| | |
|---|---|
| Title | Biofortification for better nutrition: developing and delivering crops with more impact |
| Author(s) | Hummel, Marijke |
| Publication Date | 2020-05-06 |
| Publisher | NUI Galway |
| Item record | http://hdl.handle.net/10379/15940 |

# Biofortification for better nutrition: developing and delivering crops with more impact

A THESIS SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY IN THE COLLEGE OF SCIENCE

**By Marijke Hummel**

Plant & AgriBiosciences Research Centre (PABC)
National University of Ireland, Galway
Supervisor Prof. Charles Spillane
April 2020

# Declaration

The work of this thesis is based on research carried out in the Plant and Agro Biosciences centre, NUI Galway.

No part of this thesis has been submitted elsewhere for any other degree or qualification. This thesis reports my own work, unless otherwise referenced in the text.

Marijke Hummel
Galway, April 2020

# Acknowledgements

First, I would like to thank my supervisor Charles Spillane for the chance of pursuing my PhD under his supervision. Another person that I owe a lot is Elise, you have been a great support during the entire process of my PhD, spending half a year in Colombia and now we continue working together at the WUR!

Many memories were made at the upper floor of Aras de Brun, in the lab and during many lunches and coffeebreaks. I would like to thank my colleagues at the department, especially Brendan and Colm. During my fieldtrips in Malawi, I have had great support from a lot of people. I would first like to thank all field staff and all participants of the studies in Malawi, you were crucial in doing all this work, thanks a lot for your dedication and time. Then, Zewdy and family, how exciting to be working together on our PhDs in such a beautiful and at times frustrating country. Hopefully we will graduate soon together! Daniel, Arthur and all other staff at the International Potato Centre in Lilongwe, it was a great year working with all of you. Also, thanks to Rodah Zulu and all other CIAT Malawi staff for their assistance and help with all bean-related questions. And lastly my stay in the G-rien house, which made it fun and made me never feel alone in Malawi.

What is better than being able to do your research accompanied with a good friend? Ati, many thanks for your help during the many weeks in the field. I don't know if it would have been possible without you.

Thereafter, my trip to Colombia, where again I met many wonderful people. Luis, and all other staff at HarvestPlus and the Human Nutrition lab at CIAT in Palmira, Colombia. We have cooked, tested, tasted and analysed quite some frijoles.

Karlijn, our nutrition career started way back when we both started our BSc in Wageningen. Your hard work has always been a great inspiration for me. It was not always easy to leave home for an adventure abroad. I am very happy I have been able to reconnect with many of you when I was back, and you have helped me. Alma, Lirije, Martine, Anneke, Marit, Evelien, Yaxi, Annegreet, Eline, and all others I have not seen enough the past few years, I will see you soon again and we will definitely celebrate!

Esther and Annemarie and Janny and Jurrien. You have followed me in many places on beautiful trips which we will all never forget. You have always been interested and this helped me to continue and put through. Mum, I am really finished now! Then last, but definitely not least: Rob, your support kept and will keep me going. I am sure you are as happy as I am that this thesis is done. Ready for new adventures together!

The past six years have been quite a rollercoaster, but I am so grateful for having the opportunity to go through this all. Having been able to work in Ireland, Malawi and Colombia were all unique experiences I will always remember. For all people that were not specifically mentioned, I really appreciated your contributions in any form, thanks a lot for that.

Finally, I would like to thank the Irish Research Council, CCAFS, USAID, CIAT and CIP for funding this PhD project.

Zikomo kwambiri, muchas gracias, bedankt, and thank you for all your support, wisdom and encouragement!

# Table of contents

# Table of contributions

| Chapter | Contributions/responsibilities of author in respective chapters:<br><br>(% of contribution between brackets, if not 100%) |
|---|---|
| **1** | Literature review, writing, editing |
| **2** | Writing (70%), sample handling, sample analysis (sample preparation, phytate analysis), data analysis (50%), editing (60%)<br><br>The climate modelling part in Ecocrop was not part of my contribution.<br><br>Co-authors: Brendan F. Hallahan, Galina Brychkova, Julian Ramirez-Villegas, Veronica Guwela, Bartholomew Chataika, Edna Curley, Peter C. McKeown, Liam Morrison, Elise F. Talsma, Steve Beebe, Andy Jarvis, Rowland Chirwa, Charles Spillane |
| **3** | Study design (80%), sample preparation, data analysis, writing, editing (70%)<br><br>Co-authors: Elise Talsma, Victor Taleon,Luis Londono, Galina Brychkova, Sonia Gallego, Bodo Raatz, Charles Spillane |
| **4** | Study design (80%), fieldwork, data analysis, writing, editing (75%)<br><br>Co-authors: Elise F. Talsma, Ati Van der Honing, Arthur Chibwana Gama, Daniel Van Vugt, Inge D. Brouwer, Charles Spillane |
| **5** | Study design (80%), fieldwork, data analysis, writing, editing |
| **6** | Writing, editing |

# Abstract


**Background**

Globally, around three billion people have inadequate diets and are often malnourished. Biofortification, a nutrition-sensitive approach that aims to increase the nutritional density of staple crops, has great potential to increase the nutrient intake of rural poor, whom are often relying on subsistence farming for their food. The overall aim of this thesis was to study three key elements in the development and delivery phase of biofortified crops to improve their nutritional impact. This research focussed on 1) The effect of **climate change** on the nutritional quality of beans, which was assessed in field trials in Malawi. 2) The **retention** of minerals and phytates in different types of beans when preparing common bean recipes. 3) Cultural and sensory **acceptability** of orange-fleshed sweetpotatoes (OFSP) and iron beans among households with children in Malawi, which was studied using mixed methods. Malawi was chosen as a target country as it is a top priority country for the implementation of biofortified crops.

**Results**

The field trials showed that under climate induced drought scenarios, future bean servings will have a lower nutrition quality (esp. iron) . Combining the low phytate and biofortification trait through crossbreeding could lead to a higher nutritional impact of iron beans through an increased bioavailability of iron. Considering both cultural and sensory attributes when introducing a biofortified crop can influence the acceptability of varieties and consumption amongst households with children. The invisible trait of iron beans poses challenges on recognizing and distinguishing these beans from conventional beans.

**Conclusions**

To further improve the nutritional impact of biofortified crops the studied elements (climate change effects on nutritional quality, retention and consumer acceptability) need attention. Improving the impact of biofortified crops could be reached through further climate-proofing of bean varieties, combining the *low phytic acid* trait with the iron trait in developing new bean varieties, leading to higher bioavailability of iron, and studying both sensory and cultural acceptability using mixed methods in a local context.

# Chapter 1

## General introduction

Marijke Hummel

# BACKGROUND

## The burden of malnutrition in low- and middle-income countries

Globally, around 3 billion people have inadequate diets and are often malnourished [1]. They are suffering from either undernutrition, micronutrient deficiencies and/or are overweight. The majority of these people are living in low- and middle-income countries (LMIC). To achieve the sustainable development goal (SDG) of ending malnutrition by 2030 [2], the overall diet quality and /or inadequate intakes (quantity) of people affected should be improved. The Panel on Agriculture and Food systems for Nutrition has been calling for a change: 'food systems should shift from feeding people to nourishing people', referring to the importance of overall dietary quality over quantity [1]. Specific groups disproportionally affected by micronutrient deficiencies and/or undernutrition are young children and women of reproductive age [3]. This is mainly due to the increased needs during growth, development, menarche and pregnancy [4]. Poor nutrition during the first 1000 days of a child's life can result in a child being stunted, i.e. reduced height for age, which leads to irreversible disadvantages later in life. Examples of these are impaired mental and physical development, leading to reduced labour productivity in adult life [5]. Stunted growth is affecting one in four children globally, and 1 in three children in Eastern and Southern Africa [6]. Undernutrition, including stunting explains around 45% of child deaths among children under 5 [7]. Next to undernutrition, micronutrient deficiencies can also be a result of malnutrition. These refer to an inadequate intake of vitamins or minerals. Micronutrient deficiencies are common around the world with over 2 billion people affected [6]. On a global scale the micronutrients with the lowest levels of adequate intake and highest prevalence of deficiencies are Vitamin A, Iron, Zinc and Iodine [8, 9]. These can result in anaemia in the case of iron, vitamin A deficiency and a range of other problems impeding growth, physical and cognitive development and an increased susceptibility to other diseases (eg. malaria, diarrheal diseases and tuberculosis) amongst others [10, 11]. Strong differences in the incidence between countries and regions are found [12]. The prevalence of micronutrient deficiencies due to insufficient dietary intake has decreased over the last 50 years in almost all world regions. However, the prevalence of multiple micronutrient deficiencies among children and women in Sub Saharan Africa remains high [9]. In Sub Saharan Africa over 40% of children under five suffer from vitamin A deficiency [7]. . In addition, close to 50% of young children and 70% of pregnant women are affected by anaemia of which an estimated 50% is due to inadequate iron intake [13]. Sub-Saharan Africa is the only region were dietary micronutrient density of foods has decreased [8]. This can be attributed to the increased production of staple foods, and reduced diversity in other more nutrient-rich crops. The Global Burden of Disease Study indicated that 6 out of 11 risk factors of the global burden of disease are related to the diet [14]. The global economic costs of malnutrition are high and estimated to be 11% of the GDP in Africa [15]. Investing in nutrition has a very high return of investment- it was estimated that every dollar invested results in a 16-dollar return [15].

## Interventions to alleviate malnutrition in low- and middle-income countries

A wide range of interventions- nutrition specific and nutrition sensitive- can be applied in order to alleviate (multiple) micronutrient deficiencies in the most vulnerable groups of the population. Nutrition-specific interventions address the direct determinants of malnutrition [16]. These interventions mostly focus on consumption behavior and include dietary diversification, micronutrient supplementation and fortification amongst others. It was estimated that scaling up 10 evidence-based key nutrition specific interventions to 90% coverage in 34 countries could reduce stunting by 20% [17]. None of these interventions alone have shown to be universally successful, thus nutrition specific interventions alone will not be able to meet the global targets for improving nutrition [7]. Therefore, there is still a need for new strategies, with a potential bigger impact. Nutrition-sensitive interventions address the underlying determinants of malnutrition, by integrating nutrition with other sectors [16]. The underlying determinants consist of household food security, care and feeding practices and the household environment including health services. Nutrition-sensitive interventions include interventions aimed to improve food security, social safety nets, early child development, mental health, women's empowerment, water sanitation & hygiene and health & family planning services amongst others.

## The role of agriculture in nutrition-sensitive interventions

There are different types of nutrition-sensitive agricultural interventions, examples are biofortification, homestead food production systems, livestock transfer programs, value chains for nutritious foods, and irrigation programs [18]. Agriculture is increasingly being recognized as an important component of the food systems in LMIC [7]. It has a strong potential to influence nutrition since it is directly influencing these underlying determinants of nutrition outcomes, especially in a rural LMIC setting, where the -often malnourished- population is relying on subsistence farming. Agriculture is in these cases the main source of food and income and directly contributes to nutrition and health [19]. Over the last decade, the focus has been on linking nutrition and agriculture, for its supposed benefits which include improved maternal and child nutrition [16, 20]. The available evidence from effectiveness studies on nutrition sensitive agricultural interventions has shown the many linkages between nutrition and agriculture. These studies quantified the nutritional beneficial effects of implementing these types of interventions for nutrition outcomes (for example improved dietary diversity, micronutrient status eg.) [18].

Biofortification is the present intervention of interest in this thesis, because it has shown to have a high efficacy across a large range of geographical areas and different target groups [21]. Biofortification is a relatively new nutrition-sensitive approach that aims to increase the nutrient density of the edible part of staple food crops. Improved nutritional content, but also other preferable traits as drought and pest/virus resistance can be incorporated in the improved biofortified varieties of staple crops [21]. Biofortification is one of the food-based approaches to decrease micronutrient malnutrition, and complementary to nutrition-specific approaches as fortification, supplementation and dietary diversification/modification amongst others [22]. Biofortification is not a silver bullet or meant to replace any of the other approaches but can be seen as an intervention to improve the habitual micronutrient intake.

Biofortification ranked fifth in the top ten list of solutions for global problems ranked by the Copenhagen Consensus 2008 and is the highest ranked nutrition sensitive agricultural intervention. Supplementation and fortification are ranked #1 and #2 respectively [23]. Biofortification is a sustainable, and cost-effective approach. An analysis of cost-effectiveness, which is expressed in the cost of one Disability Adjusted Life Year saved, ranks biofortification also as highly cost-effective, defined as <$200 for one DALY saved [24].

## Biofortification as a nutrition-sensitive agricultural intervention to alleviate malnutrition

Using biofortification, nutritional intake of the rural poor and often malnourished can be increased by growing and consuming these crops. The diets of these vulnerable populations are mostly staple-based and low in animal products, which makes them more vulnerable for deficiencies, due to a lower (bio)availability of the micronutrients in their diet [22]. In addition, in rural areas, access to markets is low and many people rely on subsistence farming [25]. The efficacy of biofortification has been shown for many different crops and target groups in South America, Africa and Asia. For example, iron beans have shown to lead to a significant increase in haemoglobin and total body iron stores in iron-depleted women in Rwanda[26]. In India, consumption of peal millet flat bread increased serum ferritin and total body iron of school kids[27]. Orange fleshed sweet potato was introduced in rural Uganda and led to an increased Vitamin A status in children[28].

These data suggest that biofortification can be an important intervention type to reduce malnutrition in LMICs. Despite the high prevalence of malnutrition in sub-Saharan African countries to date, more data on the effects of biofortification of crops on malnutrition in the sub-Saharan African context needs to be collected, especially when and after delivering these crops (effectiveness) and optimizing the developed crops further to increase their impact.

Biofortification can be achieved through three different methods; 1) conventional plant breeding, 2) transgenic approaches or, 3) through the use of fertilizers [21, 29]. The regular consumption of these crops will lead to measurable improvements in human health and nutrition [21]. In this thesis we will focus on the first method; the use of conventional plant breeding. This method starts with screening of the germplasm available, the living tissue from which new plants can be grown, containing the genetic resources (in this case the seed) to search for preferable traits. Micronutrient dense germplasm is identified and further crossbreeding results in possible new varieties [21]. The success of breeding for biofortified crops through conventional breeding depends on the availability of wild varieties that have specific nutrient producing traits. If these are not available for a crop or the micronutrient density is not high enough in the gene pool, the second biofortification method of transgenic approaches can be used. An example of a transgenic crop is golden rice, biofortified with Vitamin A [30]. Transgenic approaches have enormous potential since the amount of micronutrients in crops can be increased much more than through conventional crossbreeding [29], but due to the lengthy process for national approval and biosafety, crops developed through conventional breeding are currently preferred [21].

The third method for biofortification is through the use of either soil or foliar fertilizers. Foliar fertilization has shown to be more effective than soil fertilization, but the disadvantages of high costs, leaf damage, low uptake and wash-off by rainfall make it unattractive for smallholder farmers [31]. The advantage of using fertilizers is that farmers do not have to change the seeds they are using, but only have to apply the fertilizers. However, these fertilizer must be applied regularly and are expensive [32], which makes it less fit within the original philosophy of biofortification of reaching the poor through a sustainable measure. Examples of biofortified (staple) crops developed through conventional crossbreeding to date are rice, wheat, pearl millet, maize, orange-fleshed sweetpotato, common beans and cassava [21]. These crops contain increased levels of iron, zinc and beta-carotene or a combination of these, to increase the nutritional intake in people in LMICs.

In this thesis, two different biofortified crops are studied; biofortified common beans with high levels of iron and orange fleshed biofortified sweetpotato containing high levels of beta-carotene.

## Box 1. Beans and sweet potatoes as crops of focus

The two crops under study in this thesis are sweet potatoes and beans. These crops were chosen since they are both part of the dietary pattern widely adhered to throughout Sub Saharan Africa and the difference in visibility of the biofortification trait between both crops. In the next paragraphs the most important characteristics of these crops will be described, along with their consumption and production characteristics.

**Figure 1:** Pictures of crops of focus: Iron beans (left, Author’s picture) and Orange-fleshed sweetpotato (Wikimedia Commons)

### Beans & iron beans

Common beans (Phaseolus vulgaris L.) originated independently from both South and Central America [33]. Primarily the bean seed and to a lesser extent the leaves of beans are consumed throughout the world, where the crop is most popular in South America and Africa. Beans are often consumed in combination with other staples such as maize, plantain or cassava. They are referred to as “the poor people’s meat”, as a higher consumption has been shown to be related to lower income families [34, 35]. These poorer families cannot afford to consume meat, and the consumption of beans is therefore an important protein and mineral source [36]. Globally, per capita consumption of beans seems to be declining as population is growing faster than production [37]. Africa is the continent with the second highest production of pulses and dry beans globally [38]. Bean production in Southern Africa, including Malawi compromises 31% of the total bean production in Africa. These beans are mainly grown by resource-poor farmers on small-scale marginal farms. Intercropping systems are widely used, having combinations of beans with cereals as maize, millet or sorghum, banana, plantains, roots or tubers [34]. Current productivity of legumes is low in LMICs, especially in Sub-Saharan Africa [39, 40]. Conditions under which beans are grown vary widely, and result in low average crop yields, ranging from 500 to 5000 kg/ha.

The main barriers for a higher yield are bean diseases, pests, reduced soil mineral nutrition, drought and heat stress, ozon stress and/or weeds [41]. Beans are an important cash-crop and are mostly marketed on established trade routes within the country of production [34]. From an agricultural point of view, legumes are an important group of crops since they are so-called nitrogen fixing plants. In symbiosis with the rhizobium bacteria the plant can fix nitrogen from the air, which leads to increased production and better soil fertility [42]. Due to climate change bean growing areas are under threat, and production levels are estimated to decrease by as soon as 2030 in Eastern Africa [43]. Iron beans are biofortified beans that have been developed using crossbreeding, after screening available germplasm for iron content and crossbreeding these lines with other lines with favourable traits (e.g. consumer acceptability, drought resistance, yields) [44]. Table 1 shows the biofortification target levels for iron beans that are set by the HarvestPlus research program [45]. These targets are set taking into account different factors, e.g. bioavailability, edible portions, environmental factors, and cultural practices. Iron beans or biofortified beans, have shown to increase iron status over time in efficacy studies in adolescents and children in Rwanda and Mexico [26, 46, 47]. Currently, iron beans have been introduced in 13 countries and are being tested for release in another 4 countries [48].

**Table 1:** Breeding target levels for iron beans (iron content) and Orange-fleshed sweetpotato (beta-carotene content)

| | **Iron beans [49]** | **Orange-fleshed sweetpotato [49]** |
|---|---|---|
| **Baseline (μg/g)** | **50** | **0** |
| **Additional content required (μg/g)** | **+44** | **+170** |
| **Total Final content (μg/g)** | **94** | **170** |
| Portion size, grams/day | 198 | 167 |
| Retention (boiled), % | 90 | 80 |
| Bioavailability, % | 7 | 8 |
| % of the physologicalrequirement contributed by biofortification | 38 | 160 |
| % of the requirement contributed by entire micronutrient content of staple (baseline + biofortification) % | 80 | 160 |

*% of the requirement* is expressed based on the estimated average requirement (EAR) for non-pregnant non-lactacting women. Portion sizes are given in cooked product (boiled). Target levels were set based on the reasonable probability of developing varieties with a x% increase in the nutrition of interest where the the minimal increase in the EAR had to be at least 40% when consuming the average portion size/day.

## Sweetpotato & orange fleshed sweetpotato

Sweetpotato (*Ipomoea batatas (L.) Lam*) originally came from the Americas to Africa in the 1500s [50]. It is one of the world's most important crops for food and nutrition security, particularly in Sub-Saharan Africa, parts of Asia, and the Pacific Islands [51, 52]. Malawi is the main producer of sweetpotatoes in Sub-Saharan Africa, with an average production of 5.5 million ton per year in the period 2017.[38] Both leaves and roots are eaten, the vines can serve as food for livestock [52]. From a nutrition perspective, sweetpotato roots are a good source of carbohydrates, fiber and vitamins B, C and E [53]. The root is most commonly boiled, fried or steamed before consumption. Most of the varieties of sweetpotato currently grown and consumed in Sub-Saharan Africa are white- or yellow-fleshed, and contain little or no beta-carotene [52]. Sweetpotato has become a major pillar of food security in many countries in the African region for different reasons.

These include the comparatively high and reliable yields (4-6 MT/ha) even under marginal conditions and poor soils, low labor requirements, short growing cycles (4-5 months), and experiences of yield increases in smallholder systems by over 50% through improved varieties, clean planting materials and better farming practices [52]. Sweetpotato has shown to respond well to many of the productivity challenges facing smallholder farmers nowadays. It is referred to as the crop that is there when maize fails [54]. Adopting this crop is investing in long-term nutrition, economic and food security benefits. The varieties high in vitamin A, orange- fleshed sweet potatoes (OFSP) were developed and disseminated by the International Potato Centre (CIP) initially.

As a result of national breeding efforts in several sub-Saharan African countries, a set of locally adapted OFSP varieties have been released and are now available that respond to the main agronomic, ecological and market conditions across the continent. In recent years, breeding programs have developed 42 improved OFSP varieties that are a good source of beta-carotene, a precursor of vitamin A [55]. Table 1 shows the improved levels of beta-carotene in OFSP compared to the white fleshed sweetpotato. OFSP varieties stand out as a proven, cost effective tool to reduce Vitamin-A deficiency (VAD) since it can provide an additional vital nutrient to vulnerable populations. OFSP's efficacy is based on the high concentration of pro-vitamin A in roots and leaves at relatively high levels bio-available through local diets[52]. Efficacy and effectiveness studies have shown a decrease in vitamin A deficiency in young children in South Africa [55] and mother-child pairs in Mozambique [56]. OFSP varieties have been introduced in 27 countries worldwide and are being tested for release in another seven countries [48].

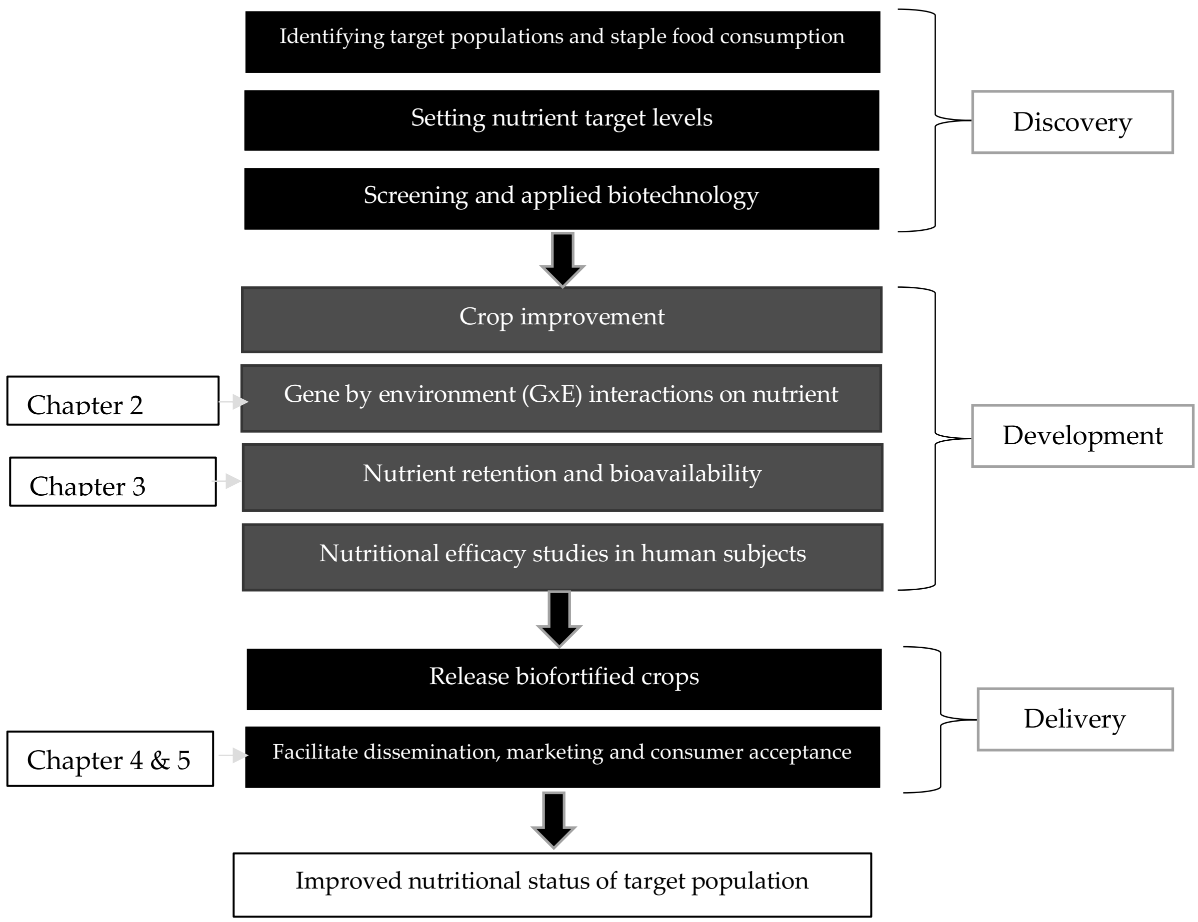
## Key elements for alleviating malnutrition using biofortified crops as intervention approach

Alongside the actual crop-development of biofortified varieties, there are other important steps needed to create the envisioned nutritional impact when implementing biofortified crops. These activities can roughly be divided in three different phases, the discovery, development and delivery phases [44, 57]. A simplified overview of this so-called 'pathway to impact' is given in Figure 2. It also shows some of the variety of aspects and professions that join to create and deliver a biofortified crop to the target population. Some of these activities are recurring, even during and after delivery of the biofortified crop, an example is acceptability research.

- **Discovery:** The discovery phase consists of identifying the target populations and assessing their habitual staple food intake. Based on this information nutrient target levels can be set. Screening and applied biotechnology is subsequently used to identify the lines that could be used to develop the biofortified crops.
- **Development:** The development phase starts with crossbreeding of the crop to develop biofortified varieties. Other key elements of this phase are testing for gene-environment interactions and verifying the retention of the nutrients in the crops after processing, amongst others.
- **Delivery:** For the delivery phase it is crucial that biofortified food crops are accepted by both farmers and consumers of the targeted population. Making delivery and uptake of the crop successful requires assessment of acceptability and adoption of the crop.

Three elements essential in the development and delivery phase will be further investigated in this thesis and are discussed in more depth in the next three paragraphs.

**Figure 2:** (Adjusted) Harvest Plus pathway to impact [58]. The figure shows the three phases: discovery, development and delivery that are taken before the biofortified crop can create its envisioned impact and highlights the different elements studied in this thesis in chapter 2-5.



## Key element 1 Gene by environment interactions and nutritional quality

The goal of studying gene-environment interactions is to see how different genotypes respond to environmental variation. In the case of biofortification, nutritional quality of crops is an important variable, but also other variables can be assessed and selected for. Examples are yield, disease resistance, plant characteristics, number of seeds etc. Gene-environment interactions are studied at research stations or farmer fields in the targeted country. Suitable varieties are grown in multi-location trials and grown over multiple seasons to assess the agronomic performance. After this step, release of the variety can be considered by the national government [21].

Climate change, defined as “change of climate that is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and that is in addition to natural climate variability observed over comparable time periods” [59] is potentially causing adverse effects on food security through negative effects on crop yields and nutritional quality [60]. It is crucial to breed new varieties that can withstand these abiotic and biotic environmental stresses and will thus be more resistant to negative effects [61]. Sub-Saharan Africa is identified as a region that is highly vulnerable to climate change [62] and the impacts on food security will be major if no adaptations are undertaken [63]. There is a general consensus that predicted changes in rainfall and temperature will lead to lower yields especially in (sub)tropical areas [60]. However, there is very limited data on whether these drought-associated reductions will also lead to changes in nutritional content in beans. It was predicted that 60% of bean-growing areas in this region needs to be transformed [64]. It is suggested that crop improvement is an important first step of this transformational adaptation in order to decrease the negative consequences of climate changes[64]. To ensure food security and better adaptation to climate change, it is therefore vital to understand more about the nutritional quality of bean crops under drought stress.

## Key element 2 Nutrient retention & bioavailability

Micronutrients in a biofortified staple food need to be bioavailable to the body in order to make a successful contribution to the micronutrient status of the individual. Bioavailability encompasses the amount of nutrient accessible for utilization, conversion or storage by the human body and can be enhanced or inhibited by the presence of antinutrients and different food processing techniques [65]. In unfortified staple crops, nutrient and bioavailability levels are mostly low [65]. It is therefore essential to study aspects influencing micronutrient bioavailability of (biofortified) crops and to learn more about two aspects specifically: the effect of household processing on the retention of nutrients in the crop and the antinutrient content of a (biofortified) crop. Retention studies are performed to measure the levels of micronutrients in crops after typical processing, storage and cooking practices [66]. It is shown that crop variety, processing method and micronutrient level influence the level of retention [65].

For this thesis we focus on the retention of (anti)-nutrients(iron, zinc and phytic acid) of the (biofortified) common bean. Limited information from retention studies in common beans shows that the minerals Fe and Zn are mostly preserved after processing [67]. However, the presence of anti-nutrients has shown to be impairing the mineral bioavailability of common beans. The most important antinutrients known are phytic acid and polyphenols [68]. These have both been studied extensively in both in vitro and in vivo studies. Series of short term isotope nutritional studies have showed that phytic acid is the main inhibitor, and polyphenols play a minor role [68]. Phytic acid is the main storage form of phosphorus in seed and chelates with mineral cations hampering them from uptake in the body. The formed salts are excreted, since humans have limited phytase activity in their digestive tract to break down these phytates [69].

The negative impact of especially phytates on bioavailability of minerals led to exploring and developing bean types with a low phytate trait. These *lpa* (low phytic acid) beans have a 90% lower phytate content compared to conventional bean varieties. The mineral retention of these *lpa* bean varieties were not yet determined. If retention of minerals remains high in these *lpa* beans this trait could possibly be combined with the biofortification trait, and lead to a new generation of biofortified *lpa* bean varieties, which have a higher bioavailability of minerals compared to the biofortified bean varieties available the moment. Promising results have been demonstrated in maize crops where *lpa* varieties of maize have shown a significantly higher Fe and Zn retention in subject fed with meals prepared with an *lpa* variant of maize, compared to conventional varieties [70-72].

## Key element 3 Consumer acceptability of the biofortified crop

A developed biofortified variety often has different sensory and/or agricultural characteristics compared to the non-biofortified varieties of the same crop. To assess and compare the willingness to consume these altered crops, acceptability and/or adoption can be assessed. Acceptability reflects the perception among producers and consumers that an intervention is agreeable. Adoption reflects the intention, initial decision or action to try a new intervention [73].

In order for producers and consumers to use the improved varieties, the first step is acceptance. Acceptability is studied using a variety of methods. Sensory acceptability studies are used to collect information on sensory attributes that influence consumer acceptance, examples are sight, odor, taste and texture and the liking of these attributes for different varieties [74]. Cross-sectional questionnaire-based surveys collect information on attitudes, constraints and facilitators of different biofortified foods. Effectiveness studies show the acceptance and adoption over a certain period of time where different ways of introducing crops are tested (e.g. Education, different marketing strategies). The last research method used to evaluate acceptance is through setting up experimental auctions of biofortified crops in so called willingness to pay studies. This is done in comparison with a locally available crop to test the need for a discount or premium when introducing the biofortified crop [74, 75].

Available evidence on the acceptance and adoption of biofortified staple crops in LMICs showed that from a sensory perspective biofortified crops seem to be acceptable in most cases [74]. However, it needs to be taken into account that most studies were done where the crops were not yet available to the population being studied. Therefore, more research should be done into acceptability of biofortified crops after introducing these and into the replacement with the traditional crops. In addition, studies should study the actual determinants of acceptance and adoption during and after wide-scale introduction of biofortified crops [74]. Any results studying acceptability are highly context-specific, based on the heterogeneity in food choices that are rooted in culture and individual specific preferences [75]. It is therefore important to conduct these studies for all different crop-country combinations [75]. The use of mixed methods is needed to unravel the socio-cultural drivers of consumers -who are often also farmers- consuming these crops. The difference in acceptability of the visible vs. invisible trait has not been studied before. In the case of iron beans, the invisible trait does not give the consumers an indication whether they are purchasing or growing a biofortified crop or not. Educating target groups on the beneficial effects of the crops and monitoring the use is then essential [75]. In the case of a visible trait (for example the OFSP) the distinguishing color can be used when promoting the crop. Different models have been developed to study health-related behavior of which the theory of planned behavior and health belief model [76, 77]. These models could provide valuable information on the cultural acceptability and socio-cultural drivers of acceptability for iron beans and OFSP.

## Aims and objectives of this thesis

Biofortification provides an opportunity to develop more nutritious staple foods that can potentially contribute to the nutritional intake of targeted populations. However, information on the effect of climate change, the acceptability of these crops and retention of nutrients in these crops is still lacking. The main objective of this PhD thesis is therefore to study/evaluate these three key elements of the development and delivery phase of biofortified crops that are important to improve their nutritional impact.

This aim can be broken down in the following specific objectives linked to the identified key elements for development:

- To study the effect of **climate change** on the nutritional content of (biofortified) beans.
- To study the iron, zinc and phytic acid **retention** levels of common, biofortified and low phytic acid beans when preparing household recipes.
- To study the **acceptability** of biofortified crops (beans and orange fleshed sweetpotato) from a cultural and sensory perspective.

These key elements are also marked in Figure 2. The pursuit of these objectives is presented in this thesis in four articles, of which three have been published in peer reviewed journals (*Scientific Reports*,*PlosOne* and *Nutrients*).

## Study area

Malawi is a landlocked country located in Southern Africa (Figure 3). The country has a total of 18.6 million inhabitants, which is expected to increase to 38.1 million by 2050 [78]. Over half of the population is under 24 years old (65%). The majority of the population is living in rural areas (85%), where the vast majority is involved in agriculture (94%) [79]. A large proportion of Malawians rely on agriculture for subsistence, 80% of the country's total workforce is employed in this sector [80]. The agricultural sector mainly relies on smallholder farmers, whom had an average size of 0.8 hectares in 2011 [81]. Over 70% of cultivated land is used for maize production [81]. A low dietary diversity and an overdependence on staples as maize can result in diets containing insufficient minerals and vitamins [82]. Malawi is among the 20 countries with the highest score on the Hidden hunger index [9]. Almost half of all children are stunted (40%) [83]. Micronutrient deficiencies are common: 21.7% of all preschool children suffer from iron deficiency and 60.4% suffer from Zinc deficiency. This not only has individual health consequences but also influences the economy; the total annual costs associated to child undernutrition in Malawi were estimated to be yearly 10.3 % of the GDP [84]. It is a top priority country for the implementation of five different biofortified crops [85]since micronutrient deficiencies are common, agriculture is the main source of income, and staple crops are consumed widely.

At the time of conducting the studies for this thesis, biofortification was still relatively unknown in Malawi. The only biofortified crop that was widely introduced through agricultural programmes led by research institutions was the orange fleshed sweet potato. Biofortified bean varieties were released in 2009, but other than small-scale projects biofortified bean seed was not produced nor disseminated yet throughout the country. Currently programs are running to promote the consumption of legumes and introduce the biofortified beans to a bigger audience (personal communication). The agricultural policy of Malawi promotes the use of nutrition-sensitive food and agriculture-based approaches for the production of more diversified foods and dietary diversification. The policy objectives explicitly mention the scaling up of biofortification, and the need for support from the Ministries of Agricultural Extension, Health and agricultural research, NGO's, Research institutions and Farmer Organizations [86].


**Figure 3:** Map of Malawi (Wikimedia Commons)

## Study context

Currrently, HarvestPlus is leading the global interdisciplinary alliance of research institutions and implementing agencies in the biofortification effort for almost all biofortified crops, developed through conventional plant breeding [21]. One exception is for the orange fleshed sweetpotato, where the development and dissemination is led by the International Potato Centre [52]. The International Centre for Tropical Agriculture (CIAT) is responsible for the work on iron beans under the Harvest Plus Programme. For the research presented in this thesis, work was conducted in conjunction with both CIAT and CIP within Malawi. These are two CGIAR organizations part of a global partnership that unites international organizations engaged in research for a food-secured future. Also Concern Worldwide, an Irish non-governmental organisation assisted in performing the studies for this thesis. This NGO, with one of their bases in Malawi, has different projects on improving food and nutrition security and introduced their farmers to different biofortified crops over the previous years in Malawi.

The NUI Galway-CIAT-CCAFS project through which part of this thesis was conducted was aiming to contribute to development and delivery and impact of iron beans in Malawi, and to identify drought tolerant beans that can be used to climate-proof bean production, utilization and nutrition amongst women and children in Malawi.

## Outline of this thesis

The present chapter introduces and clarifies the topics under study. In chapter 2 a paper is presented on the effect of drought on the nutritional content of twenty different (biofortified) bean varieties and gives an indication of future climate scenarios and climate change impacts regarding drought stress. Chapter 3 presents the results of a retention study where low phytate, biofortified and conventional varieties of beans where prepared using common household recipes. Chapter 4 and 5 further explore the acceptability of two biofortified crops in Malawi. In chapter 4 results of a sensory and cultural acceptability study on the acceptability of orange fleshed sweetpotato is presented. In chapter 5 results of a mixed method study on the acceptability of iron beans is given. The final chapter summarizes the papers presented and indicates directions for further research and implications.

## REFERENCES


1. Haddad L, Hawkes C, Waage J, Webb P, Godfray C, Toulmin C. Food systems and diets: Facing the challenges of the 21st century. 2016.
2. Assembly G. sustainable Development goals. SDGs), Transforming our world: the. 2015;2030.
3. Darnton-Hill I, Webb P, Harvey PW, Hunt JM, Dalmiya N, Chopra M, et al. Micronutrient deficiencies and gender: social and economic costs. The American journal of clinical nutrition. 2005;81(5):1198S-205S.
4. Bothwell TH. Iron requirements in pregnancy and strategies to meet them. The American journal of clinical nutrition. 2000;72(1):257S-64S.
5. Haddad LJ, Hawkes C, Achadi E, Ahuja A, Ag Bendech M, Bhatia K, et al. Global Nutrition Report 2015: actions and accountability to advance nutrition and sustainable development: Intl Food Policy Res Inst; 2015.
6. Unicef. Levels and trends in child malnutrition. eSocialSciences; 2018.
7. Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, De Onis M, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. The lancet. 2013;382(9890):427-51.
8. Beal T, Massiot E, Arsenault JE, Smith MR, Hijmans RJ. Global trends in dietary micronutrient supplies and estimated prevalence of inadequate intakes. PloS one. 2017;12(4):e0175554.
9. Muthayya S, Rah JH, Sugimoto JD, Roos FF, Kraemer K, Black RE. The global hidden hunger indices and maps: an advocacy tool for action. PLoS One. 2013;8(6):e67860.
10. Brown KH, Peerson JM, Allen LH. Effect of zinc supplementation on children growth: a meta-analysis of intervention trials. 2004.
11. FAO W. Human vitamin and mineral requirements. Report of a Joint FAO/WHO Expert Consultation, Bangkok, Thailand. Food and Nutrition Division, FAO, Rome. 2001:235-47.
12. Horwitz A, Kennedy ET, Howson CP. Prevention of micronutrient deficiencies: tools for policymakers and public health workers: National Academies Press; 1998.
13. De Benoist B, Cogswell M, Egli I, McLean E. Worldwide prevalence of anaemia 1993-2005; WHO Global Database of anaemia. 2008.
14. Vos T, Barber RM, Bell B, Bertozzi-Villa A, Biryukov S, Bolliger I, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. The Lancet. 2015;386(9995):743-800.
15. ifpri. Global nutrition report 2016: From promise to impact. International Food Policy Research Institute Washington, DC; 2016.
16. Ruel MT, Alderman H, Maternal, Group CNS. Nutrition-sensitive interventions and programmes: how can they help to accelerate progress in improving maternal and child nutrition? The Lancet. 2013;382(9891):536-51.
17. Bhutta ZA, Das JK, Rizvi A, Gaffey MF, Walker N, Horton S, et al. Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? The lancet. 2013;382(9890):452-77.
18. Ruel MT, Quisumbing AR, Balagamwala M. Nutrition-sensitive agriculture: What have we learned so far? Global Food Security. 2018;17:128-53.
19. Pinstrup-Andersen P. The food system and its interaction with human health and nutrition. Reshaping agriculture for nutrition and health. 2012;21.
20. Berti PR, Krasevec J, FitzGerald S. A review of the effectiveness of agriculture interventions in improving nutrition outcomes. Public health nutrition. 2004;7(5):599-609.
21. Bouis HE, Saltzman A. Improving nutrition through biofortification: a review of evidence from HarvestPlus, 2003 through 2016. Global food security. 2017;12:49-58.
22. Gibson RS, Hotz C. Dietary diversification/modification strategies to enhance micronutrient content and bioavailability of diets in developing countries. British Journal of Nutrition. 2001;85(S2):S159-S66.

23. Horton S, Alderman H, Rivera JA. The challenge of hunger and malnutrition. Copenhagen Consensus. 2008:3-4.
24. Meenakshi J, Johnson NL, Manyong VM, DeGroote H, Javelosa J, Yanggen DR, et al. How cost-effective is biofortification in combating micronutrient malnutrition? An ex ante assessment. World Development. 2010;38(1):64-75.
25. Cornish G. Modern irrigation technologies for smallholders in developing countries. Modern Irrigation Technologies for Smallholders in Developing Countries: Practical Action Publishing; 1998. p. 1-90.
26. Haas JD, Luna SV, Lung'aho MG, Wenger MJ, Murray-Kolb LE, Beebe S, et al. Consuming Iron Biofortified Beans Increases Iron Status in Rwandan Women after 128 Days in a Randomized Controlled Feeding Trial–3. The Journal of nutrition. 2016;146(8):1586-92.
27. Finkelstein JL, Mehta S, Udipi SA, Ghugre PS, Luna SV, Wenger MJ, et al. A randomized trial of iron-biofortified pearl millet in school children in India. The Journal of nutrition. 2015;145(7):1576-81.
28. Hotz C, Loechl C, Lubowa A, Tumwine JK, Ndeezi G, Nandutu Masawi A, et al. Introduction of β-carotene–rich orange sweet potato in rural Uganda resulted in increased vitamin A intakes among children and women and improved vitamin A status among children. The Journal of nutrition. 2012;142(10):1871-80.
29. Welch RM, Graham RD. Breeding for micronutrients in staple food crops from a human nutrition perspective. Journal of experimental botany. 2004;55(396):353-64.
30. Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G, et al. Improving the nutritional value of Golden Rice through increased pro-vitamin A content. Nature biotechnology. 2005;23(4):482.
31. Khoshgoftarmanesh AH, Schulin R, Chaney RL, Daneshbakhsh B, Afyuni M. Micronutrient-efficient genotypes for crop yield and nutritional quality in sustainable agriculture. A review. Agronomy for Sustainable Development. 2010;30(1):83-107.
32. Hirschi KD. Nutrient biofortification of food crops. Annual Review of Nutrition. 2009;29:401-21.
33. Debouck DG, Toro O, Paredes OM, Johnson WC, Gepts P. Genetic diversity and ecological distribution of Phaseolus vulgaris (Fabaceae) in northwestern South America. Economic botany. 1993;47(4):408-23.
34. Broughton WJ, Hernandez G, Blair M, Beebe S, Gepts P, Vanderleyden J. Beans (Phaseolus spp.)–model food legumes. Plant and soil. 2003;252(1):55-128.
35. Tharanathan R, Mahadevamma S. Grain legumes—a boon to human nutrition. Trends in Food Science & Technology. 2003;14(12):507-18.
36. de Jager I, Borgonjen-van den Berg KJ, Giller KE, Brouwer ID. Current and potential role of grain legumes on protein and micronutrient adequacy of the diet of rural Ghanaian infants and young children: using linear programming. Nutrition journal. 2019;18(1):12.
37. Akibode CS, Maredia MK. Global and regional trends in production, trade and consumption of food legume crops. 2012.
38. Faostat F. Statistical databases. Food and Agriculture Organization of the United Nations. 2009.
39. Pingali PL. Green revolution: impacts, limits, and the path ahead. Proceedings of the National Academy of Sciences. 2012;109(31):12302-8.
40. Nedumaran S, Abinaya P, Jyosthnaa P, Shraavya B, Rao P, Bantilan C. Grain Legumes Production, Consumption and Trade Trends in Developing Countries; Working Paper Series No. 60. 2015.
41. Graham P, Ranalli P. Common bean (Phaseolus vulgaris L.). Field Crops Research. 1997;53(1-3):131-46.
42. Giller KE. Nitrogen fixation in tropical cropping systems: Cabi; 2001.
43. Lobell DB, Burke MB, Tebaldi C, Mastrandrea MD, Falcon WP, Naylor RL. Prioritizing climate change adaptation needs for food security in 2030. Science. 2008;319(5863):607-10.
44. Bouis HE, Hotz C, McClafferty B, Meenakshi J, Pfeiffer WH. Biofortification: a new tool to reduce micronutrient malnutrition. Food and nutrition bulletin. 2011;32(1_suppl1):S31-S40.
45. Bouis HE, Welch RM. Biofortification—a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. Crop Science. 2010;50(Supplement_1):S-20-S-32.
46. Haas J, Luna S, Lung'aho M, Ngabo F, Wenger M, Murray-Kolb L, et al. Iron biofortified beans improve iron status in Rwandan University women: results of a feeding trial (646.1). The FASEB Journal. 2014;28(1 Supplement):646.1.
47. Haas JD, Villalpando S, Beebe S, Glahn R, Shamah T, Boy E. The effect of consuming biofortified beans on the iron status of Mexican school children. The FASEB Journal. 2011;25(1_MeetingAbstracts):96.6.

