


FONIO (DIGITARIA EXILIS) IN WEST AFRICA: TOWARDS IMPROVING NUTRIENT QUALITY




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This research was conducted under the auspices of the Graduate School VLAG (Advanced studies in Food Technology, Agrobiotechnology, Nutrition and Health Sciences).

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Thesis

submitted in fulfilment of the requirements for the degree of doctor

at Wageningen University

by the authority of the Rector Magnificus

Prof. Dr A.P.J. Mol,

in the presence of the

Thesis Committee appointed by the Academic Board

to be defended in public

on Tuesday 8 September 2015

at 4 p.m. in the Aula.

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Fonio (Digitaria exilis) in West Africa: Towards improving nutrient quality

180 pages.

PhD thesis, Wageningen University, Wageningen, NL (2015)

With references, with summary in English

ISBN 978-94-6257-455-7

Abstract

Hidden hunger affects a far greater percentage of the world's population with iron and zinc deficiencies being the most common, particularly affecting women of reproductive age. The primary cause of the mineral and vitamin deficiencies in developing countries is inadequate intakes of multiple and bioavailable micronutrients in common cereal-based diets, emphasizing the need for increased quality of diets. Plant genetic diversity, and also indigenous foods and/ or traditional grains as fonio may play a critical role in reduction of the problem for resource poor populations. Fonio *(Digitaria exilis)* is the most ancient West African cereal representing a key crop in food supply during crop shortfall periods. However, less is known about its potential to contribute improving nutrition and health. In this context, the thesis investigated whether the nutrient quality of fonio, especially iron, could be improved to potentially contribute to the daily intake of the population. Research was done in the framework of the EU funded FP6 Fonio project.

Investigations in this thesis comprised: *i)* the consumption pattern of fonio and its contribution to nutrient intakes (24 hr recall among 108 women aged 15-49 year-old, Bamako, Mali); *ii)* the validation of the Mali food composition database (TACAM) for assessing population level intakes of energy and nutrients (weighed food record and chemical analysis of duplicate portion among 36 women out of 108 previously selected); *iii)* the genetic diversity, nutrient content especially bioavailable iron and zinc content and the effect of processing on fonio landraces (chemical and amplified fragment length polymorphism analysis of 12 Malian fonio landraces); *iv)* the sensory variability among fonio landraces (sensory analysis of 20 fonio landraces from Mali, Guinea and Burkina Faso); *v)* improved food processing combining dephytinisation with native phytase and fortification of fonio diet with iron to increase iron absorption (iron absorption stusy among 16 women aged 18-30 years, Cotonou, Benin).

The results indicated that fonio is consumed one to three times/month by 68% of our study population with an average daily portion size of 152g when consumed. Only 5% of the study population consumed fonio dishes contributing to 16% of the daily energy intake for the consumed portion size, reflecting the low consumption of fonio related to significant barriers such as availability of cooked fonio in urban markets, lack of consistent supply throughout the year, difficult post- harvest processing, high-quality product demand, hard texture coupled with time consuming cooking process, and high cost of fonio products.

The use of the adjusted TACAM is acceptable for estimating average intake at population level for macronutrients, calcium and zinc in a low intake population, but not for carbohydrate and iron intakes which were underestimated and vitamin A which was overestimated, nor for probability of adequate intakes and nutrient densities. At individual level, significant differences were observed between estimated and analyzed intakes for all the nutrients increasing with higher intakes.

Fonio landraces in West Africa were different for their visual (colour and presence/absence of impurities) and their textural (consistency of cooked grain) charactersitics. We found no meaningful genetic diversity among the 12 landraces in Mali (indicated by the very low polymorphism level of 3.5%) and the proximate composition, iron and zinc content showed no significant differences among landraces. Traditional processing reduced iron (96% reduction) and phytate (75% reduction) content, however, the molar ratio of phytate to iron remained above the critical cut-off of >1 indicating poor iron bioavailability. Zinc concentration was hardly affected by processing and cooking.
Processing experiments confirmed that whole wheat flour is a good source of natural phytase to produce low phytic acid containing fonio porridge. Dephytinisation using intrinsic wheat phytase reduced phytate-to-iron molar ratio from 24:1 to 3:1 after only 1 hour of incubation at 50ºC with pH of 5.0, and iron fortification further reduced the molar ratio to 0.3:1. Dephytinisation with wheat phytase and fortification significantly increased iron absorption ratio from 2.6% to 8.2% in fonio porridges.

