pol.dk

Nyhed

Mirakelbønne kan hjælpe mod underernæring - 14.10.2011

Forskere har fundet en bønne i Afrika, som gror under vanskelige forhold, men alligevel har en ernæringsmæssige sammensætning, der kan hjælpe med at bekæmpe underernæring. Samtidig kan bønnen også mindske allergi i Vesten.

Underernæring og fejlernæring er et stort problem i Afrika, hvor store dele af kontinentet er dækket af ørken. Dette gør dyrkning af grøntsager besværligt for lokalbefolkningen. En afrikansk bønne ved navn marama kan dog være med til at ændre på dette.

Bønnen er blevet undersøgt nøje for dens ernæringsmæssige fordele af ph.d. studerende Mette Holse, Institut for Fødevarevidenskab, LIFE, Det Biovidenskabelige Fakultet, Københavns Universitet.

Bønnen, som vokser i områderne omkring Kalahariørkenen i Nambia, Sydafrika og Botswana, har vist sig at have en overraskende næringsrig sammensætning på trods af at den vokser i ørkenen.

Mette Holse fortæller til Politiken: "Planten har vist sig at være en glimrende kilde til både protein, fedt og kostfibre".

Bønnen indeholder 29-38% protein, 32-42% fedt og 19-27% kostfibre og kan dermed blive en vigtig næringskilde.

Mette Holse fortsætter: "Desuden er maramabønnen rig på flere vigtige vitaminer og mineraler. Fx indeholder den høje koncentrationer af E-vitamin, samt sporstofferne zink og jern, som en stor procentdel af befolkningen i Afrika har underskud af".

Maramabønner bruges allerede af San-folket, der giver bønnerne til syge, børn og gravide, som netop har brug for ekstra næring.

Udover at bønnen kan være meget nyttig i Afrika, så kan den også være til gavn i vesten, da det viser sig, at den vil kunne anvendes som erstatning for jordnød og lupin, som en betydelig del af vestens befolkning er allergiske overfor.

Grunden til maramabønnen kan bruges i stedet, er at den ikke indeholder proteiner, som folk med allergi overfor jordnødder eller lupin ville reagere på.

Maramaplanten har en stor tung knold i jorden, som kan veje op til 150 kg. Knolden opsamler den smule vand, der falder i Kalahariørkenen, hvor den gror, og kan indeholde op til 90% vand.

Læs mere om bønnen i artiklen "Ørkenens bønner" og på Marama projektets egen side

Kilder og links til yderligere læsning:

Politiken, Viden, d. 18. september 2011

Holse, M, Larsen, FH, Hansen, ÅS & Engelsen, SB (2011). Characterization of marama bean (Tylosema esculentum) by comparative spectroscopy: NMR, FT-Raman, FT-IR and NIR. *Food Research International*, 44(1), 373-384.

Holse M, Husted S, Hansen ÅS (2010). Chemical composition of marama bean (Tylosema esculentum) a wild African bean with unexploited potential. *Journal of Food Composition and Analysis*, 23(6), 648-657.