48. HarvestPlus. Biofortified crops: what is available where? 2018.
49. Bouis H, Saltzman A, Low J, Ball A, Covic N. An overview of the landscape and approach for biofortification in Africa. African Journal of Food, Agriculture, Nutrition and Development. 2017;17(2):11848-64.
50. O'BRIEN PJ. The Sweet Potato: Its Origin and Dispersal 1. American anthropologist. 1972;74(3):342-65.
51. Bouwkamp JC. Sweet potato products: a natural resource for the tropics. 1st ed. Boca Raton CRC Press, Inc.; 1985.
52. Low JW, Mwanga RO, Andrade M, Carey E, Ball A-M. Tackling vitamin A deficiency with biofortified sweetpotato in sub-Saharan Africa. Global Food Security. 2017.
53. Bovell-Benjamin AC. Sweet potato: a review of its past, present, and future role in human nutrition. Advances in food and nutrition research. 2007;52:1-59.
54. Low J, Lynam J, Lemaga B, Crissman C, Barker I, Thiele G, et al. Sweetpotato in Sub-Saharan Africa. The sweetpotato: Springer; 2009. p. 359-90.
55. van Jaarsveld PJ, Faber M, Tanumihardjo SA, Nestel P, Lombard CJ, Benadé AJS. β-Carotene–rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response test. The American journal of clinical nutrition. 2005;81(5):1080-7.
56. Hotz C, Loechl C, de Brauw A, Eozenou P, Gilligan D, Moursi M, et al. A large-scale intervention to introduce orange sweet potato in rural Mozambique increases vitamin A intakes among children and women. British Journal of Nutrition. 2012;108(01):163-76.
57. Bouis HE, Saltzman A, Birol E. Improving nutrition through biofortification. Agriculture for Improved Nutrition: Seizing the Momentum. 2019:47.
58. Bouis HE, Hotz C, McClafferty B, Meenakshi J, Pfeiffer WH. Biofortification: a new tool to reduce micronutrient malnutrition. Food & Nutrition Bulletin. 2011;32(Supplement 1):31S-40S.
59. Gregory J, Stouffer RJ, Molina M, Chidthaisong A, Solomon S, Raga G, et al. Climate change 2007: the physical science basis. 2007.
60. Scheelbeek PF, Bird FA, Tuomisto HL, Green R, Harris FB, Joy EJ, et al. Effect of environmental changes on vegetable and legume yields and nutritional quality. Proceedings of the National Academy of Sciences. 2018;115(26):6804-9.
61. Lane A, Jarvis A. Changes in climate will modify the geography of crop suitability: agricultural biodiversity can help with adaptation. 2007.
62. Connolly-Boutin L, Smit B. Climate change, food security, and livelihoods in sub-Saharan Africa. Regional Environmental Change. 2016;16(2):385-99.
63. Wheeler T, Von Braun J. Climate change impacts on global food security. Science. 2013;341(6145):508-13.
64. Rippke U, Ramirez-Villegas J, Jarvis A, Vermeulen SJ, Parker L, Mer F, et al. Timescales of transformational climate change adaptation in sub-Saharan African agriculture. Nature Climate Change. 2016;6(6):605.
65. La Frano MR, de Moura FF, Boy E, Lönnerdal B, Burri BJ. Bioavailability of iron, zinc, and provitamin A carotenoids in biofortified staple crops. Nutrition reviews. 2014;72(5):289-307.
66. De Moura FF, Miloff A, Boy E. Retention of provitamin A carotenoids in staple crops targeted for biofortification in Africa: cassava, maize and sweet potato. Critical reviews in food science and nutrition. 2015;55(9):1246-69.
67. Bechoff A, Taleon V, Carvalho L, Carvalho J, Boy E. Micronutrient (provitamin A and iron/zinc) retention in biofortified crops. African Journal of Food, Agriculture, Nutrition and Development. 2017;17(2):11893-904.
68. Petry N, Boy E, Wirth JP, Hurrell RF. The potential of the common bean (Phaseolus vulgaris) as a vehicle for iron biofortification. Nutrients. 2015;7(2):1144-73.
69. Kumar V, Sinha AK, Makkar HP, Becker K. Dietary roles of phytate and phytase in human nutrition: A review. Food chemistry. 2010;120(4):945-59.
70. Mendoza C, Viteri FE, Lönnerdal B, Young KA, Raboy V, Brown KH. Effect of genetically modified, low-phytic acid maize on absorption of iron from tortillas. The American journal of clinical nutrition. 1998;68(5):1123-7.

71. Hambidge KM, Huffer JW, Raboy V, Grunwald GK, Westcott JL, Sian L, et al. Zinc absorption from low-phytate hybrids of maize and their wild-type isohybrids. The American journal of clinical nutrition. 2004;79(6):1053-9.
72. Hambidge KM, Krebs NF, Westcott JL, Sian L, Miller LV, Peterson KL, et al. Absorption of calcium from tortilla meals prepared from low-phytate maize–. The American journal of clinical nutrition. 2005;82(1):84-7.
73. Peters DH, Tran NT, Adam T. Implementation research in health: a practical guide: World Health Organization; 2013.
74. Talsma E, Melse-Boonstra A, Brouwer ID. Acceptance and adoption of biofortified crops in low-and middle-income countries. 2016.
75. Birol E, Meenakshi J, Oparinde A, Perez S, Tomlins K. Developing country consumers' acceptance of biofortified foods: a synthesis. Food Security. 2015;7(3):555-68.
76. Janz NK, Becker MH. The health belief model: A decade later. Health education quarterly. 1984;11(1):1-47.
77. Ajzen I. The theory of planned behavior. Organizational behavior and human decision processes. 1991;50(2):179-211.
78. United Nations DoEaSA, Population Division, . World Population Prospects 2019, custom data acquired via website 2019 [
79. NSO. Integrated Household Survey 2010-2011. Household socio-economic characteristics report. Zomba, Malawi: National Statistical Office; 2012.
80. Fund IM. Malawi: Poverty Reduction Strategy Paper. Washington, D.C. USA: IMF; 2012.
81. IFAD. Republic of Malawi Sustainable Agricultural Production Programme: Programme Design Report. . Rome: International Fund for Agricultural Development; 2011.
82. Haddad L, Hawkes C, Webb P, Thomas S, Beddington J, Waage J, et al. A new global research agenda for food. Nature News. 2016;540(7631):30.
83. Ministry of Health M, Department of Nutrition HaAitOoPaCD-O, Ministry of Health (MOH), National Statistics Office (NSO), UNICEF and CDC. The national micronutrient survey 2009. Lilongwe, Malawi: : Ministry of Health, UNICEF, Irish Aid and CDC; 2011.
84. World Food Programme, United Nations Economic Commission for Africa, Commission AU. The Cost of Hunger in Africa: The Economic and Social Impact of Child Undernutrition. 2016.
85. Herrington C, Lividini K, Angel MD, Birol E. Prioritizing Countries for Biofortification Interventions: Biofortification Priority Index Second Edition (BPI 2.0).
86. Ministry of Agriculture IaWD. National Agriculture Policy. 2016.

# Chapter 2

## Reduction in nutritional quality and growing area suitability of common bean under climate change induced drought stress in Africa

Marijke Hummel
Brendan F. Hallahan
Galina Brychkova
Julian Ramirez-Villegas
Veronica Guwela
Bartholomew Chataika
Edna Curley
Peter C. McKeown
Liam Morrison
Elise F. Talsma
Steve Beebe
Andy Jarvis
Rowland Chirwa
Charles Spillane

*Scientific Reports 2018, 8 (1), 16187*
*doi:* 10.1038/s41598-018-33952-4

## ABSTRACT

Climate change impacts on food security will involve negative impacts on crop yields, and potentially on the nutritional quality of staple crops. Common bean is the most important grain legume staple crop for human diets and nutrition worldwide. We demonstrate by crop modelling that the majority of current common bean growing areas in south-eastern Africa will become unsuitable for bean cultivation by the year 2050. We further demonstrate reductions in yields of available common bean varieties in a field trial that is a climate analogue site for future predicted drought conditions. Little is known regarding the impact of climate change induced abiotic stresses on the nutritional quality of common beans. Our analysis of nutritional and antinutritional compounds reveals that iron levels in common bean grains are reduced under future climate-scenario relevant drought stress conditions. In contrast, the levels of protein, zinc, lead and phytic acid increase in the beans under such drought stress conditions. This indicates that under climate-change induced drought scenarios, future bean servings by 2050 will likely have lower nutritional quality, posing challenges for ongoing climate-proofing of bean production for yields, nutritional quality, human health, and food security.

## INTRODUCTION

Dietary deficiencies of micronutrients such as iron and zinc constitute major public health problems globally, particularly amongst women and children in sub-Saharan Africa [1]. While micronutrient supplementation and food fortification are important for improving delivery of micronutrients, staple food crop biofortification through breeding provides an additional route for increasing the supply of key micronutrients (iron, zinc, vitamin A) from staple crops to the diets of poorer communities in developing countries [2-5]. The level of micronutrients (e.g. iron, zinc) in staple crops and foods is one of the key determinants of the extent of uptake of dietary micronutrients [6, 7]. However, the presence and levels of anti-nutritionals, in particular phytic acid and polyphenols, can inhibit bio-availability and hence the level of uptake of such micronutrients [8-15]. The consideration of anti-nutritionals in biofortification breeding programs is important to ensure that efforts to increase the levels of micronutrients (e.g. iron) in crops are not compromised by inadvertent increases in levels of anti-nutritionals (such as phytic acid and/ or polyphenols) that could arise from breeding efforts [8, 9, 12, 14, 16] or from environmental stresses. A meta-analysis of 143 studies showed a negative impact of predicted mid-century elevated $CO_2$ levels on iron and zinc levels of C3 grain and legume crop plants [17], which is anticipated to aggravate the extent of iron deficiency in human diets globally [18]. The mechanism(s) responsible for this decline in iron and zinc linked to increased $CO_2$ are not yet fully clear. Carbohydrate dilution is one possible explanation where $CO_2$ -stimulated carbohydrate production dilutes other grain components (thus minerals), but this could not explain differences in decreases between minerals and other significant mechanisms must also play a role [19]. In addition to increased $CO_2$ levels, reduced and erratic rainfall will lead to increases in the incidence and frequency of drought in some regions, which in turn will lead to reductions in crop yields [20]. For common bean, it is not known whether drought-associated reductions in crop yields will also lead to changes in the nutritional quality of beans under future climate change induced drought scenarios [21].

In this study, we have used Ecocrop to model the impact of climate change induced changes in heat and precipitation by 2050, on the suitability for cultivation of common bean across a range of countries in south-eastern Africa. We hypothesize that nutronal quality (for this study iron, zinc and protein) of beans will decrease under drought conditions compared to the same varieties grown under rainfed conditions. In addition, we have combined the climate impact modelling with experimental field trials of common bean, under the extent of drought anticipated due to future climate change (by 2050), to determine the impact on both yield and the nutritional quality of common bean under climate-induced drought stress. Our results are important for efforts to climate proof cultivation of the staple crop common bean, so that varieties can be developed, which under drought stress maintain good yields and contain high levels of the dietary micronutrients iron and zinc, while containing low levels of anti-nutritional factors such as lead and phytic acid.

## MATERIALS AND METHODS

### EcoCrop modelling of drought and heat impacts on common bean in East Africa

For a more detailed description of the EcoCrop model the reader is referred to Ramirez-Villegas *et al*. [22], and Hijmans *et al*. [23]. For previous assessments climate change impacts using EcoCrop for various crops (including common beans) in Africa the reader is referred to Rippke et al. [24] and Jarvis et al. [25]. EcoCrop suitability simulations for common bean were performed for a historical period (1960–1990, chosen to be a representative baseline) and then for the 2050 period (2040–2069) under the Representative Concentrations Pathway 6.0 (RCP 6.0) [26]. RCP 6.0 was chosen since it is representative of a business as usual scenario. Climate data used for historical suitability simulations was derived from WorldClim [27], which is a global high resolution database of monthly climatological means for mean, maximum and minimum temperatures and total precipitation. For each future period, simulations were performed using 19 Global Climate Model (GCM) projections, statistically downscaled and bias-corrected [28]. For both historical and future simulations, we assume that the common bean crop is not viable when the overall suitability is below 43% [24]. The EcoCrop model numerically assesses the environmental conditions (based on a parameter set defining the optimal and marginal temperatures and rainfall at which the crop can grow is defined, ideally retrieved via statistical data analysis) related with agricultural yields to determine a potential climatic suitability rating [22]. EcoCrop is a relatively simple crop suitability model and as such is subject to various limitations, most notably, it does not include estimates of extreme climate impacts, soil fertility, pest and diseases, all of which can be critical to bean growth [29, 30]. It also does not provide information on crop productivity. Whilst the exclusion of these limitations likely means we underestimate climate change-related challenges for bean production, we argue that they are useful to identify key adaptation priorities for the region.

## Plant material

The common bean (*Phaseolus vulgaris*) varieties used in this experiment included released varieties in Malawi, a landrace and also a range of unreleased advanced lines from the CIAT's bean biofortification breeding program. This consisted of eight BC1F4 Andean Nutrition (NUA) bean lines (NUA 743, NUA 720, NUA 674, NUA 730, NUA 746, NUA 705, NUA 706 and NUA 740) and nine released varieties (A268, A197, CAL 143, A344, NUA 45, SUGAR 131, CAL 113, VTTT 294/4–4, NUA 59, UBR (92)25 and DRK 57) and one landrace (Nasaka). These varieties were tested in earlier seasons for suitability under the Malawian environmental conditions.

## Field trials

All twenty bean varieties were field trialled under both rainfed conditions and drought-stress conditions that are representative of conditions predicted by Ecocrop modeling to occur by 2050. The drought and rainfed trials of all bean varieties were conducted between 2013–2015 at the Kandiyani site of the International Center for Tropical Agriculture (CIAT) Chitedze Research Station, Lilongwe, Malawi. The crop trial was laid out in a Randomized Complete Block Design (RCBD) with three plots/replicates. Two seasons per year the beans were harvested, in the rainy season, plants were grown under natural, rainfed conditions between December and March. The drought trial was conducted in the winter/off -season which lasts from late August until November.

For each plot, four ridges were prepared, measuring 5 m long and spaced at 0.60 m. The net plot comprised of the two middle ridges with the outer ridges acting as borders. One seed was planted per planting station spaced at 0.50 cm. No fertilizer was applied in the trials. Pests and diseases were controlled using Dimethoate (Aryzta LifeScience, South Africa) and Karate (Syngenta, Basel, Switzerland) according to product label recommendations. Agronomic data collected from each plot consisted of; days to 50% flowering; days to 95% physiological maturity; plant height at harvesting; grain weight; total plot yield. Yield was determined by weight of grains

## Sampling of bean pods and grains for nutritional analysis and sample preparation

Bean samples from four harvests in the period 2013–2015 were analysed. Bean pods were sampled when mature, from every plot 10 plants were randomly selected and of each of these plants 10 pods were taken (on different sides of the plant and not touching the soil). All procedures were followed according to HarvestPlus protocols for crop sampling to ensure samples were representative and minimize any risk of sample contamination [31]. After harvest, all samples were kept in a storage room in CIAT, Malawi at room temperature until transfer to the National University of Ireland Galway (NUI Galway). On arrival in NUI Galway, beans were removed from pods and stored at −20 °C in plastic sample bags until further processing. Bean samples were then freeze-dried and a subsample of 20 beans (i.e. grains) were milled to a fine powder using a coffee grinder (Delonghi, KG49). Samples were vacuum-packed until ICP-MS analysis in Ionomics Lab, University of Aberdeen, Aberdeen, Scotland.

### ICP-MS analysis of elemental composition of common bean grains

Briefly, bean powder of each sample was analysed in duplicate with inductively coupled plasma-mass spectrometry (ICP-MS) as follows: trace metal grade nitric acid was added to the samples. Samples were then spiked with an internal standard plus hydrogen peroxide and left overnight to pre-digest. Following exposure to 115 °C for 4 hours, digested samples were diluted with Milli-Q water. Aliquots were transferred to a 96-well plate for analysis. A detailed description of ICP-MS analysis is provided in the Supplementary Information (Appendix 2).

### Protein extraction and measurement

Bean proteins were extracted in three separate stages[32, 33], namely aqueous extraction (albumins) followed by saline extraction (globulins), followed by cell-lysis extraction (other, membrane-bound proteins). Microcentrifuge tubes (2 ml) were prepared with ten 1 mm glass beads (Sigma-Aldrich) inside. For aqueous extraction bean powder was suspended in distilled water (50 mg in 400 µl; 1:8), shaken in a tissue lyser (QIAGEN) at maximum speed for 30 seconds, followed by incubation at 4 °C for 30 min (samples were shaken every 10 minutes). The sample was centrifuged at 14000 rpm for 15 min. The supernatant was transferred to new tubes. Next, the water-insoluble fraction was extracted from the sediment. The sample was treated as before, but 0.5 M NaCl solution was used instead of distilled water. The supernatant was transferred to new tubes. This should result in 75% of total protein extraction. To release all membrane-bound proteins from the sediment, the sample was treated as before but Lysis buffer (50 mM citric acid, pH 3.0, 1 M NaCl, 2% SDS, 0.5% Triton-X- 100) was used instead of NaCl solution or distilled water. The supernatant was transferred to new tubes; this is expected to result in ≥91% of total protein extraction. Samples were measured using the BCA protein assay (Thermo Fisher Scientific) on 96-well plates in triplicate. Readings were taken at 560 nm wavelength (Modulus Microplate Mulitmode Reader, Turner Biosystems). The three fractions were measured separately, and total protein content is the sum of all three fractions.

## Phytic acid analysis of common bean grains

Samples for phytic acid analysis were prepared using modified protocols of Harland *et al*. [34] and Ellis *et al*. [35]. We used the same milled samples as those for ICP-MS. In brief, 50 mg aliquots of powder were thoroughly mixed with 2 ml 2.4% HCl, incubated at room temperature (RT) for 1 hour followed by 3 min centrifugation at 13000 rpm (Thermo Scientific, Fresco17). 1.8 ml of supernatant was transferred into new 14 ml tubes and diluted with 8.2 ml Milli-Q water. To remove the inorganic phosphate, 10 ml diluted samples were applied to the 10 ml Poly-Prep Chromatography Columns (#7311550, Bio-Rad), pre-packed with 0.3 g of AG1-X4 resin, 100–200 mesh (AG 1-X4 Resin #1401341, Bio-Rad) and equilibrated with 0.7 M NaCl, followed by elution with 10 ml 0.1 M NaCl. Phytate was eluted from the columns with 0.7 M NaCl into 15 ml Falcon tubes. Each sample was analysed in triplicate to measure phytate with WADE reagent (0.03% FeCl3*6H2O and 0.3% sulfosalicylic acid in Milli-Q water, according to Latta *et al*. [36].

## Data preparation and statistical analysis

All field trial data points, yield data and nutrient data were organized on Microsoft Excel (Microsoft, WA, USA) and all statistical analysis was performed in SPSS Statistics (version 21) (IBM, NY, USA).

## RESULTS

The majority of current common bean growing areas in southeastern Africa will become unsuitable for bean cultivation by the year 2050 due to changes in temperature and precipitation.

To determine climate change impacts on common bean (Phaseolus vulgaris) suitability for cultivation in southeastern Africa, the EcoCrop model was used to produce spatially-explicit simulations of potential climatic suitability for five countries in south-eastern Africa. For each spatial unit (i.e. grid cell), EcoCrop performs separate calculations for temperature-limited (heat and cold) and precipitation-limited (waterlogging and drought) suitability, and then calculates an overall suitability for the crop [22, 23].

Figure 4 shows the spatial distribution of historical climatic suitability for common bean cultivation (Fig. 4A), as well as the spatial distribution of projected climate change impacts on bean cultivation by 2050 (Fig. 4B). The unshaded areas within the countries analysed (Malawi, Mozambique, Tanzania, Zambia and Zimbabwe) are considered unsuitable for bean cultivation (Fig. 4A). Currently, suitable areas for bean cultivation extend across most of Zambia, Zimbabwe, Tanzania, western Malawi, and northern Mozambique (Fig. 4A). Our simulated historical climatic suitability for common bean cultivation agrees well with the observed distribution of bean cultivation in Africa [37]. Our EcoCrop modelling of future climate impacts on bean cultivation suitability indicates that a significant proportion of the currently suitable areas will become unsuitable for common bean cultivation by 2050 (red areas in Fig. 4B), particularly in southern Zambia, eastern Zimbabwe, and central Tanzania. In these areas, unless appropriate climate adaptation actions (e.g. climate smart agriculture (CSA) breeding and agronomy options such as new varieties or irrigation) are put in place, it will no longer be possible to grow common beans.

### Common bean cultivation suitability differs across locations within each country dependent on changes in temperature or precipitation.

Our results further indicate that a reduction in the temperature-related suitability resulting from increased heat stress by 2050 is predicted as the main cause ( T<P) for the overall reduction in climatic suitability of bean growing areas in north western Tanzania, southern Zambia, and western Zimbabwe (dark red shading in Fig. 4C). Conversely, reductions in precipitation-related suitability by 2050 were found to be the major cause (P<T) of climatic suitability change reductions in west-ern Malawi, northern Zambia, eastern Tanzania, and southern Zimbabwe (orange shading in Fig. 4C).

Figure 4D demonstrates where future bean cultivation suitability by 2050 will be predominantly limited by temperature (red), precipitation (blue) or both temperature and precipitation (yellow).

### Reductions in yields of common bean varieties at a climate analogue field site that is representative of predicted drought conditions by 2050.

To determine the impacts of future drought scenarios by 2050 on the yields of common bean, we conducted a field trial in western Malawi of 20 common bean varieties over two growing seasons. The bean varieties used consisted of varieties commonly cultivated by smallholder farmers and also bean lines developed by the CIAT and PABRA bean breeding programs under consideration for entry into the national varietal registration and official release process. They comprise a range of different market classes (i.e. quality types) including black, red, kidney, mottled red, brown, light brown and white beans, both smaller and larger sized seed varieties. These varieties were chosen to reflect the reality of (a) what bean varieties are actually available to smallholder farmers in Malawi and (b) what bean varieties are in the regulatory pipeline in Malawi that may become accessible to smallholders within the next decade, taking account of the time lag expected for national varietal registration, certification processes and bulking up of seed by suppliers. The suitability of the field trial site area in western Malawi for common bean cultivation is projected to fall below current suitability levels by 2050, primarily due to decreased precipitation (Fig. 4B, C).

All twenty bean varieties were field trialled under both rainfed conditions and drought-stress conditions that are representative of conditions predicted by EcoCrop modelling to occur by 2050 (Fig. S1). is corresponded to four field trial seasons in total, i.e. two trials under rainfed conditions and two under drought-stress conditions. To ensure seasons could be reliably and reproducibly grouped according to "rainfed" and "drought-stress" conditions, *k*-means cluster analysis was performed on recorded weather data for the field trial site. The identified clusters were analysed using discriminant analysis for the goodness of fit of the model, and t-statistics were applied to verify the predicted *vs*. found mean of the clusters.

In addition, to further verify the climate change relevance of our drought-stress growing seasons, Simulated Weather Data for Crop Modelling and Risk Assessment (MarkSim) software V.2 was employed (http://www.ccafs-climate.org/pattern _scaling/). These analyses confirmed that our drought-stress field trial conditions can be considered as a climate analogue site for bean growing

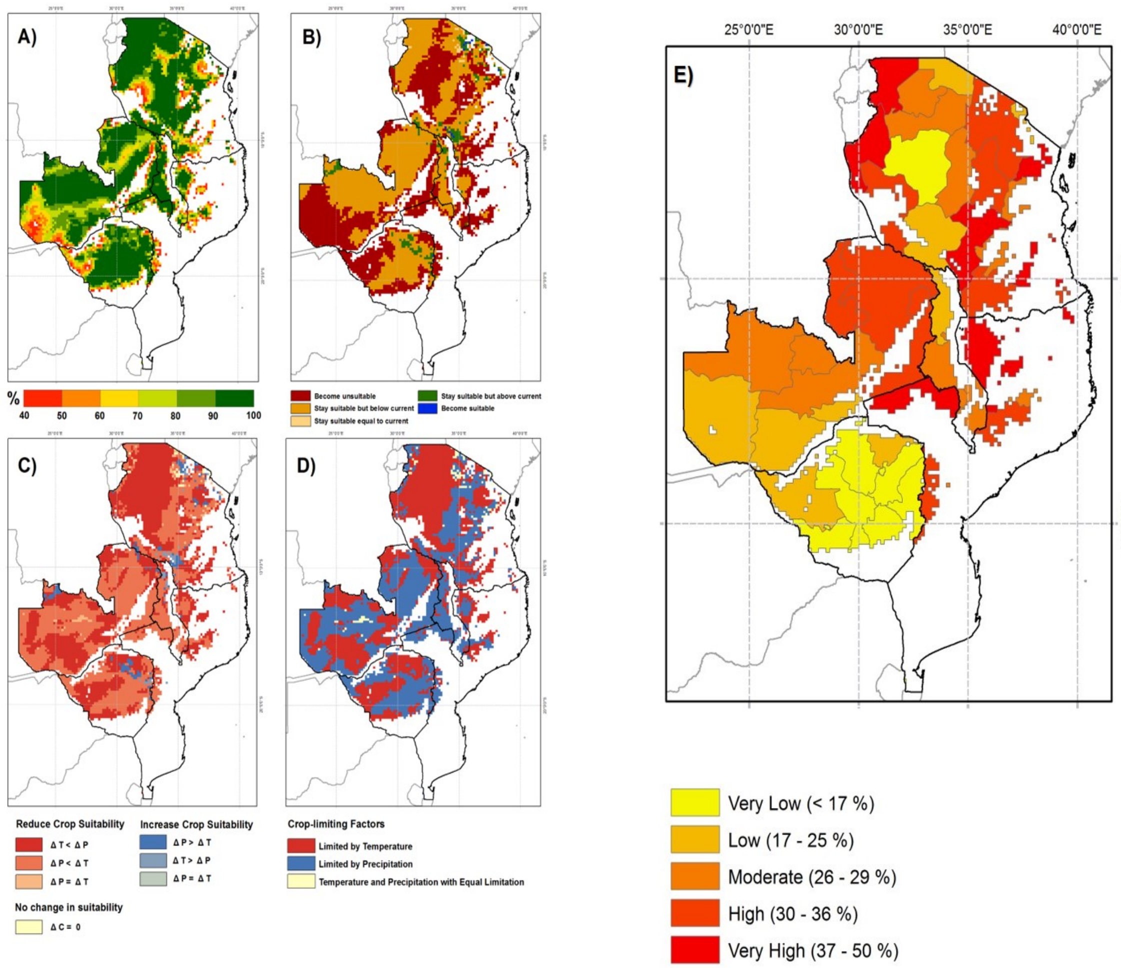



**Figure 4.** Historical and future (2050) common bean suitability simulations for south-east africa and current percentage of children underweight**; (a)** suitability of currently cultivated common bean for historical climate; **(b)** projected impact of climate change by 2050s; **(c)** driving factor of change in future climatic suitability; **(d)** factor most limiting to bean cultivation suitability by 2050; **(e)** percentage of children, under the age of 5, who are underweight (defined as weight for height <-2 standard deviations of the WHO Child growth standards) (data from ciesin), for the period 1990–2002. Red (reduced suitability), blue (increased suitability), and beige (no change in suitability) colours are used in (c) to separate directions of change. In red areas, shades of red are used to differentiate areas where suitability reductions are due to temperature changes ($\Delta t<\Delta p$), from those where suitability reductions are due to precipitation changes ($\Delta p<\Delta t$) or where temperature- and precipitation- suitability reductions are equal ($\Delta p=\Delta t$). In blue areas, shades of blue are used to differentiate areas where suitability increases are primarily driven by precipitation ($\Delta p>\Delta t$), temperature ($\Delta t>\Delta p$), or equally driven by both ($\Delta p=\Delta t$).

seasonal weather conditions in the year 2095 in Malawi, under RCP 8.5. Average temperatures during our drought field trials were 22 °C with extremes of 35 °C, closely resembling the predicted average temperatures for the bean growing season (March, April and May) of 24 °C and extremes of 32 °C in the year 2095. Likewise, the average rainfall during the bean grain filling period in 2015 was 54 mm, similar to the projected average rainfall of 58 mm (Fig. S2).

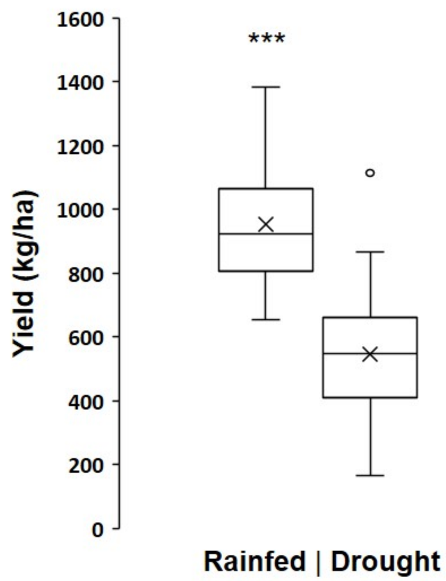



**Figure 5.** Box and whisker plot showing average grain yield of 20 common bean varieties grown under rainfed and drought-stress conditions. 'X' indicates mean value. *** P < 0.001

To determine whether the reduction in yield of the common bean varieties under drought-stress conditions at the climate analogue trial site was more influenced by genotype (variety) or weather conditions (temperature, precipitation), an F-test was performed. This revealed that the variation in yield observed at the trial site is influenced more by weather conditions than genotype. To determine whether there are any statistically significant differences between the varieties under drought stress conditions, a one-way ANOVA was performed, which showed no significant yield difference between the bean genotypes. Similarly, no significant yield differences were identified between the bean genotypes under rainfed conditions. Overall, for all bean varieties that were field trialled at the Malawi climate analogue site, the common bean grain yield decreased by an average of 43% under drought- stress conditions, which is significantly lower (two-tailed independent samples t-test, P <0.001) when compared with rainfed conditions (Fig. 5).

## While iron levels in bean grains decrease, under climate-scenario relevant drought stress conditions, zinc, lead, protein and phytic acid levels increase.

Many of the countries analysed in this study have moderate to high child underweight rates (Fig. 4E ) [38]. Micronutrient deficiencies are major contributors to the global problem of maternal and child malnutrition, which causes underweight, stunting and wasting conditions in afflicted children[1, 39].

To determine the changes in levels of dietary micronutrients between rainfed and drought-stress conditions of each common bean variety, we used inductively coupled plasma-mass spectrometry (ICP-MS) to measure the relative concentrations of twenty-two elements (B, Na, Mg, P, S, K, Ca, Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo, Cd and Pb), which included the important dietary micronutrients iron and zinc, and also antinutritional compounds such as lead (Table S1; Fig. S3). For each common bean variety under rainfed and drought conditions, we also determined the protein levels and the levels of phytic acid, a major antinutritional limiting micronutrient uptake from human diets [40] (Table S1; Fig. S3).

As seen for yield, the variations in iron, zinc, lead, protein and phytic acid under drought-stress conditions at the field site are more influenced by weather conditions than genotype (variety). To further investigate the lack of variation among genotypes for iron, zinc, lead, protein and phytic acid, a one-way ANOVA was performed. When we compared across genotypes under rainfed conditions we found no significant difference between genotypes for iron, zinc, lead, protein and phytic acid content. Similarly, when we compared across genotypes under drought conditions, we found no significant difference between genotypes for iron, lead, protein and phytic acid content. Under drought-stress conditions, one variety shows significantly higher ($P < 0.05$) zinc levels than the other nineteen varieties, but at the 5% significance level such a result would be expected by chance.

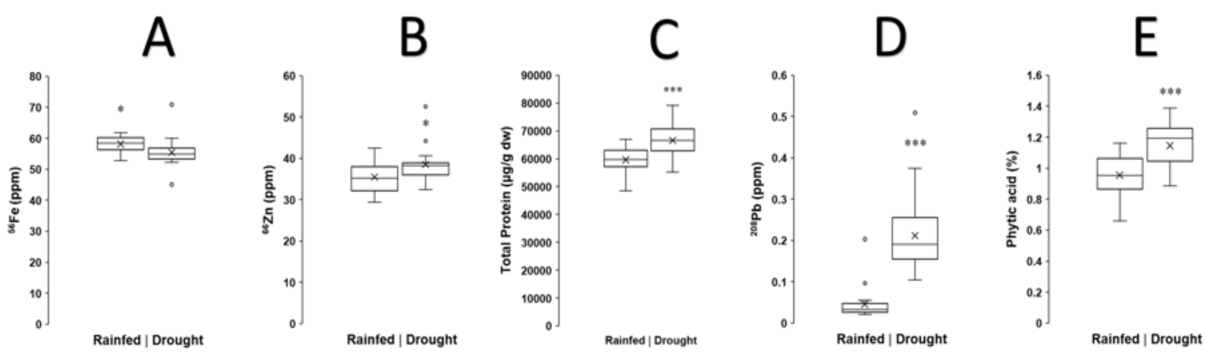



**Figure 6.** Box and whisker plot showing average grain iron (**A**), zinc (**B**), protein (**C**), lead (**D**), and phytic acid (**E**) levels of 20 common bean varieties grown under rainfed and drought-stress conditions. 'X' Indicates mean value. $*P < 0.05$ $***P < 0.001$. Results are based on dry weight.

We also compared our results for concentrations of Zn and Fe for the twenty varieties tested under rainfed or drought conditions with those recorded for the 1000 accessions in the CIAT cultivated common bean core collection (Fig. S4) and find them to be representative of the range of Fe levels, and representative (or slightly enriched) for Zn. The responses of Fe and Zn in our tested varieties are therefore likely to be similar to those of other common bean genotypes.

Overall, across all of the twenty bean varieties analyzed, the levels of iron are significantly reduced under drought stress (two-tailed independent samples t-test, $P < 0.05$), from an average concentration of 59 ppm in beans from rainfed plants to 54 ppm from drought-stressed plants (Fig. 6A). Conversely, the average concentration of zinc significantly increases under drought stress (two-tailed independent samples t -test, $P <0.05$), from 35 ppm in beans from rainfed plants to 39 ppm from drought-stressed plants (Fig. 6B). We identify a significant increase (two-tailed independent samples t-test, $P <0.001$) in lead levels in the bean grains under drought-stress conditions, from an average concentration of 0.05 ppm to 0.22 ppm, representing a fourfold increase (Fig. 6C). is exceeds the maximum level permissible (0.1 ppm) for pulses as defined by the Codex Alimentarius [41]. The average total protein concentration also significantly increases under drought stress (two-tailed independent samples t-test, $P <0.001$) from 326 mg/g of dry weight in beans from rainfed plants to 371 mg/g of dry weight in beans from drought-stressed plants (Fig. 6D). Similarly, there was a significant increase (two-tailed independent samples t-test, $P <0.001$) in phytic acid levels under drought stress, from an average level of 0.96% under rainfed conditions to 1.16% under drought stress (Fig. 6E).

**Changes in precipitation and temperature correlate with changes in yield of common bean, and also with iron, lead and protein levels, but not zinc and phytic acid levels.**

To further investigate the link between weather conditions during the grain filling period and common bean growth, multiple linear regression analysis was performed. As the differences in rainfall between the rainfed seasons and the drought-stress seasons were also accompanied by differences in air temperature (Fig. S1), the impact of temperature was included as well as rainfall. Over 50% of the variation in yield and iron could be explained by changes in temperature and rainfall between lowering date and harvest. Approx. 40% of the variation in lead could be explained by changes in temperature that occurred between flowering date and harvest, while rainfall that occurred during this time window did not improve the model.

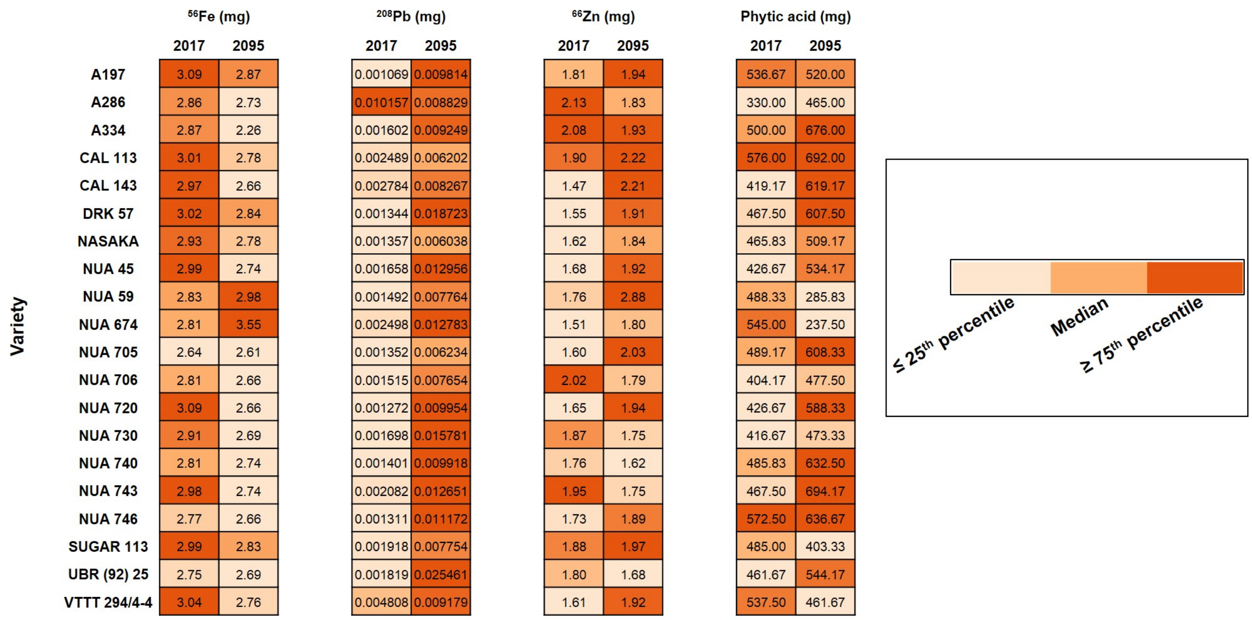



**Figure 7.** Heat map of nutritional quantity from one serving of beans harvested from present-day (2017) and predicted future (2095) conditions. Concentration of nutrients (56Fe, 66Zn, total protein) and anti-nutritionals (208Pb, phytic acid) in a 50 g serving (dry beans) of 20 common bean varieties under rainfed and drought conditions was calculated. Median, lower and upper quartile values were calculated for 56Fe, 66Zn, protein, 208Pb and phytic acid separately.

Approx. 30% of the variation in protein levels could be explained by changes in temperature and rainfall between flowering date and harvest. Finally, weather conditions during the grain filling period only explained a small part of the variation in zinc (4.0%) or phytic acid (17%), suggesting that other factors not included in the model influence zinc and phytic acid levels in the grain.

## Under climate-change induced drought scenarios, future bean servings will have lower nutritional quality.

While yield is typically measured in kg/hectare, the nutritional yield can be considered as the quantity of supply of nutritionally-important compounds per unit area [42]. To determine the supply of nutritional and anti-nutritional compounds on a per meal basis, we calculated the quantity of each dietary compound that each common bean variety would deliver per serving (50 g dry raw beans) under present day (rainfed) and future climate (drought-stress) scenarios (Fig. 7). This highlights that while future bean servings under climate change may become more zinc-rich, they will contain less iron and more undesirable anti-nutritionals (lead and phytic acid). Our results indicate that some varieties (NUA 59, NUA 674) may display promise for drought proofing dietary supplies from beans under predicted future climate-change scenarios.

## DISCUSSION

Climate change represents a threat to food security, particularly resulting from ongoing and anticipated negative impacts on agricultural productivity (yields/hectare) [43-47]. While there have been a range of studies which indicate negative impacts on yields of major staple crops [48-50], there have been fewer studies that have investigated the impact of climate change stresses (e.g. rising $CO_2$, heat, drought) on staple crop grain quality parameters [17, 51-55].

Common beans are the most important grain legume supporting food security and human nutrition globally, responsible for almost 15% of daily calories and 36% of daily protein in some countries in Africa and the Americas [56]. In sub-Saharan Africa, common beans are an important staple crop for smallholder farmers and a key nutritional component in diets of poor rural communities. Smallholder farmers in sub-Saharan Africa plant a wide range of bean varieties and landraces that they access from multiple sources, including via purchasing from formal (e.g. government distributors, commercial seed companies, agro-dealers, NGO/UN) or informal (e.g. local markets, own seed stocks, neighbour) sources [57-59]. Such smallholder farming communities are extremely vulnerable to negative impacts of climate change on their livelihoods and nutritional status, including through reductions in yields and/or nutritional quality of staple crops they both consume and trade [60, 61].

Climate change can change weather patterns, resulting in altered temperature and rainfall effects in different regions, which can have concomitant impacts on the suitability of crops for continued cultivation in climate-change impacted regions [62]. In particular, elevated temperatures (heat) and reduced rainfall (drought) can reduce crop yields [44, 63-65]. Our EcoCrop climatic suitability analyses for common bean in South Eastern Africa to 2050 indicates that predicted increases in temperature and reductions in rainfall (precipitation) will result in common bean becoming unsuitable for cultivation across the vast majority of current bean growing regions (Fig. 4D). Only in specific localised zones in northern Zimbabwe, southern and northern Tanzania, and northern Malawi, are increases in climatic suitability for bean cultivation projected. Relocation of bean cultivation to different areas beyond the current range of cultivation may be possible, but in this case careful consideration should be given to choosing varieties suited for the new area, including any change in photoperiod that occurs with changing latitude. Overall, our findings are consistent with those of previous studies where different models to EcoCrop have been used [66-68]. Future climate conditions will be associated with elevated atmospheric $CO_2$ concentrations, which may either exacerbate or alleviate the effects of increased temperature and reduced precipitation on common bean growing regions. While the EcoCrop model cannot process atmospheric $CO_2$ effects, it is noteworthy that C3 plants (of which common bean is one) have been shown experimentally to respond to drought by reducing photosynthesis, an effect which is not removed upon doubling $CO_2$ treatment[69]. We conclude that, in the absence of implementation of significant adaptation strategies to maintain yields of common bean in south-eastern Africa, it can be expected that yields of common bean will dramatically decline across the region in the period to 2050.

The adaptation strategies that will be necessary to implement at scale may be incremental (e.g. breeding new bean varieties or using agronomic practices such as irrigation) or transformational (e.g. involving changing to a different protein or high-value crop species, or finding an alternative livelihood that is more climate-resilient) [70].

In addition to yield reductions that will negatively impact on livelihoods, there is potential for climate stresses to also impact on the nutritional quality of crops. Such climate effects may frustrate biofortification efforts to breed new biofortified varieties of staple crops that have elevated levels of essential micronutrients[6]. Screening of over 1000 genotypes of common bean germplasm from the CIAT [71] gene bank revealed an average Fe concentration of 55 ppm, within a concentration range of 34 and 89 ppm. The average Zn concentration was 35 ppm, within a concentration range of 21 and 54ppm. Notably, all 20 genotypes investigated in our study have similar Fe and Zn concentrations. The average Fe concentration among genotypes used in our study (i.e. 59ppm) is statistically similar to the average observed across the primary genepool (i.e. 55 ppm). Likewise the average Zn concentration among the genotypes in our study (i.e. 35 ppm) is identical to the average across the genepool (i.e.35 ppm) ( Fig. S4, Supplementary Results File).

While there has been a previous attempt to determine the effect of water-limiting conditions on Fe and Zn levels in common bean, using a limited number of genotypes under irrigated conditions, further studies are required to determine impacts on nutritional availability under drought in key bean growing regions [56-59]. In our study we have modeled the negative impacts of climate change on common bean production in south-eastern Africa, which has revealed that reduced precipitation by 2050 will be the main limiting factor for bean cultivation. Furthermore, we conducted a multi-year field trial at a climate analogue site which experiences weather conditions similar to that predicted for Malawi in the year 2095. Our results are the first to demonstrate that the level of a key nutrient (i.e. iron) in common beans under climate change induced drought stress will significantly decline.

Studies conducted to date on iron bioavailability from high-Fe biofortified beans using Caco-2 cell models are congruent with findings from poultry models [13, 72], which in turn are consistent with human feeding trials which have shown positive nutritional impacts from consumption of high-Fe biofortified beans [8, 40, 73-75]. However, antinutritional compounds in staple crop plants can negatively affect the uptake (bioavailability) of nutritional compounds (e.g. iron, zinc) [76]. Such antinutritional compounds include phytic acid and polyphenols, which have been shown to negatively affect the bioavailability and uptake of iron and zinc from common beans, using *in vitro* (Caco-2 cell) and animal (poultry) models [8, 9, 12, 13, 16, 40, 72]. In addition, the composition of targeted diets or meal plans can affect the extent of iron uptake from high-Fe biofortified beans. For instance, some foods commonly consumed with beans (e.g. rice) can inhibit Fe bioavailability while others (e.g. potato) can increase Fe bioavailability when eaten with beans[8].

In this study we have focused on the effect of phytic acid because of its major influence on iron bioavailability, especially in the case of consuming beans in a composite meal [40]. However, we recognise that polyphenols are an additional class of anti-nutritionals that need to be considered in high-Fe bean biofortification efforts. For instance, studies in black beans have shown that total polyphenols inhibit iron uptake in Caco-2 cell assays [9]. The overall inhibitory effect of polyphenols is combinatorial, whereby some polyphenols (catechin, 3,4-dihydroxybenzoic acid, kaempferol, and kaempferol 3-glucoside) promote iron uptake while others (myricetin, myricetin 3-glucoside, quercetin, and quercetin 3-glucoside) inhibit iron uptake [9]. Because of differential potency effects between polyphenols that inhibit or promote iron uptake, it is considered that the majority of the inhibitory polyphenol compounds would need to be removed from biofortified beans in order to substantially reduce the inhibitory effect on iron uptake[16].

There are also a number of heavy metals (e.g. lead, cadmium, arsenic), which can enter the human body via diet, that can act as toxic anti-nutritionals (depending on concentration) [77]. In addition to drought-induced reductions in the levels of iron, our results demonstrate that drought-stressed common bean varieties also display increases in the levels of the antinutritional compounds phytic acid and lead. While we did not measure the levels of polyphenols, polyphenol levels can increase in plants in response to drought-stress [78, 79], and have been shown to negatively affect iron and zinc bioavailability and uptake from dietary common bean [9, 12].

The underlying physiological basis for the increases of antinutritional compounds such as phytic acid and lead under drought stress are unclear. In tropical soils, such as laterites, it has been shown that sorption values for $Pb^{2+}$ are greater than those of other bivalent cations [80]. The development of deeper rooting systems in arid soils could potentially lead to greater contact with $Pb^{2+}$ cations adsorbed within the soil. Alternatively, the observed increase could be a secondary effect of greater investment in active cation uptake when the soluble fraction of nutrient cations is insufficient to meet plant needs, reminiscent of the increased uptake of $Al^{3+}$ cations that can be observed in calcicole plants under acidic conditions. However, we cannot exclude other impacts of drought and heat on root physiology [81], and direct analysis of root growth in common bean under different climatic conditions will be required to distinguish these possibilities. This underlying physiological basis for an increase in phytic acid and lead should be further investigated through closer analysis of the (physiology of) root systems and seed of the plant and the exchange/uptake of minerals in the plants under different (drought stress) conditions.

