FACULTY OF BIOSCIENCE ENGINEERING

Moringa leaf concentrate (MLC) and moringa leaf powder (MLP): a comparative study

An analysis of their nutritional composition, bioavailability, acceptability, production techniques, economy and marketing

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DEDICATION

This dissertation is dedicated to my beloved parents, especially my late father, who has supported me all my life and who has given me the determination to complete this dissertation.

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By

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PREFACE

I was first introduced to Moringa by my father years ago. He was familiar with it in his childhood and started experimenting with Moringa leaf powder in the kitchen. The novelty of it soon wore off and we forgot about the Moringa pot in the cupboard. Nertheless, I always remained interested in this special tree and was hence immediately drawn to a thesis topic on Moringa I had discovered on the university website. At that moment, I was not due to start my dissertation yet and needed to wait one year, by which time the topic had vanished. Luckily, through a lot of digging and e-mailing, I got into touch with Prof. Dominique Bounie who then directed me to APEF. It is at this point my journey started and I am extremely grateful for the opportunity provided by APEF to work for a few months in Nicaragua, researching Moringa for my Master's dissertation.

This dissertation reflects my personal interests – a wide range of socio-economic research, accompanied by no-nonsense fact-checks in the lab. This study aims to provide a reference base regarding perceptions of Moringa, nutritional values of Moringa leaves and associated costs, albeit not exhaustive, for the consortium of NGOs working on a larger project in Nicaragua. The overall objective of this larger NGO-led project would be to spread the concepts of making leaf concentrate and/or leaf powder of Moringa, as a contribution to human diets, in particular for anaemic children in Nicaragua.

The journey to completing a dissertation is a long one, and at times, a tough one. Yet what has gotten me through these tougher times, were my reminders of why I started this dissertation on Moringa in the first place and my fond memories of my father's enthusiasm for my research.

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LIST OF ABBREVIATIONS

AMF	Arbuscular Mycorrhizal Fungi
ANF	Antinutritional Factor(s)
DM	Dry Matter
FW	Fresh Weight
GAE	Gallic Acid Equivalents
ICP-OES	Inductive Coupled Plasma Optical Emisson Spectometry
LD_{50}	Lethal Dose for 50% of subjects
M	Mass
MLC	Moringa Leaf Concentrate
MLP	Moringa Leaf Powder
ROS	Reactive Oxygen Species
PDCAAS	Protein Digestibility-Corrected Amino Acid Score
PER	Protein Efficiency Ratio
SOP	Standard Operation Procedure
TCAM	Traditional, Complementary and Alternative Medicine
QE	Quercetin Equivalents

SUMMARY

Moringa oleifera Lam. has a wide variety of uses described in literature and is a multi-purpose tree. It is gaining recognition in research, as a trade commodity and in humanitarian aid projects. The present dissertation aims to distinguish the differences and similarities of two Moringa-based leaf products, i.e. Moringa Leaf Powder (MLP) and Moringa Leaf Concentrate (MLC) and its potential for a development project in Nicaragua. Both products are analysed for their production methods, local perceptions and acceptability, nutritional value, costs and benefits.

To assess local perceptions and acceptability, both interviews and two sensorial analyses were conducted in Nicaragua. Interviews showed that Moringa was mainly associated with medicinal values and has a great diversity in cultivation methods. The sensorial analyses indicated that acceptability may depend on recipe formulation.

Nutritional compositions determined in this study were mostly in line with literature. In light of this project, MLC tended to have a higher nutritional value than MLP, thanks to a higher energy content, higher crude protein content and higher iron and zinc content. What is more, MLC also contained less antinutritional factors, i.e. condensed tannins, phenolic compounds and phytic acid, which could seemingly increase absorption of iron. However, *in vitro* digestibility experiments could not provide conclusive evidence of an increased bio-accessibility of iron and zinc in food products containing MLC. There is an indication that the effect of mineral enhancers plays a part in increasing the bio-accessibility of iron and zinc in MLC, but not in MLP.

Regarding costs, MLC is generally more expensive than MLP. The production process for MLC would need to improve in efficiency for it to become cheaper than MLP, but only when scaling both products to an equal amount of protein or dialyzable iron. On an equal weight basis, MLP remains the cheaper option. Valorisation of by-products of the MLC process, i.e. fiber and whey, could contribute in the long run to lowering the cost.

Therefore, it seems that at household level in Nicaragua, MLP seems to be more cost- and time-effective. Out of the present study, the nutritional benefits of MLC are clear, but they do not seem to outweigh the previously mentioned costs.

SAMENVATTING

Moringa oleifera Lam. heeft een brede waaier aan gebruiken die beschreven staan in de literatuur en is een boom die bestemd is voor meerdere doeleinden. De boom krijgt meer en meer erkenning in onderzoek, op de markt en ook in humanitaire projecten. Deze masterproef probeert de verschillen en overeenkomsten te duiden tussen twee Moringa bladeren-gebaseerde producten, d.i. Moringa bladpoeder (MLP) en Moringa bladconcentraat (MLC), en hun potentieel voor een ontwikkelingsproject in Nicaragua. Beide producten werden geanalyseerd voor hun productiemethoden, lokale percepties en acceptatie, nutritionele waarde, kosten en voordelen.

Bij het onderzoeken van de lokale percepties en de acceptabiliteit, werden zowel interviews als sensorische analyses afgenomen in Nicaragua. Interviews toonden aan dat Moringa voornamelijk geassocieerd werd met medicinale gebruiken en dat er een grote diversiteit was in cultivatiemethoden. De sensorische analyses gaven weer dat de acceptabiliteit van een product mogelijks afhangt van het geformuleerde recept.

De nutritionele samenstelling zoals bepaald in deze studie lag grotendeels in de lijn van de literatuur. In het kader van dit project bleek MLC een hogere nutritionele waarde te hebben dan MLP, dankzij een hogere energie-inhoud, een hoger eiwitgehalte en een hoger ijzer- en zink-gehalte. Bovendien bevatte MLC minder antinutritionele factoren, zoals gecondenseerde tannines, fenolische componenten en fytinezuur, wat de bio-beschikbaarheid van ijzer zou kunnen verhogen. Desondanks konden de *in vitro* verteringsproeven geen uitsluitsel geven over het feit of producten met MLC een hogere bio-beschikbaarheid van ijzer en zink hadden. Er is wel een indicatie dat het effect van minerale absorptiebevorderaars een rol speelt bij de bio-beschikbaarheid van ijzer en zink in MLC, maar niet bij MLP.

Wat betreft de kosten, is MLC doorgaans duurder dan MLP. Het productieproces van MLC zou efficiënter moeten worden, opdat het goedkoper zou worden dan MLP, maar enkel en alleen wanneer beide producten worden uitgedrukt in eenzelfde hoeveelheid eiwit of dialyseerbaar ijzer. Bij eenzelfde eenheid gewicht, blijft MLP de goedkopere optie. De valoristaie van nevenproducten uit het MLC-proces, d.i. vezel en wei, kunnen bijdragen aan het verlagen van de prijs op lange termijn.

Aldus zou het blijken dat op het niveau van het huishouden in Nicaragua, MLP de meer kosten- en tijdsefficiënte optie zou zijn. Uit de huidige studie komen de nutritionele voordelen van MLC duidelijk naar voren, maar ze wegen niet op tegen de eerder genoemde kosten.

INTRODUCTION

Moringa oleifera Lam. is a tree-species that has rapidly spread all over the world, thanks to its adaptability to different climates. Its reputation as a “miracle tree” – nutritionally speaking – has sparked academic interest in this species, yet research on Moringa has only picked up over the last few years, mostly with regard to its possible use as a novel food source. As such a source, mainly the leaves – be it fresh or in dried form – are of most interest.

Leaf protein concentrate, a plant-based product, is novel and has not been researched in Moringa. Leaf concentrates have been studied in animal feeds. All the same, the product and its effects on the human body are largely unknown. A research project was initiated by APEF in Nicaragua, where Moringa is already being cultivated in plantations and where there is a high incidence of malnutrition. The objective is to produce Moringa leaf powder and leaf concentrate in Nicaragua, evaluate the feasibility of production for local households or communities, determine its nutritional composition and digestibility and finally, to determine which product is more suitable to the Nicaraguan context. The working hypothesis herein is that leaf concentrate will have higher concentrations of microminerals and proteins, with a higher bio-availability, and will hence be favourable for local production and local needs. Nutritional value in this context relates to the larger objectives of the research project, i.e. having the potential to be useful in humanitarian projects regarding malnutrition.

In this dissertation, *Moringa oleifera* Lam. will be referred to as “Moringa”. This research is a joint effort between the University of Ghent, the French NGO “*Association pour la Promotion des Extraits Foliaires en nutrition*” (APEF), the American NGO “*Leaf for Life*”, the Nicaraguan NGO “*Soynica*” and Polytech Lille, in order to contribute to this relatively novel area of research.

REVIEW OF LITERATURE

Introduction to the Moringa tree

Moringa oleifera Lam. is a subtropical tree, native to the Indian subcontinent, where it was described as early as 2000 B.C. (Oluduro *et al.*, 2016). It is commonly called “Moringa” or “drumstick”, the last referring to the shape of its pods (Ramachandran *et al.*, 1980). At times, it also goes by the name of “horseradish tree” or “ben oil tree” (Oluduro *et al.*, 2016) and in Senegal, the tree is called “Nebeday”, probably derived from the English words “never die” (Fuglie, 2001). Throughout the years, it has spread to the rest of Asia, Africa and Latin-America. Moringa is well-known as the miracle tree, due to its high nutritional value, its many medicinal benefits and uses, and its disease- and drought-tolerance (Oluduro *et al*., 2016; Foidl *et al*., 2001). The leaves, pods, seeds, flowers and roots can be consumed and the bark can be used for its fiber (Ramachandran *et al.*, 1980). Furthermore, studies have found that *Moringa oleifera* Lam. seeds can purify water (Anwar *et al*., 2007) and it is because of these interesting properties that research interests have slowly picked up regarding Moringa.

History and taxonomy

Moringa was already known by the Romans, Greeks and Egyptians (Fahey, 2005). Ancient Egyptians would use Moringa oil in wrinkle removal formulas (Kleiman *et al*., 2006). A search with Google Books Ngram Viewer (20/11/2017) between 1750 and 2000 reveals that the term “Moringa” was coined around the 1780s. The species was first misclassified under the genus of *Guilandina*, a genus with similar looking leaves and flowers and part of the Fabaceae family. Now it is part of the Moringaceae family and has its own genus. The taxonomical family encompasses up to 14 different species, amongst which *Moringa oleifera* Lam. is the most studied (Mekonnen, 2002). There are several other important species, such as *Moringa stenopetala* (Baker f.) Cufod, which is an important species in certain parts of Africa (Mekonnen, 2002). *Moringa peregrina* (Forssk.) Fiori is another species originating from the more arid regions around the Red Sea and the Horn of Africa. Lastly, *Moringa pterygosperma* Gaertn. is often mentioned too, but it is the old taxonomical name of *Moringa oleifera* Lam. (Bosch, 2004).

Research on Moringa leaves is relatively limited, compared to other tropical and subtropical leaf crops. A search with Google Scholar (9/12/2017) shows that the keyword “tea” yields 3 million results, while the keywords “Moringa leaves” yield about 24 000 results. A similar search was conducted by Mbikay in 2012 when using both PubMed and Google Scholar, where the scientific interest in Moringa was rather mild as well.

Geographical distribution

The Moringa tree is native to the sub-Himalayan area, including India, Bangladesh, Afghanistan and Pakistan. Moringa is widespread nowadays in the tropics and in countries such as India, Egypt, Philippines, Thailand, Cuba, Jamaica and Nigeria (Fahey, 2005; Ramachandran *et al*., 1980), as can be seen in figure 1. It is cultivated in the Middle East, the subtropical and tropical zone and was introduced in East-Africa in the beginning of the 20th century (Foidl *et al*., 2001). According to work by Berger *et al.* in 1984, the British colonialists took the tree for ornamental purposes to Africa (as cited in Morton, 1991).

*Figure 1: Geographical distribution of Moringa in the world, indicated by the green areas (*Bioweb.uwlax.edu, 2017)

Moringa in Nicaragua

In Nicaragua, the tree was introduced in the 1920s as an ornamental tree and used for hedges. The tree, commonly known in Nicaragua as “Marango”, has mainly spread in the Western part of the country (Foidl *et al*., 2001). *Proyecto Biomasa* is an agricultural research program in Nicaragua that conducted several experiments with Moringa, such as making leaf extracts that contain plant growth hormones and Moringa’s use as green manure, livestock feed or water treatments (Price, 2007; Fuglie, 2000).

Morphology

Moringa is a perennial tree (Foidl *et al*., 2001). The tree can grow up to 15 m tall, but mostly stays in the range between 5 m and 10 m. Its trunk can be up to 25 cm thick and its branches are fragile and tend to droop (Morton, 1991). The roots, amongst which the main taproot, are tuberous and soft (Ramachandran *et al*., 1980).

Figure 2: (Left) Botanical drawing of a Moringa leaf, including a part of its fruit, the leaf stem (petiole), the rachis and the leaflets (pinnae). (Right, top left) Moringa flower panicle. (Right, top right) Moringa fruits or pods. (Right, bottom left) Moringa leaflets composed of pinnules, or secondary leaflets. (Right, bottom left) Moringa flower.

The leaves are double or triple pinnate (figure 3), are about 20-70 cm long and tend to develop mainly at the terminal end of the branches. The smaller leaflets are 1-2 cm in length (Oluduro *et al*., 2016; Foidl *et al*., 2001; Morton, 1991). When assessing the available literature, attention must be paid to the exact definition of the term “Moringa leaf” used, whether this is an indication of the pinnules, the secondary leaflets or the whole leaf structure above the petiole.

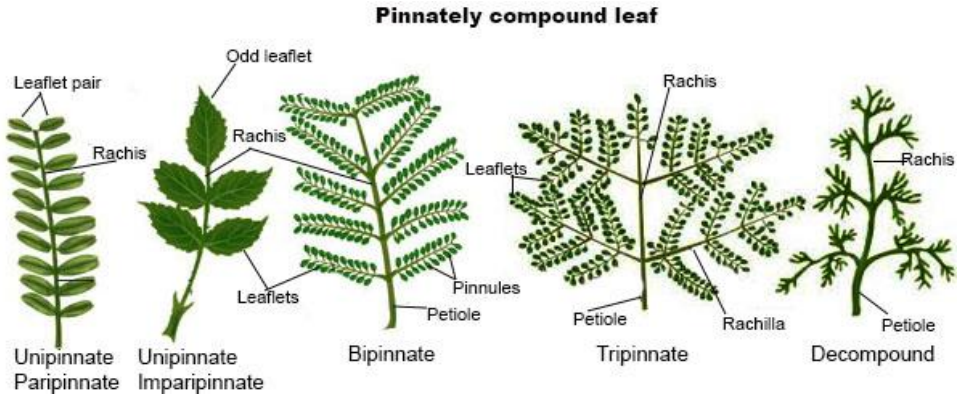

Figure 3: Overview of the botanical nomenclature of a pinnately compound leaf (Kullabs, 2017)

The flowers are white, zygomorphic, bisexual, about 2.5 cm in length and fragrant (Foidl *et al*., 2001; Ramachandran *et al*., 1980). Furthermore, the flowers form a panicle of about 15 cm in length. The tree can flower throughout the whole year in certain countries (Orwa *et al*., 2009). In India, Moringa flowers twice a year in February-May and September-November. Cross-pollination is the main form of pollination, but self-pollination can also occur. The main pollinators are diurnally active insects, mainly different bee species and the flowers contain a nectar, consisting mainly of glucose (Jyothi *et al*., 1990).

The fruits resemble long bean pods and are typically 20-60 cm long (Foidl *et al*., 2001). The fruits, resembling drumsticks, are first green and turn brown when mature (Heuzé *et al*., 2014). When the pods dry, they split open in three parts and reveal small seeds. One pod can contain up to as much as 35 seeds and an annual production of 15 000 – 25 000 seeds is possible per tree. The seeds are round, have white wing-like attachments and weigh about 0.3 g on average (Foidl *et al.*, 2001). In figure 2, a depiction of the leaves, flowers and fruits of Moringa are provided.

Ecology

The tree does not tolerate freezing temperatures or frost (Mridha, 2015). It grows well at lower levels of elevation, but can be found at heights up to 1350 m in East Africa. Furthermore, it is drought tolerant and it has been found in arid regions with as little as 500 mm rain a year (Bosch, 2004). Moringa grows in all types of soils, except heavy clay soils (Ramachandran *et al*., 1980). Nonetheless, it benefits most from fertile and well-drained soils. Moringa contributes to ecosystem services by improving the soil, controlling erosion, its ornamental value and pollution control. Additionally, in intercropping or agroforestry systems, it can provide protection from wind (Bosch, 2004).

Figure 4: Overview pictures of Moringa trees in a cultivation in Nicaragua (credit: B. Peddi)

Cultivation practices

The plant is vegetatively propagated using branch cuttings of about 1 to 2 m long or it is propagated by seeds (Mridha, 2015). Tissue culture seems to be possible too (Mridha, 2015; Islam *et al*., 2005). However, for tissue culture to be viable, the economic benefits of Moringa need to be higher.

Seeds are sown in a nursery bed or directly into the field, at a depth of about 2 cm. The seed germination rate is 80% for fresh seeds, but declines to 50% after 12 months storage. Germination can take up to two weeks and is positively influenced by shade. Initially, seedlings are watered twice a day, but this is reduced to once a day once the seedlings are about 15 cm tall. In the initial growth phase, seedlings grow up to 40 cm in 3 months and are ready for transplanting. Following transplantation, the small trees can grow up to 3 to 4 m in one year (figure 4). Flowering starts after two years for trees grown from seed, but for trees grown from cuttings, the first fruits could be expected as soon as 6 to 12 months after planting. In a Moringa monoculture as a short-duration crop, an intra- and interrow distance of 0.7 to 1 m is used, whereas for long-term production 3 to 5 m is used. For alley cropping, a distance of 2 m is commonly used (Bosch, 2004). The roots of the Moringa tree are considered to be dependent on mycorrhiza and hence inoculation with arbuscular mycorrhizal fungi (AMF) is beneficial to growth (Mridha, 2015).

The trees benefit from fertilizer application: manure or chemical fertilizers. Irrigation is necessary in the dry season. To promote branching of the trees and therefore their yield, pruning, pollarding – i.e. cutting the trees back nearly to the trunk – and coppicing are recommended. This also facilitates harvesting by keeping the trees at working height. Pruning can be done once or twice a year (Bosch, 2004). Furthermore, Moringa does not suffer a lot from diseases or pests, yet it differs according to the locality. Termites may be a problem, as are aphids, certain caterpillars, borers and fruit flies (Bosch, 2004).

Moringa leaf production has the highest yields in the rainy season. In Niger, the yields can reach up to 600 kg per month for a plot of 1000 m². In the dry season, yields can reduce to 50 kg per month, if the crop is not irrigated. Overall, the annual production of fresh leaves is about 27 ton per hectare in this setting. The harvesting is done by stripping the leaves from the branches and afterwards the leaves are packed in plastic bags for transport. Harvesting can be done up to twice a month. After harvesting the leaves, they can be dried or pulverised for storage. For seed production, the yields are about 3.3 kg for a four-year-old tree in Tanzania per year. In India, a productive tree can yield up to 1000 fruits per year. The harvest of green fruits starts about 7 months after planting, while dry fruits are ready for

harvest after about 8.5 months. Harvested seeds need to be protected from insect damage during storage (Bosch, 2004). Seeds should be stored at 3 °C with a moisture content of 5% to 8% (Orwa *et al*., 2009).

Since Moringa's centre of origin is probably in or near India, this is where the highest genetic variation can be found. The genus is highly heterogenous, mainly due to cross-pollination, resulting in high variations in reported yields. There is germplasm available in gene banks in Burkina Faso and the Philippines. Regarding breeding and developing varieties, there has been some work undertaken in India. There, a short-stem variety of Moringa has been developed called "PKM1". Farmers often grow this variety as an annual crop with two harvests a year for seeds. So far, breeding has focused on fruit yield and not so much on leaf yield. Hybridization with other Moringa species might be an option to cultivate higher-yielding varieties. The chromosome number (*2n*) of Moringa is 28 (Bosch, 2004).

Ethnobotanical uses of Moringa

Food

Young leaves are edible and are often eaten as a spinach, a soup or a salad (Joshi & Mehta, 2010; Maroyi, 2006; Foidl *et al*., 2001). However, when preparing the leaves in a leaf sauce, the leaves are boiled several times to get rid of the bitter taste, causing a loss of nutritional value (Fuglie, 2001). The bitter taste of seeds is mainly attributed to alkaloids, saponins, glucosinolates and cyanogenic glucosides (Makkar & Becker, 1997). It can be hypothesised that one or more of these components are also present in the leaves.

The bean-like pods and seeds can be boiled and eaten (figure 5), however, they must be green and a thin seed-skin must be removed before consuming seeds (Foidl *et al*., 2001). The pods can be pickled as well (Ramachandran *et al*., 1980). Dry leaves or pods seeds can be pulverised into a powder and can be used to season sauces or other preparations (Joshi & Mehta, 2010; Maroyi, 2006; Foidl *et al*., 2001). Flowers can be eaten raw in salads or can be cooked briefly (Foidl *et al*., 2001). They are sometimes used in a tea (Heuzé *et al*., 2014). Roots used to be consumed as a condiment, but this practice is no longer recommended due to the high alkaloid concentrations in the root (Maroyi, 2006). Finally, in bee-keeping practices, a honey can be produced from this tree (Maroyi, 2006; Mridha, 2015).

Figure 5: In India, Moringa fruit is used in traditional dishes, like 'sambar', as shown here (Joshi, 2018)

Oil

The oil of Moringa is commercially known as Ben or Behn oil, is edible and can be used for cosmetics or illumination (Ramachandran *et al*., 1980). It is also used as a cooking oil (Foidl *et al*., 2001). Industrially, Moringa oil is used as a machine lubricant, especially for fine machinery as used by watchmakers (Ramachandran *et al*., 1980). In the perfume industry, it is used as well due to its capacity to retain volatile compounds (Foidl *et al*., 2001). Finally, there is ongoing research in using Moringa oil in the cosmetic industry (Kleiman *et al*., 2008).

Medicine

Moringa products are used in traditional medicine for diabetes and cardiovascular diseases. There are scientific indications that Moringa holds therapeutic potential for chronic hyperglycemia and hyperlipidemia, as symptoms of diabetes and cardiovascular diseases. Its therapeutic potential is often explained by the relatively high antioxidant activity of its leaves, flowers and seeds. Reactive oxygen species (ROS) and free radicals are the main contributors to oxidative stress in cells, if these compounds are not reduced by certain pathways and oxidative stress is a major contributing factor to cardiovascular diseases and diabetes. In Moringa, mainly flavonoids seem to be responsible for the antioxidant activity of the plant (Mbikay, 2012).

The plant also seems to have anti-inflammatory properties. What is more, Moringa contains phytosterols – such as beta-sitosterol – which could reduce the dietary intake of cholesterol (Mbikay, 2012). Moringa contains pterygospermin, which has antibiotic effects and acts as a fungicide (Heuzé *et al*., 2014). The flowers are sometimes used in a concoction as a remedy for the common cold (Orwa *et al*., 2009).

Fodder

The main benefit of growing Moringa for feed and/or fodder, is that it can be grown on marginal lands, less suitable for other agricultural crops. This makes it a promising alternative when substituting commercial rations in feeds, especially in developing countries and in small-scale farming (Nouman et al., 2014; Paguia *et al*., 2014).

The leaves can be eaten by livestock, especially donkeys, camels and goats. The seed cake, which is a residual production from the oil extraction, is unfit for animal fodder due to its high alkaloid and saponin content. It is therefore mainly used as a fertilizer (Bosch, 2004). In certain African countries, Moringa leaves are used in small-scale rabbit farming. Feeding poultry, pigs and/or fish with leaves is possible, but is not done often due to its antinutritional properties (Heuzé *et al*., 2014). However, according to Paguia *et al*. (2014), the feed consumption, feed conversion ratio, egg production, amongst others, were not significantly different between hens fed on a control diet and hens on a Moringa feed diet.