We conclude that the current contribution of fonio to daily bioavailable iron intake is low due to small portion sizes being consumed in low frequency, to considerable losses during processing to mid-wet fonio, and to a high phytate-iron molar ratio. Fonio landraces from Mali, Guinea and Burkina Faso are variable in visual and textural characteristics (colour, presence of impurity and consistency of the cooked grain, respectively), determining the preference of consumers. Selecting landraces for preferred sensory properties may offer an entry point for processors who intend to promote the consumption of fonio and increase its role in diet. In absence of meaningful genetic diversity and variation in iron content in fonio landraces in Mali, there is little benefit in selecting landraces for natural high iron content. Dephytinisation using intrinsic wheat phytase could be a promising processing practice to improve iron bioavailability and fortification is required to increase the amount of absorbed iron from fonio meals.

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Chapter one

- General introduction

BACKGROUND

The role of biodiversity in improving micronutrient deficiencies

Maternal and child malnutrition remains a pervasive and damaging public-health problem in many low- and middle-income countries affecting billions of people. To date, over 165 million children under five years of age are stunted (too short for their age) and about 52 million children are wasted (too thin) and thus require special treatment [1]. Maternal under nutrition, indicated by the prevalence of low BMI among adult women (<18.5 kg/m^2), remains above 10% in Africa and Asia [1]. In addition to undernourishment, there is rising concern about the quality of diets in terms of adequate micronutrients in developing countries. Hidden hunger, which represents a lack of micronutrients, affects a far greater percentage of the world's population with iron and zinc deficiencies being the most common, particularly affecting women of reproductive age [2]. Over a quarter of the world's population is anaemic, including 293 million children younger than five years of age (47%) and 486 million non-pregnant women (30%) with the highest prevalence (over 60% and 40% respectively) in Africa [3, 4]. Anaemia is a contributing factor to over 20% of all maternal deaths among pregnant women [5]. Iron deficiency is considered the major cause of anaemia, accounting for around 50% of cases [6] and has adverse health effects on the outcome of pregnancies, infant growth, cognitive performance, psychomotor development, immune status and work capacity [7-11].

The primary cause of the mineral (especially iron) and vitamin deficiencies in developing countries is inadequate intakes and low bioavailability of multiple micronutrients in the common, monotonous cereal-based diets, emphasizing the need for increased quality of diets [2]. The potential contribution of biodiversity to food and nutrition security through improved dietary diversity and quality is increasingly recognized by the international research community [12, 13]. Plant genetic diversity has a critical role in increasing and providing sustainable production levels and nutritional diversity [13]. In many areas in the world, traditional or indigenous foods and wild varieties play a major role in supplementing the cereal-based diets [14, 15] or constitute the major part of the diet during periods of food shortage [16, 17] especially for resource poor populations. Some of these lesser-known foods and varieties have micronutrient superiority over some more common and widely-utilized food [13]. They also provide valuable genetic traits for developing new crops through breeding and selection [18, 19].