The increases in phytic acid are of particular concern as it is considered the main anti-nutritional compound in legumes. The increased phytic acid accumulation likely relates to its function in limiting oxidative stress in legumes under dryer conditions [82, 83]. Indeed, phytic acid is known to accumulate in legume seeds (e.g. chickpeas) in response to drought stress [84, 85]. It should be noted however that phytic acid in field peas has been found to be reduced under elevated $CO_2$ levels , but the mechanism that could explain these findings is not yet clear [17]. If a similar response occurs in common beans then the increase in phytic acid levels we observe could be counteracted. Growth trials combining changes in climate and $CO_2$ simultaneously will be needed to assess interactions between possibly competing effects.

Our study indicates that ongoing efforts to develop biofortified bean varieties will need to not only develop heat- and drought-tolerant beans, but will also need to ensure that such varieties also maintain elevated iron and zinc levels, and low levels of antinutritional compounds (e.g. phytic acid, lead and specific inhibitory polyphenols) under drought or other environmental stresses. To avoid unintended consequences, our results highlight that it is critically important that biofortified crop varieties (including under abiotic stresses) do not accumulate anti-nutritionals (e.g. phytic acid, lead, arsenic, polyphenols)[86]. Overall, our results demonstrate that there will be a reduction in the nutritional quality of a typical bean serving, if the common bean varieties have been cultivated under the levels of drought stress predicted for south-eastern Africa to 2050 and beyond.

## CONCLUSIONS

Both incremental and transformational climate change adaptation[2, 24, 67, 70] strategies are needed for common bean cropping systems of smallholder farmers in south-eastern Africa, whereby such farmers can have greater access to improved varieties and agronomic practices that allows their cropping systems to be more resilient to increased heat and drought conditions, while maintaining or improving nutritional composition of bean grains. Recent plant breeding progress to develop drought- and heat-adapted bean varieties indicates that genetics-based adaptation should be possible[87], which can be a component of an overall portfolio of climate smart agriculture technologies and practices to ensure resilient of common bean cultivation to climate change impacts [88]. Where farmers have access to (and widely adopt) such improved bean varieties [58], it may be possible to maintain yields in areas where cultivation suitability will be negatively impacted by climate change [24]. As the breeding, testing and dissemination of new bean varieties can take a decade or more, our results highlight the need for accelerated development and seed-system dissemination of heat- and drought-tolerant common bean varieties that can maintain yields while also improving nutritional quality (e.g. via biofortification breeding) under future climate change scenarios.

## ACKNOWLEDGEMENTS

We would like to thank John Danku and David E. Salt for performing the ICP-MS analysis. We are thankful to Alberto Fabio Guerrero and Steve Beebe for access to data on Fe and Zn content levels in the CIAT common bean core collection. This research was supported by the CGIAR Climate Change, Agriculture and Food Security (CCAFS) program. C.S. and G.B. acknowledge support from Science Foundation Ireland (SFI) Grant No. 13/IA/1820. C.S., B.H. and M.H. acknowledge funding support from the Irish Research Council (Grants No. GOIPG/2014/1021 and the CropBioFort project funded by the Irish Research Council's New Foundations program). The authors thank Camilo Barrios Perez from CIAT for providing support to the EcoCrop modelling. This work was implemented as part of the CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS), which is carried out with support from the CGIAR Trust Fund and through bilateral funding agreements, including Irish Aid. For details please visit https://ccafs.cgiar.org/donors. The views expressed in this document cannot be taken to reflect the official opinions of these organizations. The authors are also thankful to three reviewers for their useful comments and suggestions on an earlier version of the paper.

## SUPPORTING INFORMATION

**Supplementary Figures** can be found in Appendix 1

# REFERENCES

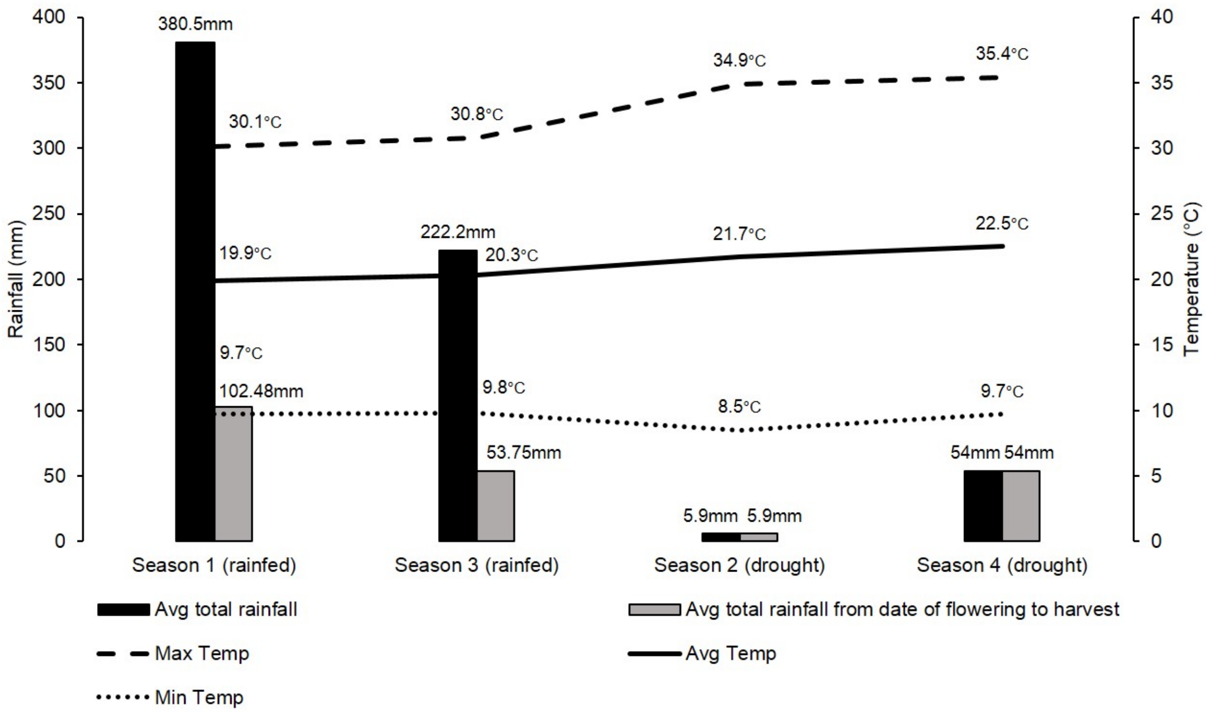

1. Bailey RL, West Jr KP, Black RE. The epidemiology of global micronutrient deficiencies. Annals of Nutrition and Metabolism. 2015;66(Suppl. 2):22-33.
2. Saltzman A, Birol E, Bouis HE, Boy E, De Moura FF, Islam Y, et al. Biofortification: progress toward a more nourishing future. Global Food Security. 2013;2(1):9-17.
3. Bouis HE, Hotz C, McClafferty B, Meenakshi J, Pfeiffer WH. Biofortification: a new tool to reduce micronutrient malnutrition. Food and nutrition bulletin. 2011;32(1_suppl1):S31-S40.
4. Imdad A, Herzer K, Mayo-Wilson E, Yakoob MY, Bhutta ZA. Vitamin A supplementation for preventing morbidity and mortality in children from 6 months to 5 years of age. The Cochrane Library. 2010.
5. Anjali V. Food fortification: a complementary strategy for improving micronutrient malnutrition (MNM) status. Food Science Research Journal. 2015;6(2):381-9.
6. Díaz-Gómez J, Twyman RM, Zhu C, Farré G, Serrano JC, Portero-Otin M, et al. Biofortification of crops with nutrients: factors affecting utilization and storage. Current opinion in biotechnology. 2017;44:115-23.
7. Sanahuja G, Farré G, Berman J, Zorrilla-López U, Twyman RM, Capell T, et al. A question of balance: achieving appropriate nutrient levels in biofortified staple crops. Nutrition research reviews. 2013;26(2):235-45.
8. Glahn R, Tako E, Hart J, Haas J, Lung'aho M, Beebe S. Iron bioavailability studies of the first generation of iron-biofortified beans released in Rwanda. Nutrients. 2017;9(7):787.
9. Hart JJ, Tako E, Kochian LV, Glahn RP. Identification of black bean (Phaseolus vulgaris L.) polyphenols that inhibit and promote iron uptake by Caco-2 cells. Journal of agricultural and food chemistry. 2015;63(25):5950-6.
10. Petry N, Boy E, Wirth JP, Hurrell RF. Review: The potential of the common bean (Phaseolus vulgaris) as a vehicle for iron biofortification. Nutrients. 2015;7(2):1144-73.
11. Tako E, Bar H, Glahn RP. The combined application of the Caco-2 cell bioassay coupled with in vivo (Gallus gallus) feeding trial represents an effective approach to predicting Fe bioavailability in humans. Nutrients. 2016;8(11):732.
12. Tako E, Beebe SE, Reed S, Hart JJ, Glahn RP. Polyphenolic compounds appear to limit the nutritional benefit of biofortified higher iron black bean (Phaseolus vulgaris L.). Nutrition journal. 2014;13(1):28.
13. Tako E, Glahn RP. White beans provide more bioavailable iron than red beans: studies in poultry (Gallus gallus) and an in vitro digestion/Caco-2 model. International Journal for Vitamin and Nutrition Research. 2010;80(6):416.
14. Tako E, Reed S, Anandaraman A, Beebe SE, Hart JJ, Glahn RP. Studies of cream seeded carioca beans (Phaseolus vulgaris L.) from a Rwandan efficacy trial: in vitro and in vivo screening tools reflect human studies and predict beneficial results from iron biofortified beans. PloS one. 2015;10(9):e0138479.
15. Tako E, Reed SM, Budiman J, Hart JJ, Glahn RP. Higher iron pearl millet (Pennisetum glaucum L.) provides more absorbable iron that is limited by increased polyphenolic content. Nutrition journal. 2015;14(1):11.
16. Hart JJ, Tako E, Glahn RP. Characterization of polyphenol effects on inhibition and promotion of iron uptake by Caco-2 cells. Journal of agricultural and food chemistry. 2017;65(16):3285-94.
17. Myers SS, Zanobetti A, Kloog I, Huybers P, Leakey AD, Bloom AJ, et al. Increasing CO2 threatens human nutrition. Nature. 2014;510(7503):139-42.
18. Smith M, Golden C, Myers S. Anthropogenic carbon dioxide emissions may increase the risk of global iron deficiency. GeoHealth. 2017.
19. Gifford RM, Barrett DJ, Lutze JL. The effects of elevated [CO2] on the C: N and C: P mass ratios of plant tissues. Plant and Soil. 2000;224(1):1-14.
20. Long SP, Ort DR. More than taking the heat: crops and global change. Curr Opin Plant Biol. 2010;13(3):241-8.
21. Beebe S, Ramirez J, Jarvis A, Rao IM, Mosquera G, Bueno JM, et al. Genetic improvement of common beans and the challenges of climate change. Crop adaptation to climate change. 2011:356-69.

22. Ramirez-Villegas J, Jarvis A, Läderach P. Empirical approaches for assessing impacts of climate change on agriculture: The EcoCrop model and a case study with grain sorghum. Agricultural and Forest Meteorology. 2013;170:67-78.
23. Hijmans RJ, Guarino L, Cruz M, Rojas E. Computer tools for spatial analysis of plant genetic resources data: 1. DIVA-GIS. Plant Genetic Resources Newsletter. 2001:15-9.
24. Rippke U, Ramirez-Villegas J, Jarvis A, Vermeulen SJ, Parker L, Mer F, et al. Timescales of transformational climate change adaptation in sub-Saharan African agriculture. Nature Climate Change. 2016;6(6):605.
25. Jarvis A, Ramirez-Villegas J, Campo BVH, Navarro-Racines C. Is cassava the answer to African climate change adaptation? Tropical Plant Biology. 2012;5(1):9-29.
26. Moss RH, Edmonds JA, Hibbard KA, Manning MR, Rose SK, Van Vuuren DP, et al. The next generation of scenarios for climate change research and assessment. Nature. 2010;463(7282):747.
27. Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. Very high resolution interpolated climate surfaces for global land areas. International journal of climatology. 2005;25(15):1965-78.
28. Ramirez-Villegas J, Jarvis A. Downscaling global circulation model outputs: the delta method decision and policy analysis Working Paper No. 1. Policy Analysis. 2010;1:1-18.
29. Araújo SS, Beebe S, Crespi M, Delbreil B, González EM, Gruber V, et al. Abiotic stress responses in legumes: strategies used to cope with environmental challenges. Critical Reviews in Plant Sciences. 2015;34(1-3):237-80.
30. Prasad P, Boote KJ, Allen LH, Thomas JM. Effects of elevated temperature and carbon dioxide on seed-set and yield of kidney bean (Phaseolus vulgaris L.). Global Change Biology. 2002;8(8):710-21.
31. Stangoulis J, Sison C. Crop sampling protocols for micronutrient analysis. Harvest Plus Tech Monogr Ser. 2008;7.
32. Jimenez O, Delgado E, Rosales S, Reveles S, Ochoa A, Nava B, et al. Efficiency in seed protein extraction from common bean cultivars grown in Mexican Northern Highlands. Annual report. 2008.
33. Oliveira AP, Andrade GF, Mateó BSO, Naozuka J. Protein and Metalloprotein Distribution in Different Varieties of Beans (Phaseolus vulgaris L.): Effects of Cooking. International journal of food science. 2017;2017.
34. Harland B, Oberleas D. A modified method for phytate analysis using an ion-exchange procedure: application to textured vegetable proteins [Soybeans]. Cereal chemistry. 1977;54(4):827-32.
35. Ellis R, Morris E. Appropriate resin selection for rapid phytate analysis by ion-exchange chromatography. Cereal chemistry. 1986;63:58-9.
36. Latta M, Eskin M. A simple and rapid colorimetric method for phytate determination. Journal of Agricultural and Food Chemistry. 1980;28(6):1313-5.
37. You L, Wood S, Wood-Sichra U. Generating plausible crop distribution maps for Sub-Saharan Africa using a spatially disaggregated data fusion and optimization approach. Agricultural Systems. 2009;99(2):126-40.
38. Center for International Earth Science Information Network - CIESIN - Columbia University. Poverty Mapping Project: Global Subnational Prevalence of Child Malnutrition. Palisades, NY: NASA Socioeconomic Data and Applications Center (SEDAC); 2005.
39. Black RE, Allen LH, Bhutta ZA, Caulfield LE, De Onis M, Ezzati M, et al. Maternal and child undernutrition: global and regional exposures and health consequences. The lancet. 2008;371(9608):243-60.
40. Petry N, Egli I, Gahutu JB, Tugirimana PL, Boy E, Hurrell R. Phytic acid concentration influences iron bioavailability from biofortified beans in Rwandese women with low iron status. The Journal of nutrition. 2014;144(11):1681-7.
41. FAO. Codex Alimentarius. General Standards for Contaminants and Toxins in Food and Feed. Food and Agricultural Organisation, Foods CoCi; 2017.
42. Cassidy ES, West PC, Gerber JS, Foley JA. Redefining agricultural yields: from tonnes to people nourished per hectare. Environmental Research Letters. 2013;8(3):034015.
43. Kang Y, Khan S, Ma X. Climate change impacts on crop yield, crop water productivity and food security–A review. Progress in Natural Science. 2009;19(12):1665-74.

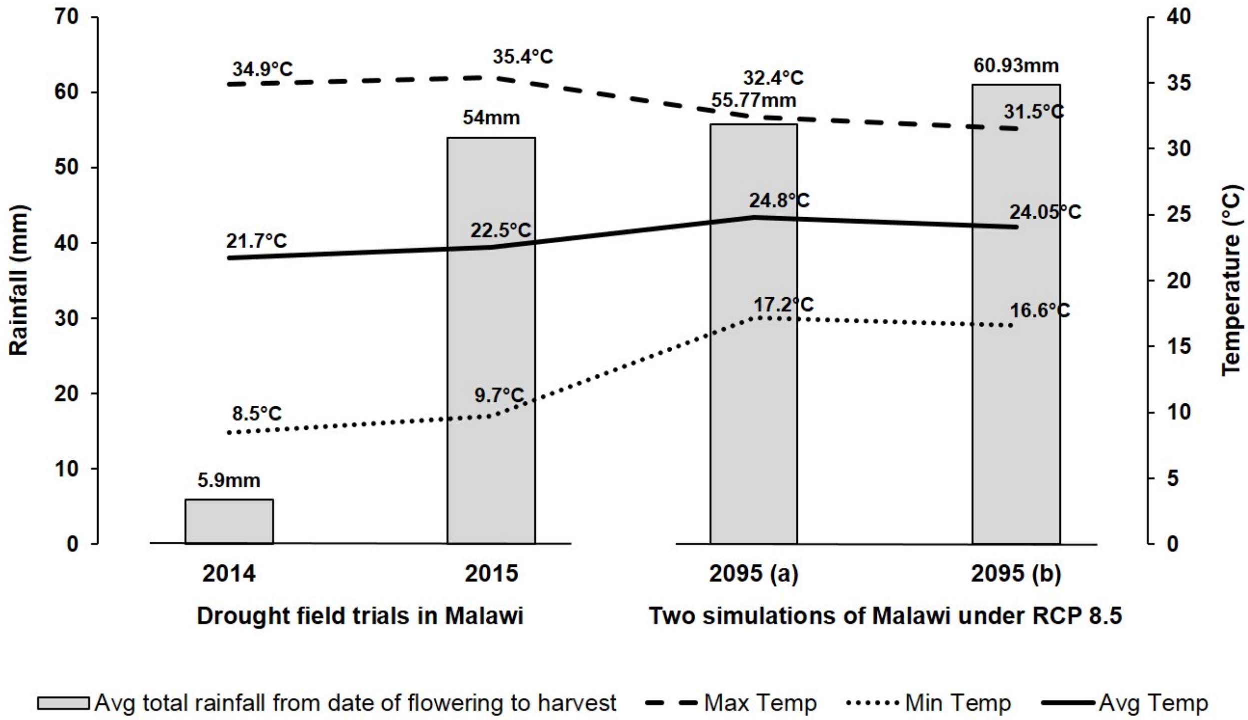
44. Lobell DB, Burke MB, Tebaldi C, Mastrandrea MD, Falcon WP, Naylor RL. Prioritizing climate change adaptation needs for food security in 2030. Science. 2008;319(5863):607-10.
45. Lobell DB, Field CB. Global scale climate–crop yield relationships and the impacts of recent warming. Environmental research letters. 2007;2(1):014002.
46. Rosenzweig C, Elliott J, Deryng D, Ruane AC, Müller C, Arneth A, et al. Assessing agricultural risks of climate change in the 21st century in a global gridded crop model intercomparison. Proceedings of the National Academy of Sciences. 2014;111(9):3268-73.
47. Challinor AJ, Watson J, Lobell D, Howden S, Smith D, Chhetri N. A meta-analysis of crop yield under climate change and adaptation. Nature Climate Change. 2014;4(4):287-91.
48. Schlenker W, Lobell DB. Robust negative impacts of climate change on African agriculture. Environmental Research Letters. 2010;5(1):014010.
49. Zhao C, Liu B, Piao S, Wang X, Lobell DB, Huang Y, et al. Temperature increase reduces global yields of major crops in four independent estimates. Proceedings of the National Academy of Sciences. 2017;114(35):9326-31.
50. Zhao C, Piao S, Wang X, Huang Y, Ciais P, Elliott J, et al. Plausible rice yield losses under future climate warming. Nature plants. 2016;3:16202.
51. DaMatta FM, Grandis A, Arenque BC, Buckeridge MS. Impacts of climate changes on crop physiology and food quality. Food Research International. 2010;43(7):1814-23.
52. Erda L, Wei X, Hui J, Yinlong X, Yue L, Liping B, et al. Climate change impacts on crop yield and quality with CO2 fertilization in China. Philosophical Transactions of the Royal Society B: Biological Sciences. 2005;360(1463):2149-54.
53. Högy P, Fangmeier A. Effects of elevated atmospheric CO2 on grain quality of wheat. Journal of Cereal Science. 2008;48(3):580-91.
54. Wang Y, Frei M. Stressed food–The impact of abiotic environmental stresses on crop quality. Agriculture, Ecosystems & Environment. 2011;141(3-4):271-86.
55. Zhu C, Kobayashi K, Loladze I, Zhu J, Jiang Q, Xu X, et al. Carbon dioxide (CO2) levels this century will alter the protein, micronutrients, and vitamin content of rice grains with potential health consequences for the poorest rice-dependent countries. Science Advances. 2018;4(5):eaaq1012.
56. Schmutz J, McClean PE, Mamidi S, Wu GA, Cannon SB, Grimwood J, et al. A reference genome for common bean and genome-wide analysis of dual domestications. Nature genetics. 2014;46(7):707-13.
57. Chirwa RM, Aggarwal VD. Bean seed dissemination systems in Malawi: A strategy. Journal of Sustainable Agriculture. 2000;15(4):5-24.
58. McGuire S, Sperling L. Seed systems smallholder farmers use. Food Security. 2016;8(1):179-95.
59. Wortmann CS. Atlas of common bean (Phaseolus vulgaris L.) production in Africa: CIAT; 1998.
60. Challinor A, Wheeler T, Garforth C, Craufurd P, Kassam A. Assessing the vulnerability of food crop systems in Africa to climate change. Climatic change. 2007;83(3):381-99.
61. Lloyd SJ, Kovats RS, Chalabi Z. Climate change, crop yields, and undernutrition: development of a model to quantify the impact of climate scenarios on child undernutrition. Environmental health perspectives. 2011;119(12):1817.
62. Ray DK, Gerber JS, MacDonald GK, West PC. Climate variation explains a third of global crop yield variability. Nature communications. 2015;6.
63. Peng S, Huang J, Sheehy JE, Laza RC, Visperas RM, Zhong X, et al. Rice yields decline with higher night temperature from global warming. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(27):9971-5.
64. Schlenker W, Roberts MJ. Nonlinear temperature effects indicate severe damages to US crop yields under climate change. Proceedings of the National Academy of sciences. 2009;106(37):15594-8.
65. Wheeler TR, Craufurd PQ, Ellis RH, Porter JR, Prasad PV. Temperature variability and the yield of annual crops. Agriculture, Ecosystems & Environment. 2000;82(1-3):159-67.
66. Ramirez-Cabral NYZ, Kumar L, Taylor S. Crop niche modeling projects major shifts in common bean growing areas. Agricultural and Forest Meteorology. 2016;218:102-13.
67. Ramirez-Villegas J, Thornton PK. Climate change impacts on African crop production. CCAFS Working Paper2015.

68. Thornton PK, Jones PG, Alagarswamy G, Andresen J. Spatial variation of crop yield response to climate change in East Africa. Global Environmental Change. 2009;19(1):54-65.
69. Hamim H. Photosynthesis of C3 and C4 Species in Response to Increased CO2 Concentration and Drought Stress. HAYATI Journal of Biosciences. 2005;12(4):131.
70. Rickards L, Howden S. Transformational adaptation: agriculture and climate change. Crop and Pasture Science. 2012;63(3):240-50.
71. Beebe S, Gonzalez AV, Rengifo J. Research on trace minerals in the common bean. Food and Nutrition Bulletin. 2000;21(4):387-91.
72. Tako E, Blair MW, Glahn RP. Biofortified red mottled beans (Phaseolus vulgaris L.) in a maize and bean diet provide more bioavailable iron than standard red mottled beans: Studies in poultry (Gallus gallus) and an in vitro digestion/Caco-2 model. Nutrition journal. 2011;10(1):113.
73. De Moura FF, Palmer AC, Finkelstein JL, Haas JD, Murray-Kolb LE, Wenger MJ, et al. Are Biofortified Staple Food Crops Improving Vitamin A and Iron Status in Women and Children? New Evidence from Efficacy Trials–. Oxford University Press; 2014.
74. Finkelstein JL, Haas JD, Mehta S. Iron-biofortified staple food crops for improving iron status: a review of the current evidence. Current opinion in biotechnology. 2017;44:138-45.
75. Haas JD, Luna SV, Lung'aho MG, Wenger MJ, Murray-Kolb LE, Beebe S, et al. Consuming Iron Biofortified Beans Increases Iron Status in Rwandan Women after 128 Days in a Randomized Controlled Feeding Trial–3. The Journal of nutrition. 2016;146(8):1586-92.
76. Siegenberg D, Baynes RD, Bothwell TH, Macfarlane BJ, Lamparelli RD, Car N, et al. Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. The American journal of clinical nutrition. 1991;53(2):537-41.
77. Vázquez M, Calatayud M, Piedra CJ, Chiocchetti G, Vélez D, Devesa V. Toxic trace elements at gastrointestinal level. Food and Chemical Toxicology. 2015;86:163-75.
78. AbdElgawad H, Zinta G, Beemster GT, Janssens IA, Asard H. Future climate CO2 levels mitigate stress impact on plants: increased defense or decreased challenge? Frontiers in plant science. 2016;7:556.
79. Varela MC, Arslan I, Reginato MA, Cenzano AM, Luna MV. Phenolic compounds as indicators of drought resistance in shrubs from Patagonian shrublands (Argentina). Plant Physiology and Biochemistry. 2016;104:81-91.
80. Chotpantarat S, Ong SK, Sutthirat C, Osathaphan K. Effect of pH on transport of Pb2+, Mn2+, Zn2+ and Ni2+ through lateritic soil: column experiments and transport modeling. Journal of Environmental Sciences. 2011;23(4):640-8.
81. Whitmore AP, Whalley WR. Physical effects of soil drying on roots and crop growth. Journal of Experimental Botany. 2009;60(10):2845-57.
82. Irvine RF, Schell MJ. Back in the water: the return of the inositol phosphates. Nature reviews Molecular cell biology. 2001;2(5):327.
83. Kido EA, Neto JRF, Silva RL, Belarmino LC, Neto JPB, Soares-Cavalcanti NM, et al. Expression dynamics and genome distribution of osmoprotectants in soybean: identifying important components to face abiotic stress. BMC bioinformatics. 2013;14(1):S7.
84. Boominathan P, Shukla R, Kumar A, Manna D, Negi D, Verma PK, et al. Long term transcript accumulation during the development of dehydration adaptation in Cicer arietinum. Plant Physiology. 2004;135(3):1608-20.
85. Joshi-Saha A, Reddy KS. Repeat length variation in the 5'UTR of myo-inositol monophosphatase gene is related to phytic acid content and contributes to drought tolerance in chickpea (Cicer arietinum L.). Journal of experimental botany. 2015;66(19):5683-90.
86. Zhao F-J, McGrath SP. Biofortification and phytoremediation. Current opinion in plant biology. 2009;12(3):373-80.
87. Beebe SE, Rao IM, Cajiao C, Grajales M. Selection for drought resistance in common bean also improves yield in phosphorus limited and favorable environments. Crop Science. 2008;48(2):582-92.
88. Lipper L, Thornton P, Campbell BM, Baedeker T, Braimoh A, Bwalya M, et al. Climate-smart agriculture for food security. Nature Climate Change. 2014;4(12):1068.

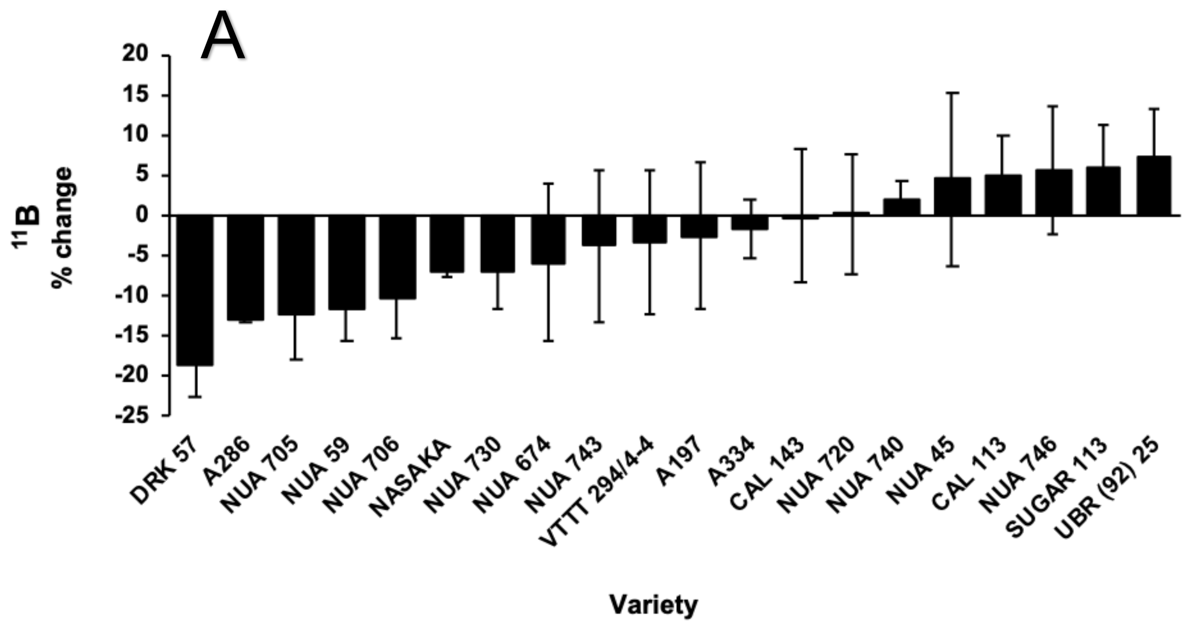
# Appendix 1 Supplementary figures

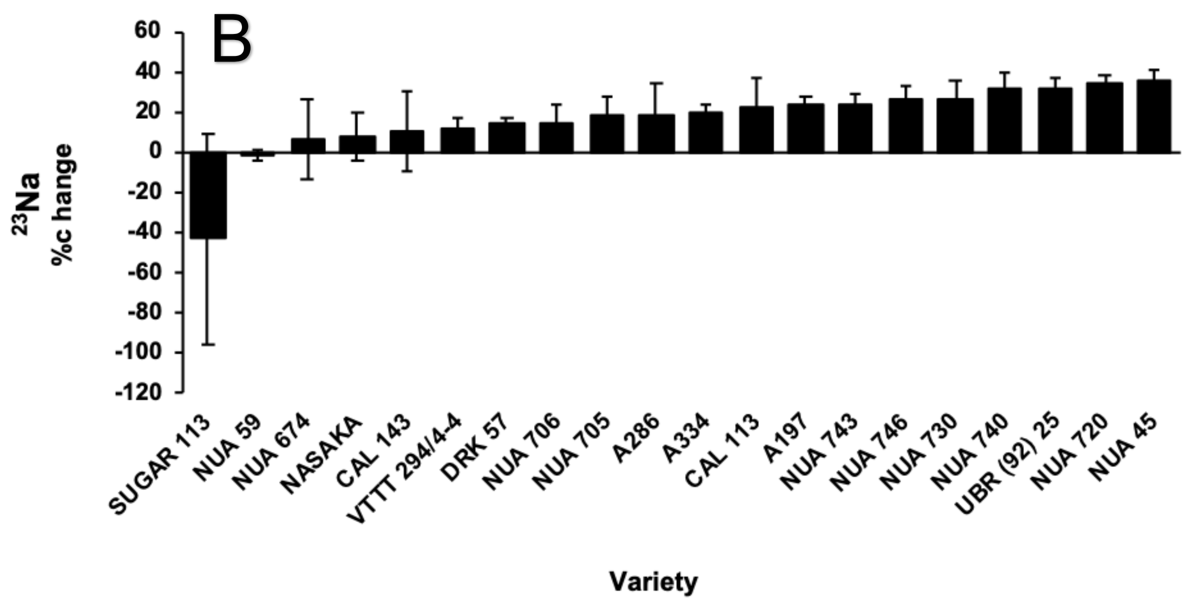



**Supplementary figure 1.** Rainfall and temperature data during crop growing period across rainfed (Season 1 and 3) and drought (Season 2 and 4) conditions. Source: Chitedze weather station.

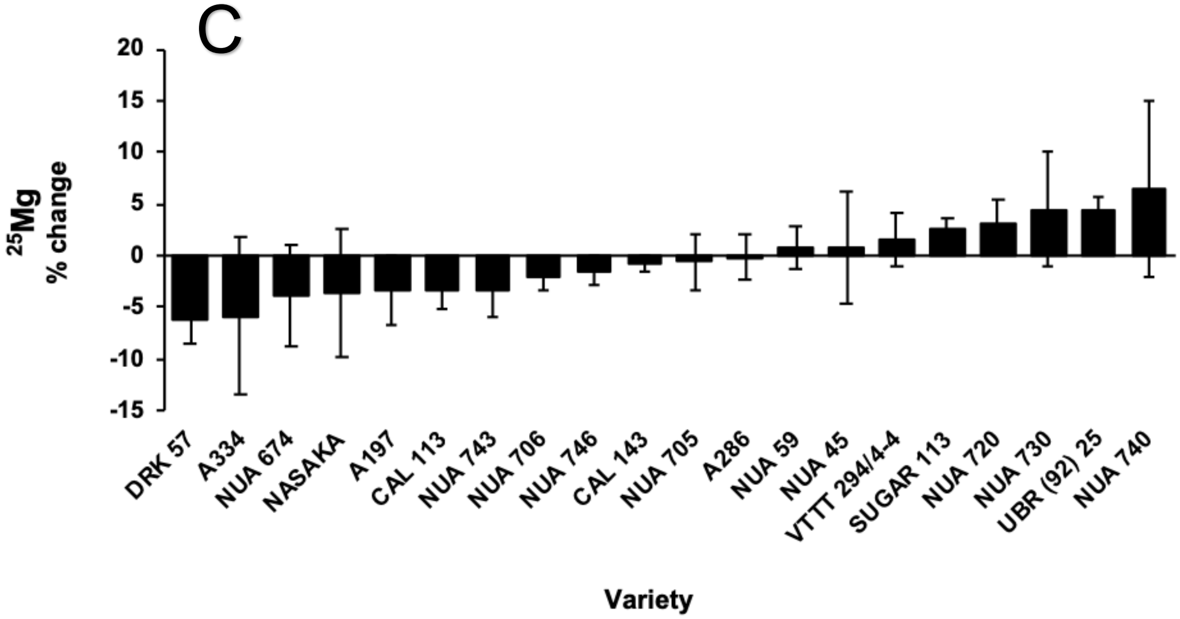


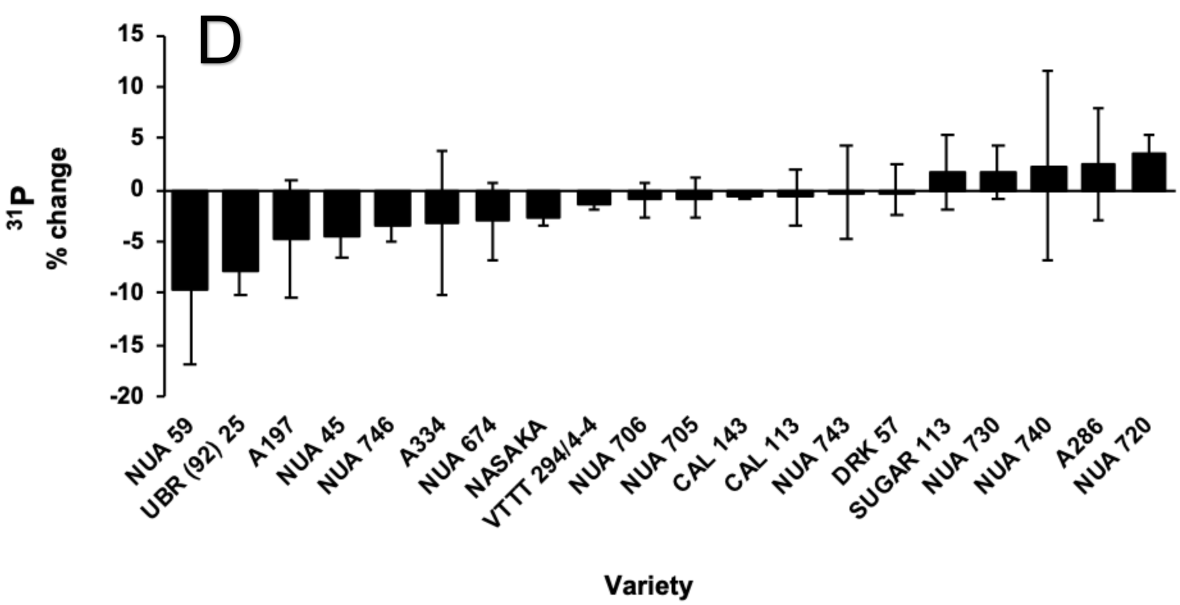

**Supplementary figure 2.** Present-day drought season weather conditions mimics future bean growing season weather conditions. Using marksim standalone V.2 software two weather scenarios were simulated in Malawi for the year 2095. Under RCP 8.5 the months of March, April and May (bean growing season) will experience an average rainfall and temperature similar to that recorded in our field trials. (a) Ensemble of 7 of the most highly cited (>400 citations) General Circulation Models (gcms). (b) Ensemble of all 17 gcms.



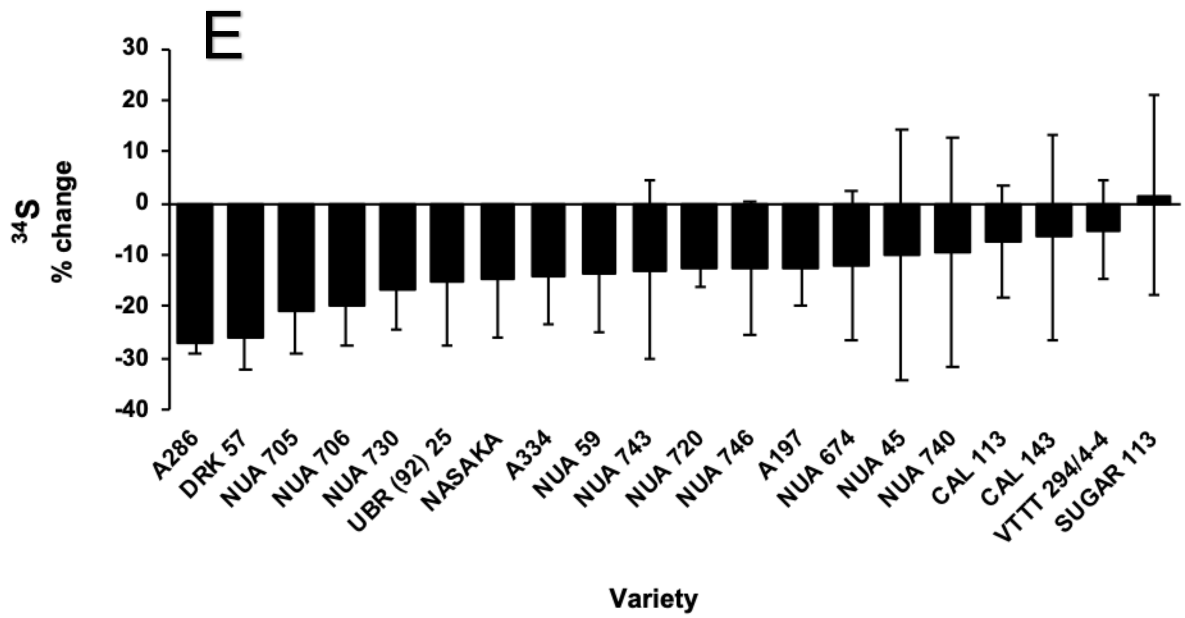



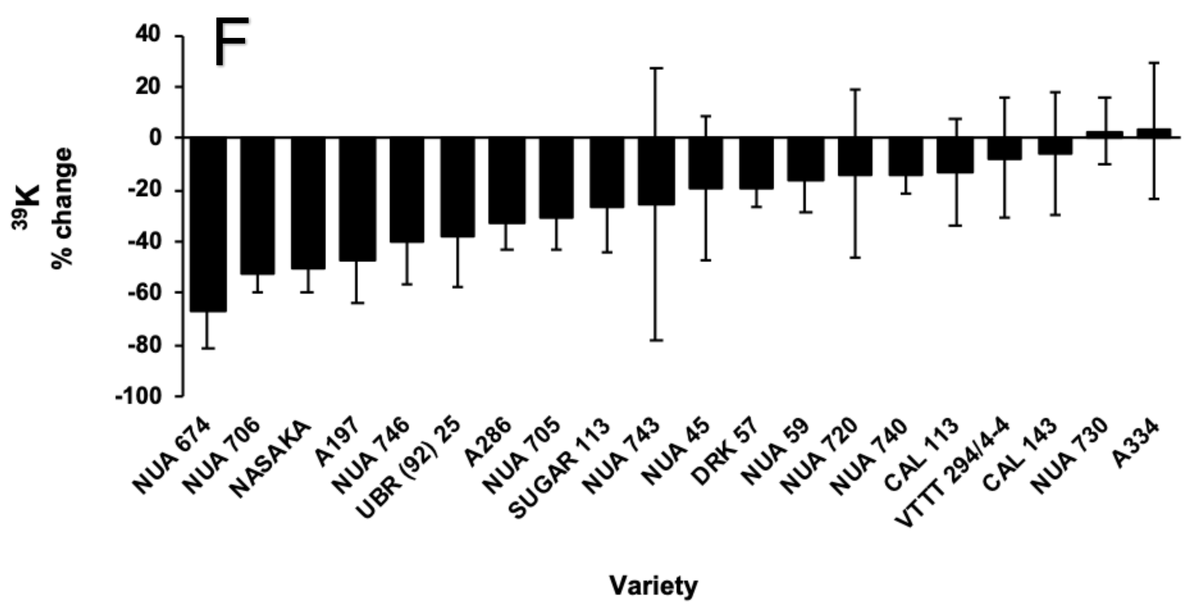

**Supplementary figure 3**. Percentage grain (A) Boron, (B) Sodium, (C) Magnesium, (D) Phosphorus, (E) Sulphur, (F) Potassium, (G) Calcium, (H) Titanium, (I) Chromium, (J) Manganese, (K) Copper, (L) Arsenic, (M) Rubidium, (N) Strontium, (O) Molybdenum, (P) Cadmium, (Q) Cobalt, (R) Nickel, (S) 57Iron, (T) lead, (U) 56Iron, (V) zinc, (W) phytic acid and protein (X) concentration change of each common bean variety under drought conditions relative to rainfed conditions.



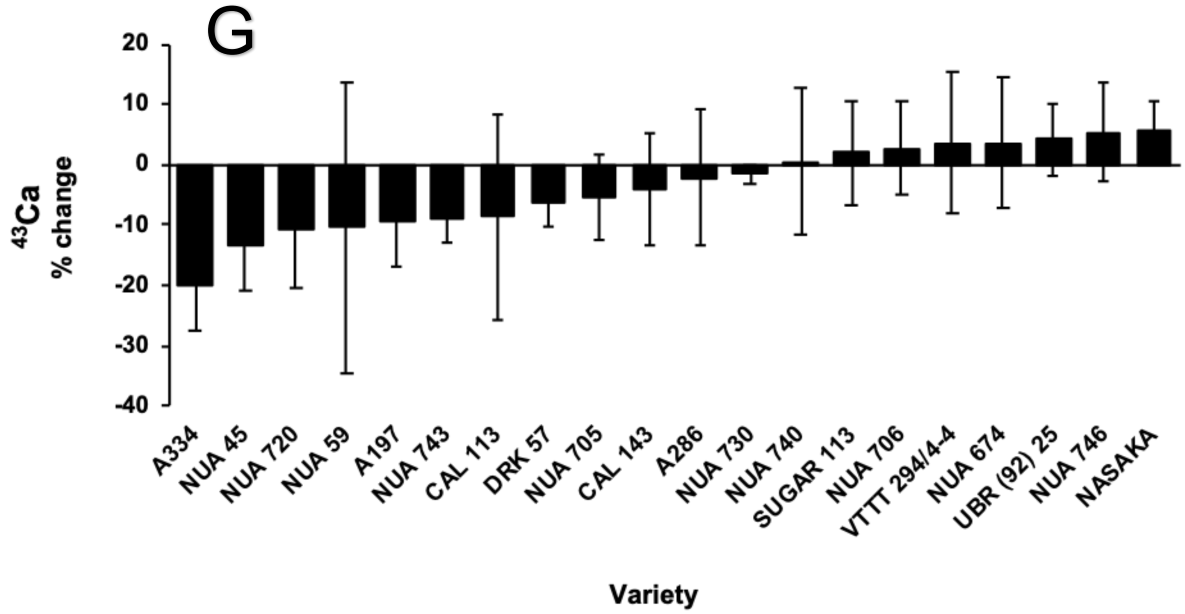



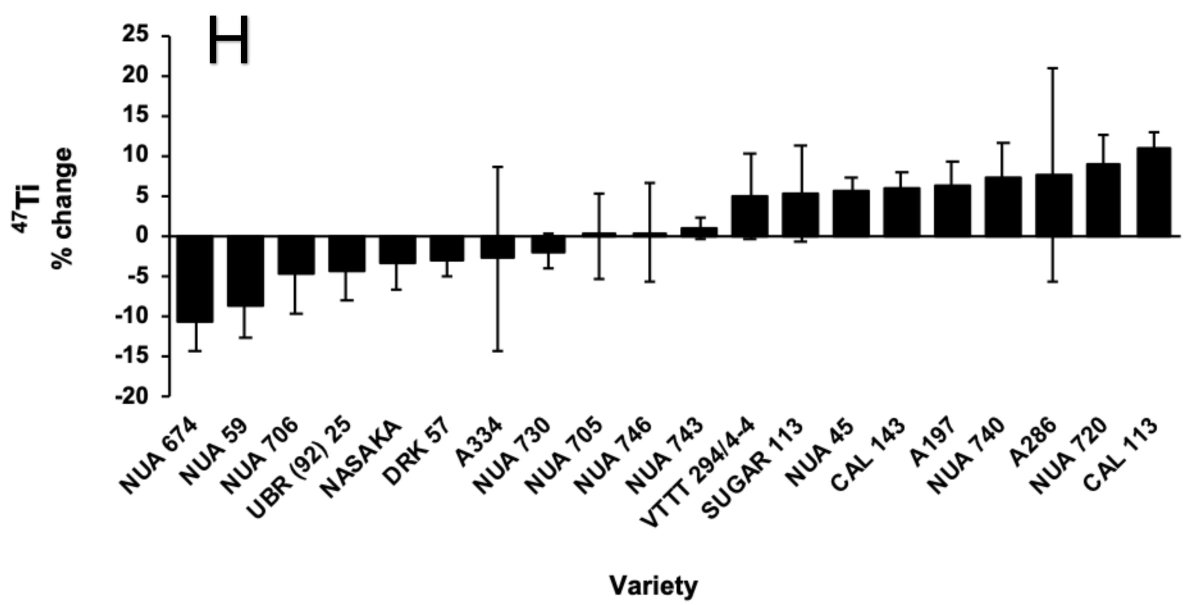

**Supplementary figure 3**. (continued) Percentage grain (A) Boron, (B) Sodium, (C) Magnesium, (D) Phosphorus, (E) Sulphur, (F) Potassium, (G) Calcium, (H) Titanium, (I) Chromium, (J) Manganese, (K) Copper, (L) Arsenic, (M) Rubidium, (N) Strontium, (O) Molybdenum, (P) Cadmium, (Q) Cobalt, (R) Nickel, (S) 57Iron, (T) lead, (U) 56Iron, (V) zinc, (W) phytic acid and protein (X) concentration change of each common bean variety under drought conditions relative to rainfed conditions.