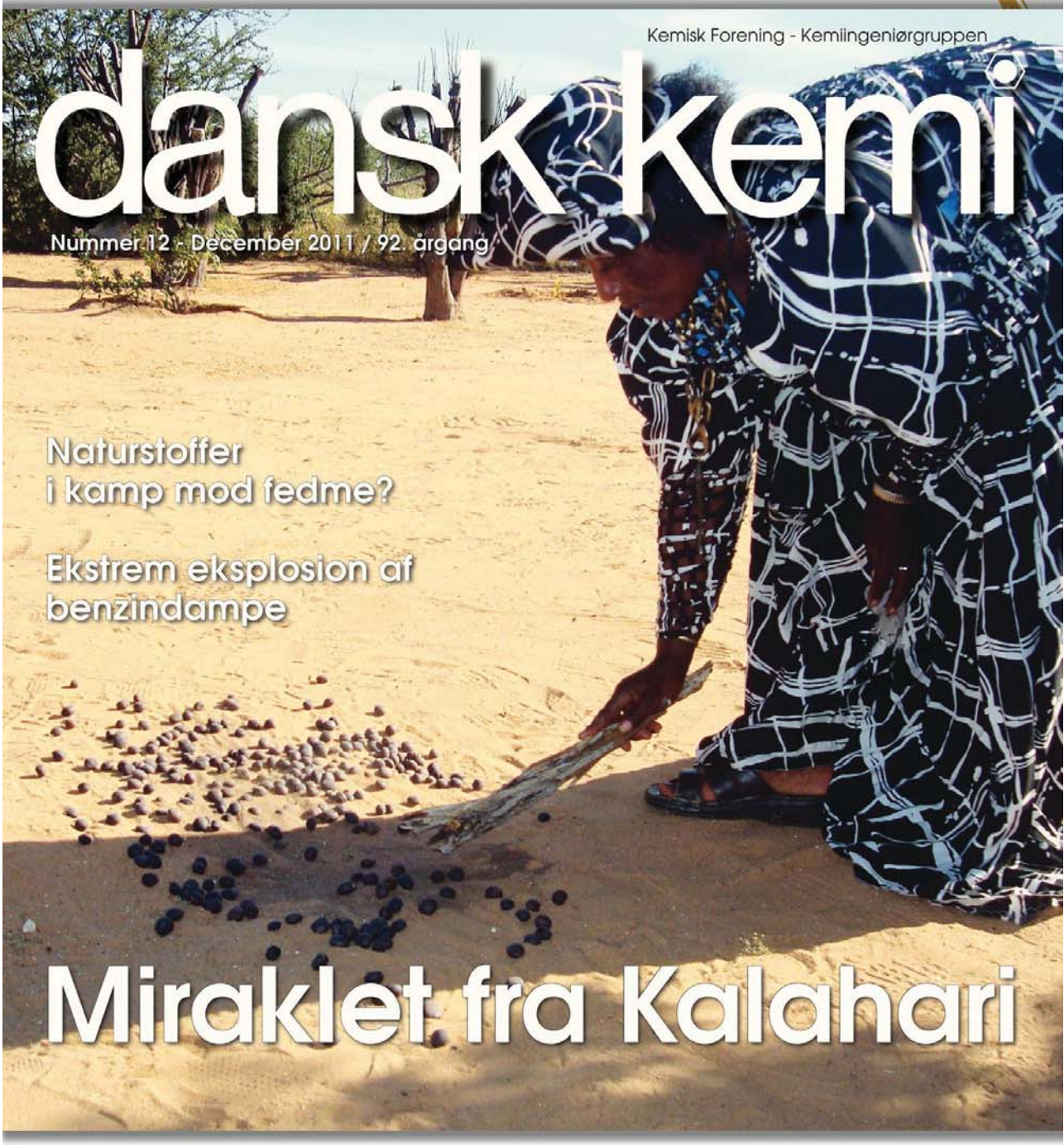
Jackson JC, Duodu KG, Holse M, Faria MD.LD, Jordaan D, Chingwaru W et al (2010). The morama bean (Tylosema esculentum): a potential crop for southern Africa. *Advances in Food and Nutrition Research*, 61(5), 187-246.

Et fagblad fra TechMedia

Kemisk Forening - Kemiingeniørgruppen

dansk kemi

Nummer 12 - December 2011 / 92. årgang



Naturstoffer i kamp mod fedme?

Ekstrem eksplosion af benzindampe

Miraklet fra Kalahari

Miraklet marama – bønner der høres

Maramabønnen er helt unik. Den er ekstremt næringsrig, om end den selv er meget nøjsom og derfor kan vokse i det barske ørkenmiljø i det sydlige Afrika, hvor det ellers er meget vanskeligt at dyrke afgrøder.

Maramaplanten med blomster (sommer).

Af Mette Holse, ph.d.-studerende, Kvalitet og Teknologi, Institut for Fødevarevidenskab, LIFE, KU

Maramabønnen vokser vildt i Kalahariørkenen i Namibia, Botswana og den nordlige del af Sydafrika. Her trives den i næringsfattig sandjord, hvor der kun er sparsom adgang til vand. Den smule regn der falder samles i en kæmpemæssig knold, som findes dybt under jorden – knolden kan veje op til 150 kg og indeholde op til 90% vand.

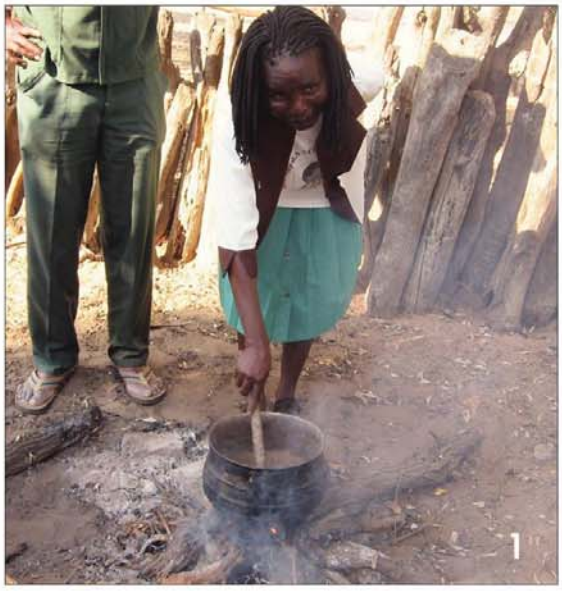
En vifte af sunde indholdsstoffer

Maramabønnen (*Tylosema esculentum*) er en glimrende kilde til både protein (ca. 38%), fedt (ca. 33%) og kostfibre (ca. 23%).

Modne (brune) og halvmodne (grønne) bælge samt modne maramabønner.

Maramaknold – kan veje op til 150 kg og indeholde op til 90% vand.

Maramabønnen vokser vildt og samles ved håndkraft. Her ses de indsamlede maramabønner.