Cereal grains contribute over 60% of the world food production and, along with pulses and oil seeds, provide a major bulk of dietary proteins, calories, vitamins, and minerals especially iron and zinc to the world population in general and to the developing world in particular [20, 21]. In sub-Saharan Africa, native traditional cereal grains encompassing sorghum (*Sorghum bicolor*), millets (*Pennisetum sp*), fonio (*Digitaria sp)*, teff (*Eragrostis tef*), and amaranth seeds (*Amaranthus caudatus*) [22] could be an effective weapon against hunger in the continent [23]. The potential of the micronutrient-value of some underutilized species has been reported in some studies along with information on ways to consider those available biological food resources in strategies to reduce malnutrition [24, 25]. Subsequently, many previously neglected species and varieties have been reconsidered to become potential vehicles for improved nutrition, as well as convenient sources of increased food supply and income among the most food-insecure population groups [22, 24, 25]. Their contribution to food requirements can be significant in many developing countries for they are subsistence-oriented food staples [22] related to their accessibility and rural availability as well as their suitability for family gardening to combat nutrition insecurity in contrast to cash crops.

A strong case for the contributions of biodiversity to nutrition and health emerges from existing data on food composition, including increasing information on the nutrient variability present within species [26]. However, due to the lack of professional attention to biodiversity and traditional or underutilized foods, large gaps exist in our knowledge of the composition and the dietary contribution of these foods to human nutrition. This has been a neglected area, or regarded as unimportant, by compilers of food composition databases and investigators involved in designing and executing food consumption surveys [27]. Therefore, the real and potential contribution of these foods to nutrition and health improvements is difficult to be judged. In addition, there is no doubt that improvement of the nutritional value of these traditional cereals could support especially resource-poor populations in improving nutrition because of the predominance of these staple foods in their diets [28]. Among these traditional and neglected cereals, fonio has an important place and offers a great potential in Sub-Saharan Africa [29], and is nowadays gradually 'rediscovered' and considered for improvement as a cultivated species [30, 31], although the evidence for its contribution to reduce malnutrition is lacking.

Micronutrient deficiencies are highly prevalent and mainly caused by low quantity and quality of food consumed. Traditional foods, such as the native sub-Saharan cereal fonio, may play a major role in reduction of malnutrition among resource poor African populations, but evidence is lacking.

Fonio, production and utilization in West Africa

Fonio is a typical West African traditional cereal cultivated across dry savannah regions along the Sudanese zone [32] from Cape Verde in the West to the Lake Chad in the East, from the edge of the Sahara in the north to the beginning of the rain forest in the South [33]. Guinea, Nigeria, Mali and Burkina Faso hold the largest fonio growing areas and production in West Africa. About 587 270 tons have been produced on 566 047 hectares in these regions in 2012 [34]. In semiarid and sub-humid areas in Guinea, Mali and Burkina Faso, fonio contributes to 17-21 % of total amount of staple cereals produced for subsistence [29].

Fonio is grown in tropical climates with a marked dry season, average temperatures of 25- 30°C and rainfall between 900 and 1000 mm [35]. As most traditional cereals, fonio is produced in traditional systems with little or no external inputs and fits perfectly into the low-input traditional farming systems of resource-poor African farmers [32, 33, 36]. In semi-arid zones, the land used for fonio cultivation represents 12% of the total land used in the rotation system after millet and sorghum and 23% of the total land used in sub-humid zones after maize, sorghum and rice [29].

Fonio is a yearly small herbaceous plant with a height of 30 to 80 cm (**Figure 1.1.A**). The seed is a caryopsis surrounded by glumes and lemmas and "dressed" as barley and rice. Morphologically, the size of the grain is very small being 1.0 mm long and 0.75 mm large with a 1.5 mm diameter (**Figure 1.1.B**), and 2000 grains are needed for 1 gram of fonio [29, 37].The grain is identical to other cereal grains but the form is variable. It is composed of bran (4-8%), germ (2-12%) and endosperm (75-85%) [37]. The germ contains the embryo which constitutes about a third of the total caryopsis. The endosperm consists of aleuronic layer and starchy endosperm. Starchy endosperm cells contain the major storage reserve of the caryopsis.