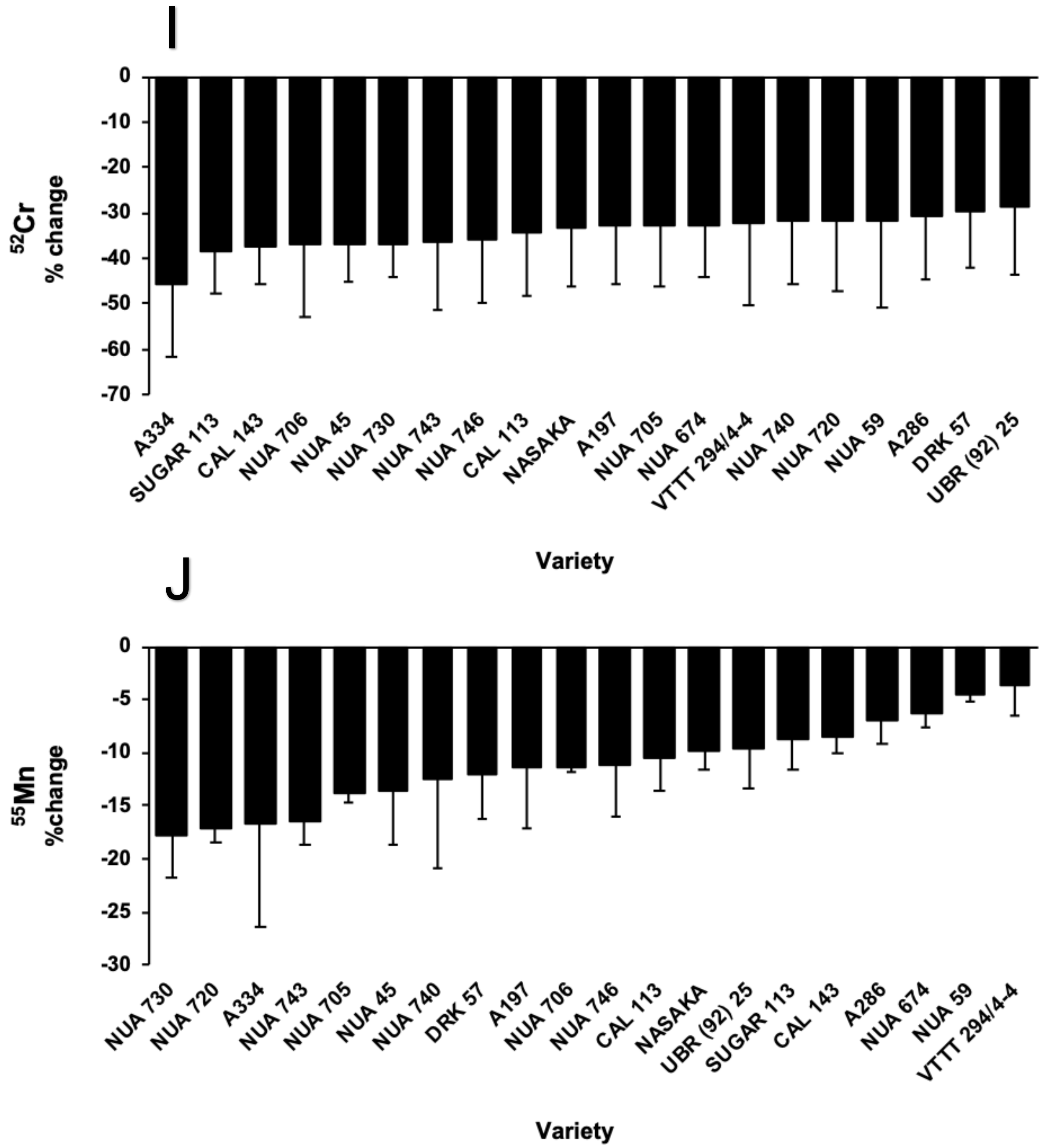




**Supplementary figure 3**. (continued) Percentage grain (A) Boron, (B) Sodium, (C) Magnesium, (D) Phosphorus, (E) Sulphur, (F) Potassium, (G) Calcium, (H) Titanium, (I) Chromium, (J) Manganese, (K) Copper, (L) Arsenic, (M) Rubidium, (N) Strontium, (O) Molybdenum, (P) Cadmium, (Q) Cobalt, (R) Nickel, (S) 57Iron, (T) lead, (U) 56Iron, (V) zinc, (W) phytic acid and protein (X) concentration change of each common bean variety under drought conditions relative to rainfed conditions.

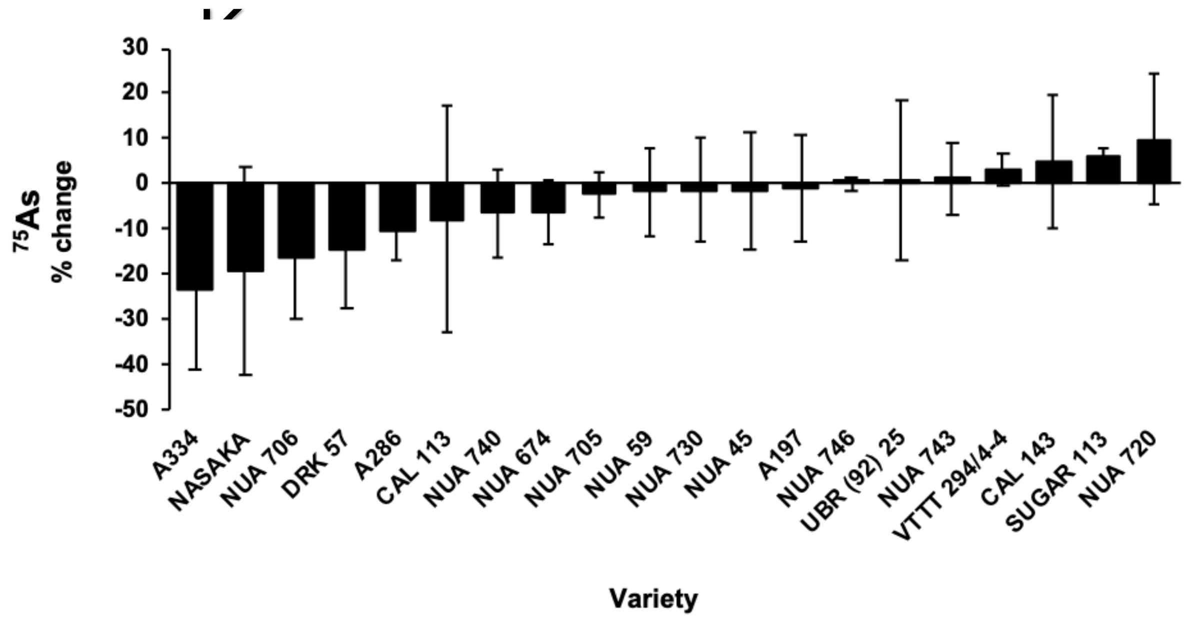


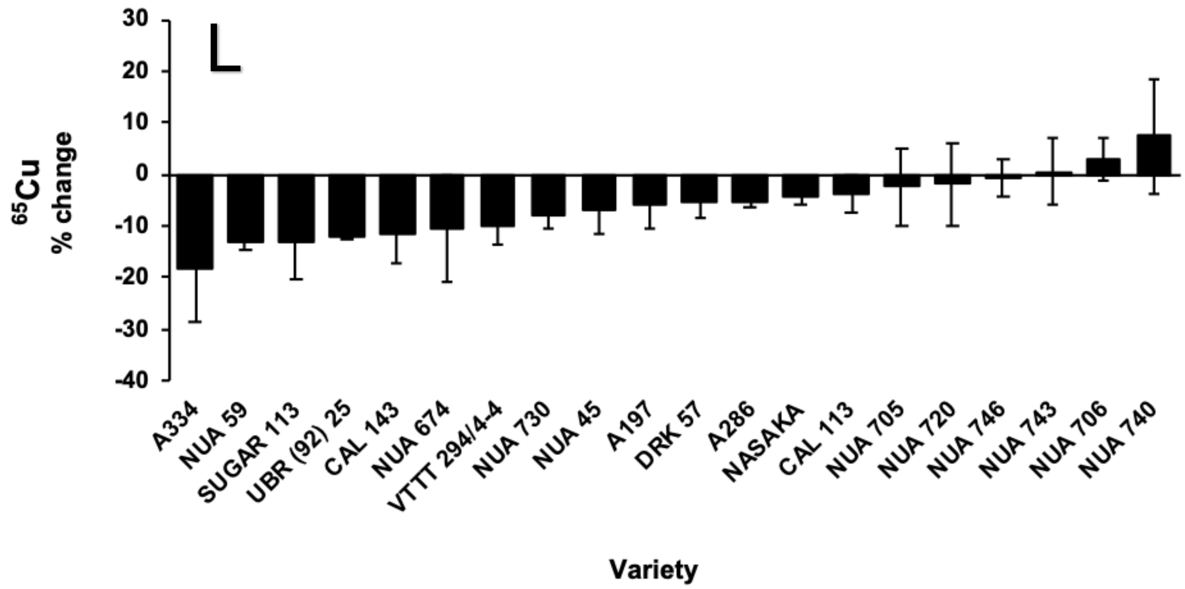




**Supplementary figure 3**. (continued) Percentage grain (A) Boron, (B) Sodium, (C) Magnesium, (D) Phosphorus, (E) Sulphur, (F) Potassium, (G) Calcium, (H) Titanium, (I) Chromium, (J) Manganese, (K) Copper, (L) Arsenic, (M) Rubidium, (N) Strontium, (O) Molybdenum, (P) Cadmium, (Q) Cobalt, (R) Nickel, (S) 57Iron, (T) lead, (U) 56Iron, (V) zinc, (W) phytic acid and protein (X) concentration change of each common bean variety under drought conditions relative to rainfed conditions.

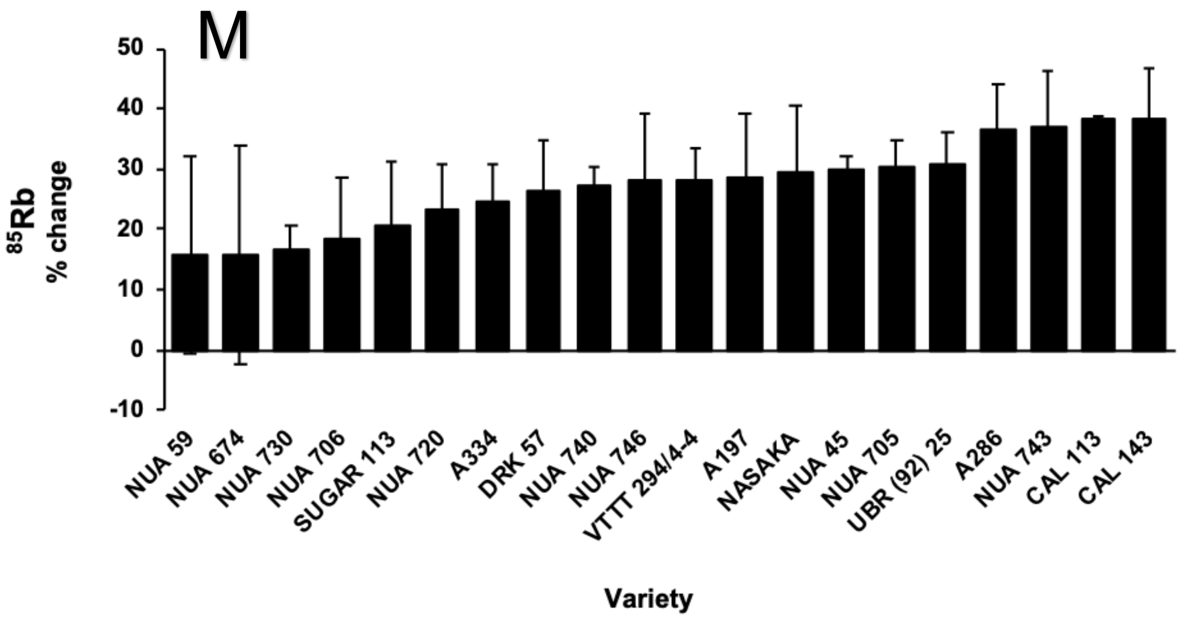


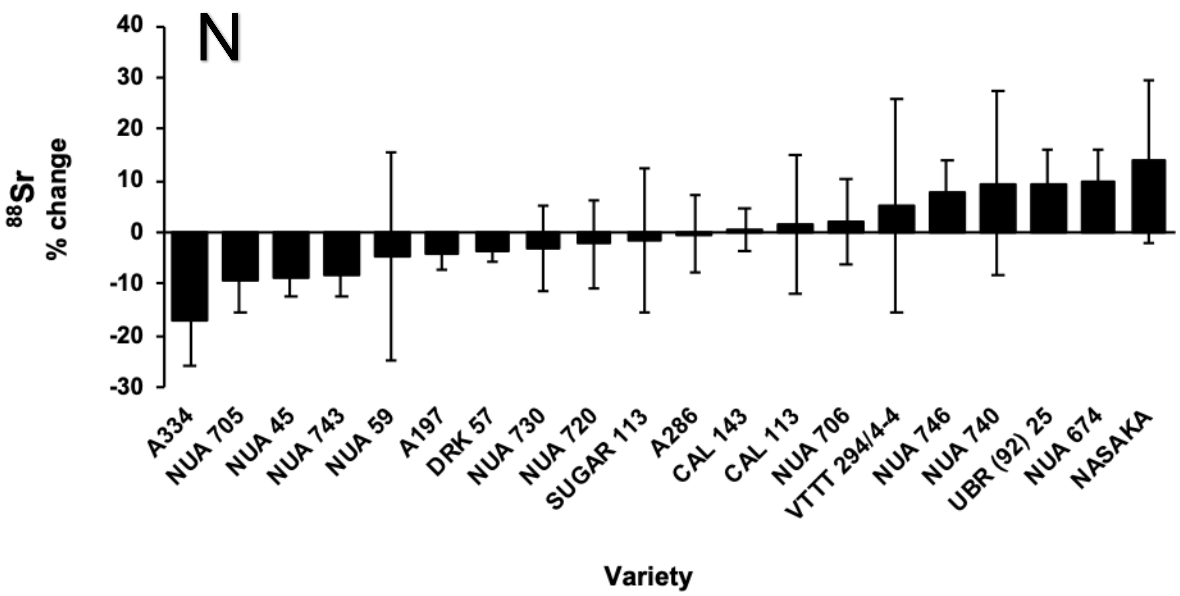

**Supplementary figure 3**. (continued) Percentage grain (A) Boron, (B) Sodium, (C) Magnesium, (D) Phosphorus, (E) Sulphur, (F) Potassium, (G) Calcium, (H) Titanium, (I) Chromium, (J) Manganese, (K) Copper, (L) Arsenic, (M) Rubidium, (N) Strontium, (O) Molybdenum, (P) Cadmium, (Q) Cobalt, (R) Nickel, (S) 57Iron, (T) lead, (U) 56Iron, (V) zinc, (W) phytic acid and protein (X) concentration change of each common bean variety under drought conditions relative to rainfed conditions.



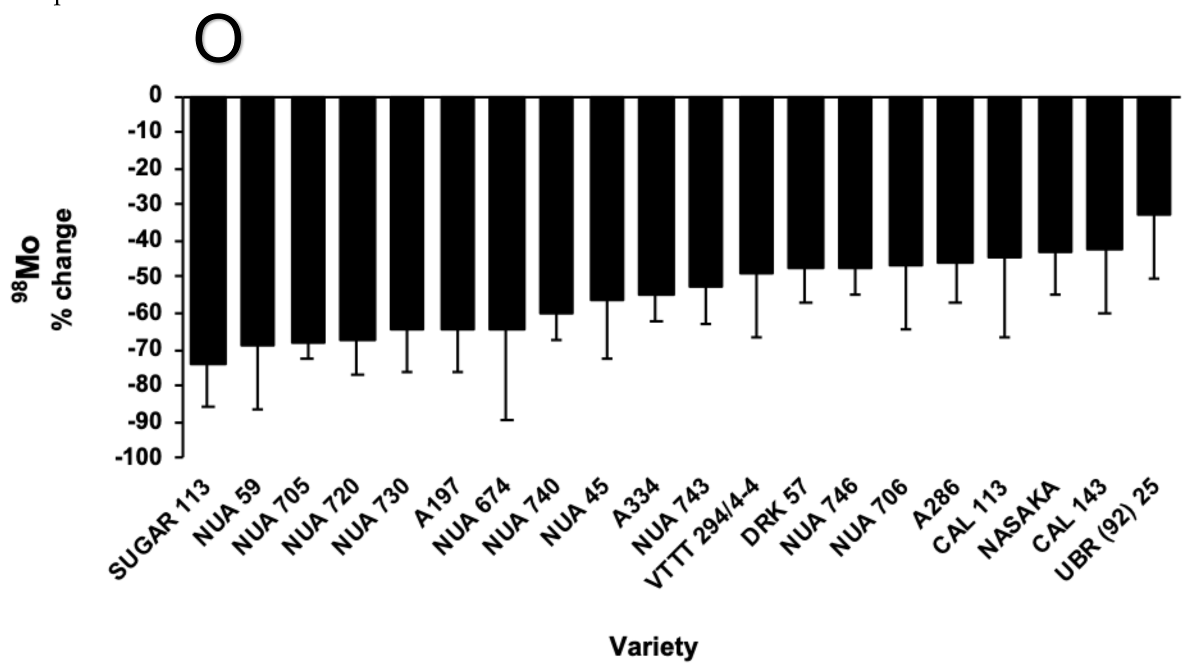



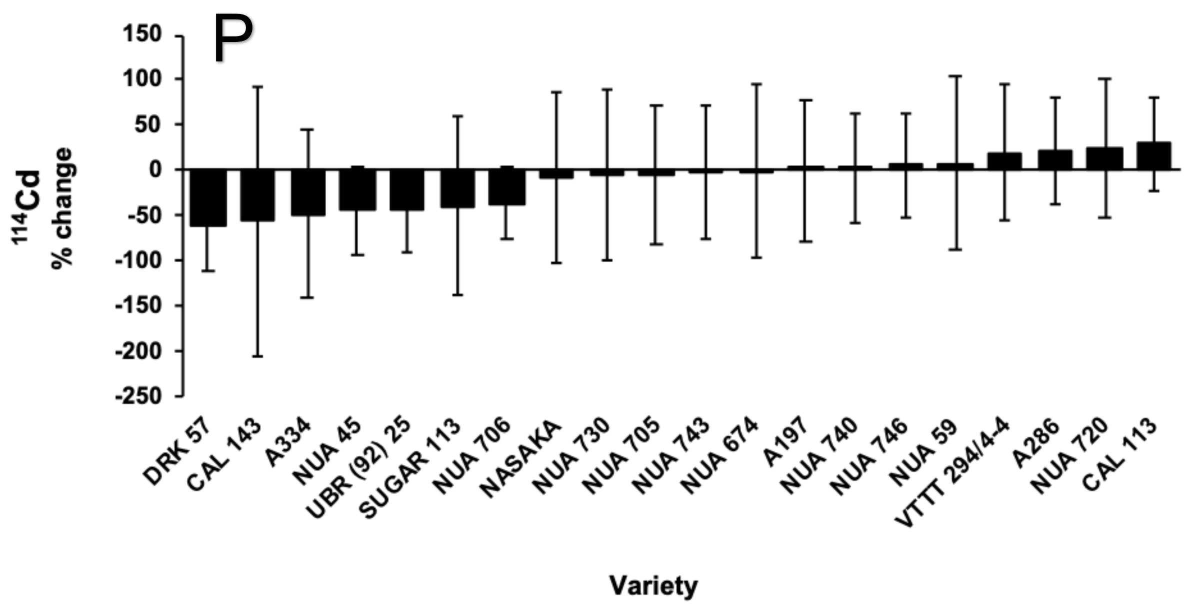

**Supplementary figure 3**. (continued) Percentage grain (A) Boron, (B) Sodium, (C) Magnesium, (D) Phosphorus, (E) Sulphur, (F) Potassium, (G) Calcium, (H) Titanium, (I) Chromium, (J) Manganese, (K) Copper, (L) Arsenic, (M) Rubidium, (N) Strontium, (O) Molybdenum, (P) Cadmium, (Q) Cobalt, (R) Nickel, (S) 57Iron, (T) lead, (U) 56Iron, (V) zinc, (W) phytic acid and protein (X) concentration change of each common bean variety under drought conditions relative to rainfed conditions.



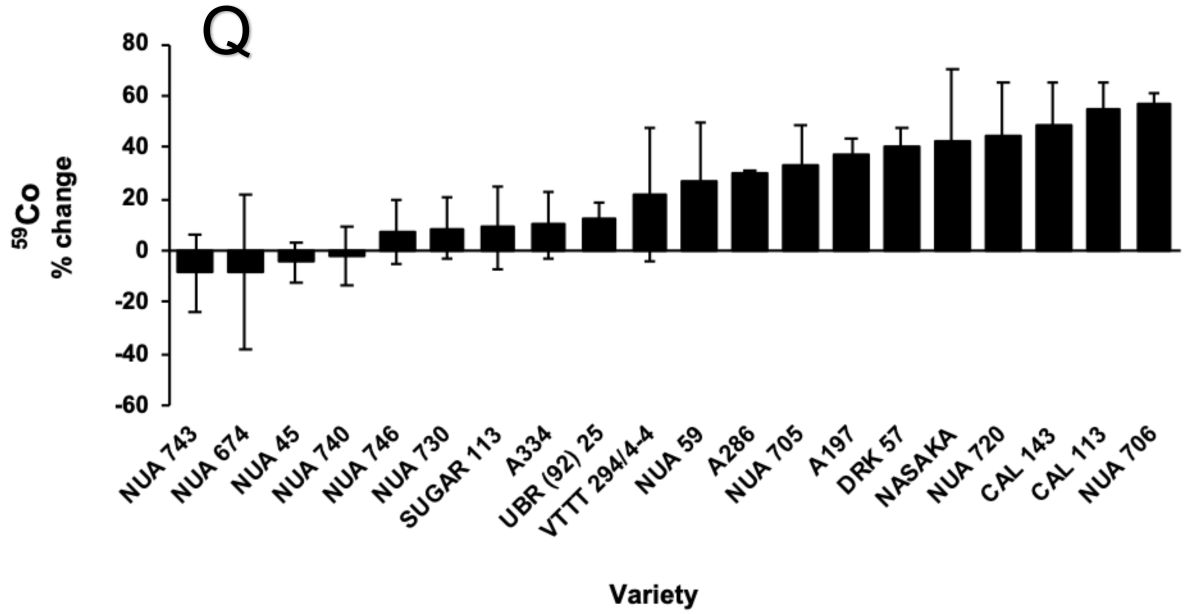



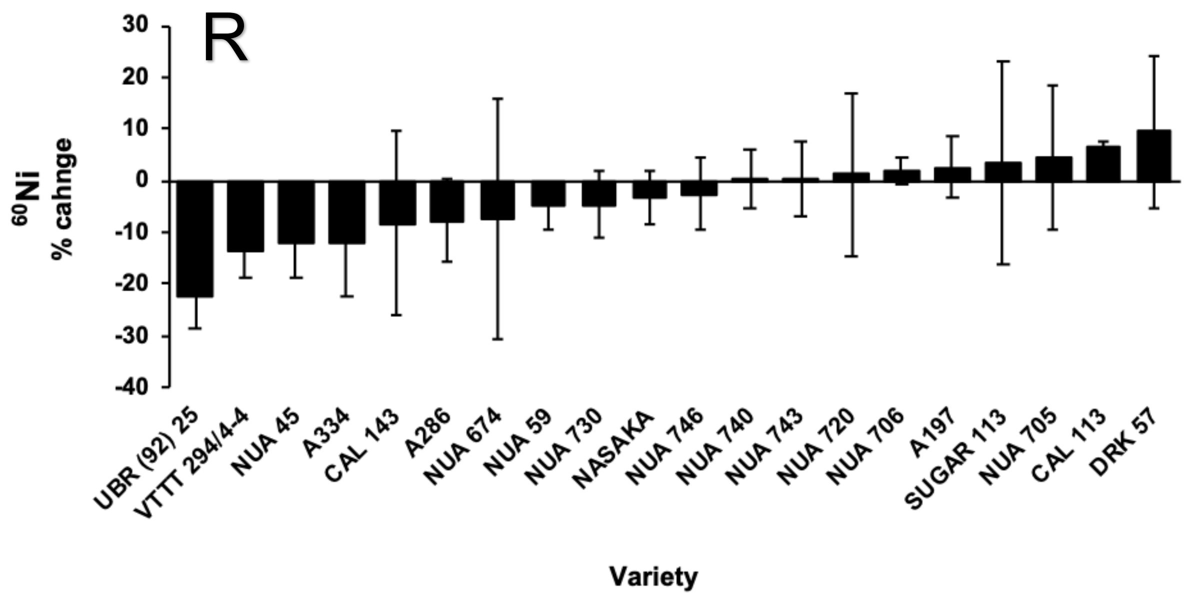

**Supplementary figure 3**. (continued) Percentage grain (A) Boron, (B) Sodium, (C) Magnesium, (D) Phosphorus, (E) Sulphur, (F) Potassium, (G) Calcium, (H) Titanium, (I) Chromium, (J) Manganese, (K) Copper, (L) Arsenic, (M) Rubidium, (N) Strontium, (O) Molybdenum, (P) Cadmium, (Q) Cobalt, (R) Nickel, (S) 57Iron, (T) lead, (U) 56Iron, (V) zinc, (W) phytic acid and protein (X) concentration change of each common bean variety under drought conditions relative to rainfed conditions.



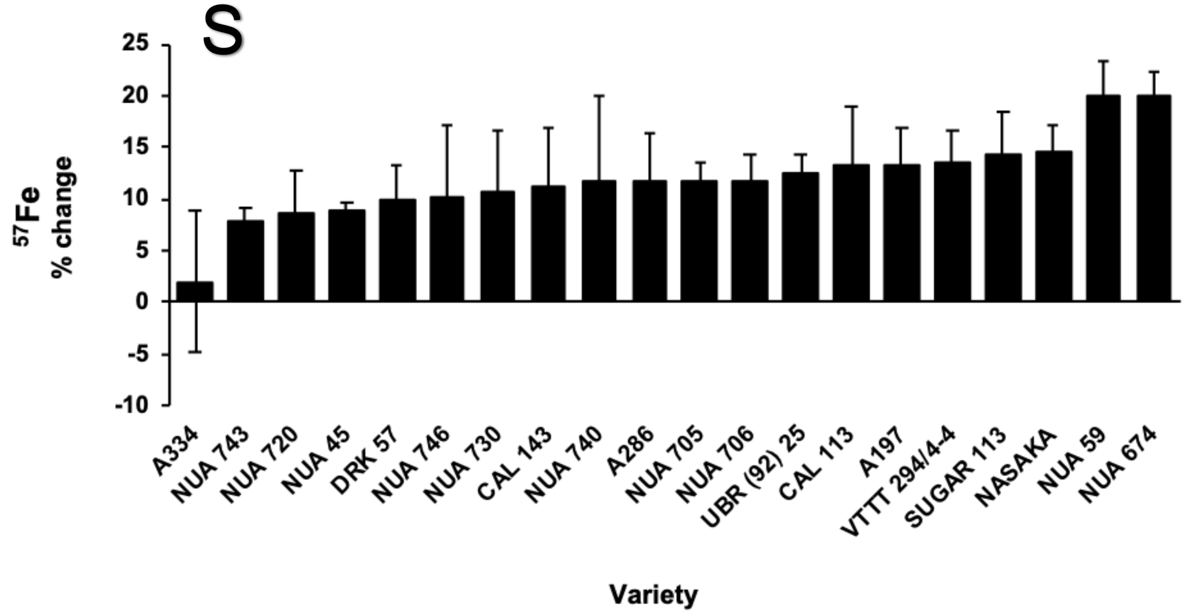



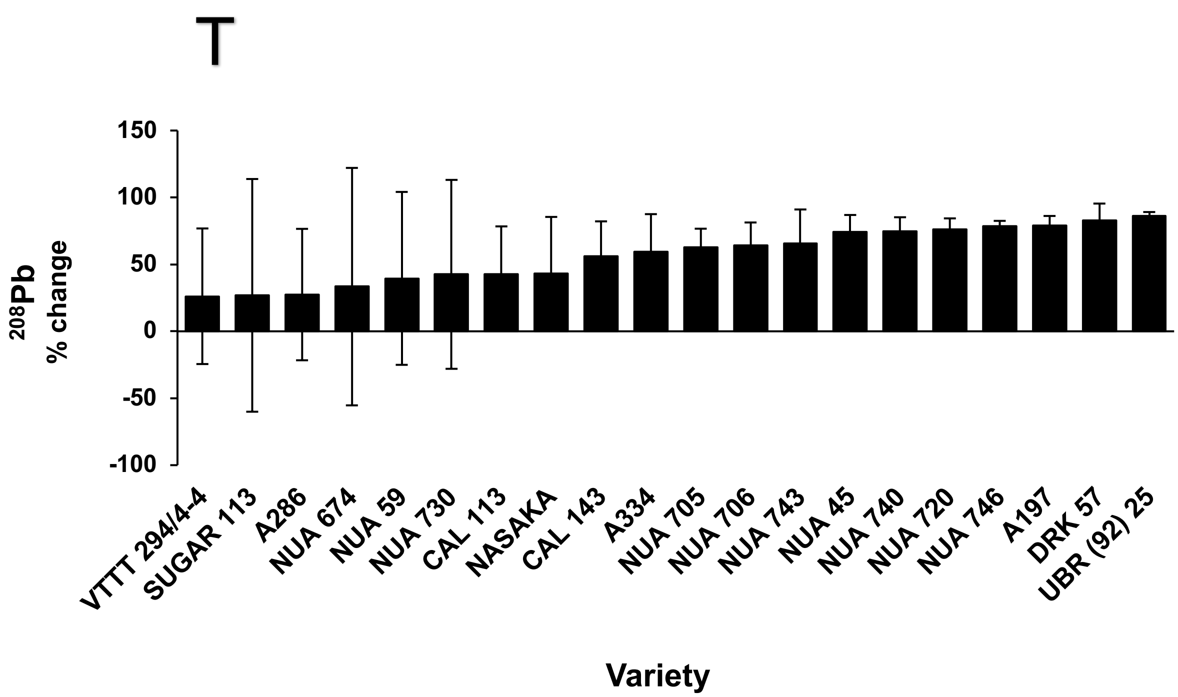

**Supplementary figure 3**. (continued) Percentage grain (A) Boron, (B) Sodium, (C) Magnesium, (D) Phosphorus, (E) Sulphur, (F) Potassium, (G) Calcium, (H) Titanium, (I) Chromium, (J) Manganese, (K) Copper, (L) Arsenic, (M) Rubidium, (N) Strontium, (O) Molybdenum, (P) Cadmium, (Q) Cobalt, (R) Nickel, (S) 57Iron, (T) lead, (U) 56Iron, (V) zinc, (W) phytic acid and protein (X) concentration change of each common bean variety under drought conditions relative to rainfed conditions.



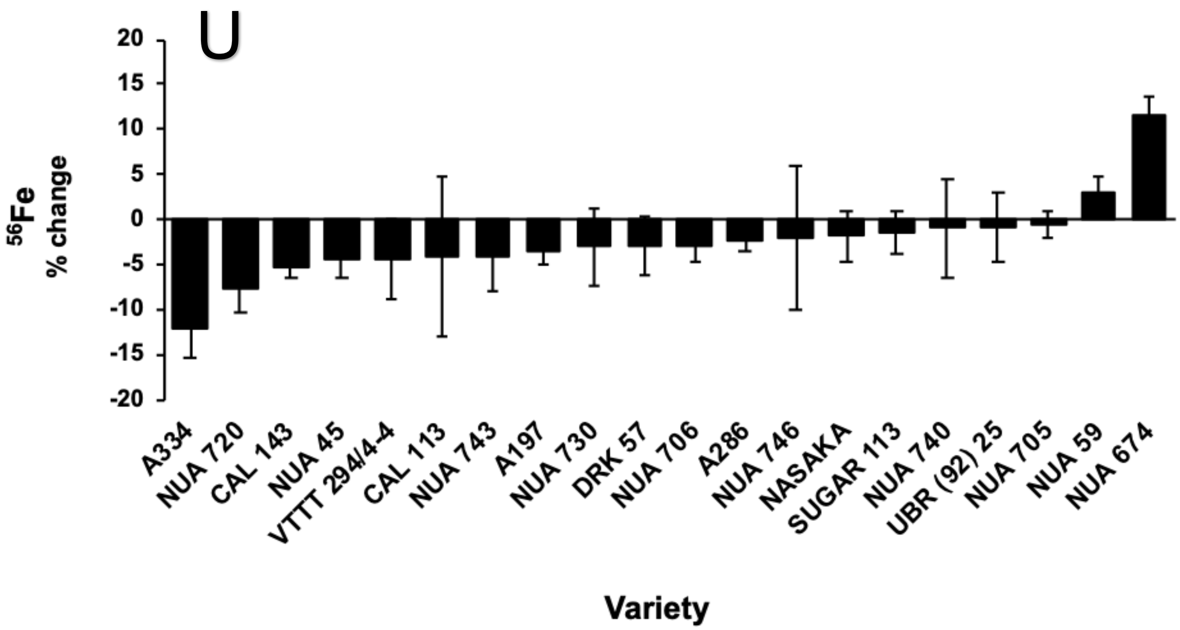



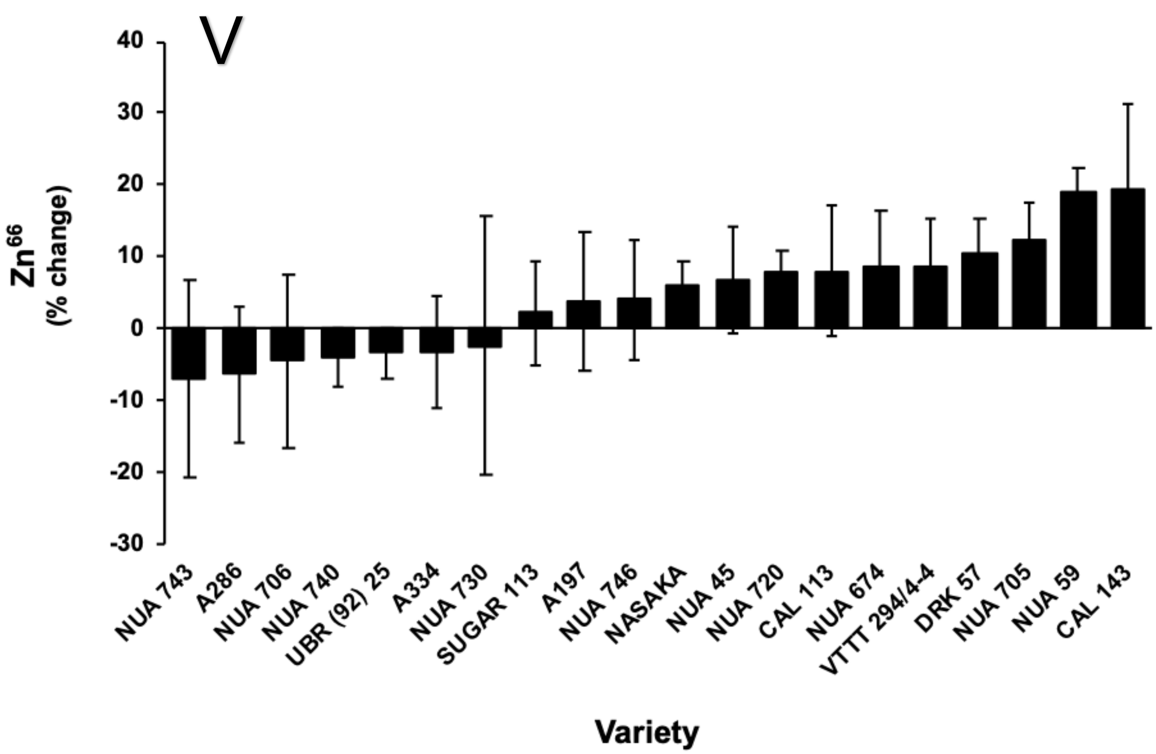

**Supplementary figure 3**. (continued) Percentage grain (A) Boron, (B) Sodium, (C) Magnesium, (D) Phosphorus, (E) Sulphur, (F) Potassium, (G) Calcium, (H) Titanium, (I) Chromium, (J) Manganese, (K) Copper, (L) Arsenic, (M) Rubidium, (N) Strontium, (O) Molybdenum, (P) Cadmium, (Q) Cobalt, (R) Nickel, (S) 57Iron, (T) lead, (U) 56Iron, (V) zinc, (W) phytic acid and protein (X) concentration change of each common bean variety under drought conditions relative to rainfed conditions.



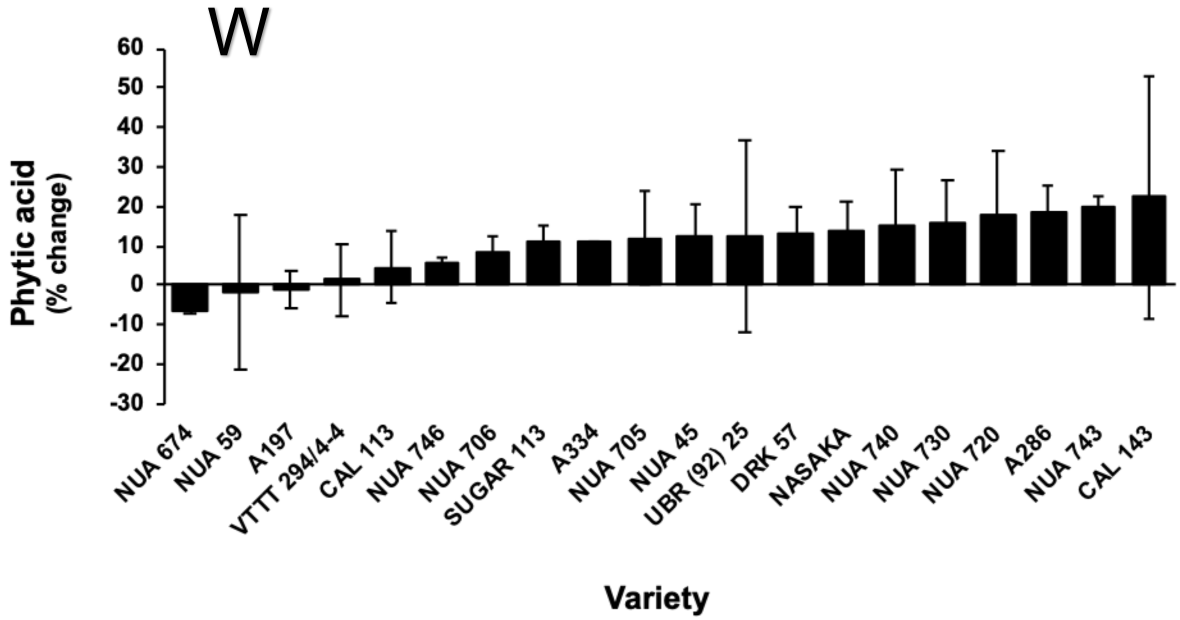




**Supplementary figure 3**. (continued) Percentage grain (A) Boron, (B) Sodium, (C) Magnesium, (D) Phosphorus, (E) Sulphur, (F) Potassium, (G) Calcium, (H) Titanium, (I) Chromium, (J) Manganese, (K) Copper, (L) Arsenic, (M) Rubidium, (N) Strontium, (O) Molybdenum, (P) Cadmium, (Q) Cobalt, (R) Nickel, (S) 57Iron, (T) lead, (U) 56Iron, (V) zinc, (W) phytic acid and protein (X) concentration change of each common bean variety under drought conditions relative to rainfed conditions.

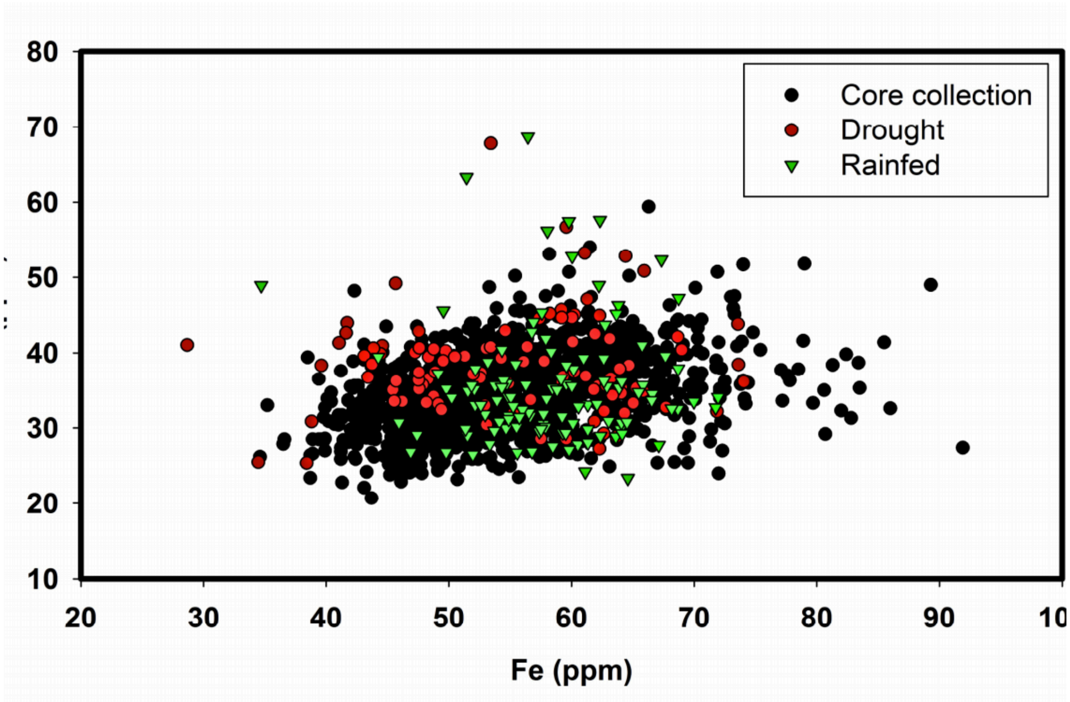



**Supplementary figure 3**. (continued) Percentage grain (A) Boron, (B) Sodium, (C) Magnesium, (D) Phosphorus, (E) Sulphur, (F) Potassium, (G) Calcium, (H) Titanium, (I) Chromium, (J) Manganese, (K) Copper, (L) Arsenic, (M) Rubidium, (N) Strontium, (O) Molybdenum, (P) Cadmium, (Q) Cobalt, (R) Nickel, (S) 57Iron, (T) lead, (U) 56Iron, (V) zinc, (W) phytic acid and protein (X) concentration change of each common bean variety under drought conditions relative to rainfed conditions.




**Supplementary figure 3**.(continued) Percentage grain (A) Boron, (B) Sodium, (C) Magnesium, (D) Phosphorus, (E) Sulphur, (F) Potassium, (G) Calcium, (H) Titanium, (I) Chromium, (J) Manganese, (K) Copper, (L) Arsenic, (M) Rubidium, (N) Strontium, (O) Molybdenum, (P) Cadmium, (Q) Cobalt, (R) Nickel, (S) 57Iron, (T) lead, (U) 56Iron, (V) zinc, (W) phytic acid and protein (X) concentration change of each common bean variety under drought conditions relative to rainfed conditions.




**Supplementary figure 4**. Scatterplot comparing combinations of Fe and Zn concentrations in the CIAT core collection of 1000 cultivated common bean accessions (black circles) with equivalent values for the twenty common beanvarieties tested in this study under rainfed (green circles) or drought conditions (red triangles).

# Chapter 3

## Mineral and phytate retention of biofortified, low phytic acid and conventional bean varieties when preparing common household recipes

Marijke Hummel
Elise Talsma
Victor Taleon
Luis Londono
Galina Brychkova
Sonia Gallego
Bodo Raatz
Charles Spillane

*Nutrients, 2020, 12 (3), 658*
*doi:* 10.3390/nu12030658

## ABSTRACT

Biofortification is an effective method to improve nutritional content of crops and nutritional intake. Breeding for higher mineral content in beans is correlated with an increase in phytates, a main inhibitor of mineral absorption in humans. Low phytic acid (lpa) beans have a 90% lower phytate content compared to conventional beans. This is the first study to investigate mineral and phytate retention after preparing common household recipes from conventional, biofortified and lpa beans. Mineral retention was determined for two conventional, three biofortified and two lpa bean genotypes. Treatments included soaking, boiling (boiled beans) and refrying (bean paste). Average true retention of iron after boiling was 77.2–91.3%; for zinc 41.2–84.0%; and for phytates 49.9–85.9%. Soaking led to a significant decrease in zinc and phytates after boiling and refrying whereas for iron no significant differences were found. lpa beans did not show a consistent pattern of difference in iron and phytate retention compared to the other groups of beans. However, lpa beans had a significantly lower retention of zinc compared to conventional and biofortified varieties ($p<0.05$). More research is needed to understand the factors that are responsible for the differences in retention between the groups of beans, especially the low retention of zinc. Combining the lpa and biofortification trait could further improve the nutritional benefits of biofortified beans, by means of decreasing the phytate:iron and zinc ratio in beans.

# INTRODUCTION

Iron and zinc deficiencies are among the most common micronutrient deficiencies globally and are estimated to affect over 2 billion people [1-3]. These deficiencies are associated with anemia (iron) [4] and impaired immunity and development (zinc) [5] and lead to a loss of human potential [6, 7]. A significant part of the population that is suffering from micronutrient deficiencies consume beans as part of their daily diet, especially in Latin America and Eastern Africa [8]. Diets of rural and poor populations in these regions are mostly plant-based, in which legumes and, more specifically, beans are an essential component [9]. Common beans (Phaseolus Vulgaris L.) are an excellent source of not only iron and zinc but also proteins, dietary fiber, and vitamins [10].