Fuel (firewood, charcoal, biogas, biodiesel), fencing and construction

In Zimbabwe, Moringa is used as firewood. It can be turned into charcoal, but is rather a poor charcoal (Maroyi, 2006). The leaves can be used for biogas, where the produced gas contains on average about 81% methane, and the oil obtained from the seeds for biodiesel (Mridha, 2015; Foidl et al., 2001). Moringa oil yields a biodiesel with the highest reported value, approximately 67, for the cetane number – an indicatory value for the combustion speed (Rashid *et al*., 2008). However, one must bear in mind that Moringa cannot be grown to suit all purposes simultaneously. A choice or trade-off will have to be made whether to grow Moringa for food and feed purposes or for fuel purposes. The wood of Moringa trees can be used for fencing or light construction work. However, the wood is susceptible to moisture and termites (Maroyi, 2006).

Water purification

Moringa seeds can purify water, reducing water turbidity between 92% and 99% as reported by Muyubi & Evison in 1995 (as cited in Anwar *et al*., 2007). The purifying capacity is due to certain coagulant proteins and antimicrobial properties that are present. The coagulation mechanism is most probably by neutralising charges of particles in the contaminated water and adsorption of these particles, causing flocculation. The seeds can also act as

biosorbents for metal ions (Anwar *et al*., 2007). Using moringa seeds could be a low-cost way to provide crude water purification systems in rural areas.

Other

The soft wood produces a blue dye and in India, the wood pulp has been used to produce paper (Bosch, 2004). Moreover, the species is often planted for ornamental purposes (Orwa *et al*., 2009). Lastly, leaf extracts can be a source of growth hormones such as cytokinins – and more specifically, zeatin –, and can be applied as a growth-enhancer in different crops (Culver *et al*., 2012).

Despite all these characteristics, one must always be careful before announcing that Moringa is a "miracle tree" and a solution for drought-prone regions. Special attention must be paid before introducing a new species in an ecosystem. This was the case in Kenya, where *Prosopis juliflora* (Sw.) DC. was deliberately introduced for its drought tolerant properties and the provision of fodder, fuel and timber. However, after introduction in Kenya, the species soon invaded grasslands and dominated the ecosystem at the expense of grasses. Browsing goats could feed only on this plant, of which the pods would get stuck between the goats' teeth. This lead to tooth decay and in serious cases, left the goats without teeth, sentencing these goats to die. Seeing as goats and livestock in general are a main part of rural livelihoods, this was a major blow to these rural communities (Morland, 2018).

Moringa and its relation to humanitarian aid

Malnutrition includes undernutrition, micro-nutrient related malnutrition, overweight and obesity (WHO, 2017). Children may suffer the most severe consequences of malnutrition, where this condition can manifest itself as marasmus or kwashiorkor – two serious conditions causing the bloating of the belly or emaciation (Mune Mune *et al*., 2016). What is more, malnutrition can cause stunting and wasting, indicators of growth impediment. In 2017, globally speaking, 22% of all children under five were stunted and 16 million children out of a total of 52 million were severely wasted. Additionally, in Africa, the number of children under 5 that is overweight has increased by 47% since 2000 (WHO, 2018a).

Several researchers and policymakers have thought of Moringa as a viable option for combatting malnutrition in parts of the world, since it is a source of nutrients in the dry season and above all, a local resource. In Senegal, in 1997, a pilot project was started to test the hypothesis that malnutrition could be prevented or even cured by consuming MLP on a regular basis. This project proved to be largely successful in training health workers, NGOs and communities in using and preparing Moringa. Furthermore, interviews revealed that those who incorporated Moringa in their diet on a regular basis, also were aware of an improvement in their general health and energy (Fuglie, 2001).

Furthermore, some researchers believe that Moringa could help combat micro-nutrient related malnutrition, such as anaemia (Busani *et al*., 2011; Maroyi, 2006). Anaemia is a medical condition wherein there is a decrease in the concentration of red blood cells that circulate in the body or a decrease in the haemoglobin concentration and hence the capacity to transport oxygen (McLean *et al*., 2008). It is estimated that about 29% of all women of reproductive age have anaemia and roughly 43% of all children suffer from it (WHO, 2015). Iron deficiency is probably the most common cause of anaemia worldwide. However, copper, folate, vitamin B_{12} or vitamin A deficiencies can also cause anaemia. Inherited disorders or diseases such as malaria can play a role as well. Its main symptoms include fatigue, weakness and dizziness (WHO, 2018b; McLean *et al*., 2008). Zinc deficiencies are also a widespread problem – about one-third of the world's population suffers from zinc deficiency.

It is related amongst others to respiratory issues, malaria and diarrhoeal diseases (WHO, 2018c).

Moringa, as a source of iron, could be a potentially beneficial supplement regarding anaemia. In Nigeria, research has been conducted regarding 'biofortification', i.e. inherently increasing certain nutritional aspects of foods. 'Amala' is a popular dish in Nigeria, based on yam flour, and was fortified with MLP. At a level of 2.5% leaf powder, the dish was still very acceptable compared to the blank and contained 11% more proteins and a higher – although not significantly – level of micronutrients such as iron (Karim *et al.*, 2013). As a supplement, it is beneficial mainly due to its nutritional density. It is also a sustainable and economically sound option, as the plant grows easily in rural areas and costs little to sow (Thurber & Fahey, 2009). Nonetheless, it must also be understood that malnutrition is not solely due to micronutrient deficiencies, but that education, poverty, famine, unsafe drinking water and parasites too come into play and need to be addressed, in order to sustainably eradicate malnutrition (Fuglie, 2001). Besides these, the acceptability of new recipes containing Moringa must be generally positive.

Moringa products as trade commodities

As an international trade commodity, Moringa is marketed as a 'superfood'. There are few statistics on export volumes or production values, but according to an online import/export platform, Zauba, India exported more than 16 000 tons of Moringa products between January 2014 and October 2016 (as cited in CBI, 2016). However, this is only one of many platforms and data provided may be a misrepresentation of reality. Most of these exports go to the United States. India, as the main global producer, heavily influences the international market price. For wholesale MLP originating from India, the price is about €5.88 per kg (CBI, 2016).

India clearly dominates this market, exporting canned and fresh fruits of Moringa, Moringa oil, seeds and leaf powder. However, some smaller African suppliers such as Kenya, Mozambique and South Africa are also entering this new market in Europe (CBI, 2016; Bosch, 2004). Main importers in Europe are the United Kingdom, Germany, the Netherlands and Austria. In Europe, the market is growing due to a growing demand from consumers for products that increase energy or help with weight management. Furthermore, there is a demand for organically produced or Fair Trade Moringa. To enter the European market, the products must comply with European legislation and Food Safety measures, such as maximum residue levels or traceability. The main market entry barriers are the scale of production – bigger production facilities in India provide fierce competition – and the logistics in the production facility to produce the products. Moringa is allowed to be marketed as a food supplement – as leaf powder or as capsules containing leaf powder – in Belgium, France and Italy (known as the BELFRIT list). Yet as a food supplement, no medicinal claims can be made for marketing (CBI, 2016).

However, there is a growing need worldwide for standards regarding food safety and environmental criteria for Moringa. When leaf powder is of poor quality, this can cause digestive illnesses. Counterfeit products can be an issue too, when, for example, different compounds are mixed into leaf powder. Guidelines regarding cultivation practices, harvesting and transportation, packaging and labelling were drafted by the Moringa Association of Ghana, for their local context and all stakeholders involved. The Ghana Standard Board also published a Good Practices Guide and an Inspection Guide (De Saint Sauveur & Broin, 2010).

Moringa-derived leaf products

Fresh leaves can be consumed directly, but have a relatively short shelf life. Especially in a tropical climate, after several hours the leaves will start to deteriorate. Therefore, it is useful to develop methods of processing fresh leaves to increase shelf life.

Moringa leaf powder

Moringa leaf powder is obtained by drying fresh Moringa leaves. Drying is one of the oldest methods to increase the shelf life of foods. Sun drying, as depicted in figure 6, is often the easiest and most practical way, but can lead to an increase in dust and microbes and hence a decrease in hygiene. It also causes a decrease in the quality of the leaves and a loss of nutrients (Oluduro *et al*., 2016). Nutrient retention is higher in shade dried leaves, compared to sun dried leaves and oven dried leaves, but not statistically significant. All leaves had a moisture content of 6%. Values for iron, phosphorus, oxalate, carbohydrates, fats, fibers, β-carotene and vitamin C were highest in shade dried leaves (Joshi & Mehta, 2010). At a rural level, drying in the shade or sun is often the most feasible method of drying.

Figure 6: A hand-made solar dryer for leaves, suitable as a low-cost option for rural communities and households. The frame is made from plywood, black bags create a heat absorbing base below and a UV-filtering plastic is placed top the dryer. The dryer is not in direct contact with the soil, but remains suspended above it (credit: B. Peddi).

Moringa leaf concentrate

Moringa leaf concentrate is obtained through a more complex process than solely drying. The process has been described by “l’Association pour la Promotion des Extraits Foliaires en la Nutrition” (APEF) and Leaf for Life, with the intended use for human consumption. First, the plant cells in the leaves are ruptured by mechanical forces, such as produced by the blades in a blender. Afterwards, the leaf juice that has leaked out of the cells is collected by filtering the obtained mass of leaves and juice. The residue of the process is called the ‘fiber’. The juice is boiled up to 100 °C to prevent microbial growth and causes the denaturation of the leaf proteins. These proteins coagulate on top of the liquid and can be skimmed from the remaining leftover juice or ‘whey’. The collected concentrate must then be filtered and dried to enhance its preservation. Figure 7 provides a clear picture of these different fractions. However, not all plants’ leaves produce a concentrate through heating – or one that provides a sufficient yield – and one must pay attention to concentrating certain antinutritional factors (APEF; Leaf for Life – *personal comm.*, 2017). Some protein concentrates are best not obtained through a thermal process, but through the addition of flocculants or through

centrifugation. This process of making leaf concentrates has already applied in the animal feed sector, to add protein to animal feeds (Baraniak, 1997).

Figure 7: (Left) Residual fibre after the process of squeezing out leaf juice. (Middle) Moringa leaf concentrate. (Right) residual whey after boiling leaf juice and filtering it (credit: B. Peddi)

Acceptability of Moringa leaf products

According to Babu & Rajasekaran (1991), the success of food and nutrition intervention programmes is largely determined by the taste of the food and hence its acceptance (as cited in Babu, 2000). Sensorial analysis of dishes containing Moringa leaves have been performed several times to assess its acceptability in different parts of the world. Glover-Amengor *et al*. (2016) conducted a trial with leaf-fortified dishes in Ghana, involving randomly selected school children (4 – 12 years old), and obtained positive results. In their study, these dishes were highly acceptable and a good source of minerals and β-carotene.

In an acceptance analysis in Malawi, Babu (2000) found that Moringa leaves were preferred over the commonly used pumpkin leaves with the majority of the participants. Moringa, when boiled with beans, was the most preferred recipe out of the four different recipes presented in this analysis. In a different study with cookies, an expert bakery panel evaluated cookies with ranging concentrations of Moringa and concluded that cookies with 10% dry leaf matter were still acceptable, but higher concentrations were not (Dachana *et al*., 2010).

In a study by Udefiagbon *et al*. (2016), different recipes of pork balls with soy and MLP were tested. Levels of soy/Moringa of 10/0.5% and 15/1.0% were most acceptable and levels of 10/0.5% also contained the highest crude protein content. This type of formulation would be less costly than 'pure' pork balls, providing a viable source of all necessary nutrients for all people in a community, even the poorest.

Nambiar *et al*. (2003) conducted a trial in India with 60 children between 1 and 5 years old. They were presented with salty snacks containing dehydrated Moringa leaves, after which facial expressions, the demand for more food and food leftovers were recorded. The results of the trial indicated that these snacks were highly accepted by the children, but also by the NGO staff and the involved authorities. Furthermore, Nambiar & Parnami (2008) found that three different common Indian recipes containing pulses and enriched with Moringa leaves were found to be acceptable by a female panel (18 to 21 years old). The most acceptable recipes contained 20 g of fresh – blanched – Moringa leaves for 100 g of cooked pulses.

Moreover, as discussed under the ethnobotanical uses of Moringa, above, some local communities already value it and incorporate it into their dishes or sauces (Mawouma *et al.*, 2017). According to certain studies examined by Thurber & Fahey (2009), the taste of Moringa leaves is perceived as varying between "tasteless" and "slightly bitter" according to the geographical origin of the leaves. Therefore, when using high concentrations of leaf powder in formulations, one must pay attention when targeting the product towards children.

Nutritional composition of Moringa leaves

Both scientific and grey literature were reviewed for results regarding the nutritional composition of Moringa leaves, as can be seen in table 1. There is a significant amount of literature on the composition of Moringa pods and seeds too, but these are beyond the scope of the current review.

It is important to keep in mind, while reading the review of the literature regarding nutritional composition of Moringa leaves, that not all phytochemicals are found in every plant part and that their presence differs according the extraction method used (Kasolo *et al*., 2010). Leaf stages and harvesting season can change Moringa leaves' nutritional values between 1.5 and 3 times, especially for iron and beta-carotene (Yang & Chang, 2006).

Table 1: Overview of reviewed literature for the proximate and mineral composition of Moringa leaves, MLP and MLC

	Analysis or review of literature?	*Origin of analysed material*	*Part of plant analysed**	*Methodology described?*
Agamou et al. (2015)	Analysis	Cameroon	LP – young and mature leaves	Yes
Castillo-López et al. (2017)	Analysis	Mexico	LP – long pod and short pod trees	Yes
Lockett et al. (2000)	Analysis	Nigeria	FL	Yes
Makkar & Becker (1997)	Analysis	Unknown	FL, St, T, Se	Yes
Moyo et al. (2011)	Analysis	South Africa	LP	Yes
Nutritive Value of Indian Foods (Gopalan et al., 1989)	Analysis	India	FL	No
Sánchez et al. (2006)	Analysis	Nicaragua	FL – at different cutting frequencies or plant densities	Yes
Shih et al. (2011)	Analysis	Taiwan	FL, St – summer and winter	Yes
Sodamade et al. (2013)	Analysis	Nigeria	LC	Yes
USDA (2016)	Analysis	Unknown	FL	No
West African Food Composition Table (Stadlmayr et al., 2012)	Literature review[1]	-	FL	Yes

**Part of plant: fresh leaves (FL), leaf powder (LP), leaf concentrate (LC), stems (St), twigs (T) or seeds (Se)*
[1]From 9 countries in West-Africa: Benin, Burkina Faso, Gambia, Ghana, Guinea, Mali, Niger, Nigeria and Senegal

Energy content

The U.S. Department of Agriculture (2016) reports an energy content of fresh Moringa leaves of 64 kcal per 100 g, while Stadlmayr *et al*. (2012) reported an energy content of 86 kcal per 100 g of edible portion. Makkar & Becker (1997) report a gross energy content of 19.35 MJ/kg DM, which can be recalculated to 462 kcal/100 g DM, as reported in table 2. In the Nutritive Value of Indian Foods list, the reported energy content was 92 kcal per 100 g of fresh leaves presumably (Gopalan *et al.*, 1989). In comparison, other common leafy vegetable such as spinach and amaranth leaves (both 23 kcal/100 g leaves) or sweet potato leaves (42 kcal/100 g leaves) contain less energy per portion (USDA, 2016).

Moisture content

Moringa leaves contain on average 81.8% moisture per 100 g of leaves, as per the reviewed literature in table 2. Lockett *et al*. (2000) report the lowest dry matter (DM) content of 4.1% and hence the highest moisture content. For leaf powder, the moisture content is on average 7.5%, based on reports by Castillo-López *et al*. (2017), Agamou *et al*. (2015) and Moyo *et al*. (2011).

Crude protein content

A value of 9.40 g/100 g raw leaves (44.05 g/100 g DM) has been reported in the United States (table 2), using a nitrogen conversion factor of 6.25 and when discarding all the stems – which made up 38% of the material presented (U.S. Department of Agriculture, 2016). It must be borne in mind, that "stems" can be interpreted in several ways, but in this dissertation, it will be assumed that only the pinnules were analysed. Agamou *et al*. (2015) on the other hand, have reported an average concentration of 23.6 g/100 g DM and 22.6 g/100 g DM for mature leaves and young leaves respectively in Cameroon. Younger leaves contain fewer proteins than mature leaves, due to their use of reserves for growth. Furthermore, protein content of leaves is influenced by the nitrogen available in the soil (Agamou *et al*., 2015). Makkar & Becker (1997) already reported a similar crude protein content of 264 g/kg DM. They used a nitrogen conversion factor of 6.25 and the amount of non-protein nitrogen reported, was 13.3%. Similar values were also reported in Nicaragua: a crude protein value of 223 g/kg DM is obtained in the first year of harvest and declines to 216 g/kg DM in the second year of harvesting, with a cutting frequency of 75 days (Sánchez *et al*., 2006). In the Nutritive Value of Indian Foods list however, a much lower protein value (nitrogen conversion factor of 6.25) of 6.7 g/100 g is reported, assumedly based on fresh weight (FW) (Gopalan *et al.*, 1989). On average, the crude protein content is 28.3 g/100 g DM, as shown in table 2.

Crude fat content

According to Agamou *et al*. (2015), the mean fat concentration for mature and young leaves was 8.64 g/100 g DM and 8.46 g/100 g DM respectively (table 2), for several sampling locations in Cameroon. In the Nutritive Value of Indian Foods list, a value of 1.7 g/100 g is reported, assumedly based on FW (Gopalan *et al.*, 1989). The fatty acids present in the leaves are mainly α-linolenic acid, comprising on average 64.5% of total fatty acids present, and palmitic acid, on average 17.2% of total fatty acids (Castillo-López *et al*., 2017; Amaglo *et al*., 2010).

Available carbohydrates

Carbohydrates are significantly higher in young leaves compared to mature leaves, due to a higher photosynthetic activity in mature leaves and hence a reduction in available carbohydrates. In young leaves, concentrations range from 33.14 g/100 g DM to 37.15 g/100 g DM and in mature leaves from 27.91 g/100 g to 30.70 g/100 g DM (Agamou *et al*., 2015). As given in table 2, the carbohydrate content is 8.28 g/100 g FW according to the U.S. Department of Agriculture (2016), where the content was calculated by the difference method – as is often done. In the Nutritive Value of Indian Foods list, a similar value of 12.5 g/100 g is reported, assumedly based on FW (Gopalan *et al.*, 1989).

Table 2: Overview of reported proximate analysis of Moringa leaves in selected literature, including energy content, moisture content, crude protein content, crude fat content, available carbohydrates content, total fibre content and ash content. In cases where the content was only given per 100 g fresh leaves, the content was calculated to 100 g DM as per the following formula: g DM = $\frac{g\ FW}{(100-moisture\ content)} x\ 100$.

Per 100g DM	**Agamou *et al.* (2015)**	**Castillo-López *et al.* (2017)**	**Lockett *et al.* (2000)**	**Makkar & Becker (1997)**	**Moyo *et al.* (2012)**	**Nutritive Value of Indian Foods (Gopalan et al., 1989)**	**Sánchez *et al.* (2006)**	**Shih *et al.* (2011)***	**USDA (2016)**	**West African Food Composition Table (Stadlmayr *et al.*, 2012)**	**Mean ± SD**
Energy (kcal)	-	-	-	462	-	382	-	-	300	366	377 ± 58
Moisture (% FW)	10.3 ± 0.4[1] 9.8 ± 0.5[2]	3.79 ± 0.48[3] 3.88 ± 0.18[4]	95.9	-	9.53 ± 0.19	75.9	-	-	78.7	76.5	81.8 ± 8.2 (7.5 ± 3.0)[***]
Crude protein (g)	22.6 ± 0.3[1] 23.6 ± 0.7[2]	36.8 ± 2.2[3] 31.7 ± 2.3[4]	20.7	26.4	30.3 ± 1.5	27.8	22.3[5] 21.6[6]	24.9	44.1	35.3 ± 3.0	28.3 ± 6.8
Crude fat (g)	8.46 ± 0.57[1] 8.64 ± 0.60[2]	8.16 ± 0.50[3] 7.57 ± 0.96[4]	12.5	-	6.50 ± 1.04	7.05	-	5.56	6.56	5.11 ± 2.13	7.5 ± 2.0
Available carbo-hydrates (g)	35.1 ± 0.7[1] 28.8 ± 0.7[2]	41.3 ± 0.5[3] 44.8 ± 4.1[4]	38.0	-	-	51.9	-	-	38.8	40.9	40.0 ± 6.3
Total fibre content (g)	21.9 ± 0.9[1] 26.9 ± 1.1[2]	-	-	-	-	-	-	-	-	-	-
ADF (g)	-		13.7	-	8.49 ± 0.35	-	-	-	-	-	11.1 ± 2.6
Dietary fibre content (g)	-	3.37 ± 1.36[3**] 4.03 ± 2.14[4**]	-	-	-	3.73[**]	-	-	9.37	8.51[**]	5.80 ± 2.59
Ash (g)	8.90 ± 0.45[1] 8.97 ± 0.50[2]	6.56 ± 0.57[3] 8.03 ± 0.48[4]	15.1	8.87	7.64 ± 0.43	9.54	-	9.77	10.6	10.2	9.5 ± 2.1

[1] Young leaves, mean values
[2] Mature leaves, mean values
[3] Long pod variety
[4] Short pod variety
[5] First year of harvest
[6] Second year of harvest
** Averages are reported of both summer and winter values given by the authors*
[**]Crude fibre
[***]Between brackets: mean moisture content reported for leaf powder by Castillo-López et al. (2017), Agamou et al. (2015) and Moyo et al. (2011). Other values are not expected to differ for leaf powder.
DM = dry matter; FW = fresh weight

Total fibre content

Total fibre content ranges between 21.92 g/100 g DM and 26.87 g/100 g DM for Moringa leaves of different development stages in Cameroon (Agamou *et al*., 2015). The USDA (2016) reports a 2 g/100 g FW total dietary fibre content, as does the West African Food Composition Table (Stadlmayr *et al*., 2012). In the Nutritive Value of Indian Foods list, a crude fibre value of 0.9 g/100 g is reported, probably based on FW (Gopalan *et al.*, 1989).

Ash

Table 2 indicates that on average, the papers that were reviewed reported an ash content of 9.5 g/100 g DM. The ash content indicates the amount of minerals present in the leaves. Mineral contents are dependent on the maturity of leaves and the locality of sampling (Agamou *et al*., 2015). In the Nutritive Value of Indian Foods list, a value of 2.3 g/100 g is reported, assumedly based on FW (Gopalan *et al.*, 1989).

Calcium, phosphorus, sodium, magnesium, potassium and sulphur are some of the main macro minerals present in plants. Their contents in Moringa leaves according to different sources are displayed in table 3. On the other hand, iron, copper, zinc, manganese, cobalt and selenium are micronutrients found in plant tissues. The latter two are not considered to be essential for plants. Cobalt contents for Moringa leaves have not been reported in reviewed literature. All other mineral contents are reported in table 4.

Vitamins and precursors

Moringa leaves are a good source of vitamins, such as vitamin C, and carotenoids (Fahey, 2005). However, vitamin C is very susceptible to heat and could be destroyed during drying processes (Agamou *et al*., 2015). The reported values from the literature are shown in table 5.

Table 3: An overview of macromineral contents reported in the literature for Moringa leaves. In cases where the content was only given per 100 g fresh leaves, the content was calculated to 100 g DM as per the following formula: $g\ DM = \frac{g\ FW}{(100 - moisture\ content)} x\ 100.$

Per 100g DM	**Agamou *et al.* (2015)**	**Castillo-López *et al.* (2017)**	**Lockett *et al.* (2000)**	**Moyo *et al.* (2011)**	**Nutritive Value of Indian Foods (Gopalan et al., 1989)**	**USDA (2016)**	**West African Food Composition Table (Stadlmayr *et al.*, 2012)**	**Mean ± SD**
Ca (g)	0.59 ± 0.04[1]	1.51 ± 0.17[3]	3.47	3.65 ± 0.04	1.83	0.87	1.85 ± 0.77	1.88 ± 0.98
	1.58 ± 0.12[2]	1.59 ± 0.12[4]						
Na (g)	0.15 ± 0.03[1]	0.14 ± 0.01[3]	-	0.17 ± 0.02	-	0.04	0.03	0.12 ± 0.05
	0.13 ± 0.03[2]	0.15 ± 0.02[4]						
Mg (g)	0.30 ± 0.02[1]	0.36 ± 0.03[3]	0.83	0.50 ± 0.01	-	0.20	0.30 ± 0.29	0.41 ± 0.18
	0.40 ± 0.03[2]	0.36 ± 0.03[4]						
K (g)	2.41 ± 0.24[1]	0.27 ± 0.03[3]	-	1.50 ± 0.02	-	1.58	1.72	1.38 ± 0.74
	1.88 ± 0.19[2]	0.30 ± 0.03[4]						
S (g)	-	-	-	0.63 ± 0.15	-	-	-	-
P (g)	0.55 ± 0.03[1]	-	0.23	0.30 ± 0.00	0.29	0.52	0.43	0.39 ± 0.11
	0.44 ± 0.02[2]							

[1] *Young leaves, mean values*
[2] *Mature leaves, mean values*
[3] *Long pod variety*
[4] *Short pod variety*

Table 4: An overview of micromineral contents reported in the literature for Moringa leaves. In cases where the content was only given per 100 g fresh leaves, the content was calculated to 100 g DM as per the following formula: $g\ DM = \frac{g\ FW}{(100 - moisture\ content)} x\ 100$.