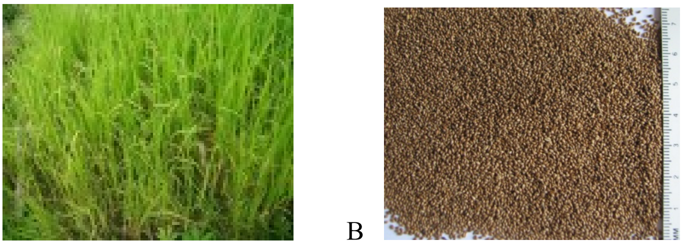
A B


Figure 1.1. *Fonio plant in field (A) and fonio paddy (B)*

Fonio is planted on light (sandy to stony), poor and degraded soils and sowed by hand using 10 to 30 kg of seed/ha [35], and normally fertilizers and pesticides are not used [33]. Fonio is performing better than other cereals under drought conditions and low soil fertility, and is less damaged by pests during storage [32].

Fonio harvesting and post-harvesting handling are reported to be the major constraints for its utilization [23, 38]. The extremely small size of the grain leads to tedious and time-consuming post-harvest dehulling and cooking processes [23, 32, 39]. From field to consumption, most of the activities are manually performed, making harvesting and post-harvest activities more laborious and time-consuming [39]. Women could spend for example 2 hours to manually dehull only 2 kg of fonio and will use up to 400 litres of water to repeatedly wash only 25 kg of fonio [40]. And although modern and new equipment have been developed to improve processing at household and industrial level [39], the problem of the high use of water in washing still remains. In the other hand, urbanization leads to an increased demand of industrially and sophisticated produced foods inducing a rapid shift to non-traditional grains [41, 42]. This resulted in a decrease in consumption of traditional foods and indigenous foods such as fonio, particularly in urban areas. Furthermore, there has been an increasing inadequacy in research investment to improve the production and processing chains of the traditional grains [43]. This resulted in a lack of improved agronomic practices, little efforts for agricultural research, low post-harvest promotion including losses, inadequate marketing channels and processing technologies, poor product quality, and no up-to-date information to upgrade the quality of the grain [29, 43]. The reduction in demand for traditional foods and the lagging investment led to a turn of farmers to the cultivation of exportable cash crops [44] bringing about a reduction of the productivity, output and harvested areas of traditional food crops such as fonio over the past years in several West African countries [43, 44]. As a consequence, fonio has been classified as "lost crop of Africa", "minor" or "neglected" and "indigenous" crop without economic potential [32].

As key-crop in agriculture and food supply in its traditional cultivation zones having a large ecological adaptability, there is a need for a renewed interest in traditional and underutilized fonio as potential vehicle for improved nutrition, as well as convenient resource of increased food supply and income.

Fonio IP6 project: Upgrading quality and competitiveness of fonio for improved livelihoods in West Africa

The interdisciplinary and collaborative research project Fonio IP6 "*Upgrading quality and competitiveness of fonio for improved livelihoods in West Africa*" was funded by the EU Specific International Scientific Cooperation Activities (INCO) to explore properties of fonio as a potential healthy and nutritive food for urban areas in Sub-Saharan Africa and European countries, while generating income for local producers. The three-year project started in 2006 and involved interdisciplinary research scientists from three European countries (France, Netherlands and Belgium) and 4 West African countries (Mali, Guinea, Burkina Faso and Senegal). The main goal of the project was to improve production, post-harvest and food technology for fonio products of high nutritional value, good sensory quality and commercial value [45]. The FONIO project focused on 3 strategies: *i*) development of farming and cropping systems to increase fonio production, the value of various uses-grain, straw and by-products as animal feed, and improving soil fertility , for creating opportunities to improve the livelihoods of producers; *ii*) investigating the nutritional quality and improving the nutritional value of fonio products for adding substantial health/nutrition value to fonio products to contribute to better nutrition and health; *iii*) investigating processing and marketing systems (women's association, small and medium enterprises) in order to facilitate economic access to local communities, and promote the development of export markets for improved/new fonio products [45]. The FONIO project aimed to contribute to alleviation of (hidden) hunger and poverty burden among women who are involved in the fonio production chain as producers, processors or traders; and to improve local living standards through a sustainable development of the fonio commodity chain from rural zones to urban or export markets. Preservation of this under-utilized crop cultivation was expected to contribute to the conservation of natural local resources and preservation of the local biodiversity [45].