Biofortification, a nutrition-sensitive agricultural intervention, is aiming to improve the nutritional status of resource-poor populations through increasing the nutrient content of food crops, by developing more nutrient-rich varieties [11]. HarvestPlus, a global interdisciplinary alliance of research agencies and implementing agencies in biofortification, use conventional crossbreeding to improve the nutritional quality of staple crops without compromising on other agronomic qualities (yield, drought resistance, etc.) [12]. Iron beans are biofortified lines of beans with increased levels of iron and zinc that have been developed by HarvestPlus and have been released in 18 countries in Latin America and 26 countries in Africa [13]. Micronutrient targets for breeding biofortified crops are established based on the food intake of target populations, nutrient losses during storage and processing, and bioavailability of the target nutrient to the human body [14]. Current breeding targets for iron beans are 94 ppm compared to an average of 50 ppm as baseline content of average varieties of beans [12].

An efficacy trial demonstrated a positive impact of iron bean consumption on iron status in Rwandese women [15]. Mineral absorption from plant foods is generally low, which is mainly due to limited bioavailability of the iron and zinc to the body [16]. Anti-nutritional compounds hamper the potential nutritional impact of consuming plant foods and iron beans, specifically [17]. Examples of these anti-nutritional compounds are phytates, polyphenols, lectins, and tannins. Research suggested that phytic acid is a significant inhibitor, whereas polyphenols play a minor role [18]. Phytic acid (Myo-inositol-1,2,3,4,5,6-hexakisphosphate), and its salt phytate are known for their negative effect on iron absorption and can decrease iron status [18]. Phytic acid is the main storage form of phosphorus and mineral storage in the bean seed and plant. It was shown that a reduction in phytic acid levels was not associated with plant health or yield [17]; hence it is possible to develop a low phytic acid (*lpa*) bean.

For biofortification to be successful, sufficient levels of retention of these micronutrients after typical processing, storage, and cooking practices must be demonstrated [19]. Also, mineral absorption of the biofortified crops should be similar or better than non-biofortified crops. Absorption of iron and zinc has shown to be limited by the antinutrient content (eg. phytic acid.). In the case of beans, the common processing methods include soaking, boiling, and refrying. Soaking has shown to reduce phytates by solubilizing them in the soaking water, but on the other hand, it can also cause the leaching of minerals [20]. Minerals are lost in preparation methods due to physical loss, through the leaking of soluble solids into water or water loss [19]. This is all captured when determining True Retention (TR), where the changes in solids of food during processing and cooking are taken into account, and in this way, it gives an accurate estimation of the actual retention during the different processes [19]. Retention studies testing conventional [21-23] and biofortified beans [19] have been published. However, these studies did not all report true retention, which makes comparing results across different studies difficult.

Low phytate mutant lines have been developed with a defective gene that prevents the storage of phytic acid in the bean [17]. Whereas other research has been studying retention in conventional bean varieties, no research on retention levels in these relatively new *lpa* lines has been published. Our hypothesis is that there is no difference in the true retention levels of iron, zinc and phytic acid between the conventional, biofortified and *lpa* bean varieties tested. If these more free or weakly bound minerals are retained in beans while being processed, this could make way for further development of combining high mineral and *lpa* bean varieties. Therefore, we aimed to assess the iron, zinc and phytate levels of *lpa*, biofortified and conventional beans and evaluated the iron, zinc and phytate retention when preparing common bean recipes using different bean varieties.

## MATERIALS AND METHODS

### Bean varieties

Seven different varieties of common beans were selected for this study. These included three biofortified varieties (BIO101, BIO107 and ICTA Chortí) two *lpa* mutant genotypes, and two conventional bean varieties (Karaote and Calima (DAN 20 breeding line)). These were two black bean grain types, 2 *lpa* mutants with medium/small brown grain, and three Calima type varieties. The control varieties were commonly used bean types grown and consumed in South America and Eastern Africa. The low phytate mutants *lpa*1 and *lpa*2 were identified at the International Centre of Tropical Agriculture (CIAT), Colombia, from a BC3 (backcross 3) population of a *lpa* mutant [17] with BAT 93. Two mutants with the lowest phytate content were selected for this study. BIO 101 and BIO 107 are biofortified varieties that were released in 2016 in Colombia [24, 25], ICTA Chortí was released in Guatemala in April 2017 [26].

**Table 2.** Overview of included genotypes of beans

| Market class (seed size) | Genotypes | Group |
|---|---|---|
| Red mottled (large) | DAN 20 - Calima | Conventional |
| Red (small) | BIO 101 – Calima | Biofortified |
| Red (small) | BIO 107- Calima | Biofortified |
| Black (small) | ICTA Chortí | Biofortified |
| Black (small) | Caroata | Conventional |
| Brown (small) | *lpa*-1 Line: *lpa*127-4<br>Pedigree: BC3 F2 127-4 x BAT 93 | *lpa* |
| Brown (small) | *lpa*-2 Line: *lpa*127-4<br>Pedigree: BC3 F2 127-4 x BAT 93 | *lpa* |

All varieties were harvested between October 2016 and March 2017 and grown in Valle del Cauca, Colombia. Exceptions were the black bean variety ICTA Chortí, which was imported from Jalapa, Guatemala, and Karaote, which was bought from a supermarket in Cali, Colombia. In Table 2, a description of all genotypes and their characteristics is provided. Beans were dried and stored in a cold room (10°C) until further processing.

### Cleaning procedure for beans and materials

Dry beans were cleaned by removing any dirt, disease-infected beans and beans with a broken seed coat. After weighing, the seeds were cleaned using ultrapure water (18 MΩ) (MilliQ® Merck-Millipore), drained and dried using paper towels for sampling, or used straight away for preparing the recipes.

All materials used for sample preparation were decontaminated from free minerals by overnight bathing in a 5% HCl solution with ultrapure water (18 MΩ). All recipes and bean cultivar combinations were prepared in duplicate and sampled at every stage, as described below. All processing and cooking of bean samples were performed at CIAT in Cali, Colombia.

## Cooking time determination

Cooking times were determined using an automated Mattson cooker, as described by Wang et al. [27]. The cooker consists of twenty-five stainless steel piercing rods, that are placed on top of twenty-five soaked (16 hours at room temperature) seeds. When the rod connects with the metal disc under the bean, the time is recorded for each of the seeds automatically. The whole device is placed into a 2 L glass beaker containing 1 L of boiling MilliQ® heated using an electrical heating plate (Waring Pro Extra Burner, SB30). Cooking time is defined as the number of minutes required for 80 % of the samples to be pierced.

## Cooking and sampling procedure

Two different recipes of beans were prepared; boiling and refried beans, using either pre-soaked or dry beans (Fig. 8). Samples for analysis were taken during all steps described below, cooled down, and stored in an ultra-freezer (-80°C) until further processing. Samples for ICP-MS, and phytate analysis were freeze-dried (Labconco, FreeZone). Scales used were Scout Pro, Ohaus, models PRO SP6000 and PRO SP402. Weights were recorded at each step, both before preparation and the finalized product.

- **Soaking procedure**

Three hundred grams of dry beans were added to 1500 ml of MilliQ (1:5) in a glass beaker and soaked at room temperature for 18 hours. Beans were drained and samples of soaked beans and the soaking water were taken. The equivalent of 200 grams of dry beans was taken to the next step for boiling.

- **Boiling procedure**

For boiling, 200 grams of dry beans or the equivalent of soaked beans were added to 1500 ml Milli-Q (1:7.5) in a glass beaker for cooking on a pre-heated electrical plate (350 °C, Corning, model PC-620D). Total cooking time ranged from 37 to 90 minutes, depending on the variety. Beans were cooked until they felt soft between fingers, after which they were drained. Samples were taken after cooling down the broth and the beans for 30 minutes at room temperature. The equivalent of 100 grams of dry beans was taken to the next step for refrying.

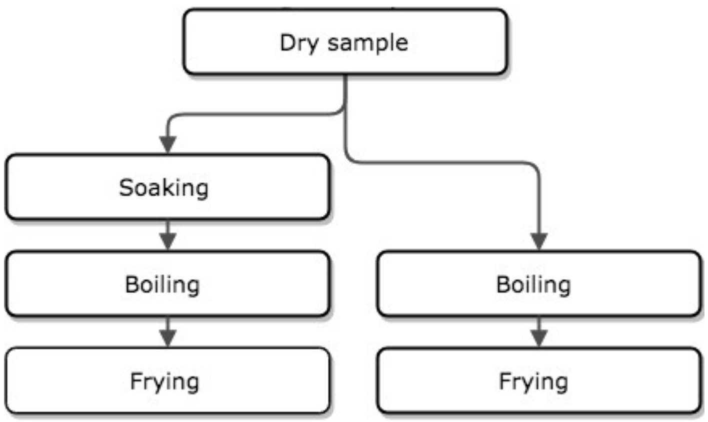



**Figure 8.** Overview of the study design with the different preparation methods

- **Refrying procedure**

A standardized recipe of refried beans was prepared using boiled beans. The equivalent of 100g of dry beans, as boiled beans, were blended (Oster, Osterizer model 4655, stainless steel) for two repeated periods of one minute, after mixing these with 200 grams of cooking broth and 20 grams of canola oil (brand Premier, Lloreda). A teflon pan was preheated for 1 min on a hot stove to an average of 210°C, after which the mass was added. The mass was continuously stirred until enough water was evaporated to form a firm mass covering around half of the pan. The mass was turned until both sides were cooked, and a light brown crust was appearing. This took on average 10 minutes and 30 seconds. Samples were taken immediately after. We corrected the calculation of true retention for the loss in mass due to not emptying the blender completely.

## Iron and zinc analysis

Inductively coupled plasma mass spectrometry (ICP-MS) analysis was conducted at Flinders University, Australia. All seed samples were gamma-irradiated at 50 kGray for sterilisation prior to release into Australia. Prior to grinding, samples were dried thoroughly at 80°C for at least 12 hours, after which samples were placed in a desiccator to keep the samples dry. Samples were ground to flour using a Retsch Ultra Centrifugal Mill ZM 200 fitted with a 12-tooth titanium rotor, titanium sieve, and pan (Retsch GmbH & Co KG, Haan, Germany). After this step, ground samples were again dried at 80°C for at least 12 hours and put in a desiccator until further analysis. A closed-tube digestion method was used for digesting samples [28]. All samples used for the validation and calibration contained <4 mg/kg Al, indicating these samples can be considered free from soil contamination as per HarvestPlus guidelines [29].

## Phytic acid analysis

Phytic acid content was based on a modified procedure of Latta and Eskin (1980) using polyprep prefilled chromatographic columns (Bio-Rad Laboratories, Richmond, CA, USA) containing an AG-1-X8 anion exchange resin (100–200 mesh chloride form, 0.8x4 cm) allowing isolation of phytic acid from bean extract. Briefly, the bean sample (0.5 g, 1.0 g for *lpa* samples) was extracted with 0.65M HCl (20 ml) for 2 h. After centrifugation (3800 RPM, 15 min), 2 ml of the supernatant was added to the column (8 ml for lpa samples). Interfering compounds and inorganic phosphorus were removed by washing with ultrapure water (18 MΩ, 5 ml) followed by 0.07 M NaCl (10 ml). Bound phytate was eluted with 0.7 M NaCl (30 ml), and an aliquot of the eluate (0.9 ml) was vortexed with 0.3 ml of Wade reagent [0.03% iron(III) chloride, 0.3% sulfosalicylic acid]. Absorbance of the salicylate–Fe(III) complex was monitored at 500 nm using a spectrophotometer (BioTek Instruments, Inc. Winooski, Vermont, USA). The concentration of phytates was calculated from a prepared standard curve obtained with potassium phytate (0–60 mg/ml; Sigma-Aldrich Canada, Oakville, ON, Canada) [30].

## Statistical analyses

True retention (TR) for all samples at all processing steps was calculated. True retention (TR) takes into account loss of dry mass (i.e., soluble solid losses and dry matter losses due to preparation) over the process. The formula used to calculate TR is (1), where Nc=nutrient content per g of cooked food, Wc=weight of cooked food (g), Nr=nutrient content per g of raw food and Wr= Weight of food before cooking (g). Meanwhile, apparent retention (AR) was calculated for the final products. apparent retention (AR) does not take into account losses of dry matter during processing and for this reason, it could be calculated if dry matter of food before and after cooking are unavailable. AR can be calculated on a moisture-free basis, using (2). TR is a more accurate method for calculating micronutrient retention compared to AR [19, 31].

$$TR\ (\%) = (Nc^*Wc) / (Nr^*Wr) * 100 \qquad (1)$$

$$AR\ (\%) = [Nc\ (\text{dry weight basis})] / [Nr\ (\text{dry weight basis})] * 100 \qquad (2)$$

All statistical analyses were conducted using SPSS (version 23.0.0.2, ICM Corp. released 2015) for Macintosh. Data were processed using analysis of variance (ANOVA) for variety, group and/or processing type. Posthoc analyses were done using Tukey's test. Statistical tests were 2-tailed (unless stated otherwise), and p-values <0.05 were considered statistically significant. Homogeneity of variances and a normal distribution of variances were checked before running the tests (where applicable), and data was transformed where needed.

## RESULTS

### Mineral and phytate content of dry beans

Iron, zinc and phytate content of the dried bean grains are presented in Table 3. The average iron content for the biofortified varieties was 88.5 ppm, well above the average of the other two groups of conventional and lpa beans (57.4–74.5 ppm), but below the current breeding targets of 94 ppm for high iron beans [12]. The Calima variety contained the lowest levels of iron with 54.4 ppm, whereas the BIO101 variety contained the most iron (90.2 ppm). Within the groups of biofortified and lpa beans the varieties did not significantly differ in iron levels. For the conventional varieties, there was a significant difference between the two varieties ($p<0.05$).

**Table 3**. Overview of iron, zinc and phytate content for the seven dry bean varieties that were selected for this study. The varieties were grouped as either conventional, biofortified or lpa varieties.

| Cultivars | Group | Iron (ppm) | | | Zinc (ppm) | | | Phytate (mg/g) |
|---|---|---|---|---|---|---|---|---|
| Calima | Conventional | 54.39 | ± | 0.10$^{a}$ | 30.17 | ± | 0.46$^{ab}$ | 17.28 ± 1.65$^{a}$ |
| Caraota | Conventional | 60.48 | ± | 1.41$^{c}$ | 31.93 | ± | 0.16$^{a}$ | 14.83 ± 0.11$^{a}$ |
| **Average conventional** | | **57.44** | **±** | **3.61$^{A}$** | **31.05** | **±** | **1.06$^{A}$** | **16.05 ± 1.71 $^{A}$** |
| BIO 101 | Biofortified | 90.23 | ± | 0.59$^{b}$ | 43.52 | ± | 0.37$^{c}$ | 14.83 ± 0.30$^{a}$ |
| BIO 107 | Biofortified | 87.27 | ± | 0.57$^{b}$ | 36.79 | ± | 0.23$^{d}$ | 21.00 ± 0.28$^{b}$ |
| ICTA Chorti | Biofortified | 87.87 | ± | 2.55$^{b}$ | 37.00 | ± | 0.14$^{d}$ | 20.05 ± 0.35$^{b}$ |
| **Average biofortified** | | **88.46** | **±** | **1.84$^{B}$** | **39.10** | **±** | **3.43$^{B}$** | **18.62 ± 2.98$^{A}$** |
| *lpa-1* | *lpa* | 73.50 | ± | 1.16$^{d}$ | 28.72 | ± | 0.38$^{b}$ | 1.05 ± 0.01$^{c}$ |
| *lpa* -2 | *lpa* | 75.44 | ± | 1.78$^{d}$ | 31.63 | ± | 1.20$^{a}$ | 1.10 ± 0.04$^{c}$ |
| **Average *lpa*** | | **74.47** | **±** | **1.66$^{C}$** | **30.17** | **±** | **1.83$^{A}$** | **1.07 ± 0.04$^{B}$** |

SD=standard deviation of the duplicates for each of the genotypes and groups. Superscripts in capitals indicate differences on group level. Superscripts in lowercase indicate significant differences between varieties (ANOVA, Tukey).

For zinc content, the average of the biofortified varieties was 39.1ppm compared to 30.2–31.1 ppm for the non-biofortified and *lpa* varieties. The *lpa*-1 variety contained the least zinc (28.7ppm), whereas the BIO101 variety contained the highest levels of zinc (43.5 ppm). Correlation between iron and zinc levels in these beans is $r=0.76$ ($p<0.05$).

Phytic acid levels in the biofortified varieties were on average 18.6 mg $g^{-1}$ compared to 16.1 mg $g^{-1}$ in the conventional varieties (no significant difference). Literature shows an increased iron content is correlated with an increased phytate content [32]. The *lpa* varieties contained on average 1.1 mg $g^{-1}$ of phytate, which is only ~6% of phytate compared to the conventional varieties, these are comparable levels as reported before in other studies on *lpa* beans [17, 33, 34].

## Cooking times

Cooking times ranged from 33.8 min for the BIO101 variety to 62.7 min for the ICTA Chorti variety as shown in Table 4. The average cooking time was 54.0 min. Both biofortified varieties had the shortest cooking time. The smallest bean genotypes (lpa mutants and Chorti) had a larger standard deviation compared to the other bean varieties. Cooking times were only determined in soaked grain because with this cooking time determination method the non-soaked seed were slipping away under the piercers of the Mattson cooker.

**Table 4.** Cooking times assessed in triplicate of the seven different bean varieties using the Mattson Cooker, soaked for 16 hours at room temperature

| Cultivars | Cooking time ± SD (min) | | |
|---|---|---|---|
| Calima | 52.1[a] | ± | 0.8 |
| Caraota | 51.8[abc] | ± | 3.2 |
| BIO 101 | 33.8[b] | ± | 1.2 |
| BIO 107 | 41.9[c] | ± | 1.5 |
| ICTA Chorti | 62.7[abc] | ± | 3.1 |
| *lpa-1* | 55.4[abc] | ± | 10.8 |
| *lpa-2* | 58.5[abc] | ± | 14.4 |

Different letters (a,b,c) indicate significant differences in cooking times between the different varieties ($p<0.05$).

## Nutrient retention in soaked, boiled and refried beans

### Iron

Table 5 presents an overview of the iron retention in different groups of beans. After **soaking,** TR values ranged from 98.8–108.4%. TR in conventional varieties was significantly higher compared to the *lpa* and biofortified varieties ($p<0.05$). Iron levels after soaking ranged from 27.5 to 39.1 ppm for fresh weight (FW) and 64.2 to 91.2 ppm based on dry weight (DW) (Supplementary table A1). TR values after **boiling** beans were 77.2–91.3%, whereas AR values were 104.8–119.6%. Conventional varieties had a significantly higher AR and TR compared to the *lpa* varieties. Biofortified varieties had a higher AR and TR compared to the *lpa* varieties after boiling, but this was not always significant ($p>0.05$). Iron levels after boiling were 21.4–

33.0 ppm in FW and 59.6–90.8 ppm DW ( Supplementary table A1). TR values after **refrying** beans were 87.3–104.5%, whereas AR values were 91.4–100.5%. Conventional beans had a significantly higher TR than the biofortified and *lpa* beans after refrying of non-soaked beans($p<0.05$). Iron levels after refrying were ranging from 18.0–27.8 ppm in FW and 48.9–74.5 ppm in DM. There were no significant differences found in the TR or AR for iron between **soaked and non-soaked** beans for both boiled and refried ($p>0.05$). Thus, soaking does not influence iron levels when boiling or refrying beans.

Overall, the iron loss after processing was low. In this study we found an average loss of 16% after boiling, and a 9% loss after refrying the beans based on TR. No major differences were found between the different groups of beans, indicating that the *lpa* beans do not show a different pattern in retention compared to biofortified or conventional beans concerning iron retention.

**Table 5.** Fresh and dry weight (ppm ± SD) and true and apparent retention (% ± SD) for iron of threegroups of beans after five different processing steps.

| | | **True retention (% ± SD)** | | **Apparent retention (% ± SD)** | |
|---|---|---|---|---|---|
| | | **Soaked** | **Non-soaked** | **Soaked** | **Non-soaked** |
| *Soaking* | Conventional | 108.4±7.0a | - | - | - |
| | Biofortified | 97.8±2.7b | - | - | - |
| | *lpa* | 98.8±5.6b | - | - | - |
| *Boiling* | Conventional | 87.8±2.0a | 91.3±9.8a | 119.3±3.2a | 119.6±11.3a |
| | Biofortified | 86.6±2.5a | 82.1±3.5ab | 118.2±3.6a | 112.4±3.14b |
| | *lpa* | 77.2±3.4b | 77.7±5.3b | 105.7±3.8b | 104.8±6.0b |
| *Refrying* | Conventional | 97.9±11.5a | 104.5±5.3a | 100.5±9.7a | 97.7±5.3a |
| | Biofortified | 93.4±5.1a | 91.2±2.9b | 97.0±2.8a | 93.0±2.7a |
| | *lpa* | 87.3±6.7a | 92.3±3.7b | 91.4±6.4a | 93.6±3.1a |

Different letters (a,b,c) indicate significant differences between the different groups in the column (per treatment). ($p<0.05$). Values are average ± standard deviation.

## Zinc

Table 6 presents the zinc retention in different groups of beans. After **soaking,** TR values ranged from 93.3 to 99.4%. On group level, no significant differences in TR were found after soaking beans. Zinc levels after soaking were 11.7–16.5 in FW and 30.1–38.5 ppm in DM ( Supplementary table A2).

TR values after boiling beans ranged from 41.2 to 84.0%, whereas AR values were between 49.3 and 96.2%. After boiling, a decrease of ~50% was found in the TR and AR of *lpa* varieties. No differences were found in the TR and AR between conventional and biofortified varieties. Zinc levels after boiling ranged from

5.4–14.0 ppm in FW and 14.9–37.5 ppm in DM. TR values after refrying beans were 63.5–100.0%, whereas AR values were 58.1–81.4%. Both groups of soaked and non-soaked *lpa* beans had different ($p<.05$) AR and TR compared to the conventional and biofortified varieties. The highest difference was in TR after refrying and soaking; a 23% lower retention was recorded in *lpa* beans compared to conventional beans. Zinc levels after refrying were 6.4–12.3 ppm in FW and 17.6–31.5 ppm in DM.

Refrying increased the TR for zinc to an average of 85% for the soaked beans and 95% for the non-soaked beans ($p<0.05$). A significant difference was found in TR after soaking the beans for both boiling and refrying ($P>0.05$), where we found a higher retention in the non-soaked beans (data not shown).

Overall, we can conclude that zinc retention is low when compared to iron retention. An average of 4.6% of zinc is lost during soaking. During boiling retention is very low for the *lpa* varieties (average loss of 56%), especially in comparison with the conventional and biofortified varieties (average loss of 20%). After refrying, zinc retention is increased but still lower in the *lpa* beans, (29% loss) compared to the other varieties (9% loss) ($p<0.05$).

**Table 6.** Fresh and dry weight (ppm ± SD) and true and apparent retention (% ± SD) for zinc of three groups of beans after five different processing steps.

| | | **True retention (% ± SD)** | | **Apparent retention (% ± SD)** | |
|---|---|---|---|---|---|
| | | **Soaked** | **Non-soaked** | **Soaked** | **Non-soaked** |
| *Soaking* | Conventional | 99.4±2.3a | - | - | - |
| | Biofortified | 93.3±4.7a | - | - | - |
| | *lpa* | 93.6±3.2a | - | - | - |
| *Boiling* | Conventional | 75.3±4.3a | 84.0±5.6a | 89.0±5.4a | 95.8±5.7a |
| | Biofortified | 77.9±2.8a | 81.1±6.3a | 92.3±2.8a | 96.2±4.4a |
| | *lpa* | 41.2±4.2b | 46.4±3.0b | 49.3±4.5b | 54.6±2.7b |
| *Refrying* | Conventional | 86.6±7.6a | 100.0±4.0a | 77.4±5.2a | 81.4±4.0a |
| | Biofortified | 85.6±2.5a | 91.3±3.7b | 77.3±3.3a | 80.8±3.9a |
| | *lpa* | 63.5±6.6b | 77.7±3.1c | 58.1±5.6b | 68.8±2.6b |

Different letters (a,b,c) indicate significant differences between the different groups in the column (per treatment). ($p<0.05$). Values are averages ± standard deviation.

**Total phytic acid**

Table 7 presents an overview of phytic acid retention in different groups of beans. After **soaking,** TR values ranged from 65.6 to 88.5%. TR for phytates were significant lower for conventional beans compared to *lpa* and biofortified beans ($p<0.005$). Total phytic acid levels after soaking were 0.4–7.0 mg $g^{-1}$ for FW and 1.0–16.0 mg $g^{-1}$ in DM. The TR values after boiling beans were 66.9–79.5%, whereas AR values were 75.5–93.4%. l*pa* beans have a significantly higher retention of phytates, compared to the other varieties. However, the absolute levels of phytic acid are still about 10% of that in the other groups of beans. Soaking beans led to a significantly lower retention of phytic acid after boiling compared to non-soaked beans ($p<0.05$) (data not shown). Phytic acid levels after boiling were 0.33–4.70 mg $g^{-1}$ for FW and 0.92–12.81 mg $g^{-1}$ in DM (Supplementary table A3). The TR values after refrying beans were 59.7–86.9%, whereas AR values were 53.6–77.0%, which means a substantial loss of 13–40% of phytic acid. Refrying increased the zinc TR with an average of 7% compared to boiling. Phytic acid levels after refrying were 0.28–3.98 mg $g^{-1}$ for FW and 0.72–11.24 mg $g^{-1}$ in DM. An effect on both AR and TR through soaking was observed ($p<0.05$), where the retention of phytic acid was lower after both boiling and refrying when the beans were soaked.

Overall, we found a higher retention of phytic acid in the *lpa* beans as compared to the other groups of beans but the *lpa* beans had very low phytate levels compared to the conventional and biofortified varieties. The lowest retention of phytic acid was found in the conventional varieties. Soaking helped to remove phytic acid, showed by a significantly lower TR phytate content when comparing soaked with non-soaked beans.

**Table 7.** Fresh and dry weight (mg/g ± SD) and true and apparent retention (% ± SD) for total phytic acid of three groups of beans after five different processing steps.

| | | True retention (% ± SD) | | Apparent retention (% ± SD) | |
|---|---|---|---|---|---|
| | | **Soaked** | **Non-soaked** | **Soaked** | **Non-soaked** |
| *Soaking* | Conventional | 65.7±16.7a | - | - | - |
| | Biofortified | 88.5±9.1b | - | - | - |
| | *lpa* | 83.9±4.7b | - | - | - |
| *Boiling* | Conventional | 49.9±2.1a | 64.0±9.7a | 59.0±3.2a | 73.2±12.0a |
| | Biofortified | 58.6±5.0b | 62.8±2.9a | 69.5±6.2b | 74.7±5.3a |
| | *lpa* | 72.1±5.8c | 85.9±4.7b | 86.3±6.1c | 101.3±6.5b |
| *Refrying* | Conventional | 59.7±9.5a | 77.3±11.7ab | 53.6±10.1a | 62.9±10.05a |
| | Biofortified | 65.6±7.2ab | 72.7±5.6a | 59.0±4.1ab | 64.3±4.0a |
| | *lpa* | 73.5±5.4b | 86.9±3.9b | 67.2±4.9b | 77.0±4.0b |

Different letters (a,b,c) indicate significant differences between the different groups in the column (per treatment). ($p<0.05$). Values are averages ± standard deviation.

## Contribution of beans to the Estimated Average Requirement (EAR) of iron and zinc intake

The contribution of beans to the mineral intake in populations with a regular bean consumption, either boiled or refried beans, was estimated for the different groups of beans. The *lpa* varieties of beans had a significantly higher zinc loss compared to the biofortified and conventional varieties. The differences in levels of iron and zinc and TR have an impact on the iron or zinc contribution to the EAR after consuming beans and depend on the preparation method used. Percentage contribution to the EAR was calculated considering an EAR of 4.1 mg $d^{-1}$ of iron [35] and 4 mg $d^{-1}$ of zinc for children aged 4–6 years old [36]. For adult women this was 8.1 mg $d^{-1}$ for iron [35] and 7 mg $d^{-1}$ for zinc [36]. The average FW iron/zinc content of soaked and non-soaked beans for each group of varieties was used. The average intake of dry beans for adults in Rwanda was 107 grams for children and 198 grams which is among the highest in the world [37]. For easy comparison throughout different preparation methods, we assume and compare here an intake of 50 grams of dry beans (~half cup, one portion), equivalent to 100 grams of cooked beans, and 125 grams of refried beans (based on our data). For children between 4–8 years old, we assume the portions are 55% compared to the adults, based on the Rwanda data. The contributions of the different groups of beans to the EAR of iron and zinc for children 4–8 years old and adult women can be found in Table 8. Results show that one portion could contribute up to 46% and 43% to the iron EAR for respectively children and adult women. For the zinc EAR this is 21% for both children and adult women. In both cases refried beans contribute slightly more to the EAR per portion and biofortified beans are the best source among the three groups of beans.

**Table 8.** Contribution of beans to iron and zinc EAR for children and adults

| | | Population | **Conventional** beans | **Biofortified** beans | ***lpa*** beans |
|---|---|---|---|---|---|
| **Iron** | **Boiled** | **Children 4-6** | 29% | 44% | 34% |
| | | **Adult women** | 27% | 40% | 31% |
| | **Refried** | **Children 4-6** | 31% | 46 % | 38% |
| | | **Adult women** | 29% | 43 % | 35% |
| **Zinc** | **Boiled** | **Children 4-6** | 15% | 19% | 8% |
| | | **Adult women** | 15% | 19% | 8% |
| | **Refried** | **Children 4-6** | 16% | 21% | 13% |
| | | **Adult women** | 17% | 21% | 13% |

Based on fresh weigh*portion size. 1 portion is defined as 55 g and 100 g of cooked and 68.75 g and 125 g of refried beans for respectively children and adult women.

## Mineral – phytic acid ratios of beans under study

Consequently, the bioavailability of the iron and zinc from the beans determine the actual improvement in micronutrient status of deficient populations [38]. This determines the contribution to the physiological requirements of the human body, influenced by the amount of iron or zinc ingested, absorption, metabolism, tissue distribution and excretion [39]. Bioavailability of iron and zinc has shown to be negatively influenced by, amongst others, the amount of phytic acid in the meal and in the whole diet [40]. One way to express the phytate to mineral concentration is calculating the ratios using the molecular weights of iron or zinc and phytic acid (MW=660). The phytic acid to mineral ratios for the beans under study are presented in Table 9 and show the very low ratios of 1:1 for the lpa beans compared to the conventional and biofortified varieties.

**Table 9.** Phytic acid to iron and zinc molar ratios for three groups of beans (conventional, biofortified and lpa).

| | | Phytate to Iron molar ratio | Phytate to zinc molar ratio |
|---|---|---|---|
| Boiling | **Conventional** | 15 | 36 |
| | **Biofortified** | 13 | 36 |
| | ***lpa*** | 1 | 6 |
| Refrying | **Conventional** | 16 | 37 |
| | **Biofortified** | 13 | 35 |
| | ***lpa*** | 1 | 4 |

## DISCUSSION

The levels of iron and zinc found in the dry beans are comparable with other studies [21, 22, 41]. Also, the positive trend between iron and zinc levels was shown by others [32, 41, 42]. Phytate levels found in conventional and biofortified beans in this study are comparable to other studies, where phytate concentration ranging anywhere from 4–26 mg $g^{-1}$ of beans were reported [18, 33, 43, 44].

Cooking times assessed using the Mattson cooker showed a large variation in the cooking times of the *lpa* genotypes. Heterogeneity of the grains could be a reason for the larger variability in cooking times within the *lpa* samples. Overall, cooking time results from this study should be interpreted with caution since storage time and temperature have shown to influence cooking time, however, the cooking time was within usual cooking time of beans [45, 46].

Our iron retention results are comparable to a study with non-soaked beans in Rwanda which showed a retention close to 100% after boiling the beans. In the Rwandan study cooking broth was not discarded in contrast to our study, this prevented the iron loss through the broth [19]. Carvhalo et al (2012) found that iron retention for both soaked and non-soaked bean grains of six different common bean cultivars led to a loss of 13–19% of iron in non-soaked and soaked beans which is similar to an average of 16% loss for both non-soaked and soaked beans in our study [21].

Refrying increased the iron TR, most certainly due to adding cooking broth to prepare the refried beans. This broth contained the iron that leaked into the cooking broth during boiling. To our knowledge, no other studies have reported iron retention after refrying beans.

Values > 100% for AR as reported in our study were also reported before by Ongol et al. [23] and Ferreira et al. [22]. The high AR of >100% for iron retention can possibly be explained by the leakage of solubles in the water (10.1–20.5%).

Retention of zinc was studied by Carvalho et al. and showed that zinc levels in broth after boiling beans did not differ between soaked or non-soaked beans [21]. Although we did not measure broth zinc concentrations, we did find a significant difference in zinc retention between soaked and non-soaked boiled beans, but this difference was small. In addition, Carvalho et al. concluded that most zinc remained in the bean after boiling and was concentrated in the cooked bean [21].

Refrying increased the zinc TR up to 100% for conventional non-soaked beans, this was most likely due to adding the cooking broth to prepare the refried beans. This broth contained the zinc that leaked into the cooking broth during boiling.

*lpa* genotypes showed substantial losses of zinc into the boiling water, which is partly reconstituted during refrying, where differences in retention are much smaller between the *lpa* and conventional group. No other studies have reported zinc retention after refrying beans. The higher affinity of zinc to phytic acid [47], the higher relatively high zinc amount trapped in the pericarp rich in phytic acid after soaking and steaming rice [48], and lower zinc retention in lpa beans during boiling soaked beans suggest that during soaking zinc from the cotyledon in non-lpa beans possibly interacted with the phytic acid retained during cooking preventing excessive zinc losses in the soaking and cooking water. However, phytic acid in lpa beans was found in relatively low quantities and the zinc from these beans may have not interacted much with the limited amounts of phytic acid remaining causing larger zinc losses in the soaking and cooking water. This suggestion should be studied.

Phytic acid levels were significantly reduced (>10%) by soaking in our study. Another study in different types of Canadian pulses showed only a slight increase in phytic acid after soaking a black bean variety (2.34%), and pinto bean (1.86%). A decrease in phytic acid was found for a dark red kidney bean, and a navy bean variety (-0.54% and -1.03%, respectively) [43]. A review of Haileslassie et al. compared 15 studies in which beans were soaked under various conditions. Results were ranging from no significant difference on phytate levels after soaking up to a 66% reduction in phytates after soaking in an autoclave [49].

In a study of Shi et al, cooking various bean varieties resulted in very modest decreases in phytic acid. Compared to the raw values for different types of beans the deceases were between -2.29% and -0.29% [43]. This is very minimal in comparison with our study where phytates were reduced up to 50% after boiling.

For the soaked samples, the soaking water was discarded and therefore higher losses of phytates were reported in comparison with the non-soaked beans when preparing boiled and refried beans. No other studies have published phytate retention after refrying beans.

Phytic acid in beans is mainly found in the protein bodies of the cotyledon where it is possibly bound to the iron present in the bean [50]. Our analysis quantified the total amount of phytates in the samples. Phytic acid could not be distinguished from various other types of dephosphorylated forms of phytates. These other compounds not necessarily inhibit mineral absorption to the same extent and could lead to an overestimation of their actual effect. Future research could identify the type of phytates present in the different types of beans, as this might be another angle of explaining the differences in retention and eventually the effect on the bioavailability of minerals to the human body [40].

The molar ratios of phytate to iron found in this study are comparable to other studies where *lpa* beans were consumed by different groups of women to compare the iron bioavailability from different types of bean seeds [33, 34]. Studies have shown that lpa beans have a higher iron bioavailability caused by the low concentration of phytates compared to conventional beans [33, 34]. No data was found on the zinc bioavailability from lpa beans.

A multiple meal isotope bean study showed that both biofortified and *lpa* beans provided more bioavailable iron in comparison with conventional beans, however there was no difference in fractional iron absorption [34]. In another single meal study, a 50–60% higher fractional absorption was found for *lpa* beans, compared to conventional beans. In addition it was reported that studies based on single meals often exaggerate the inhibiting effect of phytate on absorption of both iron and zinc [40]. One study used dephytinized beans (95% phytate reduction) and compared these to conventional and biofortified varieties for the fractional iron absorption in a multiple meal study [51]. Results showed a fractional absorption of iron of 13.2, 9.2 and 7.1% for respectively dephytinized, biofortified and conventional beans. When these results are extrapolated to findings from our study, one portion of boiled beans could contribute for 14, 16 and 23% for respectively conventional, biofortified and *lpa* beans (taken as 95% dephytinized beans) of the physiological requirements of iron in an adult woman. Hence, overall results are inconclusive but data from the here referenced studies indicate that the *lpa* trait is promising and of public health relevance, especially in settings with a high iron deficiency prevalence, a high phytate diet, and a high consumption of beans.

In addition, the phytic acid content of the whole diet has shown to be of influence on especially the zinc bioavailability from beans. Absorption from diets with a phytate to zinc ratio of 12–15 compared to a ratio of 5 was approximately 50% less [52]. For iron, an increase in bioavailability influenced by phytate ratios is only found at very low ratios of 0.4–1.0 [53]. This means that when *lpa* beans would replace conventional beans and would be added to an already low-phytate diet this could potentially increase the absorption of both iron and zinc significantly. Further research is needed to confirm and to show to what extent low phytate-mineral ratios in beans lead to a higher bioavailability of iron and zinc, when part of a whole diet.

## CONCLUSIONS

This is the first retention study on beans including *lpa* lines and comparing these with biofortified and conventional beans. Our results show a relatively high retention for the conventional and biofortified varieties after processing, consistent with literature. In contrast, *lpa* varieties have extremely low phytate levels and a much lower retention of zinc, compared to the other groups of beans. More research is needed into the 1) binding of iron and zinc in the beans by phytates and 2) the exact types of phytates in the different groups of beans. This will likely further explain our findings. Furthermore, our findings imply that soaking should be more widely promoted as a means to decrease phytate content of beans as this is likely to improve the bioavailability of iron and zinc.

There is no consensus yet on to what extent phytates influence the bioavailability of the minerals in the different types of beans and as part of a whole diet. However, different studies showed that lower phytate:iron/zinc molar ratios in beans have a higher fractional absorption of iron and therefore the *lpa* lines are promising in contributing to the iron and zinc intake.

Developing beans with an increased mineral content combined with a low phytate trait and shorter cooking times could be the next generation of biofortified beans attractive for consumers and lead to a higher nutritional intake compared to the beans currently on the markets.

## ACKNOWLEDGEMENTS

We would like to acknowledge the assistance of Gelver Patiño Lenis and Yolanda Ramirez in performing the experiment, and Juan Camilo Orozco Ágredo in performing phytate analyses. Furthermore, we would like to thank James Stangoulis and Lachlan Palmer from Flinders University, Australia for performing the iron and zinc analyses. We would also like to thank the Instituto De Ciencia Y Tecnología Agrícolas in Guatemala for providing us with the ICTA Chorti beans.

# REFERENCES


1. Wessells KR, Singh GM, Brown KH. Estimating the global prevalence of inadequate zinc intake from national food balance sheets: effects of methodological assumptions. PLoS One. 2012;7(11):e50565.
2. Stoltzfus RJ. Iron deficiency: global prevalence and consequences. Food and nutrition bulletin. 2003;24(4_suppl2):S99-S103.
3. McLean E, Cogswell M, Egli I, Wojdyla D, De Benoist B. Worldwide prevalence of anaemia, WHO vitamin and mineral nutrition information system, 1993–2005. Public health nutrition. 2009;12(4):444-54.
4. Stoltzfus RJ. Defining iron-deficiency anemia in public health terms: a time for reflection. The Journal of nutrition. 2001;131(2):565S-7S.
5. Hambidge M. Human zinc deficiency. The Journal of nutrition. 2000;130(5):1344S-9S.
6. Haas JD, Brownlie IV T. Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. The Journal of nutrition. 2001;131(2):676S-90S.
7. Welch RM, Graham RD. Breeding crops for enhanced micronutrient content. Food Security in Nutrient-Stressed Environments: Exploiting Plants' Genetic Capabilities: Springer; 2002. p. 267-76.
8. Petry N, Boy E, Wirth JP, Hurrell RF. The potential of the common bean (Phaseolus vulgaris) as a vehicle for iron biofortification. Nutrients. 2015;7(2):1144-73.
9. Broughton WJ, Hernandez G, Blair M, Beebe S, Gepts P, Vanderleyden J. Beans (Phaseolus spp.)–model food legumes. Plant and soil. 2003;252(1):55-128.
10. Hayat I, Ahmad A, Masud T, Ahmed A, Bashir S. Nutritional and health perspectives of beans (Phaseolus vulgaris L.): an overview. Critical reviews in food science and nutrition. 2014;54(5):580-92.
11. Bouis HE, Welch RM. Biofortification—a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. Crop Science. 2010;50(Supplement_1):S-20-S-32.
12. Bouis HE, Saltzman A. Improving nutrition through biofortification: a review of evidence from HarvestPlus, 2003 through 2016. Global food security. 2017;12:49-58.
13. Saltzman A, Birol E, Oparinde A, Andersson MS, Asare-Marfo D, Diressie MT, et al. Availability, production, and consumption of crops biofortified by plant breeding: current evidence and future potential. Annals of the New York Academy of Sciences. 2017;1390(1):104-14.
14. Hotz C, McClafferty B. From harvest to health: challenges for developing biofortified staple foods and determining their impact on micronutrient status. Food and Nutrition Bulletin. 2007;28(2_suppl2):S271-S9.
15. Haas JD, Luna SV, Lung'aho MG, Wenger MJ, Murray-Kolb LE, Beebe S, et al. Consuming Iron Biofortified Beans Increases Iron Status in Rwandan Women after 128 Days in a Randomized Controlled Feeding Trial–3. The Journal of nutrition. 2016;146(8):1586-92.
16. La Frano MR, Moura FF, Boy E, Lönnerdal B, Burri BJ. Bioavailability of iron, zinc, and provitamin A carotenoids in biofortified staple crops. Nutrition reviews. 2014;72(5):289-307.
17. Campion B, Sparvoli F, Doria E, Tagliabue G, Galasso I, Fileppi M, et al. Isolation and characterisation of an lpa (low phytic acid) mutant in common bean (Phaseolus vulgaris L.). Theoretical and Applied Genetics. 2009;118(6):1211-21.
18. Petry N, Boy E, Wirth JP, Hurrell RF. Review: The Potential of the Common Bean (Phaseolus vulgaris) as a Vehicle for Iron Biofortification. Nutrients. 2015;7(2):1144-73.
19. Bechoff A, Taleon V, Carvalho L, Carvalho J, Boy E. Micronutrient (provitamin A and iron/zinc) retention in biofortified crops. African Journal of Food, Agriculture, Nutrition and Development. 2017;17(2):11893-904.
20. Díaz-Gómez J, Twyman RM, Zhu C, Farré G, Serrano JC, Portero-Otin M, et al. Biofortification of crops with nutrients: factors affecting utilization and storage. Current opinion in biotechnology. 2017;44:115-23.
21. Carvalho LJ, Corrêa M, Pereira E, Nutti M, Carvalho JV, Ribeiro EG, et al. Iron and zinc retention in common beans (Phaseolus vulgaris L.) after home cooking. Food & nutrition research. 2012;56(1):15618.
22. Ferreira AS, Naozuka J, Kelmer GA, Oliveira PV. Effects of the Domestic Cooking on Elemental Chemical Composition of Beans Species (Phaseolus vulgaris L.). Journal of Food Processing. 2014;2014.
23. Ongol MP, Owino J, Lung'aho M, Dusingizimana T, Vasanthakaalam H. Micro-Mineral Retention and Anti-Nutritional Compounds Degradation during Bean Cooking Process. CURRENT RESEARCH IN NUTRITION AND FOOD SCIENCE. 2018;6(2):526-35.
24. ICA. Registro de la Variedad de Frijol FNC BIOFORTIFICADO 101. . Bogota, Colombia; 2016.

25. ICA. Registro de la Variedad de Frijol FNC BIOFORTIFICADO 107. Bogota, Colombia; 2016.
26. ICTA. ICTA Chortí -Variedad de frijol arbustivo Biofortificada con hierro y zinc. In: ICTA, editor. Villa Nueva, Guatemala2017.
27. Wang N, Daun JK. Determination of cooking times of pulses using an automated Mattson cooker apparatus. Journal of the Science of Food and Agriculture. 2005;85(10):1631-5.
28. Wheal MS, Fowles TO, Palmer LT. A cost-effective acid digestion method using closed polypropylene tubes for inductively coupled plasma optical emission spectrometry (ICP-OES) analysis of plant essential elements. Analytical Methods. 2011;3(12):2854-63.
29. Pfeiffer WH, McClafferty B. HarvestPlus: breeding crops for better nutrition. Crop Science. 2007;47(Supplement_3):S-88-S-105.
30. Park S-Y, Lee SM, Lee J-H, Ko H-S, Kweon SJ, Suh S-C, et al. Compositional comparative analysis between insect-resistant rice (Oryza sativa L.) with a synthetic cry1Ac gene and its non-transgenic counterpart. Plant biotechnology reports. 2012;6(1):29-37.
31. Murphy EW, Criner PE, Gray BC. Comparisons of methods for calculating retentions of nutrients in cooked foods. Journal of Agricultural and Food Chemistry. 1975;23(6):1153-7.
32. Hoppler M, Egli I, Petry N, Gille D, Zeder C, Walczyk T, et al. Iron speciation in beans (Phaseolus vulgaris) biofortified by common breeding. Journal of food science. 2014;79(9):C1629-C34.
33. Petry N, Egli I, Campion B, Nielsen E, Hurrell R. Genetic reduction of phytate in common bean (Phaseolus vulgaris L.) seeds increases iron absorption in young women. The Journal of nutrition. 2013;143(8):1219-24.
34. Petry N, Rohner F, Gahutu JB, Campion B, Boy E, Tugirimana PL, et al. In Rwandese women with low iron s tatus, iron absorption from low-phytic acid beans and biofortified beans is comparable, but low-phytic acid beans cause adverse gastrointestinal symptoms. The Journal of nutrition. 2016;146(5):970-5.
35. Trumbo P, Yates AA, Schlicker S, Poos M. Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington (DC): Institute of Medicine (US) Panel on Micronutrients. ; 2001. Report No.: 2212-2672 Contract No.: 3.
36. Hotz C, Brown KH. Assessment of the risk of zinc deficiency in populations and options for its control. 2004.
37. Mulambu J, Andersson M, Palenberg M, Pfeiffer W, Saltzman A, Birol E, et al. Iron beans in Rwanda: crop development and delivery experience. African journal of food, agriculture, nutrition and development. 2017;17(2):12026-50.
38. Saltzman A, Birol E, Bouis HE, Boy E, De Moura FF, Islam Y, et al. Biofortification: progress toward a more nourishing future. Global Food Security. 2013;2(1):9-17.
39. Caballero B, Trugo LC, Finglas PM. Encyclopedia of food sciences and nutrition: Academic; 2003.
40. Gibson RS, Raboy V, King JC. Implications of phytate in plant-based foods for iron and zinc bioavailability, setting dietary requirements, and formulating programs and policies. Nutrition reviews. 2018;76(11):793-804.
41. Beebe S, Gonzalez AV, Rengifo J. Research on trace minerals in the common bean. Food and Nutrition Bulletin. 2000;21(4):387-91.
42. Blair MW, Medina JI, Astudillo C, Rengifo J, Beebe SE, Machado G, et al. QTL for seed iron and zinc concentration and content in a Mesoamerican common bean (Phaseolus vulgaris L.) population. Theoretical and Applied Genetics. 2010;121(6):1059-70.
43. Shi L, Arntfield SD, Nickerson M. Changes in levels of phytic acid, lectins and oxalates during soaking and cooking of Canadian pulses. Food Research International. 2018;107:660-8.
44. El Tinay A, Mahgoub S, Mohamed B, Hamad M. Proximate composition and mineral and phytate contents of legumes grown in Sudan. Journal of food Composition and Analysis. 1989;2(1):69-78.
45. Nasar-Abbas SM, Plummer JA, Siddique KH, White P, Harris D, Dods K. Cooking quality of faba bean after storage at high temperature and the role of lignins and other phenolics in bean hardening. LWT-Food Science and Technology. 2008;41(7):1260-7.
46. Jackson GM, Varriano-Marston E. Hard-to-cook phenomenon in beans: Effects of accelerated storage on water absorption and cooking time. Journal of Food Science. 1981;46(3):799-803.
47. Chan H-c. Phytate and cation binding activity: Texas Tech University; 1988.
48. Hotz C, Kabir KA, Dipti SS, Arsenault JE, Bipul M. Rice fortification with zinc during parboiling may improve the adequacy of zinc intakes in Bangladesh. Journal of the Science of Food and Agriculture. 2015;95(2):379-85.