Per 100g DM	Agamou *et al.* (2015)	Castillo-López *et al.* (2017)	Lockett *et al.* (2000)	Moyo *et al.* (2011)	Nutritive Value of Indian Foods (Gopalan et al., 1989)	USDA (2016)	West African Food Composition Table (Stadlmayr *et al.*, 2012)	Mean ± SD
Fe (mg)	12.6 ± 0.9[1] 20.7 ± 1.4[2]	12.0 ± 0.8[3] 10.5 ± 0.9[4]	105	49.0 ± 4.9	3.53	18.7	26.0 ± 17.0*	28.7 ± 29.6
Zn (mg)	2.46 ± 0.41[1] 1.62 ± 0.27[2]	5.65 ± 0.51[3] 4.69 ± 0.49[4]	2.04	3.10 ± 0.34	-	2.81	3.83	3.28 ± 1.28
Cu (mg)	0.34 ± 0.02[1] 0.38 ± 0.02[2]	1.09 ± 0.09[3] 0.80 ± 0.03[4]	0.96	0.83 ± 0.01	-	0.49	0.68	0.70 ± 0.26
Mn (mg)	3.28 ± 0.55[1] 5.86 ± 0.99[2]	5.45 ± 0.55[3] 5.98 ± 0.44[4]	11.3	8.68 ± 0.39	-	4.98		6.50 ± 2.46
Se (mg)	-			36.3 ± 0.04	-		-	-

[1] *Young leaves, mean values*
[2] *Mature leaves, mean values*
[3] *Long pod variety*
[4] *Short pod variety*
** questionable quality, as indicated by the author*

Table 5: An overview of different vitamin contents reported in the literature for Moringa leaves. In cases where the content was only given per 100 g fresh leaves, the content was calculated to 100 g DM as per the following formula: $g\ DM = \frac{g\ FW}{(100 - moisture\ content)} x\ 100$.

Per 100g DM	Agamou *et al.* (2015)	USDA (2016)	West African Food Composition Table (Stadlmayr *et al.*, 2012)	Mean ± SD
Vitamin C (mg)	20.2 ± 1.3[1] * 50.8 ± 1.6[2] *	242	698 ± 336	470 ± 228 (35.5 ± 15.3)***
Thiamin (mg)	-	1.20	0.98 ± 0.09	1.09 ± 0.11
Riboflavin (mg)	-	3.09	3.11 ± 2.09	3.10 ± 0.01
Niacin (mg)	-	10.4	11.5 ± 2.1	11.0 ± 0.6
Pantothenic acid (mg)	-	0.59	-	-
Vitamin B6 (mg)	-	5.62	5.11	5.37 ± 0.26
Total folate (µg)	-	187	872	530 ± 342
Vitamin A (µg RAE)[1]	-	1771	3140	2456 ± 685
Total carotenoids (mg)	112 ± 1[1] 93 ± 1[2]	-	-	-
β-carotene equivalents (mg)	-	-	37.7	-
Vitamin E (mg)	-	-	13.1	-
Vitamin B12 (mg)	-	0	0	0
Vitamin D (mg)	-	0	0	0

[1] *RAE = Retinol Activity Equivalents, where precursors that can be converted into retinol are equated to 'retinol equivalents'. For example, 12 µg β-carotene equates to 1 µg RAE.*
**The author indicates that the Vitamin C contents could have been affected by the drying process.*
****Between brackets: mean vitamin C content reported for leaf powder by Agamou et al. (2015)*

Secondary plant metabolites

Kasolo *et al*. (2010) qualitatively observed the presence of phytochemicals such as tannins, steroids and triterpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugars in Moringa leaves. Depending on the extraction method, the level of concentrations (low, moderate or high) differed. No anthocyanins were detected in Moringa leaves (Bennett *et al*., 2003).

Phytate and oxalate

Phytate is the major form of storage for phosphorus in the plant. Over 80% of total phosphorus can be in the form of phytates in cereals and seeds. Phytases are the enzymes that are able to 'cleave' phytates and hydrolyse them (Reddy & Sathe, 2002). Phytates are often viewed negatively due to their chelating ability that can limit the absorption of certain essential minerals. It can form complexes with iron, copper, zinc, cobalt, manganese and calcium. However, it is suggested that phytates may also have antioxidant properties thanks to its chelating ability with iron (Burgess & Gao, 2002). Makkar & Becker (1997) report a value of 21.0 g/kg DM in Moringa leaves – present as phytic acid – in their study. Another study in Nigeria found a value of 2.59 ± 0.13 g/100 g DM in leaves (Ogbe & Affiku, 2011).

Oxalate on the other hand, often occurs in plants as intracellular calcium oxalate crystals, to maintain osmotic and ion balance. Its creation could also be a method for the plant to remove excess oxalic acid. In animals, calcium oxalate is often extracellular and associated with kidney stones (Franceschi & Horner, 1980). According to the Nutritive Value of Indian Foods by Gopalan *et al.* (1989), fresh Moringa leaves contain 101 mg/100 g FW or 419 mg/100 g DM (as cited in Joshi & Mehta, 2010). Ogbe & Affiku (2011) report a similar oxalate content of 0.45 ± 0.01 g/100 g DM. A roughly fourfold lower value of 0.99 ± 0.21 mg/g DM has also been presented, which is about 25 times lower than spinach oxalate contents, i.e. 25 – 45 mg/g DM (Yang & Chang, 2006).

Phenolic compounds

Dietary phenolic compounds may act as antioxidative, anticarcinogenic or cardioprotective compounds (Siddhuraju & Becker, 2003). According to a study by Pakade *et al*. (2013), the total phenolic content of Moringa leaves was nearly twice that of vegetables such as cabbage, spinach, peas, cauliflower and broccoli. Over a study of 4 years in South Africa, the average value found was 27.6 g gallic acid equivalents (GAE)/kg DM. Makkar & Becker (1997) report a value that is roughly twice as high – of 44.3 g/kg DM as tannic acid equivalents – in their study. However, Castillo-López *et al*. (2017) find lower values ranging between 71.08 ± 12.05 mg GAE/g DM and 76.63 ± 10.63 mg GAE/g DM for leaf powder. Yet, Moyo *et* al. (2011) find a value of 2.02 ± 0.39 g GAE/100 g DM for MLP. Nonetheless, Shih *et al*. (2011) find an average value of 191 mg catechin equivalents/100 g DM for Moringa leaves, values of summer and winter combined. Siddhuraju & Becker (2003) analysed and compared samples from Nicaragua, India and Niger for their phenolic compounds. When using a solvent extract to determine total phenolic compounds, Nicaraguan samples contained the most of these phenolic compounds. Methanol extracts led to higher contents than water or ethanol extracts, namely 12.3 ± 0.5 g GAE/100 g DM.

One distinct class of phenolic compounds are tannins. Tannins are water-soluble polyphenolic compounds and have antimicrobial properties. They are sometimes used in food processing to increase food products' shelf life. Tannins are commonly classified in two groups: hydrolysable and non-hydrolysable, or condensed, tannins (Chung *et al*., 1998). Makkar & Becker (1997) report a value of 1.20 g/100 g DM tannic acid equivalents in their study, while a tenfold higher value of 21.19 ± 0.25 g/100 g DM leaf powder was reported by

Ogbe & Affiku (2011). The method used to determine the amount of condensed tannins is unclear in this study. Moyo *et al*. (2011) report a value of 3.12 mg/g DM of leaf powder, as tannic acid equivalents.

What is more, Moringa plant parts contain flavonoids. The flowers of Moringa contain kaempferol and quercetin (Bosch, 2004) and these are also the predominant flavonols of Moringa leaves (Mbikay, 2012). Flavonoids are most known for their anti-oxidant properties nowadays (Rice-Evans, 2001). Castillo-López *et al*. (2017) found values for total flavonoids of 60.26 ± 7.21 mg quercetin equivalents (QE) per g DM for long pod varieties and 55.70 ± 7.00 mg QE/g DM for short pod varieties. The USDA (2016) reports a content of 28 mg/100 g DM for kaempferol and a content of 78 mg/100 g DM for quercetin. Sultana & Anwar (2008) on the other hand, found values of 28.10 ± 0.56 mg/100 g DM of quercetin and 4.02 ± 0.08 mg/100 g DM of kaempferol. They studied other leafy vegetables as well, such as spinach and aloe vera leaves. In spinach, no quercetin was detected and kaempferol values were 1.5 times higher than the studied Moringa leaves. In aloe vera, quercetin values were about 3 times lower and kaempferol contents were more than 6 times higher than Moringa leaves.

Castillo-López *et al*. (2017) have reported the presence of several phenolic acids, for long-pod and short-pod varieties of Moringa. These phenolic acids include gallic acid (on average 1.22 mg/g DM), chlorogenic acid (on average 0.58 mg/g DM), caffeic acid (on average 0.49 mg/g DM), *p*-coumaric acid (on average 1.10 mg/g DM) and ferulic acid (on average 0.53 mg/g DM).

Saponins

Saponins can be found in Moringa leaves and are considered to be an antinutritional factor. Makkar & Becker (1997) reported a value of 8.10 g/100 g DM as diosgenin equivalents for the Moringa leaves in their study. Compared to other Moringa plant parts, this result was the highest. Ogbe & Affiku (2011) found a value of 1.60 ± 0.05 g/100 g DM in their study of Nigerian Moringa leaves.

Other

The bark is a source of moringinine or benzylamine, an alkaloid (Mbikay, 2012; Bosch, 2004). The roots also produce spirochin and pterygospermin (Bosch, 2004). Moringa plant parts contain phytosterols and several other bioactive phytochemicals too. One of such are glucosinolates, of which glucomoringin in the most abundant in Moringa leaves. Enzymatic hydrolysis of these components leads to the formation of isothiocyanates, thiocyanates or nitriles, several which possess antihypertensive properties (Mbikay, 2012).

Toxicology of Moringa leaves

Mbikay (2012) reviewed several studies and found none that reported an acute or sub-acute toxicity after a treatment of rodents with Moringa leaves. The lethal dose for 50% of subjects (LD_{50}) has been estimated for aqueous leaf extract of Moringa by Awodele *et al*. (2012). They estimated a value of 1585 $mg.kg^{-1}$.body $weight^{-1}$ through an acute intraperitoneal toxicity test with rats. The acute oral toxicity test of aqueous leaf extract showed no mortality at its maximal dosage of 6400 $mg.kg^{-1}$.body $weight^{-1}$ in rats. However, toxicology studies mainly identify and quantify the possible hazards. A risk assessment is often not conducted, as risk is the effect times the probability of encountering this effect, which ultimately determines the potential danger to human health.

Nutritional composition of Moringa leaf powder

Moringa leaf powder should have the same nutritional composition as the fresh leaves, expressed on DM, as described above. However, it must be kept in mind that the drying process might degrade certain components, such as certain vitamins. In tables 1, 2, 3 and 4, studies by Castillo-López *et al*. (2017), Agamou *et al*. (2015) and Moyo *et al*. (2011) were included pertaining to the contents of MLP.

Nutritional composition of Moringa leaf concentrate

Only one literature source has been found pertaining to the nutritional composition of leaf concentrate of Moringa. However, other studies have studied protein extraction processes of Moringa seeds (Ghebremichael *et al*., 2005; Okuda *et al*., 1999; Gassenschmidt *et al*., 1995). The results obtained by Sodamade *et al*. (2013) in Nigeria for the proximate composition of leaf concentrate and its mineral composition are given in table 6 and 7. However, no specific units were provided in the paper and the present author has assumed due to the description that all is expressed on a DM basis.

Another study determined the nutritional components of leaf concentrates of other leafy vegetables, i.e. 'bitter leaf' (*Vernonia amygdalina*), 'African nightshade' (*Solunam africana)*, 'green tete' (*Amaranthus hybridus*) and 'fluted pumpkins' (*Telfaria occidentalis*). On average, the crude protein content of these leafy vegetables ranged between 31.7 – 34.6 g/100 g DM and the crude protein content of their leaf concentrates ranged between 35.1 – 54.9 g/100 g DM. The gross energy of these leaf concentrates averaged at 439 kcal/100 g DM, while their iron content was on average 674 mg/kg DM – the four fresh leafy vegetables contained on average 251 mg/kg DM (Aletor *et al*., 2002).

Table 6: Overview of proximate analysis of Moringa leaf concentrate as reported by Sodamade *et al*. (2013)

PARAMETER (% DM)	RESULT
Moisture (% FW)	9.00 ± 2.30
Ash	6.59 ± 0.69
Crude fat	2.67 ± 0.52
Crude fibre	5.97 ± 0.25
Crude protein	43.0 ± 0.2
Carbohydrates	42.0 ± 0.3

Table 7: Overview of mineral analysis of Moringa leaf concentrate based on report by Sodamade *et al*. (2013)

MINERAL (mg/100g DM)	RESULT
K	25.5 ± 0.3
Na	235 ± 0
Ca	795 ± 0
Mg	744 ± 0
P	5.49 ± 0.13
Fe	205 ± 0
Mn	277 ± 0
Cu	60.4 ± 0.1
Zn	602 ± 0

Digestibility and absorption of nutrients

The nutritional composition of a product is only one aspect of something as complex as human nutrition. The following will briefly discuss what happens after food intake, such as absorption of selected nutrients and their metabolism.

Antinutritional factors and mineral absorption

Several antinutritional factors can inhibit the absorption and digestion of nutritional components, including digestive enzyme inhibitors, haemagglutinins, certain plant enzymes such as lipoxygenase, cyanogenic glycosides, goitrogens, oestrogens, saponins, tannins,

amino acid analogues, alkaloids, anti-metals such as phytates and oxalates, anti-vitamins and Favism factors (Soetan & Oyewole, 2009). Makkar & Becker (1997) did not detect the presence of cyanogenic glycosides in leaves, nor any amylase or trypsine inhibitors. Ogbe & Affiku (2011), however, did find minor concentrations of trypsine inhibitors, 3.00 ± 0.04 g/100 g DM.

Mineral absorption can also be facilitated or inhibited by certain specific compounds. For example, phytic acid can limit the bioavailability of minerals such as iron and zinc (Agamou *et al*., 2015; Institute of Medicine of the National Academies, 2005). Tannins, too, can limit iron absorption (Teucher *et al*., 2004) as can calcium (Zijp *et al*., 2000). Iron absorption can be enhanced by several organic acids including vitamin C (Teucher *et al*., 2004) and by the consumption of haem-iron found in meat, fish and poultry (Zijp *et al*., 2000). On the other hand, zinc absorption is facilitated by certain animal proteins, but inhibited by calcium. High doses of iron administered in solution or as a supplement may also interfere with zinc absorption (Krebs, 2000). So far, research pertaining to Moringa leaves has focused on characterizing the presence of possible "facilitating factors" or "antinutritional factors (ANF)" for mineral absorption specifically, but has not looked at effects *in vitro* or *in vivo*.

Protein digestibility

Not only the total protein content of a product is important, but the amino acid profile and the digestibility of the proteins as well – i.e. the *quality* of the protein (Witt, 2014). Leaf flour or powder contains all essential amino acids, is high in leucine and valine and lower in methionine and cysteine. Lysine and sulphur amino acids are the most limiting amino acids. Moringa leaf proteins are also easily digested by pepsin – as found in the stomach – according to one *in vitro* study, with an average pepsin-digestibility of 41%. The pancreatic digestibility was on average 57% (Mune Mune *et al*., 2016).

Moringa leaf flour's chemical score was 72.40, a score that is based solely on the amino acid profile in the product. Moringa flour's protein digestibility corrected amino acid score (PDCAAS) was 41.42% (Mune Mune *et al*., 2016). This score not only incorporates the amino acids present, but also their digestibility (Institute of Medicine of the National Academies, 2005). The protein efficiency ratio (PER) of Moringa leaf flour is between 3.47 and 3.71 (Mune Mune *et al*., 2016). This ratio is obtained through *in vivo* experiments with rats by dividing the gain in body mass by the protein consumption (Health Protection Branch Ottawa, 1981). In comparison, according to FAO/WHO (1991), the PDCAAS score of wheat is 42%, that of chickpea 80% and that of milk powder 100% – after truncating the original value, 110% (as cited in Institute of Medicine of the National Academies, 2005). Soy, a plant well-known for its protein content, has a PDCAAS score of almost 100% (Udefiagbon *et al*., 2016).

Sánchez *et al*. (2006) report an overall *in vitro* digestibility of leaves of 682 g/kg DM at a cutting frequency of 75 days and in the first year of harvesting. During the second year of harvesting, this value declines to 658 g/kg DM. According to another study, approximately 64% of the crude protein was degradable *in vitro* and approximately 33% was potentially digestible (Makkar & Becker, 1997).

Metabolism of vitamins and phenolic compounds

During digestion, vitamins and other compounds (such as phenolics, i.e. anthocyanins), could be lost due to the influence of pH and the presence of oxygen. However, the total phenolic compounds contents in a product that are released during digestion do not always imply an increase of action in blood serum (Pérez-Vicente *et al*., 2002). Or even when the

concentration increases in the blood plasma or serum, the increase could be insufficient to influence certain processes.

A better understanding is necessary of the bioavailability of flavonoids, their circulating metabolites and their interactions with the gastrointestinal tract. It is hypothesized that these compounds are only absorbed in their aglycone forms and that they are normally present as glycosides in the diet (Rice-Evans, 2001). Overall, little to no research has been done regarding the metabolism in the human body of vitamins and phenolic compounds derived from Moringa plant parts.

Knowledge gaps and opportunities for further research

For some nutritional compounds, the concentration reported varies considerably. It would therefore be interesting to look if the underlying factors causing this variation are mainly technical or biological – i.e. genetic variation and/or environmental variation. Little genetic stability has so far been achieved with Moringa 'cultivars', if such a term can already apply to Moringa.

Secondly, there is only a limited amount of literature reporting on *in vitro* or *in vivo* human digestibility of Moringa leaves and even less that assess the digestibility of leaf products when they are formulated in certain recipes. These recipes might influence the bioavailability of nutritional compounds present in Moringa leaves.

Additionally, it could be interesting to take a look at the market potential for Moringa in certain countries. In order to achieve this, attention must be paid to improving the quality and effectiveness standards and methods of production. These practices can be targeted towards domestic production or for an international market. In the case of an upscaling Moringa production in a given area, it might be beneficial to perform an analysis of the effects and consequences of such an evolution.

In the current dissertation, further research has been conducted on nutritionally characterising Moringa leaf products such as leaf powder and leaf concentrate and the nutritional effects of their respective production processes, where previous research has mainly focused on fresh Moringa plant parts. The digestibility of these Moringa leaf products *in vitro* is assessed too – which, to the best of our knowledge, has not yet been reported for MLC. Furthermore, a sensorial analysis with both MLP and MLC has been conducted as well as an economic analysis. As far as we know, no analysis has been made so far to compare MLP and MLC in these respects.

DATA ANALYSIS

Introduction

This research was intended as a preliminary research for APEF and partners into possible applications of Moringa leaves in human diets. Concretely, APEF is planning to instigate a clinical trial in Nicaragua with anaemic children to assess the effect of the addition of Moringa to their diets. Moringa leaf powder and Moringa leaf concentrate are two Moringa-leaf based products with a high shelf-life that can be produced locally in Nicaragua. Of these two products, it is important to determine which will be the most promising for the clinical trial in terms of nutritional density and bio-availability, low-cost means of production and food acceptability.

The aim of the experimental part in this preliminary research is to assess the viability and characteristics of a MLP/MLC production in Nicaragua, nutritional composition and quality of the obtained products and their economic feasibility. Both products are compared with each other, in order to determine an optimal product for local conditions and for a follow-up project regarding anaemia alleviation in malnourished children designed by APEF.

The first part ("partim I") is about setting the scene and assessing the local Nicaraguan conditions in terms of Moringa production. This part aims to answer questions such as "*what are the production conditions of Moringa and Moringa-derived products in Nicaragua?"* and "*how do local consumers experience Moringa products?*". The second part ("partim II") focuses on analytical and quantitative data collection to assess the nutritional value of several Moringa leaf samples taken in Nicaragua. The final part ("partim III") focuses on economic viability of a Moringa leaf powder or leaf concentrate production in Nicaraguan circumstances. These different aspects – local context, scientific backing up of claims surrounding Moringa and economic viability – will make a conclusion possible as to whether MLP/MLC production and/or consumption is preferable.

As all parts of this research were intended in the first place to gain an insight in above-mentioned topics and to be preliminary to further, more in-depth research, the sample sizes were often limited and hence, no results can be generalised over a whole population.

Leaf concentrate made from other plants have in the past been proven to contain more iron. This iron was also shown to be more bio-accessible and bioavailable than in fresh leaf material (APEF – *personal comm.*, 2017). It is therefore thought that nutritionally speaking – in terms of alleviating anaemia – iron concentration and bioavailability in Moringa leaf concentrate will be higher than in leaf powder. However, leaf concentrate production is also more expensive and time-consuming, so a cost-benefit analysis must be made locally in order to assess production viability.

PARTIM I: ASSESSING THE LOCAL CONTEXT AND PERCEPTIONS OF MORINGA

Materials and methods – data collection in Nicaragua

Getting an overview on local production techniques

Informal discussions to obtain a view of common ways of cultivating Moringa in Nicaragua were held with local stakeholders in July, August and September 2017. These stakeholders include representatives from a local NGO: Soynica, which aims to promote the consumption of Moringa in Nicaragua, the main Moringa producers in the western part of Nicaragua, members of a women's association in Masaya called FUPROSUMONIC, and the Universidad Nacional Agraria (UNA).

Conversations with Moringa producers individually, a total of 4 semi-structured interviews in Spanish and/or English, allowed for an insight into commercial production of Moringa in Nicaragua. The producers interviewed included 3 of the largest producers in Nicaragua, in production volume and cultivated area. The producers were asked to provide several key parameters of how their production was organised. These parameters include the Moringa variety being grown, the plant density, the distance between rows and in rows, the age of the plantation, time since the last harvest, irrigation practices, fertilizer application and crop protection practices.

Getting a view on local knowledge on Moringa through interviews

To assess if Moringa was commonly known amongst specific groups of consumers, a total of 30 semi-structured interviews were conducted in 3 localities in Nicaragua with local market vendors and local residents living near a Moringa plantation in August 2017.

Thirty-one young to middle-aged interviewees were selected as randomly as possible in these 3 localities. The interviewer also sought to maintain a 50/50 gender ratio. People were randomly selected to participate in interviews amongst those who manned stalls during a market day in the centre of Granada (11 people) and at Mercado Roberto Huembes in Managua (10 people), as well as residents of a small village called 'Loma Alegre' (10 people), who live in close proximity to – about 5 km distance – a Moringa plantation. These groups were sought out, since they have a higher chance than other Nicaraguan citizens – excepting those who work in the sector – of having encountered Moringa. Market vendors especially encounter a high variety of plants in their line of work and through targeting market vendors, a wider geographical area of survey can be achieved. This includes a selection bias and generalisations over the entire population are not possible as the sample is not a representative sample, but it does hold an indicative value.

Selected interviewees were explained in Spanish why they were being interviewed, were asked whether they gave their consent and were shown several black-and-white pictures of different Moringa plant parts. They were told that all these pictures represented the same plant. They were then asked if they recognised the plant and if so, by what name they knew it. If the interviewee recognised the plant, but could not give its name, the interviewer provided the name and noted it if they agreed or not in that case. If the interviewee recognised the plant, they were then asked how they knew it and how they used the plant. All the respondents' answers were noted down during the interviews.