Fedtet er primært til stede i form af umættede fedtsyrer (ca. 75%), hvoraf olie- og linolsyre udgør størstedelen. På denne måde minder maramaolien kemisk set om olivenolie. Olien har en flot gul farve og en behagelig nøddeagtig smag dog med en anelse bitterhed. Olien har potentiale både som fødevare og kosmetik.

Aminosyresammensætningen af maramaprotein opfylder stort set den menneskelige organismes behov. Aminosyren lysin, der kun findes i små mængder i kornsorter, er til stede i rigelige mængder (ca. 5%) i protein fra marama. De svovlholdige aminosyrer, methionin og cystein (total ca. 1,3%), findes i større mængder end i de fleste andre bælgfrugter og i mængder der stort set er tilstrækkelige til at dække det menneskelige behov. Desuden findes der store mængder af tyrosin, asparginsyre og glutaminsyre. Proteinrigt maramamel kan derfor med fordel anvendes til berigelse af f.eks. sorghumgrød, og giver endda denne basisfødevare for mange i Afrika en bedre smag.

Kulhydraterne i maramabønner er hovedsageligt uopløselige polysaccharider. Kun 4% af kostfibrene er opløselige og der er under 1% stivelse og opløseligt sukker.

Maramabønner er rige på flere vigtige **vitaminer og mineraler**. De er en god kilde til C- og B-vitaminer og indeholder høje koncentrationer af E-vitamin, som bl.a. virker som en kraftig antioxidant i kroppen. De indeholder rigelige mængder uorganiske forbindelser med kalium, fosfat, magnesium og calcium. Sporstofferne zink og jern, som en stor procentdel af afrikanere mangler, findes i høje koncentrationer i bønnerne.

Bønnernes **lignaner** besidder en bred vifte af sundhedsfremmende virkninger.

Den næringsrige sammensætning er ekstremt vigtig for befolkningen, da både under- og fejlernæring er udbredt. Mere end 20% af den voksne befolkning er hiv-positive og derfor meget afhængige af at spise tilstrækkelige mængder af kalorier for at overleve.

Potentiale for et hav af nye maramaprodukter

Kun fantasien sætter grænser for hvilke produkter, der kan fremstilles af maramabønner.

Buskmændenes mirakelmiddel

Det er ikke noget nyt, at maramabønnen er et "mirakelmiddel". Buskmændene (San-folket) og flere andre indfødte grupper såsom Herero- og Tswanafolkene i det sydlige Afrika har igennem århundreder samlet og gemt maramabønner. Bønnerne er især blevet givet til syge, børn og gravide, som har ekstra brug for næring, men betragtes også som en delikatesse, da de har en lækker nøddeagtig smag, når de ristes.

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Foto 1, 2 og 3 viser hvordan lokale kvinder rister maramabønnerne i sand i en gryde over bål.

Et EU-finansieret projekt (Development of Innovative and Healthful Marama Bean (*Tylosema esculentum*) Products Targeting Niche Markets) er netop afsluttet og har ført til undersøgelser af produkter som koldpresset maramaolie, der kan bruges ligesom olivenolie. Maramamel med et lavt fedtindhold kan anvendes til proteinberigelse af andre fødevarer. Ristede ▶

Som erstatning for peanuts
Maramabønnen kan have potentiale på det vestlige marked. Undersøgelser har vist, at bønnen ikke indeholder proteiner, som folk med allergi over for jordnød eller lupin reagerer på. Disse fødevarer er skyld i flest allergiske reaktioner i den vestlige verden. Det betyder, at maramabønnen kan anvendes som erstatning for jordnød og lupin i mange forskellige produkter, som en betydelig del af verdens befolkning nu er allergiske over for.

Efter afkøling i sandet afskalles maramabønnerne vha. en sten.

maramabønner kan spises som en snack ligesom peanuts. Der kan fremstilles produkter i stil med dem fra sojabønner, nemlig mælk, smør, is, kød-analoger og snackprodukter som småkager, kiks og kager.

Produkterne må produceres vha. simpelt udstyr, der kan betjenes af de lokale, og skal kunne sælges. Det vil ikke blot forbedre beboernes ernæringsstatus, men også øge fødevaretilgængeligheden og muligheden for job i de fattige tørre landdistrikter i det sydlige Afrika.

Næste skridt

Der er allerede afholdt workshops i det sydlige Afrika, hvor maramabønnens gode egenskaber og anvendelsesmuligheder er blevet demonstreret for lokalbefolkningen. Mindre produktioner af maramaprodukterne er under etablering. Et større dyrkningsprojekt er etableret i Namibia, så der i fremtiden kan høstes nok bønner til at sætte en kommerciel produktion af maramaprodukter i gang.

Forhåbentlig vil bønnen i nær fremtid blive udbredt, opdyrket og anvendt til produkter, som kan være med til at forbedre levevilkårene for befolkningen i det sydlige Afrika gennem såvel bedre næring, øget fødevaretilgængelighed og flere job.

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Kilder
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