49. Haileslassie HA, Henry CJ, Tyler RT. Impact of household food processing strategies on antinutrient (phytate, tannin and polyphenol) contents of chickpeas (Cicerarietinum L.) and beans (Phaseolus vulgaris L.): a review. International Journal of Food Science & Technology. 2016;51(9):1947-57.
50. Ariza-Nieto M, Blair MW, Welch RM, Glahn RP. Screening of iron bioavailability patterns in eight bean (Phaseolus vulgaris L.) genotypes using the Caco-2 cell in vitro model. J of Agricultural and Food Chemistry. 2007;55(19):7950-6.
51. Petry N, Egli I, Gahutu JB, Tugirimana PL, Boy E, Hurrell R. Phytic acid concentration influences iron bioavailability from biofortified beans in Rwandese women with low iron status. The Journal of nutrition. 2014;144(11):1681-7.
52. Sandström B, Almgren A, Kivistö B, Cederblad Å. Zinc absorption in humans from meals based on rye, barley, oatmeal, triticale and whole wheat. The Journal of nutrition. 1987;117(11):1898-902.
53. Hurrell. Phytic acid degradation as a means of improving iron absorption. International Journal for Vitamin and Nutrition Research. 2004;74(6):445-52.

# APPENDICES

**Supplementary table A1.** Fresh and dry weight (ppm ± SD) for iron of three groups of beans after five different processing steps.

| | | Fresh weight (ppm ± SD) | | Dry weight (ppm ± SD) | |
|---|---|---|---|---|---|
| | | **Soaked** | **Non-soaked** | **Soaked** | **Non-soaked** |
| *Soaking* | Conventional | 27.50±1.42a | - | 64.18 ± 2.66a | - |
| | Biofortified | 39.05±1.97b | - | 91.28±3.11b | - |
| | *lpa* | 30.48±1.52a | - | 78.48±4.57c | - |
| *Boiling* | Conventional | 22.48±1.92a | 21.38±2.12a | 59.70±5.52a | 59.60±5.08a |
| | Biofortified | 32.05±1.14b | 32.98±1.43b | 90.75±2.18b | 86.32±1.65b |
| | *lpa* | 26.08±1.69c | 24.65±0.96c | 68.75±3.22c | 68.15±4.59c |
| *Refrying* | Conventional | 19.50±2.63a | 17.95±1.96a | 50.20±5.34a | 48.90±5.38a |
| | Biofortified | 27.78±1.09b | 27.45±0.39b | 74.48±1.66b | 71.42±1.78b |
| | *lpa* | 23.88±0.53c | 21.50±1.70c | 59.43±4.83c | 60.88±2.68c |

Different letters (a,b,c) indicate significant differences between the different groups in the column (per treatment). ($p<0.05$). Values are average ± standard deviation.

**Supplementary table A2.** Fresh and dry weight (ppm ± SD) for zinc of three groups of beans after five different processing steps.

| | | Fresh weight (ppm ± SD) | | Dry weight (ppm ± SD) | |
|---|---|---|---|---|---|
| | | **Soaked** | **Non-soaked** | **Soaked** | **Non-soaked** |
| *Soaking* | Conventional | 13.65±0.55a | - | 31.85±0.68a | - |
| | Biofortified | 16.45±1.88b | - | 38.47±4.03b | - |
| | *lpa* | 11.7±0.81a | - | 30.13±2.29a | - |
| *Boiling* | Conventional | 9.90±0.93a | 11.20±0.8a | 27.70±2.50a | 29.73±2.09a |
| | Biofortified | 13.12±1.00b | 14.00±1.46b | 36.07±2.77b | 37.53±2.84b |
| | *lpa* | 5.38±0.69c | 6.33±0.67c | 14.88±2.08c | 16.53±1.70c |
| *Refrying* | Conventional | 8.60±0.68a | 10.10±0.96a | 24.03±1.83a | 25.28±1.87a |
| | Biofortified | 11.12±1.12b | 12.27±0.83b | 30.23±2.46b | 31.52±1.71b |
| | *lpa* | 6.35±0.94c | 8.15±0.45c | 17.60±2.64c | 20.80±1.58c |

Different letters (a,b,c) indicate significant differences between the different groups in the column (per treatment). ($p<0.05$). Values are averages ± standard deviation.

**Supplementary table A3.** Fresh and dry weight (mg/g ± SD) for total phytic acidof three groups of beans after fivedifferent processing steps.

| | | Fresh weight (mg/g ± SD) | | Dry weight (mg/g ± SD) | |
|---|---|---|---|---|---|
| | | **Soaked** | **Non-soaked** | **Soaked** | **Non-soaked** |
| *Soaking* | Conventional | 4.60±0.91a | - | 10.74±1.91a | - |
| | Biofortified | 6.98±0.83b | - | 16.04±1.9b | - |
| | *lpa* | 0.40±0.00c | - | 0.96±0.03c | - |
| *Boiling* | Conventional | 3.38±0.21a | 4.38±0.42a | 9.47±0.61a | 11.66±1.06a |
| | Biofortified | 4.70±0.70b | 5.03±1.08a | 12.81±1.7b | 13.81±3.48a |
| | *lpa* | 0.33±0.05c | 0.40±0.00b | 0.92±0.09c | 1.08±0.04b |
| *Refrying* | Conventional | 3.03±0.33a | 3.98±0.43a | 8.53±0.91a | 10.02±0.82a |
| | Biofortified | 4.03±0.75b | 4.3±0.84a | 10.79±2.16b | 11.24±2.35a |
| | *lpa* | 0.28±0.05c | 0.3±0.00b | 0.72±0.07c | 0.82±0.04b |

Different letters (a,b,c) indicate significant differences between the different groups in the column (per treatment). ($p<0.05$).

Values are averages ± standard deviation.

# Chapter 4

## Sensory and cultural acceptability trade-offs with nutritional content of biofortified orange-fleshed sweetpotato varieties amongst households with children in Malawi

Marijke Hummel
Elise F. Talsma
Ati Van der Honing
Arthur Chibwana Gama
Daniel Van Vugt
Inge D. Brouwer
Charles Spillane

*PLOS One, 2018, 13(10): e0204754*
*doi:* [10.1371/journal.pone.0204754](10.1371/journal.pone.0204754)

## ABSTRACT

Biofortified orange-fleshed sweetpotato (OFSP) varieties are being promoted to reduce vitamin A deficiencies due to their higher beta-carotene content. For OFSP varieties to have impact they need to be accepted and consumed at scale amongst populations suffering from vitamin A deficiencies. We investigated the sensory and cultural acceptability of OFSP varieties amongst households with children aged between 2-5 years old in two areas in Central and Southern Malawi using an integrated model of the Theory of Planned Behavior (TPB) and the Health Belief Model (HBM). Sensory acceptability was measured using a triangle, preference and acceptance test using three OFSP varieties and one control variety, among 270 adults and 60 children. Based on a food ethnographic study, a questionnaire on cultural acceptability was developed and administered to 302 caretakers. Data were analyzed by calculating Spearman's correlations between constructs and multiple linear regression modeling. The sensory evaluation indicates that all three OFSP varieties are accepted (scores >3 on 5-point scale), but there is a preference for the control variety over the three OFSP varieties. Almost all caretakers are intending to frequently prepare OFSP for their child in future (97%). Based on regression analysis, the constructs 'subjective norms' ($\beta$=0.25, p=0.00) reflecting social pressure, and 'attitudes toward behavior' ($\beta$=0.14 p=0.01), reflecting the feelings towards serving their child OFSP, were the best predictors for caretakers' behavior to prepare OFSP for their child.

**Conclusions:** Our study shows that both sensory and cultural attributes can influence acceptability of varieties and consumption amongst households with children. Considering these attributes can improve the impact of biofortified crops in future programming, by reducing Vitamin A deficiencies through the intake of these nutrient-rich crops.

## INTRODUCTION

Sweetpotato (*Ipomoea batatas (L.) Lam*) is one of world's most important crops for food and nutrition security, particularly in Sub-Saharan Africa, parts of Asia, and the Pacific Islands [1, 2]. Malawi is the main producer of sweetpotatoes in Sub-Saharan Africa, with an average production of 3.9 million ton per year in the period 2012-2014 [2]. From a nutrition perspective, sweetpotato roots are a good source of carbohydrates, fiber and vitamins B, C and E [3]. Most of the varieties of sweetpotato currently grown and consumed in Sub-Saharan Africa are white- or yellow-fleshed, and contain little beta-carotene [2]. In recent years, breeding programs have developed improved biofortified orange-fleshed sweetpotato (OFSP) varieties that are a good source of beta-carotene, a precursor of vitamin A [4].

Vitamin A deficiency is one of the major nutritional deficiencies in the world, affecting 190 million preschool children globally [5]. Micronutrient surveys conducted in Malawi in 2001 and 2009 reported that 59% and 23% of preschool children were vitamin A deficient [6]. Recent data on vitamin A deficiency however suggests that only 4% of preschool children living in rural areas in Malawi are vitamin A deficient [7], which is defined by the World Health Organization as a mild public health problem [8]. Possible explanations for this drop in deficiency rates could be the mandatory vitamin A fortification of oil and sugar in Malawi since 2015. Only 67% of preschool children received a vitamin A capsule in the last 6 months [9], hence there remains a need for a more sustainable and cost effective approach to reduce vitamin A nutrition deficiencies.

Biofortification strategies to improve human nutrition can be complementary to supplementation, dietary diversification, and fortification initiatives to combat vitamin A deficiency. Biofortification is a food-based approach to combat micronutrient malnutrition through breeding staple crops that have higher levels of micronutrients (e.g., iron, zinc, beta-carotene). Biofortification has been shown to be effective to alleviate micronutrient deficiencies in several populations [10]. The HarvestPlus Program has met pre-set breeding goals for OFSP with a beta-carotene level of 3200 ug/100g OFSP, to meet the daily requirements for vitamin A when consuming 100 grams of OFSP per day for a child aged 4-6 years [10]. The consumed beta-carotene is converted in the human body to vitamin A, which is one of the essential micronutrients for human nutrition [11]. Hence, OFSP consumption has major potential to contribute to decreases in vitamin A deficiency rates in children as well as adults, which has been shown by both efficacy and effectiveness studies [4, 12].

The first OFSP variety Zondeni was locally available in farmers' fields and officially recognized in Malawi in 2008, followed by an additional five varieties that were released in 2011 through a breeding program [13]. The OFSP varieties have different visual phenotypes and taste than the pre-existing varieties of sweetpotato used by farmers. The orange color intensity of OFSP varieties is associated with higher beta-carotene levels and lower dry matter content [14, 15]. Such trait changes can influence sensory and cultural acceptability, where newly introduced varieties have to remain acceptable to consumers if they were to have the intended effect of improving the vitamin A status of the target consumers. As acceptability can differ due to cultural and demographic factors, it is important to conduct research on each country-crop combination [16]. Talsma *et al.* have reviewed nine studies on the sociocultural drivers and determinants of acceptance and adoption of OFSP [17]. Overall, these studies indicated that acceptability and adoption of OFSP were high in areas where it was promoted. While OFSP has been promoted throughout Malawi since 2009, no in-depth research has been published on identifying factors that can influence the acceptability of consuming OFSP. To assess such cultural acceptability an integrated model, combining the Theory of Planned Behavior (TPB) and the Health Belief Model (HBM), can be used to investigate food or health-related behavior [18].

The TPB model assumes that the intention to perform a behavior, in our case consumption of OFSP, is closely related to the behavior itself. The intention to perform this behavior can be predicted by attitudes toward the behavior, subjective norms and perceived behavioral control [19]. The HBM is used for explaining and predicting acceptance of health-related recommendations. It combines individual perceptions and modifying factors to a likelihood of action, eg. of adopting a certain behavior, in our case OFSP consumption. The most important elements are the perceived susceptibility and threat of the health problem, the cues to action to adopt the behavior and the perceived benefits of the preventive action [20]. This combined TPB/HBM model has been used to investigate the acceptance of foods such as amaranth, iron fortified soya sauce, fonio and yellow cassava, [18, 21-23], but has to date not been used to investigate the acceptability of OFSP.

The aim of our study was to investigate the sensory and cultural acceptability of OFSP amongst households with children between 2-5 years old in two areas in Central and Southern Malawi using the integrated model of the TPB and HBM.

## MATERIALS AND METHODS

### Ethics statement

Written informed consents were collected among research participants or caretakers before the start of the study and all children were asked for their verbal consent. Ethical clearance for this research project was obtained from the National University of Ireland Galway Research Ethics Committee (Reference 16/FEB/07) and the National Commission of Science and Technology in Lilongwe, Malawi (Protocol number P.06/16/114.).

### Study area

The research was conducted in Central and Southern Malawi in, respectively, the Mngwangwa location in Lilongwe district and Katuli location in Mangochi district. These rural research sites were selected based on their high production levels of sweetpotato and beans, the difference in culture (Chewa ethnic group in Mngwangwa and Yao ethnic group in Katuli) and the presence of collaborating organizations (International Potato Center and Concern Worldwide).

Mngwangwa is situated relatively close to the capital of Malawi, Lilongwe, at a distance of approximately 30 km. Katuli lies more isolated behind hills on 60 kilometers from Mangochi, and is bordering with Mozambique. Malawi has one rainy season stretching from December to April, followed by a long dry season [24]. Both locations can be described as rural areas where over 90% of the population is engaged in agriculture activities [25]. Major crops grown in both locations include maize and groundnuts. In Lilongwe, tobacco, beans and soy are also important crops. Diets are mainly cereal based, in which over 50% of calorie intake is from maize, with Nsima (maize flour mixed with water) as a staple, supplemented with starchy roots (cassava, potatoes), vegetables and beans [26]. Literacy is higher in Lilongwe (64.5%) than Mangochi (57.2%) [25].

### Study participants & sampling

This study consisted of two parts, the sensory evaluation and the cultural acceptability survey. To prevent bias, the two parts of the study were conducted in different areas within the locations. For the sensory study, five villages were identified in each location through random sampling where adults, preschool children aged 4-5 years and their caretakers were interviewed using the method of convenience sampling and central location testing [27]. Sample sizes were large enough to perform analyses stratified by location, except for the preference test for children, where all children were analyzed as one group.

For the cultural acceptability study, participants were selected using multistage sampling, by selecting five areas within the location (each location was subdivided in 20 areas) and then randomly selecting three villages in each selected area. Inclusion criteria for the cultural acceptability study were the presence in the household of a child between 2-5 years of age whom they were taking care of and previous exposure to the OFSP varieties. Therefore, villages were included if there had been any previous OFSP related activity (nutrition promotion, agricultural training, demonstration plots). The list of villages was composed and crosschecked by Agriculture Extension Development Coordinators and field staff from the International Potato Center of the respective areas who were responsible for implementing the OFSP related activities. Probability proportional to (population) size sampling was used to calculate the number of participants per village, in which the sample size for a sub-population (area/village) is weighted proportional to its size (evenly distributed over the 2 locations). Per location 15 villages were included. Participants within each village were randomly sampled using household lists. If the participant selected was not available a new randomly selected household was invited for the interview.

The study was conducted in the period between September and November 2016 by four trained enumerators for the sensory evaluation and five trained enumerators for the cultural acceptability study. Interviews took place in a communal place and were conducted in Chichewa or Yao depending on the preference of the participant. All questionnaires were developed in English and translated to Chichewa and Yao local languages. Correctness of translation was checked by back translation to English. Pretesting was done for both studies and resulted in small changes in explanation to the participants and language used. To assess whether study participants understood the modified 5 point Likert-scale with both faces and checks (i.e. √ symbols) that was used for both parts of the study, an example question was asked which was not related to the research. Caretakers decided about which child the interview was with, when there was more than 1 child eligible; this decision was based on availability of the child to attend the interview. Reasons for exclusion were if the participant did not understand the scale or if the mother could not present a proof of birth date for the child.

## Study measurements

### Sensory evaluation

For the sensory evaluation one control yellow-fleshed sweetpotato variety (Kenya) and three OFSP varieties (Kadyaubwerere, Chipika and Zondeni) were peeled and cut into roughly equal sized portions between 25 and 40 grams. All varieties were harvested in the dry season from the same irrigated farmer's field at the Chiwamba location in Lilongwe district, and stored for the same number of days before the tasting took place.

The Kenya variety is a yellow sweetpotato that is widely available in Malawi, and therefore the control variety. Kadyaubwerere is a high-yielding OFSP variety, variety Chipika is a more drought-resistant OFSP, whereas Zondeni is the oldest OFSP variety available in Malawi, and has lower yields [28]. These were boiled until the texture, assessed by a fork by the researchers, was considered right for consumption [29]. To assess sensory acceptability three different tests were done. All participants did a preference test (n=270), followed by either a triangle (n=66) or an acceptance test (n=210).

A triangle test (n=66) was conducted with the control variety and Kadyaubwerere OFSP variety to determine whether blindfolded participants could perceive a difference in the taste between two sweetpotato varieties. Previous studies indicate that 24-30 participants for a difference test are sufficient to determine a statistical significance for noticeable differences in sensory testing [30]. Three samples of sweetpotato were presented to the blindfolded participant, who was asked to taste and identify the odd sample [27, 30]. A preference test was conducted with adult participants (n=270) and with 60 children of the age 4-5 years to study the preference between the control variety and the Kadyaubwerere OFSP variety. Sample sizes over n=20 are possible to analyze, but the ideal sample size is >100 participants when the binomial distribution is very equal to the normal distribution [27]. Participants were presented two samples for tasting and they indicated whether they preferred the OFSP or the control variety and for what reasons which was noted by the enumerators. The same procedure was used for children between 4-5 years [31]. An acceptance test (n=210) was conducted to evaluate the overall liking of sweetpotato, as well as the liking of the following attributes; taste, colour, smell, texture, starchiness and sweetness. Four sweetpotato varieties were presented to the participants, one control yellow variety of sweetpotato (Kenya) and the three OFSP varieties, Kadyaubwerere, Chipika and Zondeni. The participants were then asked to rate samples for each attribute on a 5-point modified Likert-scale with both smiley faces and checks. All test samples were color-coded and presented in random order. Participants were allowed to swallow the samples and were asked to rinse their mouth with water before the test and after tasting each sample.

**Cultural acceptability**

For the cultural acceptability survey, questionnaire-based interviews were conducted. The questionnaires consisted of two parts. In part one, information on socio-demographics were gathered, followed in part two with statements according to the 13 constructs of the TPB and HBM. These statements were identified based on literature study and a food ethnographic study, consisting of focus group discussions, a food attribute and food difference study, key informant interviews and a pile sorting session [32]. In total, 109 statements were categorized into thirteen constructs as described by Sun *et al.* and Ajzen *et al*. [18, 33]. Anticipated affect was added as an construct to this model for which it was shown that it can explain additional variance in predicting the intention to perform the behavior [33]. The construct 'attitude towards behavior' consisted of a maximum of twenty-two questions, whereas there was only one question for the construct 'health behavior identity'. Respondents were asked to respond to all statements on a 5-point modified Likert scale with both faces and checks to indicate their response ranging from "I completely disagree" to "I completely agree".

For the constructs (prior) behavior and intentional behavior a different scale was used, to reflect the frequency of (intended) consumption scored from 0-5: (0) never, (1) once a month, (2) 2-3 times a month, (3) once a week, (4) 2 or more times a week and (5) every day. The items of the majority of other constructs were scored from 1 to 5. For two constructs paired questions were asked; the construct 'attitude toward behavior' consisted of behavioral beliefs and the evaluation of these beliefs. For the construct 'subjective norms' these were the normative beliefs and motivation to comply. The scores of the beliefs were scored 1-5, whereas the evaluation of these beliefs and motivation to comply was scored with -2 to 2, after which the answers were multiplied resulting in a total score between -10 and 10. Total scores per construct were calculated for each caretaker, by adding all scores of the individual statements within a construct. The adjusted combined model of the TPB and the HBM (as we used for our study) can be found in Figure 9. In our case, compared to the original model presented by Sun et al.[18], the construct 'prior behavior' was a better predictor of behavior than the construct 'behavioral intention'. Therefore, we swapped the constructs 'behavior' and 'behavioral intention' as shown in Fig 9.

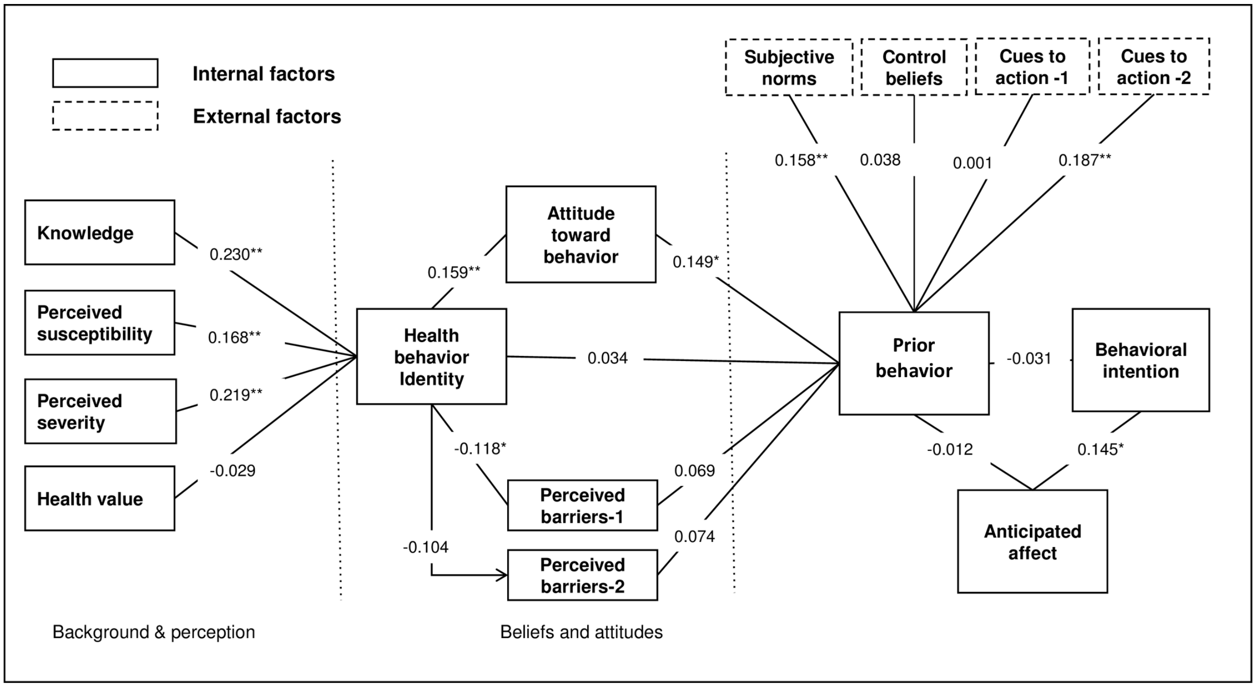



**Figure 9. Adjusted combined model of the TPB and the HBM with correlations between the various constructs.** (Based on Sun *et al.* 2006) About the model: the model is predicting behavior based on the construct 'prior behavior' (frequency of serving the child OFSP in the past when available). Prior behavior is linked to the 'behavioral intention' (intention to serve the child OFSP in future), which was the original predictor in the model of Sun et al. Both constructs can be influenced by the anticipated affect (feelings of regret when not serving the child OFSP). All other constructs are divided in three categories. 'Background and perception' consists of the constructs 'knowledge' (on vitamin A and OFSP),'perceived susceptibility' (perceptions on the susceptibility of vitamin A deficiency), 'perceived severity' (perceptions on the severity of vitamin A deficiency) and 'health value' (perceptions on the importance of health in general). These are followed by constructs around 'beliefs and attitudes', 'health behavior identity' (perception that it is healthy and good to eat OFSP), 'attitudes toward behavior' (feelings towards serving OFSP to their child) and 'perceived barriers' (perceived sensory (1) or agricultural (2)-related barriers that prevent the caretaker to serve OFSP to the child). The last category covers the external factors, 'subjective norms' (perceived social pressure on serving OFSP to their child), 'control beliefs' (perceived ability to make decisions in the household) and 'cues to action' (external triggers either (1) health-related or (2) activities that stimulate to serve OFSP to their child). *p<0.05, **p<0.01 (both two-tailed), Spearman's correlation coefficients between constructs were calculated.

**Sweetpotato measurements**

Dry matter content (%) was determined in triplicate for three randomly sampled raw roots of the four sweetpotato varieties. Dry matter content (%) was determined as the ratio of fresh weight compared to dry weight for the sweetpotato varieties. Sweetpotato samples were dried in an oven at 70 degrees °C until weight remained unchanged (on average 27 hours) [34]. Color charts were used to estimate beta-carotene levels for the different OFSP varieties [35].

## Statistical analyses

The triangle and preference tests were analyzed using a binomial distribution. Critical values to determine the number of correct or agreeing choices were retrieved from statistical tables [27]. Sample sizes were sufficient to do analyses on location level. Data from the acceptance test for individual attributes were treated as ordinal data. Non-parametric tests were used to analyze if there were significant differences between location and varieties, the Mann Whitney U test for independent samples and the Wilcoxon Rank test for paired samples. Mean liking was defined as the average of all attributes assessed.

For the cultural acceptability survey, multiple item constructs were tested for reliability using the Cronbach-$\alpha$ and item total correlation. Items were removed from the analysis in case of a low item total correlation $<0.3$ or if the Cronbach alpha of the construct increased significantly upon removal of an item. In total 25 items were excluded. For each respondent, a total score per construct was calculated by adding all individual scores. Spearman's correlation was used to calculate bivariate associations within constructs. Multiple linear regression modelling was performed to build the models. Models were adjusted for interviewer, education level, age and location (if applicable).

Statistical tests were 2-tailed and p-values $<0.05$ were considered statistically significant. All statistical analyses were performed using SPSS (version 23.0.0.2, ICM Corp. Released 2015) for Macintosh.

# RESULTS

## Sensory evaluation

To investigate the sensory acceptability of the four different sweetpotato varieties, a total of 270 adults and 60 children (who were at least once exposed to OSFSP before) participated in a range of different tests. The overall mean age of the adults was 31.9 (±10.7) years; slightly more women were included (63%). For the children, the mean age was 4.5 ±0.8 years. The percentage of participants that reported to grow OFSP was significantly different between areas, 36% in Lilongwe versus 72% in Mangochi. The consumed sweetpotatoes were mainly from own production (52%), the market (33%) or purchased from other farmers (26%). Participants reported to consume sweetpotato mostly as a breakfast dish (98%), and to a much lesser extent at lunch or dinner (respectively 14% and 13%). The most common prepared dishes were boiled OFSP (74%), boiled OFSP mixed with peanut flour (called 'Futali', 18%) and roasted OFSP (6%). As a benefit of sweetpotato, 59% of the participants reported health and nutrition related reasons, and 17% reported it as a source of income. The main reason for not consuming more sweetpotatoes was the availability (59%).

**Adults were able to discriminate the control variety from the OFSP variety when blinded**

In the triangle test, blinded participants in both areas were able to observe the difference between the orange and control sweetpotato samples, in total 49 out of 66 participants pointed out the odd sample, where 29 right answers were needed for a significant difference ($p<0.05$) (Table 10).

**Table 10.** Results for the triangle test with OFSP (Kadyaubwerere) and the control yellow sweetpotato (Kenya) variety, per location and total results

| | **Total** | **Lilongwe** | **Mangochi** |
|---|---|---|---|
| Number of participants | 66 | 36 | 30 |
| Minimum number of correct judgments needed ($\alpha$=0.05) | 29 | 18 | 15 |
| Correct judgments | 49* | 28* | 21* |
| Triangle u test: $\mu0=1/3$ | 0.74 | 0.78 | 0.70 |

* significant ($p<0.001$, $\alpha$=0.05)

**Adult consumers display preference for the non-OFSP control variety over the OFSP variety**

For the preference test all participants (n=270 adults, n=60 children) were asked about their preference for either the yellow-fleshed control variety (Kenya) or the OFSP variety (Kadyaubwerere). Amongst the adults, sixty-four participants favored the OFSP variety (24%) (Fig. 10 and S1 Table), due to sweetness (22%), odor (19%) and taste (19%). Two hundred and eleven participants favored the control variety (76%) because of sweetness (36%), starchiness (24%), and odor (13%). Color was also mentioned as a reason for preferring one of the varieties: 8% for the OFSP and 3% for the control sweetpotato variety. For the children (n=60), the OFSP variety was preferred by 35 children, although this was not statistically significant (p>0.05) (Fig. 10 and Appendix 1). No significant differences in preference were found between locations for both adults and children.

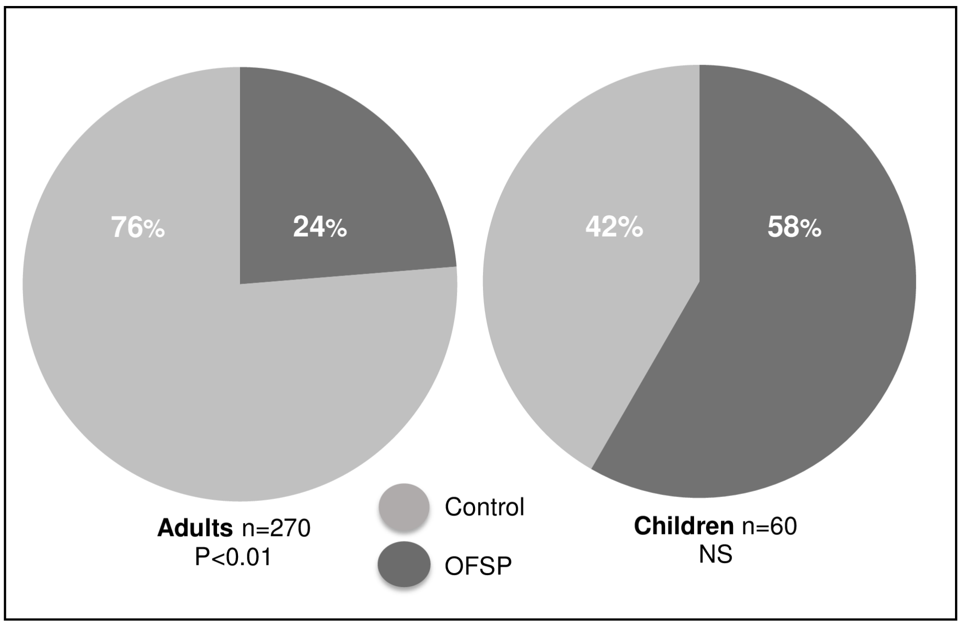



**Figure 10.** Results for the preference test with OFSP and a control sweetpotato variety. Adults significantly preferred the control variety (76.3%) over the orange variety, whereas more children preferred the OFSP (58.3%, not significant). * p<0.05

To further investigate the difference in liking between varieties for seven different attributes, an acceptance test with four different varieties of sweetpotato was conducted (Appendix 2). The color of the Zondeni and Kadyaubwere varieties were highest rated followed by the control variety Kenya; between these three no significant differences were found (p>0.05). Lowest rated was the Chipika variety, which was not significantly differently rated from the Kenya variety (p>0.05). For smell and texture only small differences were found in the liking of these attributes, for all varieties the median was 4. Hedonic scores of starchiness for the varieties Kenya and Zondeni (median 4) were significantly higher compared to Chipika and Kadyaubwerere (median 3) (p<0.05). Sweetness received a significantly different score for all varieties (p<0.05), Kenya was rated highest with a median of 5, followed by Zondeni (4), Kadyaubwerere (3) and Chipika (3). For the attribute taste, the median scores for Kenya were highest (5) and significantly higher than for the other three varieties ($p<0.05$). There was a significant difference in the scores of overall liking for all varieties ($p<0.05$), where Kenya was the preferred variety (5). No differences in liking for any of the seven attributes were found between locations for the Kenya variety. For the OFSP varieties Chipika and Kadyaubwere no difference was found for the overall liking between Lilongwe and Mangochi districts, all the other attributes were significantly differently evaluated. For the Zondeni variety a significant difference in hedonic scores between areas was found for all attributes, except for taste ($p<0.05$).

**Zondeni is the highest rated OFSP variety**

After combining all attributes into a mean score for each variety (Table 11), a significant difference was found between all varieties. Overall, the control variety Kenya is liked most. In Mangochi however, the OFSP variety Zondeni is rated higher than Kenya, but this difference is not significant. Analysis of the difference in liking between the control and the OFSP varieties shows that there is on average a 0.50-point higher rating given for the control variety. There is a significant location effect between the areas, where the scores that were given for OFSP in Mangochi are much closer to the scores given for the control varieties (mean difference -0.22) than in Lilongwe (mean difference -0.76).

**Table 11.** Results for the acceptance test with calculated means of all attributes (n=210 adults)

| | | **Total** mean (SD) | **Lilongwe** mean (SD) | **Mangochi** mean (SD) |
|---|---|---|---|---|
| **Mean of attributes** | Chipika (OFSP) | 3.28 (0.98) a | 3.08 (1.00) a | 3.49 (0.91) a* |
| | Kadyaubwerere (OFSP) | 3.51 (1.00) b | 3.34 (0.99) b | 3.69 (0.97) a* |
| | Zondeni (OFSP) | 3.98 (0.96) c | 3.60 (1.05) b | 4.04 (0.80) b* |
| | Kenya (Control) | 4.04 (0.81) d | 4.13 (0.71) c | 3.94 (0.90) b |
| | Overall liking OFSP | 3.53 (0.69) | 3.33 (0.71) | 3.74 (0.62) * |
| **Mean differences** | Chipika- Control | -0.75 (1.24) a | -1.02 (1.31) a | -0.47 (1.10) a* |
| | Kadyaubwerere - Control | -0.52 (1.23) b | -0.77 (1.13) a | -0.27 (1.28) a* |
| | Zondeni - Control | -0.21 (1.30) c | -0.50 (1.38) b | 0.09 (1.13) b* |
| | OFSP total - Control | -0.50 (1.04) b | -0.76 (1.06) | -0.22 (0.95)* |

Means with a common letter in a column do not significantly differ at $\alpha$=0.05
* Significant difference between locations $p<0.05$

**Cultural acceptability study identifies opportunities and barriers for including OFSP in children's diets by caretakers**

To investigate the cultural acceptability of the OFSP varieties, a total of 302 caretakers were interviewed in a cultural acceptability study. The mean age of study participants was 31.9 (±9.1) years, almost all women (99.7% female). A household consisted on average of 5.9 persons (±1.8), where 74% of the caretakers had more than 3 children. In total 23.6% of the caretakers were illiterate, with most of them having attended only (part of) primary school (71%). The main household income source was from farming (56%). The majority of caretakers had consumed OFSP before (92.5%), with 20% reporting having grown OFSP in the last season and 60% reporting that they have grown any variety of sweetpotato in the last season. Additional descriptive information of the study population and difference between locations can be found in Table 12.

.

**Table 12.** Socio-demographic characteristics of caretakers in Lilongwe, Mangochi and total

| | | **Total** | **Lilongwe** | **Mangochi** |
|---|---|---|---|---|
| | | n=302 | n=151 | n=151 |
| | Mean age (SD) | 31.9 (9.1) | 32.1 (8.1) | 31.8 (10.1) |
| **Education (%)** | | | | |
| | No education | 23.6 | 11.9 | 35.3 |
| | Primary school 1-4 | 35.9 | 35.8 | 36 |
| | Primary school 5-8 | 36.2 | 46.4 | 26 |
| | Secondary school | 4.3 | 6 | 2.7 |
| **Marital status (%)** | | | | |
| | Married | 82.1 | 84.7 | 79.5 |
| | Separated | 14.3 | 13.3 | 15.2 |
| | Widow | 3.3 | 2.0 | 4.6 |
| | Single | 0.3 | - | 0.7 |
| **Number of children (%)** | | | | |
| | 1-2 children | 25.5 | 23.2 | 27.8 |
| | 3-5 children | 60.6 | 62.3 | 58.9 |
| | More than 5 children | 13.2 | 13.2 | 13.2 |
| **Income (%)** | | | | |
| | Farming | 55.6 | 50.3 | 60.8 |
| | Casual labour | 21.5 | 34.9 | 8.1 |
| | Remittances/gifts | 7.4 | - | 14.9 |
| | Other | 15.5 | 14.8 | 16.2 |

Sixty percent of caretakers reported that their children ate OFSP at least once a week when it was in season (between April and August), whereas 97% had the intention to feed their child OFSP once a week or more. The intention of feeding the children OFSP was much higher than current consumption when in season. The initial outcome construct of 'behavioral intention' showed very little variation in responses, which made it unsuitable to link the different constructs of the model to. Therefore, we used the construct 'prior behavior' instead as an outcome measure, which had a more even distribution of responses (and therefore was more suitable), which was included in the adjusted model in Figure 9.

Furthermore, over half of the caretakers agreed that OFSP is rich in vitamin A (59%), that vitamin A could improve eyesight (62%), and could prevent diseases (60%). Most caretakers agreed that children in the age between 2-5 years old are at risk of developing vitamin A deficiency (69%) and almost the same proportion of caretakers also considered their own child being at risk of developing vitamin A deficiency (65%). Most caretakers agreed with the statements that 'vitamin A deficiency makes the child more frequently ill' (77%) and 'lack of vitamin A can lead to stunted growth of my child' (71%). Caretakers acknowledged that it was very important to them that their child can see properly during dusk (96%) and has a good health (99%). The majority of the caretakers (88%) were convinced that eating OFSP was good for their child, while the remaining 12% gave a neutral response to this question.

The majority of participants agreed that OFSP has an attractive color (86%) and that it tastes well (96%). Over one third of respondents indicated that they would rather sell OFSP than consume it themselves (37%). Caretakers indicated that provision of OFSP vines would make them decide to cultivate and prepare OFSP for their child (98%) and that information sessions on the benefits of OFSP would convince them to feed OFSP to their children (94%). Most caretakers agreed that other cues to prepare OFSP for their child were: (a) if their child was sick, (b) would have vitamin A deficiency or (c) would have problems with seeing properly during dusk or dawn (65-71%). The most influencing opinions on food preparation for caretakers were the opinions from health workers (96%), the child growth centers (90%) and health extension workers (80%). The opinions of friends and neighbors are much less valued in making decisions on what food to prepare for their children (both 53%). Furthermore, the majority of caretakers indicated that they would regret it if they would not give OFSP to their child (91%). Table 13 provides an overview of the different constructs. Cronbach-$\alpha$ scores ranged from 0.53 to 0.81, which demonstrated a medium reliability for most of the constructs, median scores of the constructs were ranging from 3 to 51.

**Table 13.** Internal consistency, median scores, range of score and item examples of all constructs (n=302)

| Constructs | Items | Cronbach | Median | Range | Item example |
|---|---|---|---|---|---|
| Knowledge | 4 | 0.69 | 16 | 8-20 | OFSP is rich in vitamin A |
| Perceived susceptibility | 2 | 0.58 | 8 | 2-10 | My child is at risk of developing VAD |
| Perceived severity | 8 | 0.81 | 33 | 10-40 | Lack of VA can make my child malnourished |
| Health value | 4 | 0.64 | 20 | 12-20 | The health of my child is the most important thing in my life |
| Health behavior identity | 1 | - | 5 | 1-5 | Eating OFSP is good for my child |
| Perceived barriers-1 | 3 | 0.75 | 4 | 3-15 | I worry about the orange colour of orange-fleshed sweetpotato |
| Perceived barriers-2 | 8 | 0.63 | 22 | 9-30 | I would rather sell OFSP than keep it for consumption |
| Attitude | 7 | 0.53 | 51 | 11-70 | OFSP tastes well/ I find it important that my child eat foods that tastes well |
| Control beliefs | 3 | 0.74 | 15 | 3-15 | Other people decide what food I buy for my household |
| Subjective norm | 8 | 0.81 | 22 | -16-80 | My parents advise me to prepare sweetpotato/The opinion of my parents about food is important to me |
| Cues to action-1 | 3 | 0.69 | 12 | 3-15 | When my child is sick, I will prepare OFSP |
| Cues to action-2 | 7 | 0.76 | 32 | 7-35 | Information sessions about the benefits of orange-fleshed sweetpotato Potato would encourage me to prepare OFSP for my child |
| Behavioral intention | 1 | - | 5 | 1-5 | How often do you think you will prepare orange fleshed sweetpotato for your child in the future if it is available? |
| Anticipated affect | 3 | 0.57 | 11 | 3-15 | If I don't give OFSP to my child I will regret it |
| Prior behavior | 1 | - | 3 | 1-5 | How often did your child eat orange-fleshed sweetpotato when it was in season? |

Figure 9 shows the correlations between the different constructs of the model. Within the background and perception section significant correlations were found between health behavior identity and the constructs 'knowledge'(r=0.230), 'perceived susceptibility' (r=0.168) and 'perceived severity'(r=0.219, all p<0.01). For the beliefs and attitudes section 'health behavior identity' was correlated with the construct 'attitude toward behavior' (r=0.159, p<0.01) and 'perceived barriers -1' (r=-0.118, p<0.05). Perceived barriers-1 are barriers related to color, taste and starch content of the OFSP. Only the construct 'attitude toward behavior' was significantly correlated to the construct 'prior behavior' (r=0.149 p<0.05). For the external factors, the constructs 'subjective norms'(r=0.158) and cues to action-2' (r=0.187) were significantly correlated with prior behavior (p<0.01). Cues to action-2 are related to activities and recommendations by others promoting OFSP. The construct 'anticipated affect' was significantly correlated with 'behavioral intention' (r=0.145, p<0.05), suggesting that a higher regret of not giving OFSP to the child leads to higher behavioral intention to give OFSP to the child. However, the construct 'anticipated affect' is not correlated with prior behavior (r=-0.012). No significant correlation was found between the constructs 'behavior' and 'behavioral intention'.

**Attitudes towards behavior and subjective norms can predict prior behavior in relation to caretakers serving their child OFSP**

To further assess the relationship between multiple constructs and to investigate which constructs can predict prior behavior, multiple linear regression was used. An overview of the relative contribution of the constructs to prior behavior, using three different models is provided in Table 14. Overall, the constructs could explain a small percentage of the total variance in predicting the behavior. Model 1, 'background and perception', explained 10% of the variance in 'health behavior identity'. No significant predictors were found in the total model, which was also the case when analyzed on location level. Model 2 explained 24% of the variance of the internal factors influencing the prior behavior of the caretakers to give OFSP to their children. Only 'attitudes towards behavior' ($\beta$=0.14) was a significant predictor (p=0.01) of prior behavior. When the model was predicted only for Lilongwe, 'health behavior identity' ($\beta$=0.24) was a significant predictor of prior behavior (p=0.01). For Mangochi 'attitude toward behavior' ($\beta$=0.22) was a significant predictor (p=0.01) of prior behavior. For model 3, predicting prior behavior using external factors, the construct 'subjective norms' was a significant predictor ($\beta$=0.25, p=0.00), also when the model was run for either of the two research locations.

**Table 14.** Overview of all models: predictors for health behavior identity (model 1) and prior behavior of consuming OFSP (model 2 and 3) among children of caretakers in Malawi (n=302)

| | **Total** | | | **Lilongwe** | | | **Mangochi** | | |
|---|---|---|---|---|---|---|---|---|---|
| | Standardized β | p | Adjusted R square | Standardized β | p | Adjusted R square | Standardized β | p | Adjusted R square |
| **Model 1** | | | | | | | | | |
| Dependent variable: Health behavior identity | | | 0.10 | | | 0.27 | | | 0.05 |
| Knowledge | 0.06 | 0.37 | | 0.07 | 0.45 | | 0.07 | 0.48 | |
| Perceived susceptibility | 0.02 | 0.77 | | 0.12 | 0.22 | | -0.14 | 0.16 | |
| Perceived severity | 0.10 | 0.15 | | 0.05 | 0.63 | | 0.18 | 0.07 | |
| Health value | 0.03 | 0.64 | | 0.05 | 0.48 | | -0.03 | 0.70 | |
| **Model 2** | | | | | | | | | |
| Dependent variable: Prior behavior | | | 0.24 | | | 0.12 | | | 0.41 |
| Health behavior identity | 0.09 | 0.13 | | 0.24 | **0.01*** | | -0.06 | 0.39 | |
| Perceived barriers -1 | 0.07 | 0.25 | | 0.08 | 0.39 | | 0.08 | 0.30 | |
| Perceived barriers -2 | 0.04 | 0.55 | | 0.04 | 0.65 | | 0.03 | 0.71 | |
| Attitude toward behavior | 0.14 | **0.03*** | | 0.05 | 0.67 | | 0.19 | **0.03*** | |
| Anticipated affect | 0.03 | 0.58 | | 0.06 | 0.49 | | 0.00 | 0.99 | |
| **Model 3** | | | | | | | | | |
| Dependent variable: Prior behavior | | | 0.25 | | | 0.13 | | | 0.38 |
| External control beliefs | 0.13 | 0.08 | | 0.17 | 0.16 | | 0.07 | 0.36 | |
| Subjective norms | 0.23 | **0.00*** | | 0.26 | **0.02*** | | 0.21 | **0.01*** | |
| Cues to action-1 | 0.02 | 0.81 | | 0.00 | 0.99 | | -0.03 | 0.75 | |
| Cues to action-2 | -0.08 | 0.22 | | -0.14 | 0.16 | | -0.09 | 0.32 | |

Adjusted for interviewer, age, level of education, location (only for total model) * Significant predictor in the model (p<0.05)

## DISCUSSION

For biofortified crops to have an impact on micronutrient intake, it is required that biofortified varieties are consumed in sufficient quantities by the vulnerable target populations, in particular to improve maternal and child health. In addition to dissemination barriers (e.g., ineffective seed systems) that can limit access by smallholder farmers to healthy planting materials (seeds, vines) of new biofortified varieties [36], additional barriers to acceptability and sustained consumption can arise due to biofortified varieties not having equivalent or improved sensory characteristics, or due to a lack of preference for the varieties by the caretakers of children.