Acceptability panels

Two acceptability tasting panel sessions were organised in Nicaragua in August 2017 and September 2017, to be able to indicate the preferences of consumers towards different Moringa products. Participants volunteered amongst those who happened to be present. The

first panel session was with a group of labourers, in a small kitchen of a cottage on a production site near the Pacific Coast of Nicaragua and consisted of 18 panellists, of which 2 were women and 2 were minors. The second panel session was with a group of church goers in a small village near Masaya and consisted of 17 panellists, of which 11 were women. This session was conducted in the local church.

The MLP and MLC, used for these panels were self-made. The MLP was obtained through solar drying fresh Moringa leaves for 2-3 days and then mixing these dried leaves in a blender at 500 W. This produced a powdered product. The MLC was obtained through the process of blending fresh Moringa leaves at 500 W, filtration of the leaf juice, boiling of the leaf juice and sieving of the coagulated proteins, i.e. MLC (figure 11). Fresh MLC was dried for about 2 days in a solar dryer.

Both sensorial analyses consisted of triangle tests – according to the BS ISO 4120:2004 standard – between blank products and products with MLP or MLC, or solely between MLP products and MLC products, preference tests between products with MLP and products with MLC and ranking tests, where each product was given a score on a continuous 5-point scale between 1 (very good) and 5 (very bad) for the parameters texture, smell, taste and colour. The number of panellists allowed for a full replication of all possible combinations of presenting 3 samples during these triangle tests. Products were prepared two days before the tests with equal concentrations of MLP and MLC – so the variables texture, smell, taste and colour would be independent of the concentrations added. The taste samples were stored in a fridge between 4 – 6 °C and were labelled as products “A”, “B” or “C”. Before serving, all products were brought to ambient temperature. All processing, storing and handling of the products was performed in such a way as to minimise any differences or variability between products, except for the addition of MLP/MLC.

Before conducting these tests, the panellists were informed in Spanish of the purpose of the acceptability test and consented in participating in it. Guidance and help was provided throughout the test, when panellists had questions or difficulty writing their answers on the provided papers. A copy of such an answer sheet is provided in Appendix A.1, along with the serving order of products. Before and between the tasting of different samples, assessors would clean the palate by taking a sip of water and by eating a piece of unsweetened and unsalted store-bought biscuit.

The products judged by the first panel were 3 types of brownies (figure 8): brownies that did not contain any Moringa, brownies containing MLP and brownies containing MLC. Brownies are eaten as a sweet in Nicaragua, though it is not a traditional recipe. The MLP and MLC for these batches were both obtained from location 2 (see table 9). Store-bought brownie mixture was used – about 290 g of flour – to reduce the variability of the recipe, to which 1 egg, 4 tablespoons of tap water and ½ cup of maize oil was added to produce “blank” brownies. Brownies with MLP contained more water, 8 tablespoons, and more oil, ¾ cup, along with 60g of MLP. Brownies with MLC contained 60g of MLC, but were otherwise identical to the blanks. The amount of 60g was chosen both for MLP and MLC, as future project products by the consortium would contain approximately 20g of MLP per 100 g of edible portion. The amount was identical to MLC in this sensorial analysis, as the goal was to assess the flavour differences at equal concentrations of both products. All brownies were baked for 20 min at 160 °C in a gas oven. Brownies containing MLC were particularly oily and produced green oil stains. Serving portions were cut out, of about 2 cm x 1 cm x 1 cm. Because differentiation between brownies based on colour would have been possible, panellists were blindfolded before conducting the triangle test, thus avoiding preference based on visual appearance or colour. For the triangle tests, all three products were placed

simultaneously on the table and the panellist tasted each product from left to right. For the preference and ranking test, the blindfold was removed and two brownies, one containing MLP and one containing MLC, were placed simultaneously on the table. After all the 18 panellists had participated, the information was disclosed that some of the samples contained Moringa. This test was conducted on the 24th of August 2017.

The products judged by the second panel were 3 types of fruit juices made out of red-fleshed dragon fruit or *pitaya* fruit (figure 9): without added Moringa, with added MLP and with added MLC. These fruit juices are common in Nicaragua. The MLP was obtained from location 2, while the MLC was obtained from location 3. The 'stock' juice was made by mixing the juice of 3 pitayas with 6L of tap water and 1 kg of sugar to a total volume of 8L. The stock was sieved to remove the bulk of seeds present. For the juice with MLP, 40g of MLP, the juice of 6 limes and 7 tablespoons of sugar were added to 1200 ml of the 'stock'. As for the juice with MLC, 40g of micronized MLC, the juice of 9 limes and 14 tablespoons of sugar were added to 1200 ml of the 'stock'. Micronized MLC was obtained through the use of a grinder of 1500W, in order to obtain finely ground particles. The amount of 40g MLP/MLC was chosen as for future project products by the consortium, about 10g of MLC would be added per 100 g of edible portion. In this case, it was estimated that one portion would equal to 300 ml of fruit juice. The amount of MLP and MLC were kept equal, to keep the effect of the amount constant and hence to be able to differentiate the effect of taste. Variations in the recipe regarding sugar and lime juice were made in order to obtain a similar taste in terms of sweetness and acidity, so differences in taste would be due to the taste of the added MLP/MLC. However, it must be acknowledged that this is a subjective method. Serving portions were about 30 ml per sample. As panellists on the first panel tended to get worried about the blindfold, panellists of the second panel were not blindfolded in this case, but were explicitly asked to try to not base their judgement on minute differences in product appearance. Panellists were informed beforehand that they would be tasting some products containing Moringa. This test was conducted on the 10th of September 2017. In this sensorial analysis, the ranking test was only performed by 16 panellists and not 17 panellists.

Statistical analysis

Statistical analysis for the triangle tests was done according to the principles set out in BS ISO 4120:2004. The null hypothesis was that there was no perceptible difference between samples and the alternative hypothesis that a difference can be detected. The number of assessors, *n,* was 18 in the first case, with $\alpha = 0.05$, $\beta = 0.20$ and $p_d = 50\%$. Hence, these tests have a chance of 80% of detecting a case in which 50% of the participants can detect a difference between the test samples. The number of assessors, *n,* was 17 in the second case, with $\alpha = 0.05$. Two sample t-test statistics were performed in R Studio version 3.4.3 to assess possible significant differences.

Figure 8: Brownies containing MLP (left), blank (middle) and brownies containing MLC (right). (credit: B. Peddi)

Figure 9: Pitaya juice containing MLP (left), blank (middle) and pitaya juice containing MLC (right). (credit: B. Peddi)

Sample collection in Nicaragua

Moringa leaf samples were collected during the rainy season in a total of 4 production sites in Nicaragua: in Chinandega, near Léon, in Masaya and at the Pacific Coast (figure 10). These locations will be referred to as locations 1 to 4, in random order. In each location, about 9 kg of fresh Moringa leaves were harvested. This is a considerable amount of leaves to take as a sample and is mainly because the yield of fresh leaves to process into dried MLC is as low as 2%. The characteristics of each location and the working methodology used in each location is listed in tables 8 and 9.

Figure 10: Overview of sampling locations in Nicaragua. Each red dot indicates a sampling location (Courtesy of the University of Texas Libraries, The University of Texas at Austin).

Table 8: Overview of a few production characteristics per location

Location	Moringa variety	Tree age[1] (years)	Diameter tree at 0.5 m (cm)[2]	Distance between trees	Irrigation system present?	Fertilisation	Remarks
Location 1	*Crollo***	3 – 4	15	Variable	No	None	Oldest leaves
Location 2	*Crollo*	2	17	0.3 m	No	Yes	High density
Location 3	PKM-1	2 – 3	7****	2.5 m	No	Yes	Youngest leaves
Location 4	PKM-1	8	73	3.5 m	Yes	Yes	

[1]The age of the tree only indicated the extent of growth the root system has had. All trees are pruned regularly and some are even cut back to the ground.
[2]Average of 3 repeated measurements of 3 tree random trees
[**]Crollo is a Spanish word conveying a mix of different (mostly unknown) varieties, as opposed to PKM-1, the most commercialised variety.
[***]Tree was cut back below 50 cm, hence the discrepancy between the age of the tree and the diameter of its stem.

Table 9: Overview of sampling locations and working methodology used in each location

LOCATION	*HARVEST OF LEAVES*	*PRODUCTION OF MLP*	*PRODUCTION OF MLC*	*TIME AND TEMPERATURE AT HARVEST*	*REMARKS*
LOCATION 1	Harvest is supervised by researcher	Harvested leaves were able to dry 24 hours outdoors*	Leaf juice was heated over a gas fire	9.40 am – 31 °C	Possible contamination of leaves with small insects or spiders
LOCATION 2	Harvest is supervised by researcher	Harvested leaves were able to dry 72 hours outdoors	Leaf juice was heated over an induction fire. Power was cut involuntarily for a period of time during heating	6.45 am – 26 °C	Possible contamination of leaves with small insects or spiders
LOCATION 3	Harvest is supervised by researcher	Harvested leaves were able to dry 72 hours outdoors	Leaf juice was heated over a wood fire	9.45 am – 32 °C	Contamination unlikely due to a thorough cleaning step
LOCATION 4	Leaves provided by producer	Harvested leaves were able to dry 72 hours outdoors	Leaf juice was heated over a gas fire	7.30 am – 27 °C	Very clean leaves were provided, contamination very unlikely

**Two dryers were at disposal for location 1, afterwards only 1 dryer for other locations.*

In each location, the harvesting of the leaves was done as randomly as possible and was done in threefold, either by consecutively harvesting three samples randomly in the field or by mixing one harvested lot of 9 kg into three random subsets. There was a selection bias present, albeit limited, as only a limited area of very expansive production fields is retained for sampling and producers prefer not to 'sacrifice' several trees for sampling in each field and prefer to contain sampling trees to a certain area in the field. Each location yields 3 samples to assess the yields. Collected samples were then randomised and mixed as 1 sample before the analyses performed in Belgium (partim II). Furthermore, three soil samples were taken randomly in the area of leaf harvest at a depth of 20 cm and were then also brought together as one mixed sample.

After collection, all harvested twigs per sample are stripped of their leaflets. Only the smaller subdivisions of the leaf, i.e. the secondary leaflets are retained, as these are the ones that are normally consumed. Following this leaf-stripping step, leaves are divided in three different batches intended for different production processes (leaves remain "fresh" or are processed into MLP or MLC). Of each sample lot of harvested leaves, a batch of about 100 g is set aside in a normal household refrigerator as "*fresh leaves*", a batch of about 100 g is put in a DIY-constructed solar dryer to produce "*Moringa leaf powder*" and the remaining quantity of leaves, approximately 2.8 kg is used for the production of "*Moringa leaf concentrate*". The yield of each produced product, MLP or MLC, was determined in threefold as there are three samples for each obtained product. A scale with a precision up to 1 g was used.

The MLC was made in the following manner, shown in figure 11: first the leaves were rinsed with water and then blended using a standard blender of 500W. The leaf juice was then filtered through a cloth. A lot of squeezing was required to obtain the maximal amount of liquid. The residual part left in the cloth is fiber. This leaf juice was captured in a plastic bucket. Leaf juice was then transferred in a metal pot and was brought to a boil over a heat source. The coagulation process starts at about 85 °C, but a temperature of 100 °C is sought-for to sterilise the product. The coagulated proteins were then scooped off using a plastic spoon and were placed in a filtering cloth. This was squeezed again to remove excess moisture. The excess moisture is referred to as "whey" and the residual part in the cloth is the fresh leaf concentrate or "curd". This curd or MLC was then placed in a solar dryer to dry alongside the MLP.

All produced samples of MLP and MLC were assessed by the researcher in terms of colour, taste, texture and smell by giving an integer score between "1" (very positive) and "5" (very negative) to determine the quality of produced leaf-products.

Finally, regarding storage and transport: all samples were refrigerated on-site when fresh, i.e. fresh leaves, fresh leaves awaiting drying and fresh concentrate. During transport to the central collection point of all samples, samples were kept as cool as possible. At the central collection point, fresh samples that needed drying were dried and all samples were stored in a freezer at -17 °C until transport by aeroplane to Belgium for analysis (see partim II).

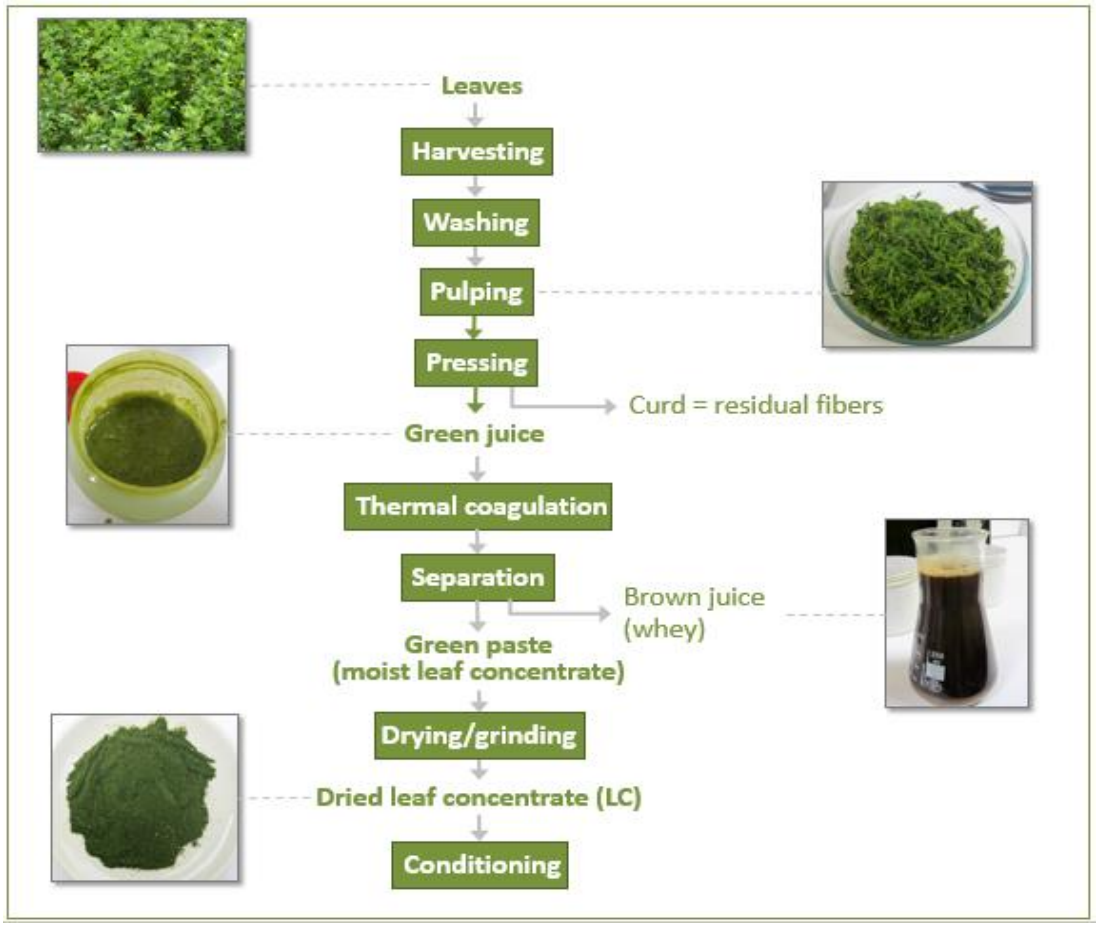

Figure 11: The MLC production process in a schematic overview (Courtesy of APEF)

Results

Setting the scene: getting an overview of local production techniques

Out of all commercial producers interviewed, one commercial producer focuses on the production of Moringa leaves. The other two produce Moringa with a focus on seed production for the oil industry but are also starting to cultivate Moringa for its leaves. On average, one third of their Moringa-cultivation area is dedicated to leaf production. Both producers would like to see this number increase in the near future. One other producer is not commercially active but keeps Moringa on a somewhat larger scale as part of an association dedicated to increase the sustainability of livelihoods for women. Therefore, the distinction is made between “producers” and “commercial producers”.

The commercial producer that focuses on leaf production, also makes use of an irrigation system. The other producers do not use any irrigation systems, but have indicated that they might invest in such systems if their leaf production acreage increases. All producers use a form of fertilisation, mostly not of a plant-based source – one producer uses a form of animal manure but would prefer to replace this with a plant-based alternative. All producers decided to produce their Moringa organically and mostly export their products. It must be noted that the definition of “organic” in Nicaragua also excludes the use of manure as fertilizer. An overview of different cultivation parameters is given in table 8.

Getting a view on local knowledge and perception of Moringa

Of the 31 interviewed, only 2 interviewees recognised the plant as “Moringa” and 13 interviewees recognised the plant as “Marango”, i.e. 48% of the people interviewed recognised the plant. This means that 52% of the people who were interviewed, could not name and/or recognise the plant instantaneously. Out of these, 7 clearly recognised the plant, but were not able to produce its name. When the name was given, they agreed vehemently, either with the name “Moringa” (1 person) or “Marango” (6 people). Finally, the 9 remaining interviewees (29% of the total number of interviewees) were unfamiliar with Moringa. Little more than half of these, i.e. 5 people, knew the name “Marango” but could not recognise the plant and 4 people did not show any sign of recognition at seeing the pictures or hearing the name.

In this context, groups are defined as the ‘Experienced’, the ‘Familiar’ and the ‘Unfamiliar’ (figure 12). The Experienced are those who recognise the plant instantaneously, provide its name – either Moringa or Marango – and can easily tell how they know the plant and how it is used. The Familiar are those in doubt, who recognise the plant but cannot produce its name, but when confronted with the name, they will acknowledge. The Unfamiliar are those who do not recognise the plant or who know the name but cannot link the plant to the name at all. They cannot easily provide uses for this plant. When somewhat familiar with the name, the uses provided are based on hear-say. The Experienced were mainly encountered in Loma Alegre, as is shown in figure 13. All Experienced people also attributed a medicinal use to Moringa, as opposed to the Familiar segment of interviewees.

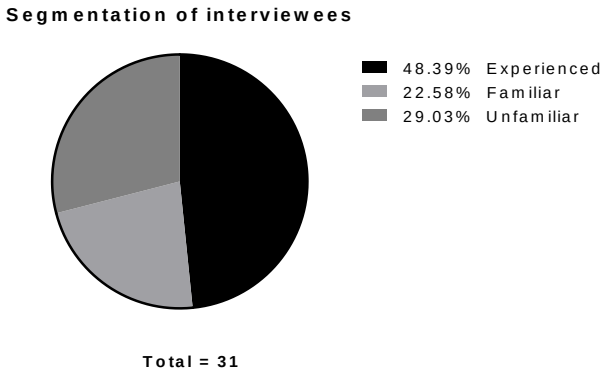

Figure 12: Overview of different groups of interviewees that were identified and their relative proportion.

A total of 19 persons of all interviewees described the plant as medicinal, of which all but one were familiar or experienced with the plant. However, the medicinal properties attributed to Moringa vary. Table 10 lists all answers given relating to medicinal uses of Moringa, provided by the interviewees, ranging from being a cough medicine to beneficial in case of diabetes. These uses were mainly associated with the consumption of its seeds and in some cases its flowers. Only a minority mentioned leaves. A common preparation method mentioned was the boiling of plant parts. A total of 5 persons out of the 31 interviewed – 2 being Unfamiliar, 1 Familiar and 1 Expert – did not provide any use for the plant and solely recognised the plant – for example, due to the plant being in their garden for ornamental purposes.

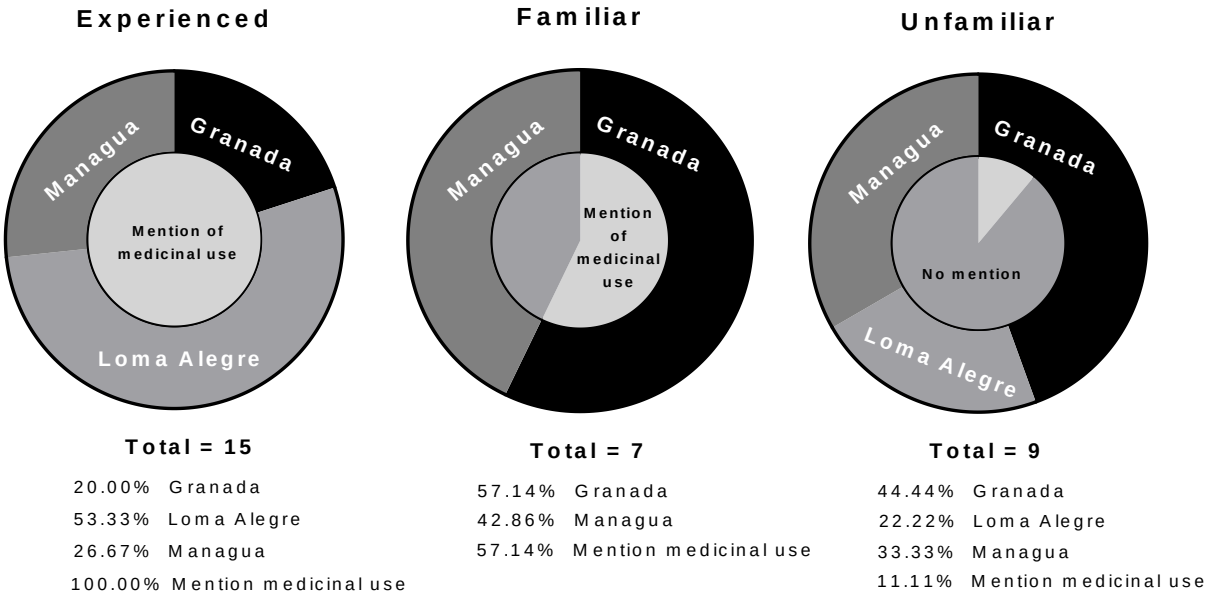

Figure 13: Overview of composition of different segments, i.e. Experienced, Familiar and Unfamiliar

Table 10: List of medicinal uses of Moringa according to interviewees

Medicinal use mentioned	***Number of times mentioned***
Eating seeds or parts of seed is medicinal	6
Seeds are eaten against diabetes	6
Seeds are eaten against pain in limbs	3
Flowers cooked as a tea and used as a cough remedy	3
Seeds are eaten to lose weight	3
Seeds are eaten against depression	1
Seeds are eaten against gastro-intestinal issues	1
Leaves are consumed against diabetes	1
Leaves as remedy against hiccups	1
Seeds eaten for heart-related diseases	1

Other uses mentioned	***Number of times mentioned***
Leaves can be consumed as a tea	1
Seeds can be eaten raw	1
Flowers can be consumed as part of egg-dish	1
No uses mentioned	12

Sensorial analysis results

According to the BS ISO 4120:2004 standard, for alpha = 0.05 and 17 or 18 assessors, a minimum of 10 correct responses is necessary to conclude that there is a perceptible difference between the samples. For alpha = 0.01, 12 correct responses are necessary at least in the case of 18 assessors present.

A perceptible difference was present between brownies containing MLP and blank brownies, as well as between brownies containing MLC and blank brownies (table 11). By calculating the upper confidence limit, there is a 95% confidence that a maximum of 70% of participants can detect a difference between MLP products and blank brownies and that a maximum of 77% can detect a difference between MLC products and blank brownies. In the case of brownies sampled, there is a slight preference (56%) for brownies containing MLP over brownies containing MLC. The order of samples did not seem to have a significant influence: 10 assessors preferred the first item presented and 8 the second item presented.

Furthermore, there is a perceptible difference between pitaya juices containing MLC and juices containing MLP. With 95% certainty, it can be claimed that a maximum of 62% can detect this difference. In the case of pitaya fruit juice, there is a clear preference (72%) for juice containing MLC over juice containing MLP. The order might have an impact too, since 12 assessors preferred the second item presented and only 4 the first item presented.

The most acceptable and hence lowest "total acceptability scores" were obtained for the pitaya juice containing MLC (table 12). However, the total scores did not differ significantly ($p>0.05$) from the juices containing MLP. Pitaya juice containing MLC scored fairly consistently across parameters assessed, with a very acceptable taste. The least acceptable "total acceptability scores" were obtained for the brownies containing MLC, though these again did not differ from brownies with MLP. Scores for taste, texture, colour and smell did not differ significantly between brownies with MLP and with MLC. However, for the juices, the MLC juice has a significantly better score for the parameters taste and smell than MLP juice.