The first objective of our study was to assess sensory acceptability of the OFSP varieties in comparison with a control variety. The difference in preference for the varieties per location highlights the importance of conducting sensory evaluation research in different areas, to be able to adjust variety dissemination initiatives to local preferences where possible, particularly to increase acceptability of varieties.

In our study, caretakers in Lilongwe significantly preferred the yellow-fleshed variety over the OFSP varieties, whereas children did not significantly prefer either. In contrast, research amongst children and mothers in Tanzania found that higher mean acceptability scores were observed for OFSP in comparison with pale–fleshed sweetpotato varieties although children gave significantly lower scores than mothers [29]. These contrasting results could potentially be due to regional differences in acceptability, and/or differences in % dry matter, color, flavor, smell and other important sensory characteristics. The preference for the control variety expressed in our study group may be of concern when promoting OFSP in Malawi, since foods liked by mothers are more likely to be offered to their children [37]. This would decrease the exposure of OFSP to children in our study population as the mothers are the primary caregivers. To address this potential barrier, it would be important to have an effective strategy to promote OFSP amongst mothers, which makes it more likely they will feed it to their children.

Dry matter has been identified as an important varietal trait when comparing OFSP to other pale-fleshed sweetpotato varieties, and has been reported as an important attribute for the liking of OFSP by consumers [38, 39]. The OFSP varieties used for the triangle test differed in their dry matter content, which has been shown to make it easier to identify the odd sample [27]. However, our aim in this study was to compare the most promising OFSP variety (based on yield and beta-carotene content), Kadyaubwerere, with the control variety used by farmers and in households. According to a study in Kenya, children have a preference for OFSP varieties with lower dry matter content, whereas adults prefer high dry matter content (>27%) [40], which was not recapitulated in our study. Analysis of the dry matter content of the OFSP varieties used in our study demonstrated that the control variety Kenya had the highest dry matter content (39.2%), followed by Chipika and Zondeni (respectively 34.6% and 34.3%), with the lowest dry matter content found for Kadyaubwerere (29.8%). In our study, the dry matter content factor alone cannot explain the difference in liking between Chipika (lowest rated OFSP) and Zondeni (highest rated OFSP). Other studies on sensory characteristics of OFSP and cream fleshed sweetpotato varieties have concluded that major varietal differences are differences in color, dry mass, sweet flavor and maltose content [38]. These characteristics could likely also explain the differences in liking of the OFSP varieties in our tests. Therefore, further research is needed to test the relationship between the hedonic test results with sensory characteristics of the different varieties to be able to explain the differences in liking in more detail. It is also important to take into account that textural traits can potentially be influenced by genotype-environment interactions, which can complicate the testing and selection of varieties for consumer acceptance and breeding for improved textural traits [39].

Another angle that provides opportunities for increasing the sensory acceptability of OFSP is researching the effect of information provision. Research showed that nutrition information combined with tasting OFSP is positively weighted and integrated by the consumer to form emotions, that can be associated with product acceptance [41]. A review summarizing acceptance studies on biofortified crops concluded that information on the health benefits is an important determinant of acceptance [42].

Our acceptance tests revealed high scores (means are >3) for all sweetpotato varieties, which indicates that all of the sweetpotato varieties are accepted. Since uptake of OFSP among a population who's source of income is mainly farming not only depends on sensory acceptability but also on production and farming system attributes (e.g. yield, resistance to pests and diseases) [43, 44], such additional factors determining adoption for cultivation and marketing should also be taken into account (see Appendix 3). While the sensory acceptability for the Zondeni variety was high, the potential yields for Chipika and Kadyaubwerere are 35 t / ha under ideal circumstances, whereas Zondeni's yield potential is only 8-16 t / ha. Therefore, from a food security point of view based on aggregate supply of sweetpotato the promotion of the Zondeni variety amongst smallholders might not be justified. On the other hand, from a nutritional perspective, the beta-carotene levels of the different OFSP varieties are also an important factor to take into account. The Chipika variety has a much lower beta-carotene content (3500 μg/100g) compared to the other OFSP varieties Kadyaubwerere and Zondeni (respectively 8900 and 9000 μg/100g) and the control variety (770 μg/100g) (see Supplementary table S3 for more data on characteristics of the different varieties used). We acknowledge that measurement of beta-carotene using the HPLC method would be favourable [42], since estimations made with color charts are less precise. However, even with our estimation approach, the differences in potential yield and beta-carotene content of the different varieties (resulting in different nutritional yields) are clear.

It is possible that the preference for the Zondeni variety might be due to an inertia effect as it was the first OFSP variety that was introduced, 3 years before the other two OFSP varieties that were tested were introduced, so participants had more exposure to this variety. It is also possible that the much higher yield of the other OFSP varieties compared to Zondeni might act as a driver for smallholder farmer adoption, that is sufficient to override the relatively small differences in liking between the varieties.

The second objective of our study was to take a cultural acceptability approach to identify the constructs that contribute most to behavior of caretakers serving their children OFSP. Our findings indicate that this behavior was strongest correlated with the constructs 'subjective norms' and 'attitudes toward behavior'. The discrepancy between the intention and prior behavior shows the caretakers' difficulty to implement the behavior possibly through various personal and environmental control factors [45, 46]. Depending on the type of behavior, the strength of the intention-behavior relationship can vary widely, and the discrepancy is larger when multiple steps have to be taken before the intention can be realized into the behavior [45].

Using prior behavior as an outcome measure, the constructs could only explain a small portion of the total variance for predicting the caretaker's frequency of serving OFSP to their children (23-29%). This is in concordance with other studies [18, 21-23], that found similar low explained variances by using the intention as an outcome measure. Therefore, an addition of the construct anticipated affect was made to the model, which explained an additional 3% of total variance. Despite its small contribution, the anticipated affect is important to take into account [33]. However, our study reveals that there remain other unknown factors that will be necessary to identify to explain the remaining variance.

Most of the respondents tended to agree with the statements in the cultural acceptability survey, and because of that, scores were high compared to the ranges possible. This high level of agreement to the statements can be due to different reasons. Firstly, it might be related to unfamiliarity to the behavior. We attempted to prevent the behavior unfamiliarity effect by only enrolling people in the survey that knew OFSP and selecting areas where it was introduced by ongoing agri-development programs and by including both negative and positively phrased questions. The intention to consume OFSP was very high, but at the same time the access to the OFSP was low, since the roots were not widely available in markets and the planting material was hard to access. Unfamiliarity with the behavior makes it less likely that there are strong beliefs towards the statements, and therefore the respondents might have had difficulty to decide on their level of agreement or disagreement. Secondly, another important factor that might have influenced our findings is that in general, respondents attempt to understand the goal of the research, in order to tailor their results which they hope will benefit themselves, their family or their community in future [47]. By giving positive responses respondents might have hoped that the survey showed their community to be a good place to continue the programming on OFSP, to be able to receive planting material or more support.

Concerning the constructs within the section background and perception it was found that the construct ‘health behavior identity’ was significantly correlated with the internal factors ‘knowledge’, ‘perceived susceptibility’ and ‘perceived severity’. However, none of these constructs were predictors in the model. In our study, we can conclude that specific knowledge on vitamin A and the threats of vitamin A deficiency are more likely to positively influence caretakers’ behavior to serve OFSP to their children than a more general knowledge on health, which is reflected by the construct ‘health value’.

The prior behavior of caretakers serving their child OFSP was predicted ($p<0.05$) by the constructs ‘attitude towards behavior’ and ‘subjective norms’. The construct ‘attitudes toward behavior’ was a good predictor within the section beliefs and attitudes, which confirms results of other studies [22, 48]. These attitudes were determined by questions about beliefs on serving the child OFSP and the importance of these beliefs for the caretakers. The most important attitudes were the (sweet) taste, the attractive appearance, that it can cure and protect against diseases, and that it is easy to prepare.

The construct ‘subjective norms’ was a good predictor within the external factors. It reflects social pressure, which can be explained as the influence other people have on the decision whether a caretaker will serve OFSP to their children or not. In particular, the opinions of the extension workers, health workers and parents were highly valued, according to the responses. It has been highlighted that Malawi is a collectivist society [49], which can mean individuals put the priority of the group above the priorities of the individual [50]. In addition, the values of extended family and the community have a major influence on the behavior of the individual. This is important to take into account when promoting OFSP, to not only focus on positive attitudes and knowledge of women, but to also include a wider range of social ‘influencers’. Other studies have found that the subjective norms were correlated. However, they were not a good predictor of the intention [22, 51] or did not find any correlation [18, 21].

The perceived economic and health benefits of OFSP in Malawi have been studied among farmers of OFSP [52]. The health benefits that were most frequently mentioned were increased energy, improved eyesight and the perception that OFSP is good for healthy bodies. From an economic perspective benefits cited were the ability to invest the income retrieved by selling OFSP (vines) in housing, livestock and food. In addition, women mentioned an increased self-esteem through the increased incomes. The most important benefits of producing and consuming OFSP can be used in information sessions and nutrition sessions where knowledge on the OFSP is communicated to potential consumers and/or farmers. These benefits would also help to create positive attitudes towards the behavior of consuming OFSP and increasing the already high acceptability of OFSP.

## CONCLUSIONS

Overall, our study reveals that biofortified OFSP varieties are well accepted in Lilongwe and Mangochi districts in Malawi from both a cultural and sensory perspective. However, we find that there is a preference for the yellow-fleshed control variety and the Zondeni variety which is high in vitamin A. Our cultural acceptability analysis indicates that attitudes toward behavior and subjective norms were correlated to, and important predictors of the caretakers' behavior of serving their child OFSP. Our study findings provide guidance and direction for improvement of ongoing and planned programs for increasing the uptake of OFSP in Malawi among households with children. We consider that there is a need to conduct a follow-on in-depth study quantifying sensory characteristics (sweetness, maltose concentration, dry matter) of the OFSP varieties for instance using a check-all-that-applies (CATA) method [53]. In addition, a more accurate quantification of the beta-carotene levels of the varieties would be needed to be able to unravel favourable traits by linking this information to the hedonic test results. Our results also indicate that there is both a need and an opportunity to promote a more diversified use of OFSP, as it is currently almost exclusively consumed as a breakfast snack (where the OFSP are mostly prepared through boiling), or in a dish called Futali. The high energy density of OFSP should be taken into account, to make sure it is a good and nutritious replacement when increasing intake or diversifying the use. Replacing with OFSP at the expense of other less nutritious crops should only be considered when it is considered a healthier alternative (in terms of nutrients and energy). The ongoing programs for promotion of uptake of OFSP varieties in Malawi will need to decide which specific OFSP varieties to promote based on criteria that include sensory acceptability, beta-carotene content or agricultural characteristics as well. For increasing adoption and consumption of OFSP to improve maternal and child health in Malawi, there is an additional opportunity to focus on positive attitudes and identify and include important influencers around the caretaker in the promotion strategy to increase the frequency of caretakers serving OFSP to their children.

Overall, while biofortified crops such as OFSP have major promise for combatting hidden hunger micronutrient deficiencies, our study highlights that consideration of sensory and cultural attributes that can influence both acceptability and consumption amongst smallholder farmers and households can improve impact pathways for biofortified crops.

## ACKNOWLEDGEMENTS

We would like to thank all adults and children that were participating in this study. We would also like to thank the interviewers for their work in the field. Furthermore, we are very grateful for being hosted at the International Potato Center (CIP) in Malawi. We would also like to express our gratitude towards Concern Worldwide and the Ministry of Agriculture, Department of Agriculture Extension Services in Katuli and Mngwangwa for supporting us with conducting the research and mobilizing research participants.

## SUPPORTING INFORMATION

**Appendix 1** **Results for the preference test with OFSP and a control yellow-fleshed sweetpotato variety among adults (n=270) and children (n=60) per location**.

**Appendix 2** **Results for the acceptance test with 3 OFSP varieties and a control variety, per area and total.**

**Appendix 3** **Beta-carotene content and dry matter (%) content for the various sweetpotato varieties**.

## REFERENCES


1. Bouwkamp JC. Sweet potato products: a natural resource for the tropics. 1st ed. Boca Raton CRC Press, Inc.; 1985.
2. Low JW, Mwanga RO, Andrade M, Carey E, Ball A-M. Tackling vitamin A deficiency with biofortified sweetpotato in sub-Saharan Africa. Global Food Security. 2017.
3. Bovell-Benjamin AC. Sweet potato: a review of its past, present, and future role in human nutrition. Advances in food and nutrition research. 2007;52:1-59.
4. van Jaarsveld PJ, Faber M, Tanumihardjo SA, Nestel P, Lombard CJ, Benadé AJS. β-Carotene–rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response test. The American journal of clinical nutrition. 2005;81(5):1080-7.
5. World Health Organization. Global prevalence of vitamin A deficiency in populations at risk 1995-2005 Geneva: World Health Organization; 2009.
6. Department of Nutrition HaAitOoPaCD-O, Ministry of Health (MOH), National Statistics Office (NSO), UNICEF and CDC. The national micronutrient survey 2009. . Lilongwe, Malawi 2011.
7. National Statistical Office (NSO), Community Health Sciences Unit (CHSU) [Malawi], Centers for Disease Control and Prevention (CDC), Emory University. Malawi Micronutrient Survey 2015-16: Key Indicators Report. Atlanta, GA, USA: NSO, CHSU, CDC and Emory University.; 2016.
8. WHO. Serum retinol concentrations for determining the prevalence of vitamin A deficiency in populations. . In: Organization WH, editor. Geneva: Vitamin and Mineral Nutrition Information System.; 2011.
9. ICF NSONMa. Malawi Demographic and Health Survey 2015-16.
. Zomba, Malawi, and Rockville, Maryland, USA: NSO and ICF; 2017.
10. Bouis HE, Saltzman A. Improving nutrition through biofortification: a review of evidence from HarvestPlus, 2003 through 2016. Global food security. 2017;12:49-58.
11. Olson JA. Provitamin A function of carotenoids: the conversion of beta-carotene into vitamin A. The Journal of nutrition. 1989;119(1):105-8.
12. Hotz C, Loechl C, de Brauw A, Eozenou P, Gilligan D, Moursi M, et al. A large-scale intervention to introduce orange sweet potato in rural Mozambique increases vitamin A intakes among children and women. British Journal of Nutrition. 2012;108(01):163-76.
13. CIP Sr. Orange Fleshed Sweetpotato Varieties for Malawi / Mitunda ya mbatata yofiira mkat yaku Malawi In: (CIP) IPC, editor. 2015. p. 12.
14. Tomlins K, Owori C, Bechoff A, Menya G, Westby A. Relationship among the carotenoid content, dry matter content and sensory attributes of sweet potato. Food Chemistry. 2012;131(1):14-21.
15. Takahata Y, Noda T, Nagatata T. HPLC determination of β-carotene content of sweet potato cultivars and its relationship with color values. Japanese Journal of Breeding. 1993;43(3):421-7.
16. Birol E, Meenakshi J, Oparinde A, Perez S, Tomlins K. Developing country consumers' acceptance of biofortified foods: a synthesis. Food Security. 2015;7(3):555-68.
17. Talsma EF, Melse-Boonstra A, Brouwer ID. Acceptance and adoption of biofortified crops in low-and middle-income countries. Nutr Rev. 2017;75(10):798-829.
18. Sun X, Guo Y, Wang S, Sun J. Predicting iron-fortified soy sauce consumption intention: application of the theory of planned behavior and health belief model. Journal of Nutrition Education and Behavior. 2006;38(5):276-85.
19. Ajzen I. The theory of planned behavior. Organizational behavior and human decision processes. 1991;50(2):179-211.
20. Janz NK, Becker MH. The health belief model: A decade later. Health education quarterly. 1984;11(1):1-47.
21. Talsma EF, Melse-Boonstra A, de Kok BP, Mbera GN, Mwangi AM, Brouwer ID. Biofortified cassava with pro-vitamin A is sensory and culturally acceptable for consumption by primary school children in Kenya. PloS one. 2013;8(8):e73433.

22. Fanou-Fogny N, van Dam B, Koreissi Y, Dossa RA, Brouwer ID. Factors predicting consumption of fonio grain (Digitaria exilis) among urban Malian women of reproductive age. Journal of nutrition education and behavior. 2011;43(4):219-28.
23. Macharia-Mutie CW, Van de Wiel AM, Moreno-Londono AM, Mwangi AM, Brouwer ID. Sensory acceptability and factors predicting the consumption of grain amaranth in Kenya. Ecology of food and nutrition. 2011;50(5):375-92.
24. Services DoCCaM. Climate of Malawi 2006 [Available from: https://www.metmalawi.com/climate/climate.php.
25. National Statistics Office. Integrated household survey 2016-2017. Malawi; 2017.
26. Aberman N-L, Meerman J, Benson T. Mapping the linkages between agriculture, food security and nutrition in Malawi: Intl Food Policy Res Inst; 2015.
27. Lawless HT, Heymann H. Sensory evaluation of food: principles and practices: Springer Science & Business Media; 2010.
28. International Potato Centre (CIP) SSA region. Orange Fleshed Sweetpotato Varieties for Malawi / Mitunda ya mbatata yofiira mkati yaku Malawi 2015.
29. Tomlins K, Ndunguru G, Stambul K, Joshua N, Ngendello T, Rwiza E, et al. Sensory evaluation and consumer acceptability of pale-fleshed and orange-fleshed sweetpotato by school children and mothers with preschool children. Journal of the Science of Food and Agriculture. 2007;87(13):2436-46.
30. Kemp S, Hollowood T, Hort J. Sensory evaluation: a practical handbook. 1st ed. Chichester: Wiley Blackwell; 2009.
31. Guinard J-X. Sensory and consumer testing with children. Trends in Food Science & Technology. 2000;11(8):273-83.
32. Den Hartog AP, Van Staveren WA, Brouwer ID. Food habits and consumption in developing countries - manual for field studies. 1st ed. Wageningen: Wageningen Academic Publishers; 2006.
33. Ajzen I, Sheikh S. Action versus inaction: anticipated affect in the theory of planned behavior. Journal of Applied Social Psychology. 2013;43(1):155-62.
34. AOAC. Official methods of analysis (18th edn, rev. 2). Gaithersburg, Marlyland, USA: AOAC International; 2007.
35. Burgos B, Carpio R, Sanchez C, Paola S, Eduardo P, Espinoza J, et al., editors. A color chart to screen for high β-carotene in OFSP breeding. 15th Triennial Symposium of the International Society for Tropical Root Crops, Lima, Peru; 2009.
36. McGuire S, Sperling L. Seed systems smallholder farmers use. Food Security. 2016;8(1):179-95.
37. Skinner JD, Carruth BR, Bounds W, Ziegler PJ. Children's food preferences: a longitudinal analysis. Journal of the American Dietetic Association. 2002;102(11):1638-47.
38. Laurie SM, Faber M, Calitz FJ, Moelich EI, Muller N, Labuschagne MT. The use of sensory attributes, sugar content, instrumental data and consumer acceptability in selection of sweet potato varieties. Journal of the Science of Food and Agriculture. 2013;93(7):1610-9.
39. Anderson L, Gugerty MK. Root, Tuber, and Banana Textural Traits A Review of the Available Food Science and Consumer Preferences Literature. 2015.
40. Hagenimana V, Low J. Potential of orange-fleshed sweet potatoes for raising vitamin A intake in Africa. Food and Nutrition Bulletin. 2000;21(4):414-8.
41. Lagerkvist CJ, Okello J, Muoki P, Heck S, Prain G. Nutrition promotion messages: The effect of information on consumer sensory expectations, experiences and emotions of vitamin A-biofortified sweet potato. Food quality and preference. 2016;52:143-52.
42. Talsma EF, Melse-Boonstra A, Brouwer ID. Acceptance and adoption of biofortified crops in low-and middle-income countries: a systematic review. Nutrition reviews. 2017;75(10):798-829.
43. Berre D, Corbeels M, Rusinamhodzi L, Mutenje M, Thierfelder C, Lopez-Ridaura S. Thinking beyond agronomic yield gap: Smallholder farm efficiency under contrasted livelihood strategies in Malawi. Field Crops Research. 2017;214:113-22.
44. Tittonell P, Giller KE. When yield gaps are poverty traps: The paradigm of ecological intensification in African smallholder agriculture. Field Crops Research. 2013;143:76-90.
45. Randall DM, Wolff JA. The time interval in the intention-behaviour relationship: Meta-analysis. British Journal of Social Psychology. 1994;33(4):405-18.

46. Glanz K, Rimer BK, Viswanath K. Health behavior and health education: theory, research, and practice: John Wiley & Sons; 2008.
47. Miller K, Msiyaphazi Zulu E, Cotts Watkins S. Husband—Wife Survey Responses in Malawi. Studies in Family Planning. 2001;32(2):161-74.
48. Nejad LM, Wertheim EH, Greenwood K. Comparison of the health belief model and the theory of planned behavior in the prediction of dieting and fasting behavior. Sensoria: A Journal of Mind, Brain & Culture. 2005;1(1):63-74.
49. Sulamoyo DS. Creating Opportunities for Change and Organization Development in Southern Africa. 1st ed. Charlotte, NC: Information Age Publishing; 2012.
50. Kâğitçibaşi Ç. A critical appraisal of individualism and collectivism: Toward a new formulation. Thousand Oaks, CA: Sage; 1994. 52–65 p.
51. Rah JH, Hasler CM, Painter JE, Chapman-Novakofski KM. Applying the theory of planned behavior to women's behavioral attitudes on and consumption of soy products. Journal of nutrition education and behavior. 2004;36(5):238-44.
52. Mudege NN, Mayanja S, Muzhingi T. Women and men farmer perceptions of economic and health benefits of orange fleshed sweet potato (OFSP) in Phalombe and Chikwawa districts in Malawi. Food Security. 2017;9(2):387-400.
53. Ares G, Jaeger S. Check-all-that-apply (CATA) questions with consumers in practice: Experimental considerations and impact on outcome. Rapid sensory profiling techniques: Elsevier; 2015. p. 227-45.

# APPENDIX 1

## Results for the preference test with OFSP and a control yellow-fleshed sweetpotato variety among adults (n=270) and children (n=60) per location

| Preference test | Caretakers | | | Children | | |
|---|---|---|---|---|---|---|
| | Total | Lilongwe | Mangochi | Total | Lilongwe | Mangochi |
| Number of participants | 270 | 140 | 130 | 60 | 36 | 24 |
| No. of responses needed for significance (α=0.05) | 152 | 83 | 77 | 39 | | |
| No. of responses favoring OFSP | 64 | 29 | 35 | 35 | 21 | 14 |
| Paired preference u test: μ0=1/2 (preference for OFSP variety) | 0.24 | 0.21 | 0.27 | 0.58 | 0.58 | 0.58 |

# APPENDIX 2

## Results for the acceptance test with 3 OFSP varieties and a control variety, per area and total

| | | Color | Smell | Texture | Starchiness | Sweetness | Taste | Overall liking |
|---|---|---|---|---|---|---|---|---|
| | | Median | Median | Median | Median | Median | Median | Median |
| **Total** | Kenya (control) | 4ab | 4a | 4a | 4a | 5a | 5a | 5a |
| | Chipika | 4b | 4b | 4b | 3b | 3b | 3b | 4b |
| | Kadyaubwerere | 4a | 4ab | 4bc | 3b | 3c | 4b | 4c |
| | Zondeni | 4a | 4a | 4c | 4a | 4d | 4c | 4d |
| **Lilongwe** | Kenya (control) | 4a | 4a | 4a | 4a | 5a | 5a | 5a |
| | Chipika | 4bc | 3b | 3b | 3b | 3b | 3b | 3b |
| | Kadyaubwerere | 4c | 4bc | 4bc | 3bc | 3b | 4b | 4bc |
| | Zondeni | 4ac | 4c | 3c | 4c | 4c | 4c | 4c |
| **Mangochi** | Kenya (control) | 4ab | 4a | 4a | 4a | 5a | 5a | 5a |
| | Chipika | 4bc | 4a | 4b | 3.5b | 4b | 4b | 4b |
| | Kadyaubwerere | 4cd | 4a | 4ab | 4b | 4b | 4bc | 4b |
| | Zondeni | 5d | 4a | 4a | 4a | 4a | 4ac | 5a |
| | | p-value | p-value | p-value | p-value | p-value | p-value | p-value |
| **Location effect** | Kenya (control) | 0.266 | 0.506 | 0.341 | 0.605 | 0.552 | 0.946 | 0.485 |
| | Chipika | 0.042* | 0.009* | 0.037* | 0.030* | 0.002* | 0.001* | 0.197 |
| | Kadyaubwerere | 0.006* | 0.001* | 0.076 | 0.131 | 0.016* | 0.022* | 0.062 |
| | Zondeni | 0.002* | 0.041* | 0.019* | 0.000* | 0.003* | 0.069 | 0.005* |

a,b,c,d represent significant similarieties/differences within column

Total n =210, n=110 for Lilongwe, n=100 for Mangochi

# APPENDIX 3

## Beta-carotene content and dry matter (%) content for the various sweetpotato varieties

| Varieties | Beta-carotene content (µg/100g) | Dry matter (%) | Potential yield (t/ha) [1, 2] | Beta-carotene * yield |
|---|---|---|---|---|
| Chipika | 3500 | 34.6 (2.0) | 35.0 | 122,500 |
| Kadyaubwerere | 8900 | 29.8 (1.5) | 35.0 | 311,500 |
| Kenya (control) | 770 [3] | 39.2 (0.1) | 30.0 | 23,100 |
| Zondeni | 9000 | 34.3 (3.8) | 8.0-16.0 | 108,000 |

1. Ministry of Agriculture and Food Security. Cassava and Sweetpotato Production Handbook. 2nd ed. Lilongwe: Ministry of Agriculture and Food Security; 2010.
2. International Potato Centre (CIP) SSA region. Orange Fleshed Sweetpotato Varieties for Malawi / Mitunda ya mbatata yofiira mkati yaku Malawi 2015.
3. Kathabwalika D, Chilembwe E, Mwale V. Evaluation of dry matter, starch and beta-carotene content in orange-fleshed sweet potato (Ipomoea batatas L.) genotypes tested in three agro-ecological zones of Malawi. African Journal of Food Science. 2016;10(11):320-6.

# Chapter 5

## Exploratory study on the barriers and enablers for implementation and consumption of (biofortified) beans in Malawi

Marijke Hummel

## ABSTRACT

Iron beans are biofortified beans, meaning that the nutritional quality of this crop is increased through conventional crossbreeding with higher levels of iron present. Biofortified crops can be used as a approach in increasing the micronutrient intake of deficient target groups. Malawi is a high priority country for the implementation of iron beans. Two varieties of iron beans were released in Malawi in 2009, but the adoption of these beans still remains low. Therefore, this study aimed to get an understanding of the barriers and enablers for implementation and consumption of (iron) beans in Malawi, focused on households with children. This was assessed by evaluating consumer acceptability from a sensory (acceptance test with six different bean varieties) and cultural perspective (focus group discussions) and through mapping the views of stakeholders (interviews).

The results showed that the most important areas of attention include 1) getting the concept of biofortification more widely known among stakeholders and farmers/consumers, 2) tackling the existing beliefs that could be barriers for consumption and 3) to closely monitor the invisible iron trait in the (biofortified) beans available.

**Conclusions:** To our knowledge, this is the first in-depth study into the barriers and enablers for implementation of iron beans and the consumption of (iron) beans in households with children in rural Malawi. The qualitative aspect of this study illustrated both the challenges faced and the utility of using this type of research to understand them. Taking into account the recommendations of this study could further improve the impact and delivery of iron beans to consumers in Malawi.

## INTRODUCTION

Globally, two billion people suffer from micronutrient deficiencies [1], which are most prevalent in children and women of childbearing age. Deficiencies of iron, zinc, iodine and vitamin A are the most prominent forms, especially in developing countries [2]. The malnourished populations are mostly living rural and depending on their own crop production for food and income [3]. Therefore, agricultural interventions have a strong potential to further improve nutrition indicators since agriculture can influence underlying determinants of malnutrition [4]. Biofortification is rooted in agriculture since it aims to increase the nutritional quality of crops and can be used as a complementary approach next to the other food-based approaches (examples are supplementation, fortification, promoting dietary diversity). Increasing the nutritional value of crops can be reached through either conventional crossbreeding, hybridization or applying micronutrient rich fertilizers [5]. Biofortification is considered a cost-effective and sustainable complementary approach to increase nutritional intake, since food crops, once developed, can be disseminated and grown or consumed by the rural target population themselves [6]. Examples of biofortified crops are sweet potato, maize, rice, cassava wheat, pearl millet and beans. In this chapter, we will discuss the latter.

Common beans, part of the legume class of vegetables, are an excellent source of proteins, minerals, dietary fiber and vitamins [7]. Beans are often referred to as the meat of the poor as they are the main protein source for people relying on a plant-based diet, which is the case in rural and poor populations [8]. Beans are of higher nutritional quality than for instance maize [9], and are grown both for consumption and as a cash crop [8]. Africa is the continent with the second highest production of pulses and dry beans, after Asia [10]. From an agricultural point of view, legumes are an important group of crops since they are so-called nitrogen fixers. In symbiosis with the rhizobium bacteria the plant can fix nitrogen from the air, which leads to increased production and better soil fertility [11]. Due to climate change bean growing areas are under threat, and production levels are estimated to decrease already by 2030 in Eastern Africa [12].

Iron beans are biofortified beans and have been developed using crossbreeding, after screening available germplasm for iron content and crossbreeding these lines with other lines with favorable traits (eg. consumer acceptability, drought resistance, yields) [13]. Different generations of iron beans have been developed, current goals for iron content of new bean varieties have been set to 94 ppm, aiming for an increase of 44 pm on top of the 50 ppm baseline iron content in conventional bean varieties [6]. Iron beans have been shown to improve iron status in Rwandese women over time in an efficacy trial [14]. In addition, the women's cognitive performance was also improved after consuming iron beans for 128 days [15].

Since beans are mainly consumed from own production in rural areas [16], sensory and cultural acceptability are major aspects and needed for farmer acceptance and consumption in the community [17]. Biofortification can result in an altered appearance and sensory characteristics of the crop, due to the increased nutrient content. However, in the case of iron beans there is no visible trait and thus cannot be discriminated from a non-biofortified variety by sight only [18]. Research studying the acceptability of biofortified crops and more specific iron beans shows that they are generally well accepted. The level of acceptance depends among others on population segment and nutrition information provided [19]. Furthermore, research showed that a good support program in dissemination leads to better adoption [19]. Most acceptability studies were done before actual implementation of biofortified beans, research on the effectiveness, the acceptability and use of iron beans in communities after implementation, has not been published [19].

The Biofortification Priority Index (bpi) tool has been developed to make investment decisions on introduction of biofortified crops and assigns a priority level to each country for the different crops. The ranking of countries using this tool is based on iron deficiency prevalence, consumption, and production levels of beansfor the iron beans . Malawi has been rated using this tool as top priority country for implementation of iron beans [20, 21].

Iron deficiency can result in iron deficiency anemia when severe, and leads to a loss in health, but also economic potential [22]. Iron deficiency is present in 21.7% of preschool children in Malawi and can be a cause of anemia, of which 28.2% of all pre-school children are suffering from [23]. Iron deficiency anemia is a moderate public health problem in Malawi according to the WHO guidelines [24]. Costs for society caused by iron deficiency and anemia remain high, and results in a weakened workforce in a country where over 80% of the population is depending on agriculture for livelihoods [25].

Beans are an important source of proteins in the Malawian diet, where an average of 7.3 kg per capita of common beans per year is consumed. From 2004/05 to 2010/11 the per capita consumption of pulses and vegetables and fruits declined (respectively a 22 and 9 % decrease), in urban areas the decline in consumption was less (Table 15) [26]. Possible explanations for this drop could be the nutritional transition, an increased intake of animal protein, decreasing popularity of beans but also low seed availability, drought, and low yields. Agriculture is an important sector in Malawi, with a share of over 30% of the country's GDP [27]. Bean yield in Malawi is very low (average 400-600 kg/hectare), about 25% of the possible yields under ideal circumstances [28]. This can be attributed to low fertilizer use, disregard for appropriate integrated soil management practices, including the use of quality seed of improved varieties [29].

**Table 15.** Contribution of common beans to the diet of Malawians [26]

| | **Quantity g/d** | | | **Calories kcal/day** | | |
|---|---|---|---|---|---|---|
| | 2004/05 | 2010/11 | Change (%) | 2004/05 | 2010/11 | Change (%) |
| **Average diet** | 25 | 20 | -22 | 84 | 61 | -28 |
| **Urban diet** | 28 | 28 | -2 | 90 | 84 | -6 |
| **Rural diet** | 25 | 18 | -26 | 83 | 57 | -32 |
| | **Iron intake** | | | **Zinc intake** | | |
| | 2004/05 | 2010/11 | Change (%) | 2004/05 | 2010/11 | Change (%) |
| **Average diet** | 1.8 | 1.3 | -26 | 0.7 | 0.5 | -28 |
| **Urban diet** | 2.0 | 1.8 | -6 | 0.8 | 0.7 | -6 |
| **Rural diet** | 1.8 | 1.2 | -31 | 0.7 | 0.5 | -32 |

There is low access to quality seed, where farmers mostly recycle their mixed seed [30]. Therefore, a farm input subsidy program was set up by the government, to support vulnerable small-scale famers with fertilizer and quality seed at subsidized rates [31]. Still less than six percent of the areas are planted with certified bean seed in 2014 (personal communication). As an extra challenge, Malawi is vulnerable to climate change [32, 33], which poses an extra threat on production in future and stresses the need for improved and more drought tolerant varieties.

To tackle some of the challenges mentioned, over 30 improved bean varieties have been introduced since 1996. The first generation of biofortified iron beans in Malawi, varieties NUA 45 and 59, were released by the Department of Agricultural Research Services (DARS) in 2009. Since then seed has been produced by government and contracted seed companies to make the seed available in small quantities for NGOs, traders, governmental organizations and farmers. The seed has been used in different farmer programs and distributed in farmer groups, but it has not been scaled up countrywide until date.

In this context, a mixed methods study was conducted to get an understanding of the barriers and enablers for implementation in general and specifically consumption of (iron) beans in Malawi, focused on households with children. This was assessed by evaluating consumer acceptability from a sensory and cultural perspective and through interviewing stakeholders. In this way, recommendations for future programs promoting biofortified iron bean production and consumption in Malawi can be given.

## MATERIALS AND METHODS

### Ethics statement

Written informed consents of research participants or caretakers were collected before start of the study and all children were asked for their verbal consent. Ethical clearance for this research project was obtained from the National University of Ireland Galway Research Ethics Committee (Reference 16/FEB/07) and the National Commission of Science and Technology in Lilongwe, Malawi (Protocol number P.06/16/114.).

### Focus group discussions: study area and study participants

This research was conducted in Central and Southern Malawi in, respectively, the Mngwangwa location in Lilongwe district and Katuli location in Mangochi district. These rural research sites were chosen based on their high production levels of sweetpotato and beans, the difference in culture (Chewa ethnic group in Mngwangwa and Yao ethnic group in Katuli) and the presence of collaborating organizations (International Potato Centre and Concern Worldwide). Participants were identified through convenience sampling of 2 existing farmer groups within each location. Participants were recruited by either agricultural extension workers or field personnel from the NGO Concern Worldwide. Inclusion criteria for participating in the FGD were that they needed to be caretakers of children between 2-4 years old. Community health volunteers were identified and recruited by agriculture extension workers to join a separate FGD.

Mngwangwa is situated relatively close to the capital of Malawi, Lilongwe, at a distance of approximately 30 km. Katuli lies more isolated behind hills on 60 kilometres from Mangochi, and is bordering with Mozambique. Malawi has one rainy season stretching from December to April, followed by a long dry season [34]. Both locations can be described as rural areas where over 90% of the population is engaged in agriculture activities [35]. Major crops grown in both locations are maize and groundnuts, in Lilongwe also Tobacco, beans and soy are important crops. Diets are mainly cereal based, in which over 50% of calorie intake is from maize, with Nsima (maizeflour mixed with water) as a staple, supplemented with starchy roots (cassava, potatoes), vegetables and beans [36]. Literacy is higher in Lilongwe (64.5%) than Mangochi (57.2%).

#### Focus group discussions: study measurements

Our study used in-depth interviews and focus group discussions as major data collection methods, food pile sorts and paired comparisons provided additional data. An overview of the FGD is provided in table 16. Four data collectors were trained to conduct the focus group discussions. They were all proficient in English and Chichewa and had all completed at least a bachelor's degree and had previous experience in conducting focus group discussions. Written informed consent was obtained from all participants.

**Phase 1: In-depth interviews with stakeholders and (potential) implementing partners**

In-depth interviews with fifteen different stakeholders and implementing partners were conducted to provide an overview of the primary issues and barriers associated with implementing and adopting the biofortified bean varieties. These interviews were conducted in English by the primary investigator (Marijke Hummel), with key individuals from collaborating organizations, private sector, multi-lateral organizations and NGO's focused or involved in agriculture-nutrition programming.

**Phase 2: Focus group discussions**

Existing literature, emerging themes from the in-depth interviews and the framework of the integrated model of the theory of planned behavior and the health belief model were used to establish the discussion points for the focus group discussions [37]. This model has been used before to assess the acceptance of different foods. Eight focus group discussions were held with a group size of between 7 and 10 persons, where each discussion lasted about 1 hour and 20 minutes. The groups were either mothers, grandmothers, fathers or community health volunteers, only the community health volunteers were mixed gender. The participants were recruited through contacting existing women farmer groups with help of the agricultural extension worker in the area. The research team moderated the sessions in the local language Chichewa to identify the main barriers and enablers these groups perceived for bean production and consumption. Additionally, iron beans were shown to participants and discussed, together with a discussion about iron as a nutrient. Two sessions included a pile sorting exercise and discussion on food attributes and food differences. For the pile sorting, the thirty most consumed food items were brought, and each individual was asked to make groups in the way they felt made sense and this was noted and discussed with the participant. The food attributes and food differences sessions were done plenary, and participants were asked to compare foods and describe differences between the two and explain what the foods were meaning to them.

**Table 16.** Overview of data collection methods used for qualitative analysis

| Type of FGD | Number of FGD | Number of participants FGD | Food attributes and differences study | Pile sort study |
|---|---|---|---|---|
| Mothers | 4 | 33 | 24 | 24 |
| Grandmothers | 1 | 7 | | |
| Fathers | 1 | 9 | | |
| Community health volunteers | 2 | 15 | | |
| **Total** | **8** | **64** | **24** | **24** |

**Focus group discussions: method of analysis**

In depth interviews were all summarized by the PI. All focus group discussions were recorded in local languages, translated into English and transcribed. The analysis of the transcribed focus group discussions was done using a codebook created by the primary investigator. Codes were generated using a grounded approach and matched with attributes within the integrated model of the theory of planned behavior and the health belief model. This was all logged in the DeDoose qualitative data computer software.

## Sensory evaluation: study area and study participants

The sensory evaluation was conducted in June 2015 in two different areas in Northern and Central Malawi, divided over three different communities. All communities can be described as rural communities, where people mainly rely on subsistence agriculture. Chosen areas were identified as major bean growing areas and the presence of farmer groups of Concern Worldwide and Catholic Relief Services.

Six bean varieties (Phaseolus Vulgaris L.) were selected to be included; two recently released varieties; NUA 45 and NUA 59 and three locally most used varieties (found in an earlier survey, not published), Phalombe, Kalima and Kholopethe. The last variety included was the best marketable variety, but not widely grown or adopted in this area: the Kabalabala bean. All varieties used for the study were purchased on local markets or from farmers growing the beans in one of the areas researched and were beans harvested in the last rainy season. In Table 17 and Figure 11 an overview of the different bean varieties is given.

**Table 17.** Overview of tested bean varieties

| Local Name | Genotype | Used in community? | Seed color |
|---|---|---|---|
| *Kabalabala* | UBR 92 (25), Haricot (Navy) bean | No | White, small seed |
| *Kholopethe* | Sugar 132, Sugar bean | Yes | Brown mottled, medium seed |
| *Kalima/ Napilira* | Calima | Yes | Red mottled, medium seed |
| *NUA 45* | Calima | No | Red mottled, medium seed |
| *NUA 59* | Calima | No | Red mottled, medium seed |
| *Phalombe* | Common red kidney bean | Yes | Red, big seed |

## Sensory evaluation: study measurements

Enumerators were trained, and a pre-test was done to see whether the questions were clear to participants. A double blind color-coded randomized survey was set up to assess the preferences of the participants for the different varieties. A randomization schedule was made using the RAND() function in Microsoft Excel. Five different attributes/terms were used to describe these preferences; color, smell, broth thickness, chewiness of the bean and taste. These attributes were chosen based on other literature [38, 39]. Samples were served in separate closed containers and were between 30-40 grams of cooked sample. Participants were asked to rinse their mouth with water after each sample. All participants were interviewed in Chichewa by trained enumerators. A five point- facial Likert scale was used by participant to rate the samples. For the survey, both adult men and women were included. As a starting point already established women groups were invited to participate, and these women were invited to also bring over their husbands and other friends. A sample size of over 60 participants is needed in hedonic testing to be able to find a significant difference between varieties which is representative of the target group [18]. Therefore, in 3 sessions as many men and women as was logistically possible were asked to participate.

**Figure 11.** Picture of the included six bean varieties. Starting up, left to right: 1: NUA 45, 2: NUA 59, 3: Kalima, 4: Phalombe, 5: Kabalala, 6: Kholophete (Author's picture)

## Iron and zinc analysis

Samples used for mineral analysis were retrieved from the sensory evaluation, the beans were bought in local markets or from farmers directly. Beans were cleaned three times using MilliQ water after which they were dried using a cloth. Samples were kept in a cool and dark room until further analysis. X-ray Fluorescence spectroscopy (XRF) analysis was performed at the Nutrition lab of the International Centre of Tropical Agriculture (CIAT) based in Cali, Colombia. Exact methods are described in Guild et al. [40]. Inductively coupled plasma mass spectrometry analysis (ICP-MS) was performed at Flinders University, Australia. Seed samples were irradiated at 50kGray (5Mrad) for sterilization prior to release into Australia. Seed samples were ground using a Retsch Mixer Mill MM 400 fitted with ZrO grinding jars and balls (Retsch GmbH & Co KG, Haan, Germany). Digestion was performed using the method of closed-tube digestion [41].

## Statistics

Data from the sensory evaluation for the five individual attributes were treated as ordinal data. Non-parametric tests were used to analyze the differences between location and varieties. The Kruskal-Wallis test was used for location testing, and the Wilcoxon signed-rank test was carried out to assess differences in single attributes. The overall mean hedonic liking was calculated as the average of all attributes assessed. Statistical tests were two-tailed and p-values $<0.05$ were considered statistically significant. All statistical analyses were conducted using SPSS (version 23.0.0.2, ICM Corp. Released 2015) for Macintosh.

## RESULTS

### (Potential) stakeholders and implementing partner views on iron beans

Stakeholders in nutrition and agriculture were interviewed and asked about their views on the implementation of iron beans in Malawi. The majority of stakeholders interviewed, were not directly related to the development and production of iron beans, and not aware of the existence and meaning of biofortification or iron beans. There is a lack of knowledge and a need for better understanding of the term. It was mentioned that '*Biofortification sounds like fortification, which suggest the crop is modified, but I understand it is not'.* Other actors producing seed or working with the biofortified varieties mentioned that a more common name in local language would make it easier to promote. Currently the variety name (either NUA 45 or NUA 59) is mostly used. Seed companies can come up with their own names, but to gain popularity it is not desirable to get a situation with several names for the same variety according to several stakeholders.

Another topic that needed clarification with interviewees was on the leaders of this initiative and who is responsible for the implementation. Research and development of these biofortified varieties is led by The International Centre for Tropical Agriculture (CIAT) who have access to different biofortified varieties and tests these genotypes for suitability in the test-fields. The department of Agricultural Research Services (DARS) is responsible for the process of variety release in Malawi, and leading the production of high quality basic seed. Since DARS does not always have capacity to produce sufficient quantities of seed, they contract private companies to produce for them. These companies sell part of the seed produced to NGO's who are using this seed to supply farmer(groups)s. There is always a high demand for this high-quality seed, and there is not enough high-quality bean seed available in Malawi. In the case of biofortified beans for many organizations the willingness to work with biofortified varieties is there, but quantities of seed available are still too low.

A major advantage of iron beans mentioned by stakeholders, is that these would be easily integrated within existing programs. Agricultural focused programs are increasingly including a nutrition component. However, there remains a need for a clear and holistic extension and promotion message, including the different benefits from a nutritional and agricultural viewpoint of these beans. It was mentioned that if the message is too focused, this can have drawbacks, when farmers and/or consumers will only use it for the purpose promoted. Problems regarding implementation foreseen are mainly around the visibility of the iron trait, since it is not possible to discriminate a non-biofortified variety from a biofortified bean.

## Focus group discussions results

**Socio economic and demographic characteristics of the FGD participants**

Participants of focus group discussions were mostly women (77%). Most mothers attended primary school (71%), where 23% of this group did not attend any education. Over half of the fathers (56%) did not attend any education, the rest of this group finished primary school (44%). Average household size for all FGD participants was 5-6 members. Almost half of the mothers (48%) indicated that their main household income came from farming, for the group of fathers this was 89%.