Table 11: Results of both triangle and preference tests for all sensorial analyses conducted

	Triangle test			Preference test
	Number of correct responses MLP-blank	Number of correct responses MLC-blank	Number of correct responses MLP-MLC	Number of times MLP product was preferred over MLC product?
Test brownies	11	12	NA	10
Test pitaya juice	NA	NA	10	5

NA = not applicable

Table 12: Overview of results obtained in the ranking test of both sensorial analyses

	Ranking test – mean scores									
	Taste		*Texture*		*Colour*		*Smell*		*Total*	
	MLP product	MLC product	MLP product	MLC product	MLP product	MLC product	MLP product	MLC product	MLP product	MLC product
Test brownies	2.7^{a}	2.6^{a}	1.9^{b}	2.1^{b}	2.2^{c}	2.1^{c}	2.4^{d}	2.8^{d}	9.2^{e}	9.5^{e}
Test pitaya juice	2.3^{a}	1.5^{b}	2.2^{c}	1.7^{c}	1.9^{d}	1.7^{d}	2.4^{e}	1.7^{f}	8.7^{g}	6.5^{g}

Different letters besides numbers indicate a significant difference ($p<0.05$) for the same parameter and within one test. The total score is the sum of the scores for taste, texture, colour and smell. Scores range between 1 (very positive) and 5 (very negative).

Yield and acceptability assessments in Nicaragua

The yield of MLP on a FW basis is about ten-fold that of MLC. The highest yield for MLP on a FW basis was obtained in location 2, whereas the highest yield for MLC was obtained in location 3 (table 13). Table 14 indicates the acceptability assessments as performed by the researcher, as an indicative value of possible spoilt samples. Location 4 seems to have a worse score for taste and smell as compared to the other locations and these samples could have been spoilt.

Table 13: Overview of yield results obtained in Nicaragua for both MLP and dried MLC

Location	Average Yield MLP (%)	Average Yield dried MLC (%)
1	17.33	2.04
2	23.82	1.94
3	20.22	2.71
4	13.94	1.81
Mean	18.83	2.13

Table 14: Overview of mean acceptability results obtained in Nicaragua for both MLP, fresh MLC and dried MLC

Location	Mean score for colour			Mean score for smell			Mean score for texture			Mean score for taste		
	MLP	MLC, fresh	MLC, dry	MLP	MLC, fresh	MLC, dry	MLP	MLC, fresh	MLC, dry	MLP	MLC, fresh	MLC, dry
1	2.7	4.0	5.0	1.7	3.0	1.7	3.0	3.7	4.0	2.7	3.3	2.7
2	3.0	4.0	5.0	2.3	3.3	2.7	2.7	3.0	5.0	3.0	3.7	2.3
3	2.3	3.0	4.0	2.3	1.7	3.3	3.0	2.0	2.3	2.3	3.3	2.0
4	3.0	3.3	4.0	3.7	3.3	4.7	2.7	2.3	4.3	5.0	4.3	2.3
Mean	2.8	3.6	4.5	2.5	2.8	3.1	2.8	2.8	3.9	3.3	3.7	2.3

Mean acceptability scores range between 1 (very positive) and 5 (very negative), n=3.

Discussion

The gathered data on local production methods is mostly a reflection of how Moringa is produced commercially or on a larger scale. Out of conversations with a range of stakeholders, what jumps out, is the diversity of production methods of Moringa in Nicaragua. Moringa is grown commercially, but is also grown in backyards and gardens or is found along the streets or in hedgerows. Some producers use a small part of their land that cannot be used for anything else, to plant some Moringa. What is more, there is no one distinct and optimised method for cultivation. There is a high variability in the method of cultivating Moringa, ranging from the distance that is maintained between trees between different plots – even on the same production site, different types of plots can be cultivated – to the frequency and method of pruning. Most of these choices are motivated by the intended end-use of the tree, be it seed or leaf production, wood production or added ornamental value. Moringa remains a multipurpose tree in this context.

Regarding the perception of Moringa by selected potential consumers, there was a selection bias of people who had a higher chance of knowing Moringa. It is therefore expected that the selected interviewees should have a higher chance of knowing Moringa. It is noteworthy that, even in this group, 29% is not familiar with Moringa. It can be surmised that, on the whole of the population in the selected town and cities, this number is equal or higher perhaps. The proportion of Experts, Familiar and Unfamiliar may shift, but those that are familiar with Moringa, will probably associate it with the same uses in a same degree of ranking. Some interesting indications are present regarding the general perception of Moringa, when people are familiar with the tree: most of the interviewees described the tree as having medicinal properties and only a minority would consume plant parts regularly as a food commodity. For food-uses, leaves and flowers are mentioned as being eaten as dishes or drinks by two different interviewees. Seeds as a food- use was mentioned by one interviewee who did not recognise the plant, only its name. Therefore, it is probable this interviewee only knew from hear-say that seeds were consumed but could not recall the purpose of this, which would probably be medicinal as this was mentioned by all other interviewees regarding Moringa seeds.

In a study in Senegal in 2008, the use of local plants against diabetes was assessed, as well as the general belief in the efficacy of these plants. A majority of about 65% of the people questioned believed that these medicinal plants were useful in combatting diabetes and also reported that they made use of Moringa leaves and roots for this purpose (Dièye *et al*., 2008). In a Ugandan study, rural communities also reported a variety of medicinal uses for Moringa leaves, including *Diabetes mellitus*, heart burn, the flu and malnutrition (Kasolo *et al*., 2010). This is partly different to the perceptions of those questioned in the present study in Nicaragua, where Moringa seeds and not the leaves were perceived to be the most adequate for medicinal properties. It could be possible that the local perception of Moringa's medicinal properties is linked to the bitter taste of these seeds. Previous studies indicate that there is a cultural-dependent relationship between the perception of taste and medicinal uses (Pieroni & Torry, 2007; Etkin, 2006). The perception by the selected interviewees that Moringa seeds are medicinally beneficial for diabetes is rooted in some scientific basis, yet there is also a gap present between perceptions and scientific evidence. Aqueous leaf extracts of Moringa seem to have antidiabetic and antioxidative effects in rats (Yassa & Tohamy, 2014; Gupta et al., 2012) and the antimicrobial effect of seed and leaf extracts have also been studied, which seem to inhibit growth of organisms (Bukar *et al*., 2010). Nonetheless, there is a lack of supporting clinical trials of the effectivity *in* vivo (Fahey, 2005). The lack of medicinal properties or the isolates of medicinal compounds has not been reported so far (Gopalakrishnan *et al*., 2016; Farooq *et al*., 2012; Anwar *et al*., 2007; Fahey, 2005), possibly being the result of a publication bias. Hence, such studies do not necessarily indicate that Moringa has medicinal properties in each case.

In Nicaragua, about 5-20% of the total population is "native indigenous". According to WHO, these populations tend to rely on traditional medicinal practices (Bodeker *et al*., 2005). Furthermore, in 2005, Nicaragua had about 6 doctors and 3 nurses per 10 000 population (Bodeker *et al*., 2005), implying that certain rural areas would have more difficult access to health facilities. Rural areas may tend to rely on traditional, complementary and alternative medicine (TCAM) and related practices, as demonstrated in Canada (Hollenberg *et al*., 2013). What is more, a study conducted in a "barrio" or poorer neighbourhood in Managua, the capital of Nicaragua, regarding the use of TCAM there showed that the majority of households resorted to these (Ailinger *et al*., 2004). Therefore, as herbal remedies are used in Nicaragua, certain misconceptions regarding Moringa as a herbal remedy could also have negative effects, i.e. when the consumption does not relieve ailments and urgent

professional medical help is necessary. In the present small ethnographic study, there was a clear link with interviewees from the village, Loma Alegre, that was located close to a Moringa plantation and their familiarity with Moringa. Still, not all interviewees from this village explained their familiarity with the tree by seeing it in a plantation and many could name at least one medicinal use. This could indicate that persons from the 'more rural area' in this study – as market vendors from Granada and Managua would also be from rural areas, but in closer proximity to an urban area –, were also more familiar with Moringa and its medicinal uses. Nevertheless, two studies in the U.S. and Nepal found that urban areas seem to have higher values for self-medication with herbal medicine (Barnes *et al*., 2004; Shankar *et al*., 2002), as opposed to the results here. However, larger validating research would be necessary to draw any conclusions regarding the medicine use between rural and urban areas in Nicaragua.

Finally, it must be kept in mind that a large proportion of interviewees could not provide any uses for the tree, either because they did not recognise the tree or because they could not specify any. Furthermore, consumer perceptions about Moringa have repercussions for marketing strategies or intended development programs.

The perception of the taste, texture, smell and colour of MLP and MLC products shows that it is dependent on the recipe formulation, as the preference for MLP or MLC products differed whether it was added to brownies or pitaya juice. For example, pitaya juice would naturally contain small black seeds, which is generally appreciated and common. The added MLC to this fruit juice would mimic these seeds, explaining the higher acceptability as compared to pitaya juice with MLP. Similarly, brownies with MLP had a texture that was closer to the blank. Therefore, it is possible that assessors preferred the product that deviated the least from the constructed idea of the product. Additionally, the preference for MLC pitaya juice seems to be mainly influenced by the taste and smell, or it could be influenced by parameters not studied in this analysis, such as peer pressure or the order of presentation.

The yields of MLP were different in each location, most probably due to differences in the drying rate and the extent of drying or the amount of residual moisture. The yields of MLC could differ due to the extent of drying, but also due to differences in the MLC production process in each location – i.e. the type of heat source used will determine the rate of heating and coagulation – or the amount of proteins in the leaves. The accumulation of proteins in leaves is higher in younger leaves, which could explain the high yield of MLC in location 3. However, the yields were lowest in location 4, but the leaves were also relatively young in this location. Since the amount of extractable protein is not only influenced by the age of leaves at harvest, but also by the amount of nitrogen fertilizer, seed rate and the climate (Arkcoll & Festenstein, 1971), differences in cultivation methods could perhaps also explain this observation. In general, an effect of location on a certain variable can relate to differences in soil and/or climate conditions, production or drying process, site-specific cultivation methods of Moringa or genetic differences. The possible spoilage of samples of location 4 could have been due to a longer transportation period and high temperatures. Spoilage could, amongst others, be due to leaf senescence brought on by the developmental age of the leaf or environmental factors such as heat, drought, salinity and other factors (Khanna-chopra, 2012). However, as will be observed later on, this possible spoilage did not have any bearing on analysed nutritional components in partim II.

PARTIM II: ASSESSING THE NUTRITIONAL VALUE

Materials and methods – data collection in Belgium

Table 15 shows an overview of all the different analyses that were performed on all the samples collected from Nicaragua, including fresh Moringa leaves, MLP, MLC, soil samples and test samples of the sensorial analyses.

Table 15: Overview of types of analyses performed on different samples

SAMPLE	MOISTURE CONTENT	ASH	CRUDE PROTEIN	CRUDE FAT	MINERALS	COND-ENSED TANNINS	PHYTATE CONTENT	TOTAL PHENOLIC COMPOUNDS	IN VITRO DIGESTI-BILITY
MORINGA FRESH LEAVES	✓	✓	✓	✓	✓	✓	✓	✓	✓
MLP	✓	✓	✓	✓	✓	✓	✓	✓	✓
MLC	✓	✓	✓	✓	✓	✓	✓	✓	✓
SOIL	✓	✓	×	×	✓	×	×	×	×
ACCEPT-ABILITY TEST	✓	✓	×	×	✓	×	×	×	✓

Sample preparation

For each location, all three frozen samples of a certain type (fresh, MLP or MLC) were mixed as one. Each location hence had 1 sample of fresh leaves, 1 sample of MLP and 1 sample of MLC to be analysed. Defrosting was avoided when possible in order to maintain freshness of samples and to avoid degradation of certain compounds. All analyses were performed in the Laboratory of Food Microbiology and Biotechnology of UGent Campus Kortrijk.

Moisture content

Dry matter content was determined according to the ISO 1442-1973 standards. Moisture content is calculated as $100\% - DM\ (\%)$.

Ash

The ash content was determined following a standard operation procedure (SOP). Crucibles were washed in 1% nitric acid solution, dried and weighed (M0). The sample was added to the crucible and both were weighed again (M1). Afterwards, the sample was ashed in a muffle oven at 550 °C overnight. The ashed sample and crucible were weighed again (M2). The ash content is calculated as $\frac{(M2-M0)}{(M1-M0)} * 100\%$.

Crude protein content

Crude protein content was determined using the Kjeldahl method (ISO 937-1978). Assuming a nitrogen content in proteins of 16%, protein content in g per unit of fresh sample weight was calculated as $\frac{((V_{HCl,\ sample} - V_{HCl,\ blanc}) * 14 * N * 6.25)}{(FW * 1000)} * 100\%$ with V the volume of hydrochloric acid titrated to the alkaline solution and N the normality of this hydrochloric acid solution, 0.1N.

Crude fat content

Crude fat content was measured using the Soxhlet method (ISO 1444-1973) and using the samples attained after the dry matter analysis. All samples were extracted for exactly 6 hours. The fat content in g per unit of dry sample weight was calculated as follows: $\frac{(M_{flask+extracted\ fat} - M_{flask})}{M_{sample}} * 100\%$.

Carbohydrate content

The carbohydrate content was calculated as the difference between total mass and analysed constituents (water content, ash content, crude protein content and crude fat content).

Energy content

Energy contents (kcal/100 g DM) were calculated by multiplying the amount of proteins by 4 kcal per gram, the amount of fat by 9 kcal per gram and the amount of fiber by 2 kcal per gram. The amount of fibers is assumed to be 80% of the total amount of carbohydrates, based on the average carbohydrate and total fiber values for younger and older leaves provided by Agamou *et al*. (2015).

Mineral analysis

Mineral analysis was performed using the SOP for mineral analysis by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Ashed samples were dissolved in 4 ml of nitric acid if the samples were soil samples and 3 ml in all other cases. Dissolved ashes were filtered and then diluted to their correct dilutions to be able to accurately analyse them. All samples for micro-elements such as iron and zinc had a dilution factor of 25. For macro-element such as calcium, sodium and magnesium, dilutions varied more according to the sample. Fresh leaves were diluted 2000 times, while MLP and MLC were diluted 1000 times. Acceptability test samples had a dilution factor of 200 for determining these macro-minerals. These dilution factors are based on preliminary tests and optimisation. Table 16 shows an overview of the instrument parameters. Both iron and zinc were measured axially, while macrominerals were measured radially.

Table 16: Overview of parameters set for ICP-OES measurements

	Radial	Axial
Instrument name	iCAP 7200	iCAP 7200
UV Exposure Time	15	15
UV RF Power	1150	1150
UV Neb Gas Flow	0.5	0.5
VIS Exposure Time	5	5
VIS RF Power	1150	1150
VIS Neb Gas Flow	0.5	0.5
Cool Gas Flow Rate	12	12
Aux Gas Flow Rate	0.5	0.5
UV Exposure Time	15	15
UV RF Power	1150	1150

Condensed tannins

Condensed tannins were determined according to a SOP. The method is based on Price *et al*. (1978) using vanillin reagent. For this method, methanol extracts were made of leaf and leaf-derived samples. The extracts were mixed using an ultra-turrax (45 seconds at 10000 rpm) and centrifuged (15 minutes, 4000 rpm, 4 °C). Both the pellet and the supernatans were collected. The analysis was performed on the collected supernatans.

Phytic acid content

The method for this analysis is described by Reichwald & Hatzack (2008) and is based on determining phytic acid through indirect spectrophotometry, as the absorbance by light of non-complexed iron to phytate is measured. A standard curve with concentrations ranging between 0 mg/ml and 140 mg/ml was constructed using phytic acid dodecasodium salt. The absorbance was measured at 540 nm.

Total phenolic compounds

The analysis for total phenolic compounds was done on the supernatans of methanol-extracts of leaf-samples using the Folin-Ciocalteu phenol reagent. This method only determines the amount of unbound phenolic compounds. Samples were diluted by a factor 4 and a gallic acid standard was used with concentrations ranging between 3 mg/L and 60 mg/L. The absorbance was measured at 760 nm.

In vitro digestibility

To assess the *in vitro* digestibility of samples, a standardised procedure was implemented as described by Minekus *et al*. (2014). As the final goal is to measure iron dialyzability, all glassware was rinsed with nitric acid and bidistilled water was used whenever necessary. The amount of the sample to be weighed for the digestions was calculated using the DM content – at least 1.5 g DM was required for this analysis: ±20 g for blank pitaya juice, ±10 g for other pitaya juices, ±8 g for fresh leaves and ±2 g for all remaining samples to be analysed. These *in vitro* digestions yield three fractions, i.e. an insoluble fraction, a soluble yet non-dialyzable fraction and a soluble dialyzable fraction. The dialyzable fraction is the food matter that was able to pass through the selective membrane simulating the human intestine. The three fractions obtained by the digestion were stored at -20 °C before ashing all these samples and performing the analysis for minerals – iron and zinc in particular. For this analysis, fresh leaves were crushed using a pestle and mortar to simulate the systematic breakdown of food by chewing.

Soil analysis

Soil samples were analysed to determine their effect on mineral content in foliage. Soil texture was determined by approximation using a ribbon test. All soil samples were also analysed for moisture content, ash content and mineral content as described previously.

Statistical analysis

Statistical analysis included one-way ANOVA analyses, to determine if multiple means differed significantly ($p<0.05$) for different production processes – being fresh leaves, MLP or MLC – and for different locations. Further statistical analysis included descriptive analysis using boxplots. Correlations were determined using Pearson's correlation test. All statistical analyses were performed in R Studio version 3.4.3.

Results

Proximate analysis

The analysed DM content for fresh Moringa leaves ranged between 15.48% for location 4 and 23.99% for location 2 and was significantly lower – about four- to fivefold – from that of MLP and MLC for each location (table 17). Hence, there was a significant effect of the type of product made in a given location on DM content (*$p = 3.26*10^{-6}$, $p = 6.57*10^{-8}$, $p = 1.57*10^{-6}$, $p = 1.22*10^{-7}$; respectively location 1, location 2, location 3 and location 4*). There also was a significant impact of the location on DM content, when looking at a given sample type (*$p = 0.0003$, $p = 2.82*10^{-5}$, $p = 0.004$; respectively fresh leaves, MLP, MLC*). The same can be said for the ash content with regard to MLP samples ($p = 3.79*10^{-5}$), while for the ash content of fresh leaves there seems to be a trend whereby the location influences the ash content (*$p = 0.050$*). For MLC ash contents however, location did not have any significant impact (*$p = 0.851$*) and the mean ash content was 2.75 g/100 g DM. In addition, for each location, there was a significant relation between different samples (*$p = 0.0001$, $p = 0.0001$, $p = 3.42*10^{-8}$, $p = 4.92*10^{-9}$; respectively location 1, location 2, location 3 and location 4*). The ash content of fresh leaves and MLP did not differ significantly, but they were significantly higher than that of MLC – approximately three or more times higher. For the crude protein, the location did not have any significant impact (*$p = 0.243$, $p = 0.808$, $p = 0.839$; respectively fresh leaves, MLP, MLC*). Hence, the average crude protein content for all locations for fresh leaves was 31.5 g/100 g DM, for MLP 30.1 g/100 g DM and for MLC 64.8 g/100 g DM. Like the ash contents described previously, there was a clearly significant relationship between samples for each location (*$p = 0.008$, $p = 0.007$, $p = 0.006$, $p = 0.006$; respectively location 1, location 2, location 3 and location 4*). For each location, MLC crude protein contents were significantly higher – about twice as high – as those of fresh leaves and MLP. These last two did not differ significantly in any location. Furthermore, for the crude fat content, the location had a significant impact on the contents found in MLC (*$p = 0.179$, $p = 0.356$, $p = 0.014$; respectively fresh leaves, MLP, MLC*). The fat content of MLC location 2 was higher than in other locations. Also, there were significant differences present between samples in a given location (*$p = 0.039$, $p = 0.001$, $p = 0.019$, $p = 0.174$; respectively location 1, location 2, location 3 and location 4*). The overall trend was that fresh leaves contained less crude fat than MLP and that MLP contained less fat than MLC. The fat content of MLC was also consistently significantly higher than that of fresh leaves and sometimes than that of MLP, except for location 4, where no statistical differences were observed between samples. The highest crude fat content found, was for MLC from location 2, i.e. 13.12 g/100 g DM. Finally, for the calculated carbohydrate contents of fresh leaf samples, there was an impact of the location, but not for the other samples (*$p = 0.011$, $p = 0.220$, $p = 0.993$; respectively fresh leaves, MLP, MLC*). Fresh leaves from location 2 had a significantly higher carbohydrate content. For MLP and MLC, the mean carbohydrate contents were 55.5 g/100 g DM and 23.3 g/100 g DM respectively. What is more, each location had significant differences between samples (*$p = 0.015$, $p = 0.005$, $p = 0.008$, $p = 0.014$; respectively location 1, location 2, location 3 and location 4*). For each location site, MLC samples have a more than 50% lower carbohydrate content than fresh or dried leaves – as the difference method was used to calculate carbohydrate contents and MLC contained higher crude protein and crude fat contents.

Lastly, the energy content does not tend to differ between different locations (*$p = 0.222$, $p = 0.107$, $p = 0.478$; respectively fresh leaves, MLP, MLC*). For fresh leaves, locations 2 and 4 differ significantly in energy content. The average energy contents for fresh leaves, MLP and MLC were 153 kcal/100 g DM, 164 kcal/100 g DM and 342 kcal/100 g DM respectively.

Between samples in a given location, there were significant differences (*p = 0.005, p = 0.002, p = 0.001, p = 0.010; respectively location 1, location 2, location 3 and location 4*). The energy content of MLC was significantly higher than that of MLP or fresh leaves – it provides more than double the amount of energy per 100 g DM.

Mineral analysis

The different locations did not have a significant impact on the iron contents of the same sample types (*p = 0.284, p = 0.108, p = 0.053; respectively fresh leaves, MLP, MLC*) and only an impact on MLC zinc contents (*p = 0.800, p = 0.264, p = 0.005; respectively fresh leaves, MLP, MLC*), where MLC zinc contents from location 1 were significantly higher than in other locations (table 18). The iron contents for fresh leaves were hence on average 8.61 mg/100 g DM over all locations, while they were on average 4.31 mg/100 g DM for MLP and 13.93 mg/100 g DM for MLC. Furthermore, there were significant differences present regarding iron content between samples (*p = 0.020, p = 0.005, p = 0.001, p = 0.01; respectively location 1, location 2, location 3 and location 4*). The MLC samples' iron contents were consistently two- or threefold the MLP iron contents for each location. There was a clear trend present, where iron contents of MLP samples were lower than those of fresh leaves and contents of fresh leaves were lower than those of MLC samples. However, MLC iron contents and fresh leaf iron contents do not always differ significantly. On the other hand, for zinc contents, there does not seem to be a significant impact of the production process in each location (*p = 0.056, p = 0.318, p = 0.054, p = 0.050; respectively location 1, location 2, location 3 and location 4*). There is a trend nonetheless, where fresh leaves have the highest zinc content, followed by MLC and MLP. The only exception to this rule was location 4, where MLC contents were lower than those of MLP.

As for calcium, there was a significant effect of the location for fresh leaves and MLC (*p = 0.035, p = 0.333, p = 0.015; respectively fresh leaves, MLP, MLC*). In location 2, the calcium content was significantly lower than in location 1 for fresh leaves, while in location 1, the calcium content of MLC was significantly higher than for all the other locations. Only for location 4 significant differences were present between samples (*p = 0.111, p = 0.288, p = 0.073, p = 0.046; respectively location 1, location 2, location 3 and location 4*). The trend for calcium contents in each location was that fresh leaves' contents were highest, followed by MLP and then only MLC. However, only in location 4, there was a significant difference between MLC and fresh leaves. Calcium contents of fresh leaves tend to be twice as high as those of MLP and more than three times as high as those of MLC. Furthermore, for sodium, there was a significant impact of the location for MLP and MLC (*p = 0.682, p = 0.015, p = $3.37*10^{-5}$; respectively fresh leaves, MLP, MLC*), where MLP contents in location 3 and 4 were lower than other locations and MLC contents of location 3 were much higher than in other locations. What is more, there does not seem to be any significant effect of the sample production process in each location (*p = 0.069, p = 0.340, p = 0.516, p = 0.465; respectively location 1, location 2, location 3 and location 4*). Yet there was an observable trend: MLC contents were lower than those of MLP and in turn, MLP contents were lower than those of fresh leaves. This holds true for all locations, except for location 3. In this case, MLC contents were higher than MLP sodium contents. Amounts of sodium in fresh leaves can be more than ten times as high as those found in MLP or MLC. Lastly, the observations for magnesium were more variable. There was a significant impact of location for fresh leaves and MLC (*p = 0.001, p = 0.178, p = 0.003; respectively fresh leaves, MLP, MLC*). Magnesium contents found in location 1 in fresh leaves were significantly higher than in other locations. For MLC from locations 3 and 4, magnesium contents were also significantly higher than other locations. There was a significant impact of the production process in most locations (*p*

= 0.001, p = 0.008, p = 0.125, p = 0.031; respectively location 1, location 2, location 3 and location 4). Here, similarly to both calcium and sodium, the trend seems to be that fresh leaves contain the highest contents, followed by MLP and MLC. These differences were not always significant. Magnesium contents of fresh leaves were 1.5 to 2 times higher than MLP contents and were four to even eighteen times higher than MLC contents.

Antinutritional factors

In the case of condensed tannins contents, the location plays no significant part in influencing the tannin content of a given sample (*p = 0.455, p = 0.554, p = 0.243; respectively fresh leaves, MLP, MLC*) (table 19). The mean tannin contents were then: 229 mg catechin equivalents/100 g DM for fresh leaves, 93.2 mg catechin equivalents/100 g DM for MLP and 67.7 mg catechin equivalents/100 g DM for MLC. Significant differences between samples per location were present, except for location 4 (*p = 0.019, p = 0.024, p = 0.049, p = 0.332; respectively location 1, location 2, location 3 and location 4*). The trend is clear: fresh leaves contain the highest amount of tannins, albeit not always significantly so. Tannin contents present in MLP and MLC seem to be similar, even though MLP contents exceed those of MLC in two locations.

Secondly, for the content of total phenolic compounds, these contents were influenced by the location in which they were sampled (*p = 0.047, p = 0.049, p = 0.015; respectively fresh leaves, MLP, MLC*). Nevertheless, these differences seem to be minimal. For each, there was a significant effect however between samples (*p = 0.004, p = $6.18*10^{-7}$, p = $2.30*10^{-6}$, p = 0.0002; respectively location 1, location 2, location 3 and location 4*). The highest contents of phenolic compounds were found in fresh leaves, followed by MLP and then MLC. The amount of MLP phenolic compounds were about 20% lower than those of fresh leaves, while for MLC the amounts can be up to 8 times lower than fresh leaves but were generally in the range of 3 to 4 times lower.

Finally, in terms of contents of phytic acid, the location tends to moderately influence the contents of fresh leaves and MLC, but not of MLP (*p = 0.013, p = 0.417, p = 0.021; respectively fresh leaves, MLP, MLC*). For fresh leaves' contents, location 4 had a significantly higher amount of phytic acid, while for MLC, locations 2 and 4 had lower amounts than the other locations. For each location except location 2, there was a significant effect however between samples (*p = 0.008, p = 0.155, p = 0.034, p = 0.003; respectively location 1, location 2, location 3 and location 4*). The overall trend is clear: fresh leaves tend to have a phytic acid content that was more than twice as high than contents found in MLP and MLC – yet these two do not tend to differ much. In locations 1 and 3, the MLC phytic acid content was higher than that of MLP, while in locations 2 and 4, it was the other way around.

Table 17: Overview of proximate composition of different samples

Location	Sample	Sample Code	DM (g/100g FW)	Ash (g/100g DM)	Crude protein (g/100g DM)	Crude fat (g/100g DM)	Carbohydrate content (g/100g DM)	Energy content (kcal/100g DM)
1	Fresh	1.1	18.37 (0.42) *a,**a***	9.09 (2.44) *ab,**a***	34.36 (0.81) *a,**a***	2.17 (1.58) *a,**a***	55.96 (2.01) *a,**a***	247 (14) *a,**a***
	MLP	1.2	93.63 (0.04) *a,**b***	9.91 (0.50) *a,**a***	30.74 (2.49) *a,**a***	5.18 (0.15) *a,**ab***	54.22 (2.62) *a,**a***	256 (4) *a,**a***
	MLC	1.3	94.11 (1.22) *ad,**b***	2.83 (0.46) *a,**b***	66.22 (7.63) *a,**b***	6.48 (0.04) *a,**b***	24.72 (8.22) *a,**b***	363 (17) *a,**b***
2	Fresh	2.1	23.99 (0.23) *b,**a***	7.32 (1.95) *a,**a***	29.84 (3.02) *a,**a***	2.02 (0.57) *a,**a***	62.30 (0.94) *b,**a***	237 (5) *a,**a***
	MLP	2.2	90.12 (0.02) *b,**b***	8.36 (0.32) *b,**a***	28.64 (2.04) *a,**a***	5.06 (0.19) *a,**b***	58.11 (2.25) *a,**a***	253 (3) *a,**a***
	MLC	2.3	95.06 (0.23) *a,**c***	2.72 (0.09) *a,**b***	61.04 (5.97) *a,**b***	13.12 (0.93) *b,**c***	23.18 (6.86) *a,**b***	399 (21) *a,**b***
3	Fresh	3.1	20.67 (0.84) *c,**a***	9.88 (0.50) *ab,**a***	29.91 (0.96) *a,**a***	2.80 (0.05) *a,**a***	57.79 (1.24) *ab,**a***	237 (2) *a,**a***
	MLP	3.2	94.94 (0.18) *c,**b***	9.01 (0.64) *ab,**a***	30.76 (2.38) *a,**a***	6.06 (1.79) *a,**ab***	53.76 (0.40) *a,**a***	264 (7) *a,**a***
	MLC	3.3	89.53 (0.45) *bc,**c***	2.63 (0.53) *a,**b***	65.23 (6.84) *a,**b***	9.26 (0.11) *ab,**b***	23.10 (7.52) *a,**b***	381 (14) *a,**b***
4	Fresh	4.1	15.48 (0.36) *d,**a***	10.89 (0.67) *b,**a***	31.97 (2.58) *a,**a***	4.77 (1.36) *a,**a***	52.82 (1.16) *a,**a***	255 (4) *a,**a***
	MLP	4.2	93.18 (0.26) *a,**b***	11.20 (0.59) *c,**a***	30.11 (2.90) *a,**a***	3.00 (2.46) *a,**a***	56.00 (1.25) *a,**a***	237 (13) *a,**a***
	MLC	4.3	91.80 (0.01) *cd,**c***	2.82 (0.27) *a,**b***	66.56 (6.69) *a,**b***	8.02 (1.95) *a,**a***	22.50 (9.06) *a,**b***	374 (30) *a,**b***

Different letters before and after comma indicate significant differences (1) between different locations for the same sample type and (2) between different sample types for the same location respectively (p<0.05), within the same column. All values are mean (SD), total n=2 for all parameters except Ash (n=4).

Table 18: Overview of mineral composition of different samples

Location	Sample	Sample Code	Fe (mg/100 g DM)	Zn (mg/100 g DM)	Ca (g/100 g DM)	Na (g/100 g DM)	Mg (g/100 g DM)
1	Fresh	1.1	10.37 (1.89) *a,**ab***	2.72 (0.60) *a,**a***	5.21 (1.92) *a,**a***	0.33 (0.16) *a,**a***	0.71 (0.06) *a,**a***
	MLP	1.2	5.78 (0.67) *a,**a***	1.26 (0.11) *a,**a***	2.17 (1.91) *a,**a***	0.05 (0.003) *a,**a***	0.26 (0.03) *a,**b***
	MLC	1.3	12.94 (0.44) *a,**b***	2.24 (0.13) *a,**a***	0.33 (0.03) *a,**a***	0.01 (0.001) *a,**a***	0.04 (0.004) *ad,**c***
2	Fresh	2.1	8.31 (0.96) *a,**a***	2.93 (1.63) *a,**a***	0.75 (0.49) *b,**a***	0.27 (0.29) *a,**a***	0.24 (0.04) *b,**a***
	MLP	2.2	4.70 (0.44) *a,**a***	1.40 (0.11) *a,**a***	0.42 (0.01) *a,**a***	0.02 (0.004) *ab,**a***	0.11 (0.03) *a,**b***
	MLC	2.3	16.74 (1.81) *a,**b***	1.44 (0.01) *b,**a***	0.19 (0.03) *b,**a***	0.01 (0.01) *a,**a***	0.02 (0.001) *a,**b***
3	Fresh	3.1	7.39 (0.24) *a,**a***	2.09 (0.13) *a,**a***	1.28 (0.38) *ab,**a***	0.38 (0.52) *a,**a***	0.26 (0.05) *b,**a***
	MLP	3.2	3.30 (0.92) *a,**b***	1.00 (0.28) *a,**b***	0.53 (0.23) *a,**a***	0.01 (0.01) *b,**a***	0.18 (0.10) *a,**a***
	MLC	3.3	13.66 (0.08) *a,**c***	1.43 (0.32) *b,**ab***	0.37 (0.03) *a,**a***	0.10 (0.001) *b,**a***	0.07 (0.005) *bc,**a***
4	Fresh	4.1	8.41 (1.53) *a,**ab***	2.34 (0.61) *a,**a***	1.32 (0.37) *ab,**a***	0.02 (0.01) *a,**a***	0.21 (0.03) *b,**a***
	MLP	4.2	3.47 (1.09) *a,**a***	0.91 (0.32) *a,**a***	0.52 (0.14) *a,**ab***	0.01 (0.01) *b,**a***	0.15 (0.04) *a,**ab***
	MLC	4.3	12.37 (1.14) *a,**b***	0.75 (0.08) *b,**a***	0.37 (0.03) *a,**b***	0.01 (0.001) *a,**a***	0.05 (0.005) *cd,**b***

Different letters before and after comma indicate significant differences (1) between different locations for the same sample type and (2) between different sample types for the same location respectively ($p<0.05$), within the same column. All values are mean (SD), total n=2.

Table 19: Overview of the content of antinutritional factors in Moringa samples

Location	Sample	Sample Code	Condensed tannins (mg catechin eq./100g DM)	Total phenolic compounds (g GAE/100g DM)	Phytic acid content (mg/100g DM)
1	Fresh	1.1	270 (49.8) *a,**a***	9.66 (0.68) *ab,**a***	1178 (22.4) *ab,**a***
	MLP	1.2	93.97 (25.49) *a,**b***	7.10 (0.59) *a,**b***	546 (134) *a,**b***
	MLC	1.3	93.80 (9.03) *a,**b***	3.41 (0.49) *a,**c***	647 (7.76) *a,**b***
2	Fresh	2.1	202 (39.6) *a,**a***	10.84 (0.06) *a,**a***	726 (329) *a,**a***
	MLP	2.2	108 (3.05) *a,**ab***	7.61 (0.02) *ab,**b***	301 (107) *a,**a***
	MLC	2.3	56.93 (19.4) *a,**b***	2.37 (0.03) *ab,**c***	195 (90.8) *b,**a***
3	Fresh	3.1	265 (92.6) *a,**a***	9.80 (0.07) *ab,**a***	918 (164) *a,**a***
	MLP	3.2	49.83 (4.37) *a,**a***	8.51 (0.03) *b,**b***	271 (85.7) *a,**b***
	MLC	3.3	56.08 (26.6) *a,**a***	2.40 (0.08) *ab,**c***	425 (139) *ab,**ab***
4	Fresh	4.1	178 (54.9) *a,**a***	9.36 (0.00) *b,**a***	1888 (76.2) *b,**a***
	MLP	4.2	121 (94.8) *a,**a***	7.70 (0.23) *ab,**b***	322 (261) *a,**b***
	MLC	4.3	63.87 (6.10) *a,**a***	1.13 (0.52) *b,**c***	272 (29.0) *b,**b***

Different letters before and after comma indicate significant differences (1) between different locations for the same sample type and (2) between different sample types for the same location respectively ($p<0.05$), within the same column. All values are mean (SD), total n=2.

Proximate analysis of test samples of sensorial analysis

For the brownies, there was a significant effect of the treatment – either blank, MLP or MLC – on the DM and the ash content (*p = 0.002, p = 1.67*10^{-7}, respectively*). Brownies with MLC had a significantly higher amount of moisture, about 1.6 times higher, than blank brownies or brownies with MLP (table 20). Brownies with MLP also had a significantly higher amount of ash, compared to the blank and brownies with MLC. There was also a significant effect of the treatment on iron contents, but not on zinc contents (*p = 0.003, p = 0.608, respectively*). Brownies with MLP did not differ significantly with the blank regarding iron or zinc contents. However, MLP brownies and MLC brownies differed significantly regarding their iron content, with brownies with added MLC containing on average more than twice as much iron. For these brownies, 60g of MLP or MLC was added – 54.1 g DM and 57.0 g DM respectively (see table 17). This equates to 2.5 mg Fe and 0.8 mg Zn added by MLP of location 2 to the brownies and to 9.5 mg Fe and 0.8 mg Zn added by MLC of location 2 to the brownies (table 18). These amounts are the theoretical amounts of iron and zinc added to 290 g of flour, as is shown in table 22 too. Table 20 allows for a calculation of the 'actual' values of these minerals. It is calculated that 0.02 mg Fe per gram of FW was added by MLP, i.e. 5.8 mg Fe for 290 g of flour, and that 0.04 mg Fe per gram of FW was added by MLC, i.e. 11.5 mg Fe for the total amount of flour. For zinc, 0.004 mg Zn per gram of FW was added by MLP, i.e. 1.2 mg Zn for 290 g of flour, while 0.003 mg Zn per gram of FW was added by MLC, i.e. 0.9 mg Zn for all of the flour. All of these 'actual' values were higher than the theoretical, expected values, probably due to the addition of additional tap water containing iron and/or zinc in the case of MLP, small differences in the mineral contents of the eggs added or small measurement errors. For brownies with MLP, the discrepancy was highest, but these brownies also had a considerable amount of water that was added to them.

On the other hand, for the pitaya juices, there was a significant effect of the treatments on DM content and ash content, but not on the mineral contents (*p = 0.002, p = 0.026, p = 762, p = 0.277; respectively DM, ash, iron and zinc*). The amount of moisture in blank pitaya fruit juice samples was significantly higher than that of juice with MLP or MLC (table 21). Furthermore, pitaya juice with MLC has a significantly lower amount of ash or total amount of minerals present than blank fruit juice. Nonetheless, the iron and zinc contents did not differ significantly between different juice samples. For these juices, 40g of MLP or MLC was added – 36.0 g DM and 35.8 g DM respectively. This equates to 1.7 mg Fe and 0.5 mg Zn added by MLP of location 2 to the juice and to 4.9 mg Fe and 0.5 mg Zn added by MLC of location 3 to the juice. As with the brownies, these amounts are the theoretical amounts of iron and zinc added to 1200 ml of stock pitaya juice – which is equalled to 1200 g of juice (table 22). Table 21 allows for a calculation of the 'actual' values of the microminerals in the juice. It was calculated that 0.002 mg Fe per gram of FW was added by MLP, i.e. 2.4 mg Fe for 1200 g of juice. Furthermore, 0.003 mg Fe per gram of FW was added by MLC, i.e. 3.6 mg Fe for all of the juice. For zinc, it was calculated that 0.0005 mg Zn per gram of FW was added by MLP, i.e. 0.6 mg Zn for 1200 g for the total amount of juice, while 0.0004 mg Zn per gram of FW was added by MLC, i.e. 0.5 mg Zn for 1200 g of juice. These 'actual' values for zinc were in line with what was expected theoretically. For pitaya juice with MLP, however, the actual iron values were higher than expected, probably due to the addition of extra lime juice. For pitaya juice with MLC, the actual iron values were lower than expected, probably due to the heterogenous nature of the juice – the MLC did not mix well.

In this section, it was not possible to develop precise mass balances for each product, as precise amounts of volume of water or lime juice added were not measured during the execution of the recipe.

Table 20: Overview of results obtained for samples from the sensorial analysis with brownies

Sample	Sample Code	DM (g/100g FW)	Ash (g/100g DM)	Fe (mg/100 g DM)	Zn (mg/100 g DM)
Brownie-blank	B1	91.93 (0.56)*a*	1.00 (0.10)*a*	2.49 (0.35)*a*	0.37 (0.09)*a*
Brownie-MLP	B2	91.81 (0.30)*a*	2.03 (0.08)*b*	2.11 (0.14)*a*	0.44 (0.06)*a*
Brownie-MLC	B3	87.12 (0.36)*b*	1.18 (0.11)*c*	4.56 (0.10)*b*	0.39 (0.02)*a*

Different letters denote a significant difference between recipes (p<0.05), i.e. per column. All values are mean (SD), total n=2 for all parameters except Ash (n=5).

Table 21: Overview of results obtained for samples from the sensorial analysis with pitaya fruit juice

Sample	Sample Code	DM (g/100g FW)	Ash (g/100g DM)	Fe (mg/100 g DM)	Zn (mg/100 g DM)
Pitaya-blank	P1	8.27 (0.86)*a*	1.64 (0.30)*a*	1.39 (0.84)*a*	0.34 (0.08)*a*
Pitaya-MLP	P2	21.87 (1.53)*b*	1.27 (0.22)*ab*	0.84 (0.11)*a*	0.22 (0.01)*a*
Pitaya-MLC	P3	17.89 (0.09)*b*	0.97 (0.08)*b*	1.51 (1.36)*a*	0.24 (0.08)*a*

Different letters denote a significant difference between recipes (p<0.05), i.e. per column. All values are mean (SD), total n=2 for all parameters except Ash (n=3).

Table 22: Theoretical and actual values for iron and zinc contents, calculated based on previous tables 17, 20 and 21

	'Theoretical' value		'Actual' value	
Sample	Fe (mg/290 g flour)	Zn (mg/290 g flour)	Fe (mg/1200 g juice)	Zn (mg/1200 g juice)
Added by MLP to brownies	2.5	0.8	5.8	1.2
Added by MLC to brownies	9.5	0.8	11.5	0.9
Added by MLP to juice	1.7	0.5	2.4	0.6
Added by MLC to juice	4.9	0.5	3.6	0.5

In vitro digestibility

It must be observed that the total amount of iron or zinc present in the samples is an overestimation of the true contents as reported in table 17. Therefore, a focus is laid on relative differences and not on absolute amounts measured, as is shown in figure 14 and 15.

When looking at the leaf material, of the total iron and zinc in fresh Moringa leaves, the highest proportion of the dialyzable fraction for iron and zinc was found in MLC of location 3, although the zinc of fresh leaves had a similar dialyzability to that of the MLC of location 3 (figure 14). Nevertheless, the highest proportion of the soluble non-dialyzable fraction was found in MLP for both iron and zinc. For MLC, the proportion of soluble non-dialyzable iron and zinc is remarkably lower than for the fresh leaf samples or MLP. The same observations can be made when looking at the sum of the dialyzable and soluble non-dialyzable relative fractions (figure 15). Fresh leaves and MLP had a similar summed proportion for both iron and zinc, but the summed proportion for MLC of location 2 was about 50% lower. For MLC of location 3, for iron, the value was similar to fresh leaves and MLP, while for zinc, they were roughly 50% lower.

Observations regarding brownies and pitaya juice samples tell a different story. Both brownies with MLP and MLC had similar proportions of dialyzable iron, that were in turn somewhat higher than that of blank brownies. Brownies with MLC had the highest proportion

of dialyzable zinc, followed by brownies with MLP and then by the blank. The reverse is true when looking at soluble non-dialyzable proportions for brownies, both in the case of iron and zinc. The summed proportions for brownie samples also follow this trend. In the case of pitaya juice samples, the most remarkable observation was the high proportion of iron and zinc dialyzability in pitaya juice with MLC – approximately 2 to 5 times higher than other samples. For both pitaya juice with MLP and the blank, the proportions of soluble non-dialyzable minerals are higher than the juice with MLC. Therefore, the summed proportions for the three types of juices are similar for both iron and zinc.

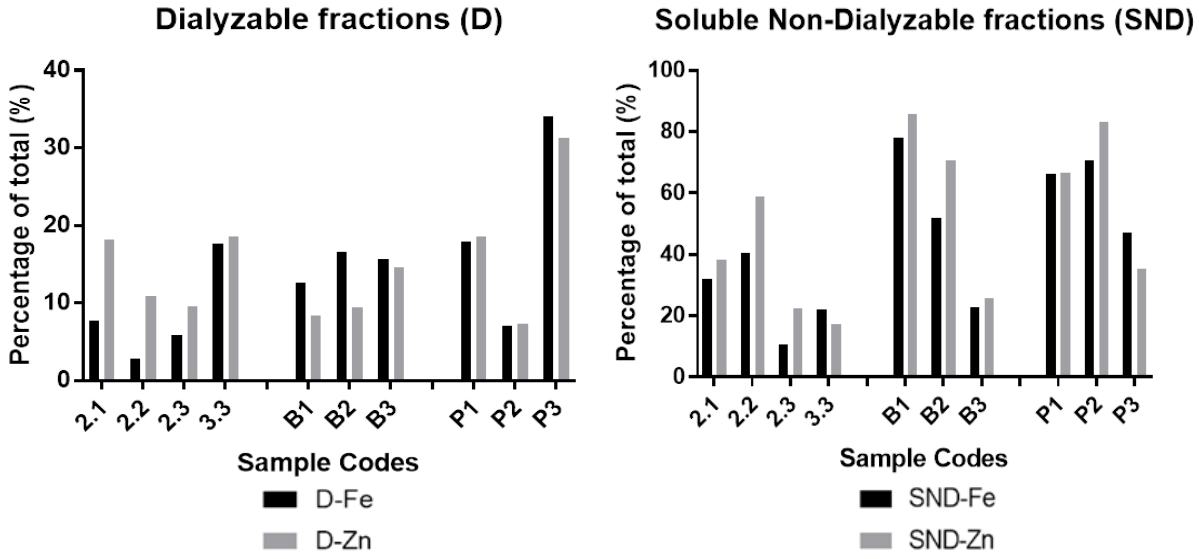

Figure 14: Overview of dialyzable and soluble non-dialyzable fractions for iron and zinc, as a percentage of the total amount of iron and zinc present in the sample. The total amount of iron, respectively zinc, is calculated as the sum of the iron, respectively zinc, contents of the three fractions. Different samples were analysed: B1 = blank brownies; B2 = brownies with added MLP; B3 = brownies with added MLC; P1 = blank pitaya fruit juice; P2 = fruit juice with added MLP; P3 = fruit juice with added MLC; 2.1 = fresh leaves from location 2; 2.2 = Moringa leaf powder from location 2; 2.3 = Moringa leaf concentrate from location 2; 3.3 = Moringa leaf concentrate from location 3.

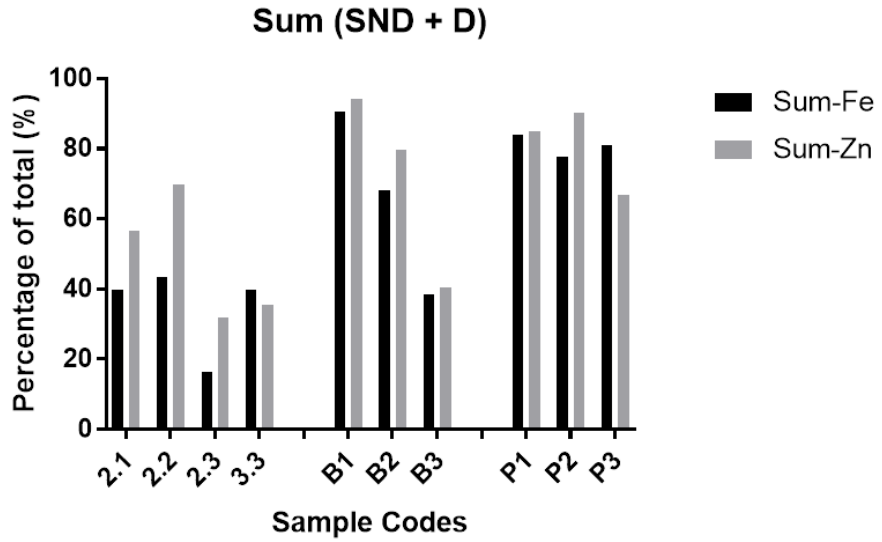

Figure 15: Overview of the sum of soluble non-dialyzable and dialyzable fractions for iron and zinc, expressed as a percentage of the total amount of iron and zinc present in the sample. Different samples were analysed: B1 = blank brownies; B2 = brownies with added MLP; B3 = brownies with added MLC; P1 = blank pitaya fruit juice; P2 = fruit juice with added MLP; P3 = fruit juice with added MLC; 2.1 = fresh leaves from location 2; 2.2 = Moringa leaf powder from location 2; 2.3 = Moringa leaf concentrate from location 2; 3.3 = Moringa leaf concentrate from location 3.