Several perceptions, barriers and enablers regarding (iron) bean consumption for children were identified during the focus group discussions. Differences in responses between the target groups existed, but the results summarized here contain the synthesized, most prominent themes unless otherwise specified.

**Beans in the diet/frequency**

Frequency of beans consumption was ranging from one time per week to consuming beans every day, depending on the time of the year and the growing seasons. Where in most cases farmers have one growing season, in areas with wetlands beans can be planted and harvested up to three times a year. During times when own production is not possible or available, beans are scarce, both due to low availability in markets and affordability. Beans are also regarded as a good cash crop and provide a source of income. Beans are mostly prepared in the morning and need several hours to cook and therefore consumed mostly during lunch or dinnertime. Beans are mostly eaten without additions or prepared as a relish, mixed with cooking oil, onions and tomatoes. Different types of beans have different colors, and each type of beans also comes a different smell. Thickness of the soup is also an important attribute, where a thicker soup is preferred. Participants prefer red varieties, since they have a good taste and strong smell. Beans were preferred over pigeon peas, but not as nice as rice or sweet potato.

**Barriers and enablers for bean consumption & production**

Beans are seen as a healthy and nutritious food and tasting nice. Several times it was referred to as a good stomach filler, especially in times when other foods are not available in the household. A recurring health problem mentioned in the discussions related to the consumption of beans, were eye problems. Eating beans frequently could result in bad eyesight according to participants. Therefore, community health volunteers advised their community to not consume beans if having eye problems.

This was sometimes related to consuming white or black beans only, but not in all cases. Also, people with stomach problems and/or ulcers were not supposed to eat beans, as these could aggravate the situation, according to participants. Children of 2 years of age are too young to eat beans as a whole, according to the participants. However, eating these mashed, only the soup or bean flour added to the porridge were several options for a child to consume.

From a sensory perspective, participants mentioned that bean varieties that have a good smell and are cooking well, are favorable. Beans with a tough skin need more cooking and are also not preferred because of the taste. It was mentioned several times that the taste of eating beans feels like eating meat, and since meat is often not accessible for the household, a good replacement. However, all participants prefer to consume meat over beans. Problems with bean production were a barrier for accessing beans for consumption, due to having little or no harvest over the last years. The main reason for this were the erratic rains, but also scarcity of seed and low soil fertility were a contributor to low production, according to participants.

When asked about decision-making within the household, women indicated that food was most often bought by themselves, whereas men indicated they were in charge of this. It was indicated that men are mostly responsible for buying more expensive foods (meat, fish) and women will buy vegetables if there is no money available for more expensive foods. For cooking both women and men indicated that women are responsible for cooking. The decision on what to cook is mostly based on what food items are available and is sometimes based on a joint decision with the husband and/or children.

Mothers are taking care of most caring tasks of the children, especially for feeding. When the mother is not available, the father, other siblings and grandparents are helping out.

**Lack of knowledge on iron among participants**

Iron status was referred to in the discussion as having more or less blood, the mineral was not known by participants, possibly because there is no literal translation in the local language Chichewa. It was acknowledged that having less blood could cause problems, and this resulted most in a pale skin, weakness and swelling up. Having enough blood is noticed when people are in good health. Vulnerable groups mentioned were pregnant women, children and elderly. When the groups were asked if they thought their children were at risk of iron deficiency, they all, except one group, felt this was not a problem. They did not see their child suffering from any of the symptoms mentioned, and see their children as healthy, shown by the child being happy, active, and playing well. Different food sources of iron were identified, but meat was only in one group mentioned as a good source of minerals. Sources that were mentioned most frequent were dark green leafy vegetables, avocado (leaves), and tomatoes. Beans were only mentioned twice.

## Enriched foods and biofortified beans

Adding nutrients to food was mostly understood when asked: *'do you know any foods where minerals/vitamins are added?'*. Examples given by participants were cooking oil, iodized salt, milk and margarine. Biofortified foods were not known by participants, therefore the term was explained to participants as: *'Have we ever heard of crops (here: beans) with added minerals which help in eg. blood supply. We mean food crops that you grow, not the ones you buy in the shop?'*

Iron beans were not known by the above explantion among participants. When **iron beans** were presented, participants did relate the beans to other bean varieties they liked. In Mangochi they were related to the Kachiyata variety (A Mozambiquan variety), and in the Lilongwe to CARE beans. Iron beans were mostly positively commented on, based on the characteristics of the lookalike variety they know. Most frequent responses recorded were that the type of beans taste nice, cooks fast and has a sweet aroma. Furthermore, the participants said these beans are healthy, give energy, contain vitamins and add blood to the body. Most participants would eat them often. It was also mentioned by one group that the leaves are delicious as well. According to the participants the best ways to promote the iron bean varieties was by making the seed available to the community and educating about the right way of growing and consuming. It was suggested that seed could be passed on between the different community members themselves. Health workers, extension workers, and the chief can help by organizing meetings to spread these educational messages in the communities on the benefits of the crop. Other channels of promotion mentioned were through a drama, posters and demonstration gardens.

## Sensory evaluation

In total 125 adults participated in the acceptance test of six different bean varieties, mostly women (76%), who consumed on average between 2-4 times beans in the week before the evaluation (66%). Mostly, beans are eaten as a relish (82%), mixed with cooking oil, spices, onion and tomato. The most important source of beans is from own consumption (83%). More characteristics can be found in Table 18.

**Table 18.** Characteristics of sensory evaluation participants broken down per location

| **Location** | **Chitikula** | **Kavikula** | **Mkuta** | **Total** |
|---|---|---|---|---|
| n | 44 | 33 | 48 | 125 |
| Female (%) | 30 (68%) | 24 (73%) | 41 (85%) | 95 (76%) |
| Age (SD) | 34.9 (10.3) | 44.5 (13.5) | 40.9 (15.5) | 39.8 (13.9) |
| **Frequency consuming beans last week** | | | | |
| Every day | 2 (5%) | - | 1 (2%) | 3 (2%) |
| 5-6 times | - | 3 (9%) | 4 (8%) | 7 (6%) |
| 2-4 times | 26 (59%) | 22 (67%) | 34 (71%) | 82 (66%) |
| 1 time | 12 (27%) | 5 (15%) | 5 (11%) | 22 (18%) |
| Not consumed | 4 (9%) | 3 (9%) | 4 (8%) | 11 (9%) |
| **Preparation of beans** | | | | |
| Plain | 18 (41%) | 2 (6%) | 2 (4%) | 22 (18%) |
| Tomato/Onion/oil/spices | 26 (59%) | 31 (94%) | 46 (96%) | 103 (82%) |
| **Major source of beans** | | | | |
| Homegrown | 42 (96%) | 27 (82%) | 35 (73%) | 104 (83%) |
| Purchased | 1 (2%) | 6 (19%) | 13 (27%) | 20 (16%) |
| Gift | 1 (2%) | - | - | 1 (1%) |

An acceptance test was performed to investigate the difference in liking between the six selected varieties for five different attributes (results in Table 19). The mean of the five attributes was calculated to reflect the overall liking. There was no significant difference between the liking of the color of five out of the six varieties (median 4), except for the white Kabalala variety which was rated significantly lower (median 3).

Broth thickness of the Phalombe and Kalima variety were liked best by the participants with a median score of 4. For the attribute taste, Phalombe was the highest scoring variety (median 5), whereas Kabalala was rated lowest (median 3), both were significantly different rated from the other varieties.

The smell of the varieties Phalombe and NUA 45 (median 4) were significantly preferred over the other varieties ($p<0.05$), whereas the Kabalala and Kholophete scored with significant difference lowest (median 3, $p<0.05$). The texture of Phalombe, Kalima and NUA 45 were highest evaluated (median 4). Overall liking of the six varieties was between 3.01 (Kabalala) and 3.98 (Phalombe). Location differences were found for the attributes broth thickness, taste and overall liking for the variety Kabalala. Kabalala was rated with a median of 2 in Mkuta for both attributes, whereas in Chitikula and Kavikula a median of 4 was given. For broth thickness in Mkuta the variety Phalombe was rated high (median 5), whereas in Chitikula and Kavikula respectively a 4 and a 3 were medians. Also, overall liking was higher on average for Phalombe (average 4.25) in Mkuta, and lower for Kabalala (average 2.70) than in the other two locations (respectively 3.89 and 3.71, and 3.10 and 3.33).

**Table 19.** Acceptance test results of six bean varieties among 125 adults in three different areas in Malawi

| | | **Color** | **Broth thickness** | **Taste** | **Smell** | **Texture** | **Overall mean hedonic score** |
|---|---|---|---|---|---|---|---|
| | | Median | Median | Median | Median | Median | Average |
| **Total n=125** | Kabalabala | $3^a$ | $3^a$ | $3^a$ | $3^a$ | $3^a$ | $3.01\ (1.12)^a$ |
| | NUA59 | $4^b$ | $3^a$ | $4^{ab}$ | $3^b$ | $4^a$ | $3.44\ (0.91)^b$ |
| | NUA45 | $4^b$ | $3^a$ | $4^b$ | $4^{bc}$ | $4^b$ | $3.60\ (0.88)^b$ |
| | Kalima | $4^b$ | $4^{ab}$ | $4^b$ | $4^b$ | $4^{ab}$ | $3.55\ (0.91)^b$ |
| | Kholophete | $4^{ab}$ | $3^a$ | $4^{ab}$ | $3^{ab}$ | $4^a$ | $3.39\ (0.98)^b$ |
| | Phalombe | $4^b$ | $4^b$ | $5^c$ | $4^c$ | $4^b$ | $3.98\ (0.91)^c$ |
| **Location effect** | | p-value | p-value | p-value | p-value | p-value | p-value |
| **Differences between locations** | Kabalabala | 0.05 | 0.01* | **<0.001*** | 0.67 | 0.99 | **0.05*** |
| | NUA59 | 0.21 | **<0.001*** | 0.89 | 0.14 | 0.12 | **0.04*** |
| | NUA45 | 0.06 | 0.30 | 0.79 | 0.88 | 0.89 | 0.70 |
| | Kalima | 0.89 | 0.11 | 0.50 | 0.72 | 0.06 | 0.50 |
| | Kholophete | 0.64 | 0.18 | 0.77 | 0.76 | 0.91 | 0.74 |
| | Phalombe | 0.32 | **<0.001*** | 0.12 | 0.38 | 0.06 | **0.02*** |

* significant difference in liking between the three locations. Medians/averages that do not share the same superscript in the column differ at $p<0.05$

## Mineral content of beans

Table 20 gives an overview of all XRF and ICP-MS results for iron and zinc content of the beans included in the sensory study. Iron levels range between 57 - 84 ppm, whereas zinc levels range from 23 - 29 ppm. The variety with the highest iron level is Kabalala (84 ppm), whereas Kholophete contains the highest level of zinc (29 ppm). Correlations between ICP-MS results and XRF are r=1.05 for iron and r=1.08 for zinc.

**Table 20.** Iron and zinc content for the six bean varieties used in the acceptance test

| | **Fe (XRF)** | **Fe (ICP-MS))** | **Zn (XRF)** | **Zn (ICP-MS)** |
|---|---|---|---|---|
| | **ppm** | **ppm** | **ppm** | **ppm** |
| **Kabalala** | 88.6 (0.9) | 83.7 | 27.3 (0.3) | 25.7 |
| **Kalima** | 57.1 (0.4) | 57.2 | 26.4 (0.7) | 23.1 |
| **Kholopethe** | 70.5 (0.1) | 69.0 | 28.9 (1.1) | 28.6 |
| **NUA 45** | 67.6 (0.3) | 69.1 | 22.6 (0.7) | 22.7 |
| **NUA59** | 67.9 (0.8) | 66.8 | 27.2 (1.2) | 25.6 |
| **Phalombe** | 57.7 (0.3) | 56.6 | 27.4 (1.0) | 26.0 |

Method of analysis is given in parentheses in the header. Values are reported as mean (Standard deviation). For the ICP-MS no standard deviations are available . Analysis based on dry matter of raw dry beans.

## DISCUSSION

To our knowledge, this is the first paper researching and summarizing the barriers and enablers for implementation of (iron) beans in Malawi and the consumer acceptability in households with young children in Malawi. The results of this study highlights the variety of barriers and enablers identified by stakeholders, farmers and consumers and shows the benefits of using qualitative methods to get a better feel for the root causes of possible barriers for acceptance.

From the stakeholder interviews, we found that overall stakeholders have an interest in learning more about biofortified crops while having reservations on some aspects of the approach, mostly because the concept is not known. Also, the importance of a leading organization to promote biofortification was mentioned by different stakeholders. In a review of evidence from HarvestPlus it was also mentioned that multi-stakeholder platforms are crucial to scaling up the early uptake [6]. These platforms should consist of many of the stakeholders interviewed, including the private and public sectors. Also, three critical elements were mentioned in this review for biofortification to reach its full potential; Supply, policy and demand. This encompasses supply in terms of sufficient knowledge on breeding for high minerals and enough planting material. Secondly, policies should explicitly mention biofortification as an approach to combat malnutrition. In Malawi, biofortification has been recognized in the strategy to end malnutrition by 2025. The third element, the need for demand, is created by consumers; in both rural and urban areas, who see the value of the biofortified staple crops and are willing to consume these.

From the focus group discussions, we found that in general the knowledge of mothers on iron, sources and recognizing iron deficiency in their kids was limited. These findings can be discussed using the Health belief model, a behavior change model that is used to explain and predict the likelihood of performing health-related behaviors [42]. The components 'perceived susceptibility' and 'perceived severity' score low since almost all most mothers did not see any problems with iron deficiency in their children, but on the other hand mothers did understand the benefits of having enough iron ('perceived benefits').

Beliefs of eye problems due to consuming beans are 'perceived barriers' that could lead to a lower chance of accepting and for the parents to let their child consume beans. It is not clear where these beliefs are based on, or where these stories originate from.

In general, focus group participants were very positive about adopting iron beans, however receiving these beans and learning about the benefits were essential according to participants to be able to serve the beans to their children ('cues to action').

Our acceptance test revealed high scores for all bean varieties (scores >3), which indicates that all of the bean varieties are accepted by the 125 adult participants of this test in rural areas of Malawi. Although all varieties received a score between 3-4, there was a significant lower liking of the Kabalala variety, whereas the Phalombe variety was liked most. For the Kabalala variety the difference in liking between locations could be explained by a different appreciation of the taste of this variety, as this was the only attribute that showed significant differences in rating between locations. The white, small seed sized variety was least liked (Kabalala), possibly because of its size and also because it was linked in the focus group discussions to causing bad eyesight (white beans in general). The red, large sized bean variety was most liked (Phalombe), this was also concluded from the focus group discussions as the most preferred variety. The bigger sized varieties (amongst others Phalombe) are also more expensive on the markets and therefore could have a higher liking. This was shown in a study in Tanzania, that showed a higher premium was paid for large sized beans [43]. The differences in liking between locations of the Phalombe variety could only be explained by the attribute broth thickness. All Calima-type varieties (NUA45/59 and Calima) were rated similar in each location for the different attributes.
In the focus groups it was also mentioned that a thicker soup was preferred. The broth thickness of both the Kalima variety and Phalombe variety were preferred.
The biofortified varieties, NUA 45 and NUA 59 are not significantly different rated for overall liking compared to most other varieties (except for Phalombe and Kabalala). This is positive for further dissemination, where based on agricultural characteristics further promotion can be done based on other possible favorable traits of these beans (beyond the scope of this study).
To our knowledge no acceptance test on different bean varieties in Malawi have been published. However, a small study among 74 beantraders showed the most demanded bean type in the market was Phalombe (63.5% agreed) [44].
Strong points of this acceptance test were that we used an ordinal scale in combination with the appropriate test for non-parametric statistical analysis [45]. Mean difference testing of single Likert-items is more a function of the total sample size rather than the attitude of the respondents, leading to false results followed by incorrect conclusions [46]. Furthermore, for this test a 5- point Likert -scale was used instead of a more elaborate 9-point Likert scale which could have shown smaller differences

between varieties. The 5-point scale was chosen since the majority of participants was illiterate. The combination of numbers, with pictures of checks and crosses and smiley faces gave options to the respondents to choose which scale was most clear to them. Interviewers were instructed to check with a control question whether respondents were able to understand the scale. Due to the high illiteracy rates, it was not possible to let people fill out the questionnaire themselves. Therefore, all interviews were performed by trained interviewers. The setting of the test was following the principles of central location (eg. in a school, community building or church) testing [47]. It was tried to give people as much privacy and time as was logistically possible.

The mineral analysis showed that the iron content of the varieties marked as biofortified (NUA 45 and NUA 59) had an average iron level of 68ppm and were lower than the target level (94 ppm) set by HarvestPlus for iron beans. The "gold standard" for mineral analysis of plant samples is to use ICP-OES, which is an expensive and labour-intensive method which can only be used for a limited amount of samples[48]. Recently a quicker and cheaper option has been developed by HarvestPlus using XRF. This method can process larger amounts of samples and does not require preprocessing [40]. The correlation between the two methods was high for our samples and XRF thus can be considered as a good option with lower associated costs as was suggested before in literature [40].

All mineral analyses of the bean seeds had to be done outside Malawi. The difficulty of measuring very low levels of minerals, which requires to use sensitive, expensive equipment and technical staff as described before which is not available in the country. This poses serious challenges in monitoring the biofortification trait, as was also shown in this study. The 'biofortified' varieties were bought from reliable traders, but analysis did not show the high iron levels in the beans.

This also leads to a concern when implementing these beans on a wider scale. Since there is no visibility trait, there is currently no quality control possible within Malawi which can unintentionally lead to a decreased impact of these beans, but also intentional fraud will be easy. A clear strategy has to be developed before these beans can be disseminated further. HarvestPlus suggests that the primary approach has been to gain market share for the biofortified bean varieties for their superior agronomic and consumption qualities and not necessary the iron trait alone [6]. As the Malawian market is slowly evolving and there is a lack of quality bean seed this option should not focus on the traditional agro-dealers when dissemination at a large scale is the goal. Farmers do recycle seed and trade within their own informal seed networks and the most effective approach to promote and a high adoption among farmers should be researched extensively to create an effective strategy.

In table 21 an overview of the barriers and enablers from both the stakeholder and household perspective on the implementation, production and consumption of iron beans can be found. A variety of different barriers will need to be taken into account to make implementation more successful in Malawi. Experiences from two other countries (Rwanda and DRC Congo) where iron beans have been implemented and adopted on a large scale can also help to tackle some of the barriers identified in our study [49]. Examples are the use of payback systems where farmers produce seed and return it under a scheme to increase the bean seed production and introducing a wide variety of different iron beans in terms of color and seed type to serve preferences of consumers in different areas.

**Table 21.** Overview of results: barriers and enablers identified for stakeholders and households (production and consumption) when implementing iron beans

| Topic | Barriers | Enablers |
|---|---|---|
| **Stakeholders - Implementation** | No local names of the varieties<br>No understanding of the terms biofortification/ iron beans<br>No clear promotors/ leaders of the initiative in Malawi<br>Low capacity of producing high quality seed | Easily implemented through existing agricultural programs<br>Broad target groups |
| **Households – production** | Lack of access to seeds<br>Recycling of bean seeds<br>Problems with producing beans | Cash crop |
| **Households – consumption** | Low availability in markets in low season<br>Expensive to buy<br>Beliefs on negative health effects of beans (especially black/white beans)<br>Decreasing trends in consumption of beans<br>Limited knowledge on iron sources | Part of the diet, consumed >1 serving per week on average<br>Familiarity with the type of beans that is biofortified<br>Overall liking of beans is high<br>Most beans consumed are homegrown |

The generalizability of the results of this study to other locations in Malawi should be done with caution and with the following remarks. Combining results from the focus groups and the acceptance test show a preference for the same type of beans in the areas we studied, thus we do expect similar results in similar age group and context within other rural settings in Northern/Central Malawi. Different ethnic groups in Southern Malawi might have different preferences for the types of beans as this area was not part of this study. Also, very specific beliefs as found in focus groups might differ among different locations (specific health effects of beans for example).

One of the strengths in this study was the respondent's diversity, represented by mothers, fathers, grandmothers and community health volunteers. This gave a good reflection on the community knowledge of the topics reflected on. There were also some methodological challenges in our study that we would like to address. First, data triangulation is a commonly used method to increase rigor and trustworthiness in qualitative studies where multiple methods or data sources are used to develop a comprehensive understanding of a certain phenomenon [50, 51]. Although in our study we used multiple methods to research the acceptability of iron beans, we think our methods could be improved by using the used methods in the same group of participants, which was not the case in this study and which could have given a more comprehensive view on the different topics addressed in this study. Another improvement could be made through focusing the research question more on one aspect (eg. acceptability or agricultural characteristics), as we felt the information retrieved for this study was very broad and therefore not always specific enough, but this was due to the fact that this study was part of a broader research study.

The relatively small sample size in the qualitative part of this study could have constrained our ability to examine the intracultural diversity [52]. This means that the results here are presented are central tendencies, but there is no easy fix to prevent overgeneralizing. It is clear from our results that there is a diversity in for instance the level of nutrition knowledge. These differences should be examined in greater depth in future studies in a way that also the consequences of these differences for the implementation of biofortification programs become clear.

Lastly, sampling of participants was not solely random and based on farmer groups and central location testing where we relied on extension workers to identify and invite people in the different target areas using our instructions for selecting within inclusion criteria a diverse sample. Although we felt that this has not affected our outcomes, we cannot rule out the possibility that to some extent selection bias might have influenced the results.

<u>Recommendations for implementing iron bean programs in the Malawian context</u>

- Inform and explain the concept of biofortification and iron beans to all related stakeholders.
- Make the leaders, project partners and supporters of biofortification in Malawi known.
- Develop a local name for the different varieties of iron beans in cooperation with stakeholders/consumers. This also helps with marketing the different varieties.
- Iron beans should be implemented as part of a multi food approach and integrated in other agricultural programs where possible.
- Nutrition education on the need for iron and dietary iron sources need to be improved. This could be done through either the health surveillance assistants or in combination with agricultural messaging when promoting iron beans through the agricultural extension system.
- Information provision to consumers remains important, but it is important to decide how to focus the message: only based on agronomic characteristics or also focused on nutritional benefits?
- Most preferred type of beans are red and large sized. White or black beans should be avoided as these are linked to negative health consequences by respondents.
- A monitoring system should be set up to ensure biofortified beans have high iron levels. This should possibly include a collaboration with a lab that is able to measure micronutrient content using XRF in a relatively short time.

Future research should be set up to create a workable system for monitoring iron levels of the existing varieties and to clear up the reasons for the low iron levels found in the beans in this study. Monitoring has shown to be crucial, as varieties tested in study were not classified as biofortified varieties based on their iron content. Following from this it should be further defined how to deal with the invisible trait in terms of marketing the bean seed and distinguishing these from conventional varieties.

For this study the participants were not exposed to biofortified beans before. It would be interesting to follow up on how people already working with and consuming the new varieties would adopt these, to understand more about the barriers and enablers formed at a later stage.

## CONCLUSIONS

In summary, the results showed a wide variety of possible barriers, enablers and the necessity of qualitative research in understanding the underlying reasons better. Areas of attention include amongst others the existing beliefs that could be barriers for adoption, monitoring the invisible iron trait in the beans and getting the concept of biofortification known among stakeholders and farmers/consumers. Overall beans are widely consumed and are a substantial part of the diet. Taking into account the recommendations of this study could further improve the impact and delivery of iron beans to consumers in Malawi.

## ACKNOWLEDGEMENTS

First, we would like to thank all respondents in the stakeholder interviews, the focus group discussions and the acceptance testing. Furthermore, we would like to thank CIAT Malawi and CIP Malawi for their assistance in setting up this study and hosting the researchers involved in this project. We would also like to thank Concern Malawi for helping us to mobilize study participants.

We are very grateful for receiving funding from CIAT and CIP for conducting this research.

# References


1. FAO I. WFP (2015), The State of Food Insecurity in the World 2015. Meeting the 2015 international hunger targets: taking stock of uneven progress. Food and Agriculture Organization Publications, Rome. 2016.
2. Black R. Micronutrient deficiency: an underlying cause of morbidity and mortality. SciELO Public Health; 2003.
3. Pinstrup-Andersen P. The food system and its interaction with human health and nutrition. Edited by Shenggen Fan and Rajul Pandya-Lorch. 2012:21.
4. Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, De Onis M, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. The lancet. 2013;382(9890):427-51.
5. Saltzman A, Birol E, Oparinde A, Andersson MS, Asare-Marfo D, Diressie MT, et al. Availability, production, and consumption of crops biofortified by plant breeding: current evidence and future potential. Annals of the New York Academy of Sciences. 2017;1390(1):104-14.
6. Bouis HE, Saltzman A. Improving nutrition through biofortification: a review of evidence from HarvestPlus, 2003 through 2016. Global food security. 2017;12:49-58.
7. Hayat I, Ahmad A, Masud T, Ahmed A, Bashir S. Nutritional and health perspectives of beans (Phaseolus vulgaris L.): an overview. Critical reviews in food science and nutrition. 2014;54(5):580-92.
8. Broughton WJ, Hernandez G, Blair M, Beebe S, Gepts P, Vanderleyden J. Beans (Phaseolus spp.)–model food legumes. Plant and soil. 2003;252(1):55-128.
9. de Jager I. Nutritional benefits of legume consumption at household level in rural areas of sub-Saharan Africa. Background document. 2013.
10. Nations FaAOotU. Faostat Statistical databases. . Rome2009.
11. Giller KE. Nitrogen fixation in tropical cropping systems: Cabi; 2001.
12. Lobell DB, Burke MB, Tebaldi C, Mastrandrea MD, Falcon WP, Naylor RL. Prioritizing climate change adaptation needs for food security in 2030. Science. 2008;319(5863):607-10.
13. Bouis HE, Hotz C, McClafferty B, Meenakshi J, Pfeiffer WH. Biofortification: a new tool to reduce micronutrient malnutrition. Food and nutrition bulletin. 2011;32(1_suppl1):S31-S40.
14. Haas JD, Luna SV, Lung'aho MG, Wenger MJ, Murray-Kolb LE, Beebe S, et al. Consuming Iron Biofortified Beans Increases Iron Status in Rwandan Women after 128 Days in a Randomized Controlled Feeding Trial–3. The Journal of nutrition. 2016;146(8):1586-92.
15. Murray-Kolb LE, Wenger MJ, Scott SP, Rhoten SE, Lung'aho MG, Haas JD. Consumption of iron-biofortified beans positively affects cognitive performance in 18-to 27-year-old Rwandan female college students in an 18-week randomized controlled efficacy trial. The Journal of nutrition. 2017;147(11):2109-17.
16. Cornish G. Modern irrigation technologies for smallholders in developing countries. Modern Irrigation Technologies for Smallholders in Developing Countries: Practical Action Publishing; 1998. p. 1-90.
17. Nestel P, Bouis HE, Meenakshi JV, Pfeiffer W. Biofortification of Staple Food Crops. 2006.
18. Birol E, Meenakshi J, Oparinde A, Perez S, Tomlins K. Developing country consumers' acceptance of biofortified foods: a synthesis. Food Security. 2015;7(3):555-68.
19. Talsma EF, Melse-Boonstra A, Brouwer ID. Acceptance and adoption of biofortified crops in low-and middle-income countries. Nutr Rev. 2017;75(10):798-829.
20. Asare-Marfo D, Birol E, Gonzalez C, Moursi M, Perez S, Schwarz J, et al. Prioritizing countries for biofortification Interventions using country-level data. International Food Policy Research Institute (IFPRI). 2013.
21. Biofortification Priority Index: A Global Strategy Tool for Investing in Crop Bioforitfication [Internet]. 2015.
22. Horton S, Ross J. The economics of iron deficiency. Food policy. 2003;28(1):51-75.
23. National Statistical Office Malawi, Community Health Sciences Unit, Centers for Disease Control and Prevention, Emory University. Malawi Micronutrient Survey 2015-16: Key Indicators Report. Atlanta, GA, USA: NSO, CHSU, CDC and Emory University; 2016.
24. WHO. The global prevalence of anaemia in 2011. Geneva: World Health Organisation; 2015.

25. (IMF) IMF. Malawi: Poverty Reduction Strategy Paper. . 2012.
26. Ecker O, Pauw K. Changes in food and nutrition security in Malawi: Analysis of recent survey evidence: Intl Food Policy Res Inst; 2014.
27. OECD/FAO. OECD-FAO Agricultural Outlook 2016-2025. Paris; 2016.
28. Mloza-Banda H, Ferguson A, Mkandawire A. The common bean (Phaseolus vulgaris L.) genetic resources in Malawi: Implications for conservation, utilization, and production. Bunda J Agric Envron Sci Tech. 2003;1:1-13.
29. Scott J, Maideni M. Socio-economic survey of three bean growing areas of Malawi. 1998.
30. Ferguson AE, Kambewa P, Mkandawire R. Bean production and use practices: Results of the 1990-91 socio-economic research in the three regions of Malawi. East Lansing, USA: Bean/Cowpea CRSP Michigan State University. 1991.
31. Dorward A, Chirwa E. The Malawi agricultural input subsidy programme: 2005/06 to 2008/09. International journal of agricultural sustainability. 2011;9(1):232-47.
32. Davis CL, Vincent K. Climate risk and vulnerability: A handbook for Southern Africa. CSIR; 2017.
33. Abson DJ, Dougill AJ, Stringer LC. Spatial mapping of socio-ecological vulnerability to environmental change in Southern Africa. Sustainability Research Institute, School of Earth and Environment, The University of Leeds, Leeds, UK. 2012.
34. Services DoCCaM. Climate of Malawi 2006 [Available from: https://www.metmalawi.com/climate/climate.php.
35. National Statistics Office. Integrated household survey 2016-2017. Malawi; 2017.
36. Aberman N-L, Meerman J, Benson T. Mapping the linkages between agriculture, food security and nutrition in Malawi: Intl Food Policy Res Inst; 2015.
37. Hummel M, Talsma EF, Van der Honing A, Gama AC, Van Vugt D, Brouwer ID, et al. Sensory and cultural acceptability tradeoffs with nutritional content of biofortified orange-fleshed sweetpotato varieties among households with children in Malawi. PloS one. 2018;13(10):e0204754.
38. Oparinde A, Birol E, Murekezi A, Katsvairo L, Diressie M, Nkundimana J, et al. Consumer acceptance of biofortified iron beans in rural Rwanda: Experimental evidence. 2015.
39. Carrillo-Centeno P , M. S-R. Sensorial Evaluation Study of a Variety of Nutritionally Improved Beans (INTA Ferroso) vs. a Conventional Variety (INTA Norte) in the Northern Zone of Nicaragua . Cali, Colombia,: HarvestPlus LAC; 2014.
40. Guild GE, Paltridge NG, Andersson MS, Stangoulis JC. An energy-dispersive X-ray fluorescence method for analysing Fe and Zn in common bean, maize and cowpea biofortification programs. Plant and Soil. 2017;419(1-2):457-66.
41. Wheal MS, Fowles TO, Palmer LT. A cost-effective acid digestion method using closed polypropylene tubes for inductively coupled plasma optical emission spectrometry (ICP-OES) analysis of plant essential elements. Analytical Methods. 2011;3(12):2854-63.
42. Champion VL, Skinner CS. The health belief model. Health behavior and health education: Theory, research, and practice. 2008;4:45-65.
43. Mishili FJ, Temu A, Fulton J, Lowenberg-DeBoer J. Consumer preferences as drivers of the common bean trade in Tanzania: a marketing perspective. Journal of international food & agribusiness marketing. 2011;23(2):110-27.
44. Chirwa R, Phiri M. Factors that influence demand for beans in Malawi. 2006.
45. Jamieson S. Likert scales: how to (ab) use them. Medical education. 2004;38(12):1217-8.
46. Clason DL, Dormody TJ. Analyzing data measured by individual Likert-type items. Journal of agricultural education. 1994;35(4):4.
47. Lawless HT, Heymann H. Sensory evaluation of food: principles and practices: Springer Science & Business Media; 2013.
48. Vinoth A, Ravindhran R. Biofortification in Pearl Millet: From Conception to Dissemination. Nutritional Quality Improvement in Plants: Springer; 2019. p. 413-28.
49. Mulambu J, Andersson M, Palenberg M, Pfeiffer W, Saltzman A, Birol E, et al. Iron beans in Rwanda: crop development and delivery experience. African journal of food, agriculture, nutrition and development. 2017;17(2):12026-50.

50. Jick TD. Mixing qualitative and quantitative methods: Triangulation in action. Administrative science quarterly. 1979;24(4):602-11.
51. Leech NL, Onwuegbuzie AJ. An array of qualitative data analysis tools: a call for data analysis triangulation. School psychology quarterly. 2007;22(4):557.
52. Pelto PJ, Pelto GH. intra-cultural diversity: some theoretical issues 1. American ethnologist. 1975;2(1):1-18.

# Chapter 6

## General discussion

Marijke Hummel

Biofortification has shown to be effective in increasing the nutritional intake of vulnerable target populations. In this thesis we presented research on three key elements around the development and delivery of biofortified crops to further improve the nutritional impact: climate change, retention and acceptability. The overall aim was to present findings that could further improve the nutritional impact of biofortified crops. Many different disciplines are part of the development and delivery phase when developing biofortified crops. Knowledge gaps that are addressed in this thesis are: Can biofortified iron beans overcome the future climate scenarios that are predicted? How does drought potentially influence the nutrient content of beans? What is the mineral retention of low phytic acid, biofortified and conventional beans? What is the acceptability of biofortified crops in terms of cultural and sensory acceptability in Malawi? In order to fill these knowledge gaps, these questions were studied for the crops of interest: beans and orange-fleshed sweetpotato.

This chapter summarizes the main findings of the research and discusses the main findings in a broader context. The chapter finishes with implications of our research for further development of biofortified crops and directions for future research. The main findings of this thesis are summarized in Table 22.

**Chapter 2** demonstrates by crop modelling that the majority of current common bean growing areas in south-eastern Africa will become unsuitable for bean cultivation by the year 2050. Furthermore, it was demonstrated through a field trial in a climate analogue site for future predicted drought conditions that there will be reduction in yields of available common bean varieties. Analysis of the nutrition and antinutritional compounds revealed that iron levels in common bean grains are reduced under climate-induced drought scenarios, therefore future beans servings by 2050 will likely have lower nutrition quality, posing challenges for ongoing climate-proofing of bean production for yields, nutritional quality, human health and food security.

**Chapter 3** describes the results of a retention study, where two different household recipes (boiled and refried beans) were prepared using biofortied, lpa and conventional beans. We found that retention was higher among the conventional and biofortified beans. Lpa varieties had very low phytate levels and a much lower retention of zinc compared to the other two groups of beans. Furthermore, pre-soaking beans significantly reduced the amount of phytates and zinc after processing. Nevertheless, soaking is recommended as it decreases the phytic acid levels with an average of >10% and could thereby potentially improve the bioavailability level. Our research showed that biofortified refried beans contribute most to the EAR when a standardized portion is consumed. The effect of the extremely low mineral:phytate ratios that were found in the lpa beans on bioavailability of minerals in humans remains to be clarified. Combining the lpa and biofortication trait could potentially further improve the nutritional impact of consuming beans.

The last key element that was studied for this thesis was acceptability of biofortified crops for which both the acceptability of iron beans and orange fleshed sweetpotato was studied in rural areas in Malawi using a mixed method approach.

In **Chapter 4** the acceptability of OFSP among households with children in rural areas in Malawi was assessed. Sensory acceptability showed a preference for the control variety (yellow fleshed variety) over the OFSP varieties; however, all varieties were accepted. Cultural acceptability showed that especially the subjective norms and attitudes toward behaviour were best predictors for the caretakers' behaviour in this context. Almost all caretakers had the intention to prepare the orange fleshed sweet potato for their children. Considering both cultural and sensory acceptability can improve the impact of biofortified crops in future programming.

**Chapter 5** describes a study that aimed to get an understanding of the barriers and enablers for implementation and consumption of (iron) beans in Malawi, focused on households with children. This was assessed by evaluating consumer acceptability from a sensory (acceptance test with six different bean varieties) and cultural perspective (focus group discussions) and through mapping the views of stakeholders (interviews). The results showed that the most important areas of attention include 1) getting the concept of biofortification more widely known among stakeholders and farmers/consumers, 2) tackling the existing beliefs that could be barriers for consumption and 3) to closely monitor the invisible iron trait in the (biofortified) beans available. Taking into account the recommendations of this study could further improve the impact and delivery of iron beans to consumers in Malawi.

The most important methodological issues for the individual chapters have been highlighted in the discussion part of the chapters itself. In addition, we would like to discuss the scope and generalizability of this research.

The scope of this research highlights only three of the key elements of development and delivery of biofortified crops. We do acknowledge there are many more topics that need to be studied, but due to budget and time constraints could not be taken into account. The chosen key elements highlight the variety of topics and the complexity and interrelatedness between some of the topics.

Three of the chapters included in this thesis specifically focussed on Malawi which is one of the highest priority countries for the implementation of biofortified crops. However, the findings from all chapters show specific methods that can also be applied in other settings.

**Table 22:** Summary of the research key findings

| Chapter | Objectives | Key findings |
|---|---|---|
| 2<br>Climate modelling and fieldtrials | To assess the impact of climate change induced changes in heat and precipitation in 2050 on the suitability for cultivation of common beans in Eastern Africa | • Common bean cultivation suitability differs across location, within each country dependent on changes in temperature or precipitation.<br>• Iron levels in bean grains decrease under climate induced drought conditions.<br>• Zinc, lead, protein and phytic acid levels increase under climate induced drought conditions.<br>• Under climate induced drought scenarios, future bean servings will have lower nutritional quality |
| 3<br>Lab study | To assess retention of iron, zinc and phytates in conventional, biofortified and low phytate beans after soaking, boiling and refrying beans | • Soaking does not influence the iron retention but does lead to a lower retention of zinc and phytates after processing.<br>• Retention of zinc is significantly lower in the lpa varieties of beans.<br>• Biofortified beans have the highest contribution to the EAR of iron per portion (44%).<br>• Combining the low phytate and biofortification trait through crossbreeding could lead to a higher nutritional impact of iron beans |
| 4<br>Mixed methods | To assess the cultural and sensory acceptability of orange-fleshed sweetpotato among households with children (0-5 years) in Malawi | • From a sensory perspective all OFSP varieties are accepted, but there is a preference for the control variety over the OFSP varieties.<br>• From a cultural perspective subjective norms and attitudes toward behaviour were the major predictors for caretaker's behaviour to prepare OFSP for their child.<br>• Considering both cultural and sensory attributes when introducing a biofortified crop can influence the acceptability of varieties and consumption amongst households with children. |
| 5<br>Mixed methods | To understand the barriers and enablers for implementation and consumption of (iron) beans in Malawi, focused on households with children | • The term iron beans is largely unknown by both potential stakeholders and consumers.<br>• Negative beliefs on the effects of bean consumption could limit the consumption of certain types of beans.<br>• Monitoring the iron content of iron beans is essential to make sure that the beans have the envisioned nutritional impact in target populations. |

## Development: Breeding for more climate-resilient beans with a higher bioavailability of nutrients

As indicated by our research, climate change through the effect of changes in temperature and precipitation, will have negative consequences on the yield but also on the nutritional quality of beans. Future changes in temperature and precipitation will however not be the only environmental consequence of climate change. Other environmental factors as part of climate change that are reported are the effect of changes in tropospheric carbon dioxide, ozone concentrations and salinization. Their effect on yield and nutrition quality are also important to take into account when studying environmental changes in future. A systematic review summarizing all studies done on the effect of all these environmental changes and their effects on yields of vegetable and legume yield on nutritional quality found 174 relevant papers studying either one of the factors [1]. It was concluded that to minimize potential reductions in vegetable and legume yields and their associated health effects it is of major importance to prioritize agricultural development by researching the effects of the mentioned factors, and where possible study combinations of factors.

In addition, chapter 3 concluded that the retention of iron, zinc and phytate differs in the conventional, biofortified and *lpa* beans. The phyate content has an effect on the bioavailability of the nutrients in humans. Therefore, we propose that more research should be done to further unravel the effect of antinutritional factors specifically focussing on phytic acid, and the combination with variable levels of mineral content. This could be done in vitro through the use of Caco-2 cells, but essential is also to assess the in vivo bioavailability of minerals in humans measured using isotope labelling [2]. Another angle of research to increase the bioavailability of iron and zinc is to study processing methods to further reduce and remove phytic acid content of beans before consumption (eg. sprouting, pressure cooking, addition of enzymes).

In conclusion, during the development phase, emphasis should be on further climate proofing and understanding the interactions between anti-nutritionals and minerals for an improved impact of biofortified crops at the implementation stage.

## Delivery: Assessing the acceptability of biofortified crops and preparing a dissemination strategy

During the delivery phase first a plan of action for disseminating the crop is prepared. In this thesis we studied the acceptability from a sensory and cultural perspective of which the results could be used as input for preparing a plan of action. A mixed method approach will lead to a more thorough understanding of the enablers and barriers for adoption and acceptability of crops. This thesis has both studied a crop with a visible trait (OFSP) and an invisible trait (iron beans). To make comparisons in acceptability between the crops in our study would not be valid, as there are many other factors influencing the overall acceptability of the crops. From our research we can conclude that a clear dissemination strategy is needed before the actual dissemination of the crops. As we saw in the case of iron beans there was no knowledge or use of iron beans, caused by a lack of a driving force promoting the beans actively. In addition, we found that even if beans are presented as iron beans the beans do not necessarily have a high iron content. Therefore, monitoring is essential to keep the iron beans and biofortification in general trustworthy. It was suggested by Harvestplus to gain market share for biofortified beans due to their agronomic and consumption quantities, where the emphasis is less on nutritional aspects [3]. In the case of developing countries with low access to monitoring the iron trait in an accessible and fast way this seems to be the way forward for now.

## Implications of findings from this thesis for increasing the impact of biofortified crops

To increase the nutritional impact of biofortified crops, the following **recommendations and future research areas** for developing and delivering biofortified crops are formulated based on the findings of this thesis:

- Further climate proofing of crops is essential to minimize reductions in yields and nutritional quality higher (chapter 2).
- Soaking should be more widely promoted as a practice when preparing beans to increase the nutrient bioavailability (chapter 3).
- Continue promoting the use of biofortified beans as preferable type of bean for consumption as these contribute most to the EAR or both iron and zinc in adults and young children (chapter 3).

- Further development of breeding beans combing the biofortification and *lpa* trait to increase bioavailability of minerals (chapter 3).
- Conduct additional studies to understand the low zinc retention from *lpa* beans (chapter 3).
- Conduct in vivo studies to fully understand the bioavailability of minerals in humans from different types of beans (chapter 3).
- A further exploration on the use of the nutritional yield metric (the quantity of supply of nutritionally-important compounds per unit area) to define what are acceptable biofortified varieties to promote from a nutritional perspective and to be able to compare different varieties [4] (chapter 4).
- The use of mixed methods to conduct acceptability and/or adoption research to understand more about enablers and barriers before and after dissemination of biofortified crops (chapter 4 and 5).
- Further validating the use of psychosocial models as a means to assess the cultural acceptability of biofortified crops in a specific setting (chapter 4).

## Conclusions

This thesis demonstrated that to further improve the nutritional impact of biofortified crops, the studied elements (climate change effects on nutritional quality, retention and consumer acceptability) need attention. Improving the impact of biofortified crops could be reached through further climate-proofing of bean varieties, combining the *lpa* trait with the iron trait in developing new bean varieties, leading to higher bioavailability of iron, and studying both sensory and cultural acceptability using mixed methods in a local context.

## REFERENCES


1. Scheelbeek PF, Bird FA, Tuomisto HL, Green R, Harris FB, Joy EJ, et al. Effect of environmental changes on vegetable and legume yields and nutritional quality. Proceedings of the National Academy of Sciences. 2018;115(26):6804-9.
2. Petry N, Boy E, Wirth JP, Hurrell RF. The potential of the common bean (Phaseolus vulgaris) as a vehicle for iron biofortification. Nutrients. 2015;7(2):1144-73.
3. Bouis HE, Saltzman A. Improving nutrition through biofortification: a review of evidence from HarvestPlus, 2003 through 2016. Global food security. 2017;12:49-58.
4. DeFries R, Fanzo J, Remans R, Palm C, Wood S, Anderman TL. Metrics for land-scarce agriculture. Science. 2015;349(6245):238-40.

# Publications and conferences attended

**Journal articles**

Hummel, M., Hallahan, B. F., Brychkova, G., Ramirez-Villegas, J., Guwela, V., Chataika, B., ... & Beebe, S. (2018). Reduction in nutritional quality and growing area suitability of common bean under climate change induced drought stress in Africa. *Scientific reports*, *8*(1), 16187. *Published*

Hummel, M., Talsma, E. F., Van der Honing, A., Gama, A. C., Van Vugt, D., Brouwer, I. D., & Spillane, C. (2018). Sensory and cultural acceptability tradeoffs with nutritional content of biofortified orange-fleshed sweetpotato varieties among households with children in Malawi. *PloS one*, *13*(10), e0204754. *Published*

Hummel, M., Talsma, E.F., Taleon, V., Londono, L., Gallego, S., Raatz, B., Spillane, C. Mineral and phytate retention of biofortified, low phytic acid and conventional bean varieties when preparing common household recipes. *Nutrients* 12 (3), 658. *Published*

**International conference presentations**

- Title: Mineral and phytate retention of low phytic acid, biofortified and conventional bean varieties when preparing common household recipes. Micronutrient Forum Bangkok, Thailand March 2020 (accepted poster presentation)
- Title: Sensory acceptability of iron biofortified beans and orange flesh sweet potato in Malawi, 21st ICN International Congress of Nutrition, on 15-20th October 2018 in Buenos Aires, Argentina (oral presentation)
- Title: Acceptability of biofortified crops in Malawi, Nutrition dissemination conference in October 2016, Lilongwe, Malawi organized by department of Nutrition, HIV and AIDS. (poster presentation)

**Professional courses attended**

- Selected participant of the European Nutrition Leadership Programme (ENLP) on 11-17th April 2019 in Luxembourg
- Production and use of food composition data in nutrition, 2-week course organized by VLAG International Advanced Courses in Wageningen, The Netherlands (October 2015)

**International Conferences and other meetings attended**

- Planetary Health conference, on 29th May-1st of June 2018 in Edinburgh, United Kingdom
- Agriculture, Nutrition and Health Academy Week, on 20-24 June 2016 in Addis Abeba, Ethiopia
- Participant of the Compact 2025, Roundtable discussions on ending hunger and undernutrition by 2025 in Malawi, organized by IFPRI in May 2016

  2nd International congress on Hidden Hunger, March 3-6th 2015, Stuttgart, Germany