Soil analysis

The soil textures were different in each location, ranging from a high percentage of sand to a high percentage of clay (table 23). There was a statistically significant effect of the location on DM content and ash content of soil samples, but not on the mineral contents (*p = 0.0002, p = 4.59*10^{-6}, p = 0.215, p = 0.053, p = 0.28; p = 0.124; p = 0.187; respectively DM, ash, iron, zinc, calcium, sodium and magnesium*). Yet, there was a statistically significant higher amount of zinc in location 2 than location 4. The mineral contents in the soil were correlated to the mineral contents found in fresh Moringa leaves. For iron, this correlation was moderate (*r*=0.55), while for sodium there was a strong negative relationship (*r*=-0.96). For zinc, calcium and magnesium, the correlations were higher, respectively *r*=0.59, *r*=0.66 and *r*=0.89.

Table 23: Overview of data collected on soil samples

Location	Soil texture	DM (g/100g FW)	Ash (g/100g DM)
Location 1	Sandy clay loam	72.10 (0.33)*a*	90.29 (0.87)*a*
Location 2	Clay	77.87 (0.32)*b*	87.80 (1.91)*ac*
Location 3	Silty clay	73.03 (0.26)*a*	91.59 (0.32)*ad*
Location 4	Loam	78.39 (0.61)*b*	99.46 (2.81)*b*

Location	Fe (mg/100 g DM)	Zn (mg/100 g DM)	Ca (g/100 g DM)	Na (g/100 g DM)	Mg (g/100 g DM)
Location 1	0.42 (0.13)*a*	0.67 (0.04)*ab*	0.13 (0.04)*a*	0.01 (0.00)*a*	0.03 (0.01)*a*
Location 2	0.43 (0.06)*a*	1.08 (0.02)*a*	0.11 (0.01)*a*	0.01 (0.00)*a*	0.02 (0.00)*a*
Location 3	0.29 (0.05)*a*	0.76 (0.21)*ab*	0.11 (0.02)*a*	0.01 (0.00)*a*	0.02 (0.00)*a*
Location 4	0.26 (0.07)*a*	0.53 (0.14)*b*	0.08 (0.01)*a*	0.03 (0.02)*a*	0.02 (0.01)*a*

Different letters denote a significant difference between locations (p<0.05), i.e. per column. All values are mean (SD), total n=2 for all parameters except Ash (n=4).

Discussion

The amount of moisture found in the present study for fresh Moringa leaves is in line with previous studies by USDA (2016), West African Food Composition Table (Stadlmayr *et al*., 2012) and the Nutritive Value of Indian Foods (Gopalan et al., 1989), albeit on the slightly higher side. The reported moisture content for MLP was in line with the content reported by Lockett *et al*. (2000), Moyo *et al*. (2011) and Castillo-López *et al*. (2017). Small differences in moisture contents of fresh leaves could be due to natural variation. The moisture content of MLP and MLC was influenced by the climatological conditions present during the drying process, hence the significant influence of the location and its associated drying process. When observing the ash contents – an indication of the total amount of minerals present – there seems to be a loss of minerals in MLC. This is reflected in the lower amounts of macrominerals, i.e. calcium, sodium and magnesium present in MLC as compared to MLP and fresh leaves. This is as expected, as fibers and “whey” have been removed during the MLC process and hence certain minerals will have been removed too. Fresh leaves and MLP ash contents do not differ significantly, as is to be expected, since drying normally does not appear to affect mineral contents in leaves or at most concentrates them. This has been

confirmed for rosemary leaves (Arslan & Özcan, 2008) and Moringa leaves (Moyo *et al*., 2011; Joshi & Mehta, 2010). However, the sum of all minerals for each sample in the present study only accounts for a minority of their respective ash contents, with the exception of fresh leaves of location 1. Therefore, a discrepancy between the ash contents and mineral contents is observed. A mineral that was not studied in the present dissertation, must probably be present in a higher concentration than expected, but this study offers no conclusions as to which mineral(s). Analogous results are present in the literature, as the ash contents found in fresh leaves and MLP were in line with previous studies and this discrepancy between ash and studied mineral contents was present too in these studies (Castillo-López *et al*., 2017; USDA, 2016; Agamou *et al*., 2015; Stadlmayr *et al*., 2012; Shih *et al*., 2011; Makkar & Becker, 1997; Gopalan *et al*., 1989). The only exception where this discrepancy is not present, is the study by Moyo *et al*. (2011). They reported higher contents of calcium than the other studies and included the sulphur content of fresh leaves. Nevertheless, ash contents reported by Moyo *et al*. (2011) are similar to those reported in the present study.

Iron contents found in fresh leaves in this study were similar, albeit lower, to values reported for young leaves by Agamou *et al*. (2015) and by those of Castillo-López *et al*. (2017). Iron contents for fresh leaves were also about half of those reported by USDA (2016) and one-third of those reported by Stadlmayr *et al*. (2012), but roughly double those reported by Gopalan *et al*. (1989). As the leaves of location 1 were older than other locations, it could perhaps explain the higher amount of iron found compared to other locations. Zinc contents in fresh leaves were in line with values reported for young leaves by Agamou *et al*. (2015), values by USDA (2016), Moyo *et al*. (2011) and those of Lockett *et al*. (2000). However, higher values have also been found by Castillo-López *et al*. (2017) and Stadlmayr *et al*. (2012). Differences in zinc content could be due to differences in mineral contents in the soil, as there seems to be a certain trade-off between iron and zinc uptake by many plants (Cakmak, 2000). As for magnesium and calcium, location 1 deviates from the other locations. Values for location 1 are similar to those reported by Lockett *et al*. (2000) and Moyo *et al*. (2011). It is possible that this is due to the age of the leaves or specific cultivation, climatic or soil related conditions in this location. For example, in kiwi fruit, the amount of light can influence the accumulation of calcium (Montanaro *et al*., 2006). Furthermore, calcium is an immobile element in leaves and would tend to accumulate in older leaves (Maathuis, 2009), as would be the case for location 1. Values for calcium in all other locations are more in line with reports made by Castillo-López *et al.* (2017), USDA (2016) and Agamou *et al*. (2015). Values for magnesium are like the finding of USDA (2016). Finally, sodium contents from location 4 deviated from other locations. This value was in line with reports by USDA (2016) and Stadlmayr *et al*. (2012). However, for the other locations, sodium levels were twice as high as those reported by Agamou *et al*. (2015) and Castillo-López *et al*. (2017). Sodium is typically taken up by the plant through passive mechanisms and K^+-transporters. Plants aim to maintain a high cytosolic K^+/Na^+ ratio, so Na^+-ions will be actively extruded from the cell (Blumwald *et al*., 2000). Hence, sodium levels could depend on individual variations in these processes or on mineral – both sodium and potassium – availability in the soil matrix. Out of the soil analysis, there seems to be a negative correlation between the availability of sodium in the soil and sodium found in fresh leaf tissue. However, with increasing salinity, the concentration of leaf-sodium increases according to a previous study. The study also found that the crude protein content, calcium and magnesium content decrease with increasing salinity (Nouman *et al*., 2012). Therefore, this correlation probably gives a distorted picture, as the values for both soil-sodium and leaf-sodium did not differ significantly between the different locations. Neither did the values for calcium and magnesium in the soil. It would

then be more likely that climatological factors or biological variations determine the differences observed in these fresh leaf contents.

The amount of iron was highest in MLC, while it was lowest in MLP. The concentration of iron in MLC is probably due to a complex formed with certain proteins, as iron-binding components like phytic acid and fiber decreased in MLC. The latter is expected from the production process of MLC due to the physical removal of fiber in the first step and from the lower carbohydrate content. The iron-protein units may have (partially) withstood heat, allowing for an accumulation of iron in MLC. Protein-iron complexes have been described in the past, for example in the case of ferritin present in plants' plastids, which acts as an iron source for proteins or enzymes involved with photosynthesis (Goto *et al*., 2000; Briat & Lobréaux, 1997). This would also match with the observation that no other minerals except iron are concentrated in MLC, as compared to fresh leaves. For MLP, the iron-protein bonds would not be present or would be broken during the drying process, as iron was able to "leak" out of the cell and the protein content was not significantly different from that of fresh leaves. This leakage could perhaps happen due to tensile stresses that cause small ruptures in the cell. These tensile stresses in the cell can happen during the drying process due to heat and mass transfers occurring simultaneously (Lewicki, 1998). It could also be a case of "vacuolar cell death" as described by van Doorn *et al*. (2011), which involves the rupture of the tonoplast. This leaking could also involve the loss of other compounds other than iron, on the condition that they are present in the vacuole. In the case of ferritin, for example, degradation occurs and iron is "freed" when the protein is damaged by free radicals and hence more sensitive to proteolysis (Briat & Lobréaux, 1997). Free radicals may form during oxidative processes set on by the wounding of a plant part or senescence (Thompson *et al*., 1987). It might be possible that these same processes occurred with MLP. Yet for zinc, these above-mentioned processes do not seem to come into play. Zinc is stored in the plant by storage proteins, such as metallothioneins (Zimmermann & Hurrell, 2002). The bond between both might have been less strong than that of the iron-protein complex and possibly did not withstand heat as well as with the case of bound iron, causing a loss of zinc both in MLC and MLP. This zinc might also have been removed along with phytate. Lastly, macrominerals tend to be reduced the most – with one exception – in MLC, compared to MLP. Magnesium and calcium tend to be bound as inorganic salts in plants, such as phytin and oxalate (Reddy & Sathe, 2002), and would therefore most probably be removed in MLC when the fibers or "whey" are removed during the production process. Magnesium is also an important part of chlorophyll molecules (Maathuis, 2009) and these have partially been removed with the fiber. Calcium occurs in very low concentrations in the cytosol, but physical damage, biotic or abiotic factors and stomatal changes might affect the amount of "free calcium" in the cell cytosol (Maathuis, 2009). Therefore, the cut Moringa leaves might have been susceptible to the leaching of calcium out of the leaf during drying, as with the other minerals. This leaching would then not be a uniform process in all leaves, hence the variability observed in MLP. It is also possible that an acidic pH of the cytosol could contribute to the solubility of minerals and their "free" presence in the cytosol.

Furthermore, the drying process did not have an influence on the protein content, as the protein contents of fresh leaves and MLP did not differ significantly. An accumulation of protein in MLC is due to the production process of MLC, due to a concentration of proteins by coagulating these at high temperatures. The higher amount of protein does not necessarily mean the amino acids compounds are more bio-available or bio-accessible. The amount of proteins found in fresh leaves was in line with studies by Moyo *et al*. (2011), Stadlmayr *et al.* (2012) and Castillo-López *et al*. (2017). Some studies, however, reported lower protein contents – being 0.10 to 0.30 times lower (Agamou *et al.*, 2015; Shih *et al*., 2011; Sánchez *et*

al., 2006; Lockett *et al*., 2000; Makkar & Becker, 1997; Gopalan *et al*., 1989). This could be explained by, as mentioned before, differences in leaf age at harvest for example. The protein amount found in fresh Moringa leaves and MLP in this study is comparable to the amount of proteins found in dried lentils, dried chickpeas or dried soy beans (Internubel, 2018). The amount of non-protein nitrogen has not been quantified in this study, yet out of a previous study, it appears that this amount is approximately 13% (Makkar & Becker, 1997), which means that Moringa leaves contain a lot of proteins. Similar to crude protein contents found, the amount of crude fat is also highest in MLC, possibly due to coagulation along with the proteins, as fats are insoluble in water. The fat content of MLP also seems to be higher than those of fresh leaves. Plant cell lipids are mainly found in the plant cell walls as phospholipids or in spherosomes in the cell as reserve fat (Sorokin, 1967). These components would not be able to "leak" out with water and would hence be concentrated in dried leaves. The results for crude fat in fresh leaves reported here are often more than twice as low as those reported in literature (USDA, 2016; Castillo-López *et al*., 2017; Agamou *et al*., 2015; Stadlmayr *et al*., 2012; Moyo *et al*., 2011; Shih *et al*., 2011; Lockett *et al*., 2000; Gopalan *et al*., 1989).

The total amount of carbohydrates, of which it is assumed that the majority of these are fibers, is lower in MLC as was to be expected. During the production process of MLC, fibers are removed as much as possible by pressing blended leaves through a straining cloth. The energy content of MLC also indicates that this Moringa-derived product is a better source of energy than fresh leaves or MLP. As for fresh leaves, the found energy contents are lower than observed literature (USDA, 2016; Stadlmayr *et al.*, 2012; Makkar & Becker, 1997; Gopalan *et al*., 1989), since the amount of crude fat in this study was lower.

As for MLC, contents of iron and zinc reported by Sodamade *et al*. (2013) seem to be 15 and 400 times higher respectively and values for macrominerals are also considerably higher. Sodamade *et al*. (2013) also reported a higher ash content, but lower protein, fat and carbohydrate – including fibers – contents. Main differences between the MLC analysed in the present study and other leaf concentrates analysed by Aletor *et al*. (2002) are that crude fat is concentrated in MLC and not in other leaf concentrates, and that in other leaf concentrates the majority of minerals tend to be concentrated on a DM basis.

When examining the results of analysed antinutritional factors, results for phytate content of fresh leaves are on average half of those reported in literature (Ogbe & Affiku, 2011; Makkar & Becker, 1997). Results for total phenolic compounds in fresh leaves are in line with – but higher than – Castillo-López *et al.* (2017) and in line with – but lower than – Siddhuraju & Becker (2003). The discrepancy with other results by Pakade *et al.* (2013) and Makkar & Becker (1997) – which are 2.5 to 3.5 times lower – could be due to the location of sampling or the extraction method used, such as acetone used by Pakade *et al*. (2013). Siddhuraju & Becker (2003), for example, observed an effect of location and extraction method – methanol, water or ethanol. Additionally, results for condensed tannins in fresh leaves are considerably lower than reported, but this is due to differences in measuring methods (Ogbe & Affiku, 2011; Makkar & Becker, 1997). The lower concentrations in these antinutritional factors for both MLP and MLC are of interest. In the case of polyphenols and phytic acid, a study with cassava leaves found that sun drying lowered their amount by 36% and 59% respectively compared with their fresh counterparts (Fasuyi, 2005). Phenolic compounds degradation, amongst which condensed tannins, could be possible due to heat treatment at temperatures of 100 °C (Larrauri *et al*., 1997; Yu *et al*., 1996), as present during the MLC production process. The temperatures reached during the MLP production process might have also caused some degradation of these compounds. Phytates would have been

expected to be higher than fresh leaves in both MLP and MLC, as – according to Reddy & Sathe (2002) – phytases are heat-sensitive and phytates are heat-resistant. Even so, concentrations are lower than fresh leaves, probably due to the removal of the fibers in MLC and the occurrence of “free phytic acid”, that was able to leach in MLP.

These previous results would imply that the bio-accessibility of other compounds – more specifically iron and zinc – should probably increase in MLP and MLC products. The main reason for this would be the decreased calcium contents, phytic acid contents and tannin contents, which can inhibit absorption of iron and zinc. Nonetheless, there is also an interaction between zinc and iron absorption in some cases (Krebs, 2000). In the case of fresh leaves and MLP, a possible interaction between iron and zinc may be present, as they seem to be part to a certain degree of a “trade-off” – their dialyzable proportion of iron is at least twice as low as their proportion of dialyzable zinc. Furthermore, for MLC, the results are variable, ranging from a lower to a higher dialyzable proportion of minerals than those of fresh leaves or MLP samples. Therefore, no definite conclusions as to whether the ingestion of MLC as such improves the bio-accessibility of iron and zinc. Nevertheless, the amount of soluble non-dialyzable iron and zinc is remarkably lower in MLC samples than in fresh leaves or MLP. This implies that a large quantity of these minerals, that would otherwise be potentially dialyzable, are bound in the insoluble fraction. It is possible that the reduction of inhibitory factors to mineral absorption – as identified earlier – is simply not high enough, in order to drastically improve mineral accessibility. For example, for corn, a reduction of phytates by one-third was necessary to increase iron absorption by 50% (Weaver & Kannan, 2002). In the case of soy, only a low amount of phytates is sufficient to inhibit iron absorption. Less than 10 mg phytates per meal is necessary in order to drastically improve iron accessibility here (Weaver & Kannan, 2002). However, for Moringa, this type of threshold has not yet been established. For MLC, the sum of dialyzable and soluble non-dialyzable iron and zinc is remarkably lower than its counterparts. Consequently, fresh leaves and MLP have a larger potential than MLC for iron and/or zinc absorption in this experiment. Given more *in vitro* digestive time, the minerals in the soluble fraction might very well still be absorbed, but *in vivo*, these minerals might also be lost to the body if minerals cannot be absorbed during passage through the ileum in the intestine.

In the case of brownies, similar trends are observed as with the leaf material and can probably be explained in a similar fashion. For pitaya juices, the higher proportion of dialyzable iron in pitaya juice with MLC could be due to the addition of more lime juice than for the blank or juice with MLP. Lime juice, like pitaya juice, has a low pH and contains vitamin C and other organic acids, which could enhance mineral absorption compared to the other juices. However, for the juice with MLP, which contained more lime juice than the blank, this enhancing effect is lacking. When looking at the sum of what is potentially bio-accessible and what has already dialyzed, the treatment did not seem to have any effect.

Lastly, brownies with MLP had a significantly higher amount of ash, compared to the blank, but did not differ significantly regarding iron or zinc contents. This would imply that other elements, which have not been analysed in the present study, were present in higher amounts in these brownies. For pitaya juice with MLC, the significantly lower amount of ash could be attributed to the heterogeneity of the sample. These results suggest that the addition of MLP or MLC to the recipes did not cause significant effects in the iron and zinc contents. Only brownies with added MLC seemed to have a significantly higher amount of iron. In order to see considerable increases in iron or zinc contents, higher concentrations would need to be added without decreasing acceptability of recipes. For zinc, the choice to

add MLP or MLC did not make a difference. On the other hand, MLC provides a higher amount of iron to the brownies and juice.

In conclusion, when comparing MLC to MLP nutritionally, MLC seems to be a better source in terms of gross energy, proteins and microminerals. Out of the present study, it is not clear whether the addition of MLC to a product or the ingestion of MLC tends to increase *in vitro* accessibility of minerals. The addition of mineral enhancers seems to have a beneficial effect on the bio-accessibility of iron and zinc in MLC.

PARTIM III: ASSESSING THE ECONOMICAL FEASABILITY

Materials and methods – Economics of Moringa leaf processing in Nicaragua

Based on the information gathered during partim I and partim II, an economic analysis is developed of the production of MLP and MLC in Nicaragua. A SWOT-analysis is performed based on key interviews with relevant stakeholders from partim I and based on relevant data from partim II regarding nutritional differences between MLP and MLC. A cost-benefit estimation analysis is executed as well, for which relevant information concerning wages and expenses was obtained through an internet search and by verifying with local citizens from Nicaragua. Other parameters such as processing time were obtained through the author's personal experiences with timing the process in Nicaragua. It is estimated which production method would lead to a minimisation of costs and under which circumstances and conditions. However, only the costs which would be different between both production processes are defined. Costs that are constant for both, such as the production of the Moringa leaves or the transport and distribution costs, are not explicitly estimated. Therefore, the costs determined only have a relative value and no absolute costs are given. Lastly, an assessment regarding the effectiveness of both strategies is made regarding the intended end-goals of the project. An overview of the calculation of costs can be found in Appendix A.2. Prices in these calculations are expressed as córdoba (c$), the Nicaraguan currency, and are calculated to euros (€). All prices and exchange rates date from 1st of May 2018.

Results

SWOT analysis

For Leaf Powder production

For MLP, the main strengths of the product are its long shelf-life and preservation, while investing little time, effort and money in achieving this better preservation quality (figure 16). The process of creating MLP is straight-forward and can be implemented easily on different scales of magnitude. The small-scale producer or home-grower can use various easy-to-achieve sun drying methods or even small drying equipment, such as food dryers. At an intermediate scale, solar drying can still be a method of choice or investments can be made in larger food dryers. At an industrial scale, typically industrial dryers can be used. All these methods do not require a large amount of labour and are easy to implement. However, different methods of drying produce different qualities of end-products, depending on whether the goal is to achieve a homogenous rate of drying or a minimum rate of deterioration of certain nutritional compounds. Furthermore, there will inevitably be a loss of nutritional aspects, as compared to fresh leaves. For example, vitamin C content is expected to be lower (Joshi & Mehta, 2010). Compared to MLC, the amount of proteins and certain minerals in MLP is lower too (see partim II).

Opportunities for the future, commercially, include an increasing demand in the export market. Demand in Europe is expected to grow by 9.5% between 2015 and 2020 (CBI, 2016). Improvements in the overall quality and the quality management in the production process could be made. In the case of Nicaragua, it could be an opportunity to position the country as a leading high-quality provider of MLP. However, the local Nicaraguan market may lag behind due to the shared view amongst consumers of Moringa as being for non-food use only. Even so, there is a growing interest by Nicaraguans in healthy foods, as can be observed by a high number of health-promoting outlets (*personal observation*). Furthermore, as the product is a powder, there are threats of fraudulent practices as the consumer cannot

easily assess the quality and authenticity of a powdered product. A possible threat may also be the political instability of the country and the presence of India as an international competitor.

For developmental projects in Nicaragua, as could be implemented by APEF, SoyNica and Leaf for Life, these points would largely be similar. The main opportunity lies in the cooperation with existing Moringa producers and the government – which shows a high interest in Moringa too –, while the greatest threat would be the perception of "intervening" as outsiders.

Figure 16: Overview of strength, weaknesses, opportunities and threats of MLP production

For Leaf Concentrate production

The main strength of MLC as a product is its higher nutritional value – in terms of gross energy, proteins and microminerals (figure 17). Its main weakness is its high costs in terms of investment of money, time, labour and effort. Its opportunities lie with the possibility of creating a novel high-quality market for the product by Nicaraguan producers. These producers are looking for opportunities that could define them – or Nicaraguan Moringa products – worldwide. From a development economics perspective, the involvement of local know-how in developing this production process and its necessary equipment would create an added value for the local communities involved and help embed the product in its local setting and thus creating unique branding. Furthermore, opportunities are there to develop new products, such as new food condiments or herbs – MLC bears a visual resemblance to pepper corns. The possible threats, however, include the current political instability in Nicaragua or a low interest by producers in investing in this process. Another threat could be not obtaining a food safety clearance, necessary for commercialisation. Specifically, for follow-up projects by the consortium of NGOs, a threat could be the lack of cooperation of local stakeholders in developing MLC on a large scale – since it comes with a great cost as mentioned before.

Figure 17: Overview of strength, weaknesses, opportunities and threats of MLC production

Cost/benefits estimation

When processing both products on a small scale, as was done in partim II in Nicaragua, the cost of 1 kg of MLC is estimated to be 28 times as expensive as 1 kg of MLP. As Moringa is often lauded for its high protein contents and is therefore often consumed for this purpose (De Saint Sauveur & Broin, 2010; Price, 2007), it might be useful to scale the costs to an equal amount of protein. When scaling both products to an equal amount of protein, protein derived from MLC remains more expensive than protein from MLP – 13 times more so. Even when both products are scaled to an equal amount of iron, MLC iron is about 9 times more expensive than MLP.

However, when accounting for economies of scale, it would be possible for MLC-protein and iron to become as costly or less costly than its MLP counterpart. To achieve this, it would be beneficial to improve the yield of MLC by at least 1% and to invest in efficient processing tools and equipment, so that the number of labourers and the time they need to attend to turn 1 kg of Moringa leaves into dried concentrate, lowers drastically. If the yield of MLC is improved to 3%, instead of 2%, and if the working time of making dried concentrate out of 2 kg of Moringa leaves is reduced to only half an hour, only then would the cost of MLC-protein and MLC-iron be less than that of MLP-protein and MLP-iron. However, the total cost per kg for MLC would still be higher than for a kg of MLP.

Cost/effectiveness estimation

As the ultimate goal of this dissertation is providing a solid reference base for future clinical research by the research consortium, it would be beneficial to relate the intended effectiveness of each product regarding anaemia alleviation to its costs. For anaemia alleviation, the most important parameters are the total amount of iron content and its approximated digestibility *in vivo*. When scaling both 1 kg of MLP and 1 kg of MLC to an equal amount of *in vitro* dialyzable iron, the cost of MLC-iron is 4 times that of MLP according to the processing methods as defined in partim II. When considering the extremely efficient

processing of MLC, the cost of dialyzable iron in MLC would be 1.7 times lower than that of MLP. When scaling both 1 kg of MLP and 1 kg of MLC to an equal amount of *in vitro* dialyzable and soluble non-dialyzable iron, the cost of MLC-iron is 22 times higher than that of MLP, according to the processing methods as defined in partim II. For the more efficient method, MLC would be approximately 3 times more expensive as that of MLP.

Discussion

The cost analyses demonstrate that improvements still need to be made in the production process of MLC to make it more cost-effective. Attention could be spent in improving the yields obtained of dried MLC, working at larger scales – since larger batches would imply fewer losses due to transferring batches in different recipients. With a higher through-put, the whole process would be less labour-intensive and time-consuming. It would also be beneficial to invest in the valorisation of waste streams of the MLC production process. However, this would be the most challenging strategy, as the value of these waste streams as a potential fertilizer or as animal feed would still need to be demonstrated and a demand needs to be created from farmers or other possible stakeholders. This demand will only be present if certain farmers in Nicaragua need a cheaper alternative to chemical fertilizers or imported feed, i.e. if the necessity is present. Another possibility could be for farmers who use manure fertilizers and who would prefer to enter the “organic” market in Nicaragua. They would then be a possible consumer base for these MLC-fertilizers. Alternatively, the producer of MLC could supply it to, or use it in his/her own, Moringa plantation as part of a closed nutrient cycling loop. However, the current methods of production already use low-cost fertilizers, i.e. the prunings or other waste streams of the cultivation process.

This approach for MLC would call for larger scales of implementation. A small-scale production unit, i.e. at household level, would then become too expensive to be sustainable. However, in Nicaragua, stimulating the cultivation of Moringa in home-gardens would be an effective approach in reaching large target groups. A study in Nicaragua showed that home-gardens are an important occupation for some families, especially women, with high labour investments and a large source of income coming from these gardens (Méndez *et al*., 2001). At the time of the investigation, Moringa was not yet cultivated in these home gardens. For this type of small-scale production, it would perhaps be more effective to consume fresh Moringa leaves or MLP. The MLC-process would probably be too time-consuming for the yield that is obtained and too expensive. Even on a village scale, for example through a communal cooperative, it would be difficult to invest in machinery when the end-use is for home consumption. Furthermore, as shown in partim I, it would be important to distribute or market MLP or MLC as being derived from “Marango”, as this is the most common name for Moringa in Nicaragua.

What is more, a study in Finland has shown the effect of “neophobia” when introducing new foods. Functional foods – foods that have proven beneficial effects – are often associated with medicine. Furthermore, they aroused suspicion amongst some of the participants and may seem unnatural. Five main dichotomies that come into play when assessing new food were mentioned in the study: trust, safety, natural or not, pleasure or necessity, and past or present (Bäckström *et al*., 2003). These effects will also come into play when designing an intervention plan, for example, in Nicaragua regarding Moringa leaves.

MAIN CONCLUSIONS

Consumption of Moringa leaves seems to have potential in Nicaragua, where interest is picking up. However, people who are familiar with Moringa mainly associate it with medicinal uses and therefore the introduction of Moringa leaves for daily consumption might be more difficult. The perception of its taste, smell, colour and texture as MLP or MLC appears to be acceptable to the limited number of participants in this study, but recipes need to be devised that enhance the acceptability of both products while maintaining their nutritional value. The aspect of neophobia is an important factor that would also need to be taken into account.

It has been established that MLC indeed has an added value nutritionally speaking, containing more energy, proteins, microminerals and less antinutritional factors. It has also been shown that, although antinutritional factors decrease, the bio-accessibility *in vitro* does not increase drastically. The main restriction for the introduction of MLC production into households would therefore be the high investments that are needed regarding time and cost, while obtaining a marginal benefit regarding iron and zinc intake. Especially when considering these economic factors, a balance between the consumption of MLP and fresh leaves would seem to be more suitable for household development interventions. At a community level, higher investments are possible, yet the fact remains that it would require a large effort to produce MLC, when its benefits are not that high to offset the benefits that come with MLP.

It is also important to stress that a healthy and varied diet is key. Moringa leaves might have beneficial effects, but must be part of a wholesome diet. It cannot replace food elements, but can supplement proteins, for example. Finally, the main limitations of this study are the small sample size and the use of non-probability sampling in partim I and II, which does not allow for any generalisations over the entire population studied. Validating research is still necessary.

Further avenues of research could include the calculation of mass balances for nutrients in the leaves and products derived from it, to precisely determine the flows in the process that contribute to losses or gains. It would also be beneficial to record temperatures and relative humidity during the drying process or to standardise the drying process and to quantify these effects. Experiments using controlled environments could perhaps also yield interesting results, as in the present study, no distinction could be made between soil, climatological, cultivation or other effects on the composition leaf samples. Another interesting avenue would be to test hypotheses regarding the “ash discrepancy” observed, i.e. measuring a wider range of minerals in samples. Regarding future sensorial analyses, savoury recipes could also be included. An additional avenue would be to study the interaction between iron-MLP and iron-MLC with iron absorption enhancers – if these compounds could partly compensate the large inhibitory effect of the antinutritional factors in MLP and MLC. Finally, researching practical methods to improve the cost efficiency of the MLC process might be interesting as well, including if valorisation of waste streams as potential animal feed or fertilizer is possible.

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APPENDIX A.1

ADDENDA TO PARTIM I

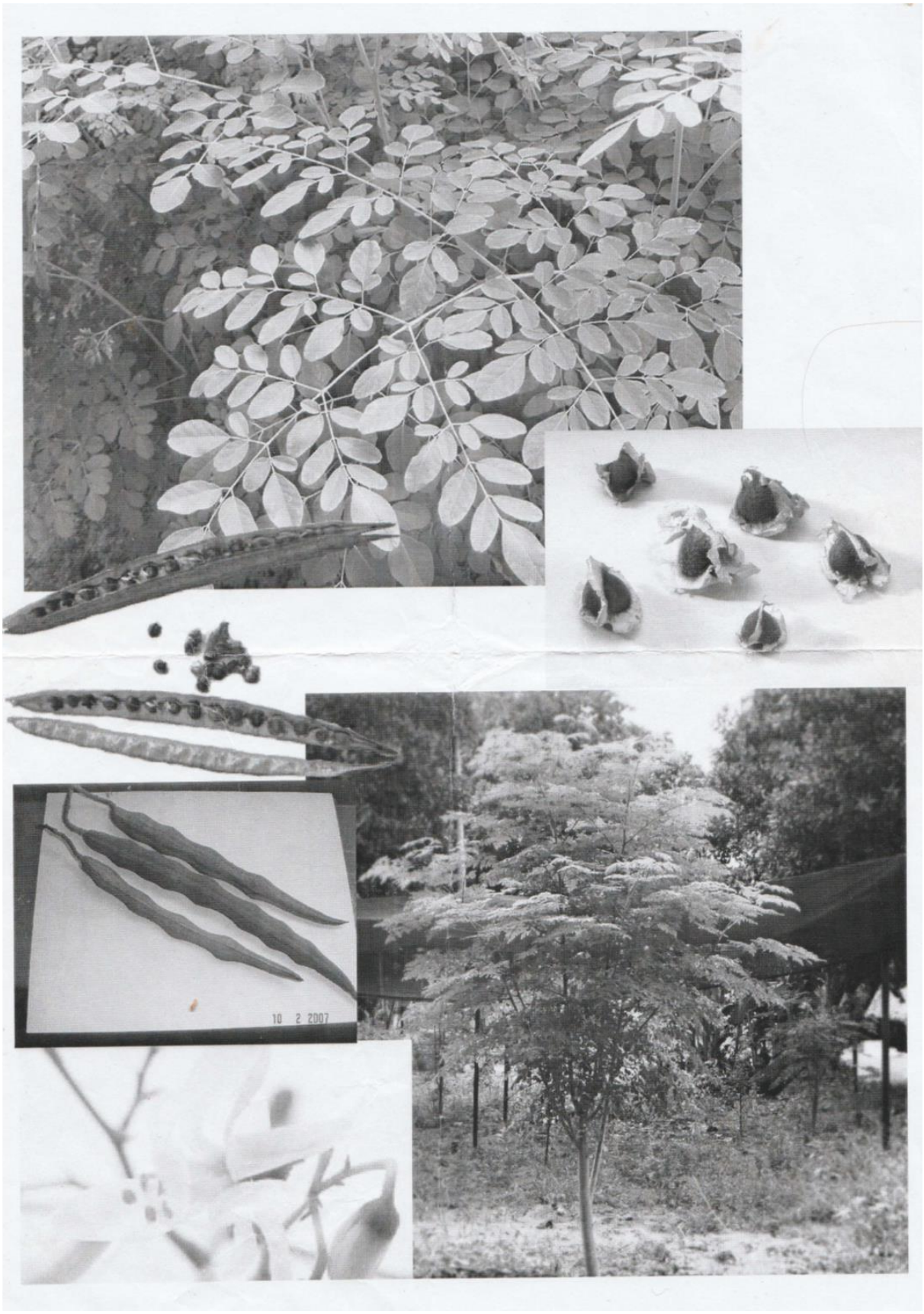

Figure 1: Pictures as shown to interviewees for partim I

Table 1: Questions asked during interview in partim I

QUESTION ASKED IN SPANISH	ENGLISH TRANSLATION
¿Conoces esta planta?	Do you know this plant?
(en caso de "sí") ¿De dónde/Cómo/Para qué?	(if "yes") How or from where do you know it? For what do you use it?
(en caso de reconocimiento, pero no puede dar el nombre) ¿La conoces como Moringa? O Marango?	(if recognition, but name cannot be given) Do you perhaps know it as "Moringa"? Or as "Marango"?

Table 2: Sample order for sensorial analysis with brownies. Letters denote the following: A = with MLP; B = blank; C = with MLC.

Assessor n°	Sample order test 1	Sample order test 2	Sample order test 4 (preference)
1	AAB	CCB	AC
2	BBA	BBC	CA
3	ABA	CBC	AC
4	BAA	BCC	CA
5	ABB	CBB	AC
6	BAB	BCB	CA
7	AAB	CCB	AC
8	BBA	BBC	CA
9	ABA	CBC	AC
10	BAA	BCC	CA
11	ABB	CBB	AC
12	BAB	BCB	CA
13	AAB	CCB	AC
14	BBA	BBC	CA
15	ABA	CBC	AC
16	BAA	BCC	CA
17	ABB	CBB	AC
18	BAB	BCB	CA

Table 3: Sample order for sensorial analysis with pitaya juices. Letters denote the following: A = with MLP; B = with MLC.

Assessor n°	Order test 1	Sample coding	Order test 2	Sample coding
1	AAB	128/171/227	AB	636/126
2	BAB	115/539/984	BA	232/234
3	ABA	937/194/689	BA	325/066
4	BBA	729/595/212	AB	833/369
5	BAA	589/120/550	AB	052/613
6	ABB	452/847/466	BA	564/618
7	AAB	721/179/566	AB	833/604
8	BAB	920/789/865	BA	723/632
9	ABA	615/257/144	BA	600/188
10	BBA	032/925/514	AB	378/374
11	BAA	810/751/235	AB	122/817
12	ABB	237/620/358	BA	175/818
13	AAB	596/308/615	AB	200/641
14	BAB	962/816/475	BA	099/191
15	ABA	136/291/498	BA	072/429
16	BBA	497/169/224	AB	520/479
17	BAA	705/151/951	AB	893/433
18	ABB	883/360/998	BA	073/377

EXAMPLE

An example of a scorecard used for the sensorial analysis with pitaya juice is given, in Spanish, but with English translations. Values for the dotted lines were completed before the test by the researcher. The scorecard for the sensorial analysis with brownies was very similar to the given one. English translations were not on the actual scorecards given to the assessors.

Tarjeta de puntuación n ° .....
Score card n° ……
Fecha: .....
Date ……

Asesor n ° .....
Assessor n° ……
Ensayo n ° …….
Test n° ……

Ahorita se le presentan 3 muestras. De estas 3 muestras, 1 es diferente que las otras dos. Primero se le pedirá que evalúe todas las muestras individualmente, de izquierda a derecha. Al final, se le preguntará si puede determinar cuál es diferente. La prueba entera debe tomar cerca de 10-15 minutos por 2 preguntas. No puedes hablar durante toda la prueba.

Right now, 3 samples are presented. Of these 3 samples, 1 is different than the other two. First you will be asked to evaluate all the samples individually, from left to right. In the end, you will be asked if you can determine which is different. The entire test should take about 10-15 minutes for 2 questions. You cannot talk during the entire test.

---

1. Primero tome un sorbo de agua. A continuación, pruebe la muestra 1 (n ° .....).
First take a sip of water. Next, try sample 1 (n ° .....).
Tome un bocado de la galleta y un sorbo de agua. Luego pruebe la muestra 2 (n ° .....).
Take a bite of the cookie and a sip of water. Then try sample 2 (n ° .....).
Tome un bocado de la galleta y un sorbo de agua. Luego pruebe la muestra 3 (n ° .....).
Take a bite of the cookie and a sip of water. Then try sample 3 (n ° .....).
Ahora ha probado las 3 muestras y las ha evaluado. ¿Cuál es diferente?
Now you have tested all 3 samples and have evaluated them. Which one is different?
(Cruce lo que no encaja)
(Cross out the one that does not fit)

Muestra 1 / Muestra 2 / Muestra 3
Sample 1 / Sample2 / Sample 3

Comentarios (Escriba aquí cualquier otra cosa que desee añadir. Explique también por qué hizo la elección que hizo antes):
Comments (Write here anything else you wish to add, also explain why you made the choice you made earlier):

---

2. Ahora se le presentan 2 muestras.
Now you are presented with 2 samples.
Primero tome un sorbo de agua. A continuación, pruebe la muestra 1 (n ° .....). ¿Cómo calificaría la muestra 1 para **SABOR** en una escala de 5 puntos, donde 1 es muy bueno y 5 es muy malo:
First take a sip of water. Next, try sample 1 (n ° .....). How would you rate sample 1 for TASTE on a 5-point scale, where 1 is very good and 5 is very bad:
(Por favor, coloque una "X" en la línea en la partitura que le da esta muestra, tenga en cuenta que las puntuaciones intermedias también son posibles)
(Please put an "X" on the line for the score that you give this sample, keep in mind that intermediate scores are also possible)

1 2 3 4 5

¿Cómo calificaría la muestra 1 para **TEXTURA** en una escala de 5 puntos, donde 1 es muy bueno y 5 es muy malo:
How would you rate sample 1 for TEXTURE on a 5-point scale, where 1 is very good and 5 is very bad:
(Por favor, coloque una "X" en la línea en la partitura que le da esta muestra, tenga en cuenta que las puntuaciones intermedias también son posibles)
(Please put an "X" on the line for the score that you give this sample, keep in mind that intermediate scores are also possible)

1 2 3 4 5

¿Cómo calificaría la muestra 1 para **COLOR** en una escala de 5 puntos, donde 1 es muy bueno y 5 es muy malo:
How would you rate sample 1 for COLOUR on a 5-point scale, where 1 is very good and 5 is very bad:
(Por favor, coloque una "X" en la línea en la partitura que le da esta muestra, tenga en cuenta que las puntuaciones intermedias también son posibles)
(Please put an "X" on the line for the score that you give this sample, keep in mind that intermediate scores are also possible)

1 2 3 4 5

¿Cómo calificaría la muestra 1 para **OLOR** en una escala de 5 puntos, donde 1 es muy bueno y 5 es muy malo:
How would you rate sample 1 for SMELL on a 5-point scale, where 1 is very good and 5 is very bad:
(Por favor, coloque una "X" en la línea en la partitura que le da esta muestra, tenga en cuenta que las puntuaciones intermedias también son posibles)
(Please put an "X" on the line for the score that you give this sample, keep in mind that intermediate scores are also possible)

1 2 3 4 5

Comentarios (Escribe aquí cualquier otra cosa que quieras añadir):
Comments (Write here anything else you want to add)

Tome un sorbo de agua. A continuación, pruebe la muestra 2 (n ° .....). ¿Cómo calificaría la muestra 2 para **SABOR** en una escala de 5 puntos, donde 1 es muy bueno y 5 es muy malo:
Take a sip of water. Next, try sample 2 (n ° .....). How would you rate sample 2 for TASTE on a 5-point scale, where 1 is very good and 5 is very bad:

(Por favor, coloque una "X" en la línea en la partitura que le da esta muestra, tenga en cuenta que las puntuaciones intermedias también son posibles)
(Please put an "X" on the line for the score that you give this sample, keep in mind that intermediate scores are also possible)

1 2 3 4 5

¿Cómo calificaría la muestra 2 para **TEXTURA** en una escala de 5 puntos, donde 1 es muy bueno y 5 es muy malo:
How would you rate sample 2 for TEXTURE on a 5-point scale, where 1 is very good and 5 is very bad:

(Por favor, coloque una "X" en la línea en la partitura que le da esta muestra, tenga en cuenta que las puntuaciones intermedias también son posibles)
(Please put an "X" on the line for the score that you give this sample, keep in mind that intermediate scores are also possible)

1 2 3 4 5

¿Cómo calificaría la muestra 2 para **COLOR** en una escala de 5 puntos, donde 1 es muy bueno y 5 es muy malo:
How would you rate sample 2 for COLOUR on a 5-point scale, where 1 is very good and 5 is very bad:

(Por favor, coloque una "X" en la línea en la partitura que le da esta muestra, tenga en cuenta que las puntuaciones intermedias también son posibles)
(Please put an "X" on the line for the score that you give this sample, keep in mind that intermediate scores are also possible)

1 2 3 4 5

¿Cómo calificaría la muestra 2 para **OLOR** en una escala de 5 puntos, donde 1 es muy bueno y 5 es muy malo:
How would you rate sample 2 for SMELL on a 5-point scale, where 1 is very good and 5 is very bad:

(Por favor, coloque una "X" en la línea en la partitura que le da esta muestra, tenga en cuenta que las puntuaciones intermedias también son posibles)
(Please put an "X" on the line for the score that you give this sample, keep in mind that intermediate scores are also possible)

1 2 3 4 5

Comentarios (Escribe aquí cualquier otra cosa que quieras):
Comments (Write here anything else you would like):

¿Cuál de las 2 muestras prefiere? (Teniendo en cuenta: el sabor, el color, la textura y el olor) Hay que hacer una elección, pero en la sección de observaciones puede anotar observaciones y por qué hizo la elección.
Which of the 2 samples do you prefer? (Taking into account: taste, color, texture and smell) You have to make a choice, but in the observations section you can write down observations and why you made the choice.

Muestra 1 / Muestra 2
Sample 1 / Sample 2

Comentarios (Escriba aquí cualquier otra cosa que le gustaría añadir. Por favor, explique por qué hizo la elección que hizo antes)
Comments (Write here anything else you would like to add, please explain why you made the choice you made before)

APPENDIX A.2

ADDENDA TO PARTIM III

Table 1: Cost analysis estimation for Moringa leaf powder and Moringa leaf concentrate

Cost analysis			
Cost parameters	***Value***		***Remarks***
Yield MLP		19%	See partim I
Yield dried MLC		2%	See partim I
Processing MLP - solar drying as done in experiment			
Cost parameters	***Value***		***Remarks***
Min. labour cost (c$ per month)	C$	3 773.82	WageIndicator.org - Nicaragua, 2018
Min. labour cost (c$ per hour)	C$	18.14	Total of six 8-hour working days every week, total of 26 working days per month (U.S. Library of Congress, 1993)
Min. labour cost (€ per hour)	€	0.48	1 NIO = 0.0266446267 Euros
Number of labourers		1	
Estimated amount of work per labourer to dry fresh leaves - spreading leaves in dryer, monitoring and collection of dried leaves (hours)		1	
Rest (hours/day)		1	
Total cost for drying	*€*	*0.54*	Formula: Min. Labour cost * Amount of labourers * (Estimated amount of work + (Rest/8))
	C$	20.41	Independent of amount of leaves, if amount of leaves falls within a range of 0-10 kg
			If amount > 10kg, add 1 hour of work for every 20 kg
Processing MLC - as done in experiment			
Cost parameters	***Value***		***Remarks***
Min. labour cost (c$ per hour)	C$	18.14	
Min. labour cost (€ per hour)	€	0.48	

Number of labourers for concentrate process (including drying)		2	
Estimated amount of work for labourers to make concentrate out of 1 kg of fresh leaves, including drying and associated activities (hours)		1.5	Based on most efficient timings obtained in Nicaragua
Rest (hours/day)		1	
Estimated amount of costs for electricity, gas and water per hour	€	0.08	Numbeo, 2018
Total cost for concentrate process	€	1.70	
	C$	63.66	

Total Costs			
Cost parameters	***Value***		***Remarks***
Total cost for 1 kg fresh leaves processed into MLP (±200g)	*€*	*0.54*	
	C$	20.41	
Total cost for 1 kg MLP	*€*	*2.89*	Taking yield into account
	C$	108.40	
Cost of 1 g MLP protein	€	0.01	Average protein content 30.06% DM or 28.09%FW (mean DM content of 93%) (see partim II)
Cost for 1 mg of iron	€	0.07	Average iron content 4.31 mg/100 g DM or 4.03 mg/100 g FW (mean DM content of 93%) %) (see partim II)
Cost for 1 mg of dialyzable iron	€	2.56	On average: 2.8% of iron is dialyzable
Cost for 1 mg of (D + SND) iron	€	0.17	On average: 43.4% of iron
Total cost for 1 kg fresh leaves processed into MLC (±20g)	€	1.70	
	C$	63.66	

Total cost for 1 kg dried MLC	€	79.63	Taking yield into account
	C$	1 494.30	
Cost of MLC protein	€	0.13	Average protein content 64.67% DM or 60.44%FW (mean DM content of 93%) %) (see partim II)
Cost for 1 mg of iron	€	0.61	Average iron content 13.93 mg/100 g DM or 13.02 mg/100 g FW (mean DM content of 93%) %) (see partim II)
Cost for 1 mg of dialyzable iron	€	10.54	On average: 5.8% of iron is dialyzable
Cost for 1 mg of (D + SND) iron	€	3.71	On average: 16.5% of iron

Processing MLC - on a larger and more efficient scale, with machinery			
Cost parameters	***Value***		***Remarks***
Min. labour cost (c$ per hour)	C$	18.14	
Min. labour cost (€ per hour)	€	0.48	
Number of labourers for concentrate process, including drying		1	
Estimated amount of work for labourers to make concentrate out of 2 kg of fresh leaves, including drying and associated activities (hours)		0.75	
Rest (hours/day)		1	
Estimated amount of costs for electricity, gas and water per hour	€	0.08	Numbeo, 2018
Total cost for concentrate process	€	0.49	
	C$	18.22	

Total Costs – on a more efficient scale			
Cost parameters	***Value***		***Remarks***
Total cost for 1 kg fresh leaves processed into MLP (±200g)	€	0.54	
Total cost for 1 kg MLP	€	2.89	Taking yield into account
Cost of 1 g MLP protein	€	0.01	Average protein content 30.06% DM or 28.09%FW (mean DM content of 93%) %) (see partim II)
Cost for 1 mg of iron	€	0.07	Average iron content 4.31 mg/100 g DM or 4.03 mg/100 g FW (mean DM content of 93%)
Cost for 1 mg of dialyzable iron	€	2.56	On average: 2.8% of iron content is dialyzable
Cost for 1 mg of (D + SND) iron	€	0.17	On average: 43.4% of iron
Total cost for 1 kg fresh leaves processed into MLC (±20g)	€	0.49	
Total cost for 1 kg dried MLC	€	11.40	Taking yield into account
	€	8.09	If yield can be improved to 3%
Cost of MLC protein	€	0.01	Average protein content 64.67% DM or 60.44%FW (mean DM content of 93%)
Cost for 1 mg of iron	€	0.06	Average iron content 13.93 mg/100 g DM or 13.02 mg/100 g FW (mean DM content of 93%)
Cost for 1 mg of dialyzable iron	€	1.51	On average: 5.8% of iron is dialyzable
Cost for 1 mg of (D + SND) iron	€	0.53	On average: 16.5% of iron