The FONIO project has been initiated with the main goal to develop research on production, post-harvest and food technology to develop fonio products of high nutritional value, good sensory qualities and commercial value.

RECONSIDERING POTENTIAL OF FONIO FOR BETTER NUTRITION

To evaluate the potential of fonio to improve nutrient, it is essential to have knowledge on the consumption patterns and the role of fonio in diets. In addition, information on the nutritional value of fonio from valid food composition tables is needed to estimate the potential contribution of fonio to nutrient intake.

Fonio consumption

Previous investigations on the dietary role of fonio in West Africa societies reported that the grain was essentially cultivated for home consumption in rural areas [29, 32, 33, 35]. In production areas, fonio is consumed by every age group, three times a day in different kinds of preparations [46]. In West Africa urban areas, on average the consumption frequency of less than once a month has been reported for 54% of fonio consumers [46]. In Burkina Faso and Guinea, 4% of people consume fonio frequently per week [46]. In urban areas of Mali, fonio accounts for less than 1% of the cereals eaten, and the average consumption is 0.5 to 1.0 kg per person annually [46]. It is mainly consumed as porridges, couscous, traditional/alcoholic beverages, fatty fonio, salads, cakes, doughnuts, cookies and bread.

The use of fonio in the diets of West African communities is often related with religious and sociocultural beliefs and practices [32, 33, 39, 46]. In Senegal, Mali, Guinea, Burkina Faso and Togo, fonio is reserved particularly for chiefs, royalty and special occasions such as Ramadan [47, 48]. For some traditional communities in Togo, fonio is the most important food during women's initiation ceremonies, weddings, and the traditional baptism of newborn children. Fonio is believed to help prevent blood clotting after women give birth [47], and the porridge based fonio is recommended for breastfeeding women to stimulate milk production [49]. The Akposso and Akebou people prepare fonio with beans in a dish that is reserved for special occasions in southern Togo [32]. In the Dominican Republic, fonio is used for its assumed aphrodisiac attributes. It can also be used as a cheap source of methionine to detoxify the cyanides in cassava [50].

However, information on fonio consumption is mostly based on anecdotal information reported in descriptive socio-economic studies [46] and so far, no systematic food consumption studies have been carried out on the consumption of fonio. Assessment of the consumption of fonio in West African urban areas is an essential step to evaluate the potential of fonio and fonio products to contribute to improve nutrient intakes.

Food composition table for assessing nutrient intakes

In food consumption surveys, the role of food composition databases (FCDB) is fundamental for estimating dietary intakes of energy and nutrients, the probability of adequacy of these intakes and the contribution of fonio to these [27]. High quality, comprehensive, and country-specific nutrient values of foods are required as inadequate food composition data may lead to a failure in understanding relationships between nutrient intake and health or disease [51]. Databases are often incomplete for key foods and nutrients which may result in large errors in some key nutrient intake estimation [52]. In Mali, the current Mali national food composition database (Table de Composition des Aliments du Mali / TACAM) is used to estimate energy and nutrient intakes of women in reproductive age in Bamako [53, 54]. The database provides composition data for 268 food items, nutrient contents of 59 food items were analyzed in Norway and South Africa [55]. Composition data of the other 209 food items were taken from other sources, mostly from FAO food composition table for use in Africa [56]. The quality of the data in TACAM is not yet assessed and necessitates looking deeply into the origins of the nutrient values. This requires going far more back than the laboratory and the analytical methodology, all the way to the compilation process and sampling protocols, the actual sampling, and handling, preparation and storage of the samples [27]. The quality assessment of data in TACAM is depending on the availability of thorough documentation of the nutrient values and this documentation is missing as is often the case for most FCDBs, especially in Africa. Therefore, a comparison of energy and nutrient intakes based on the use of TACAM compared with direct chemical analysis will support the evaluation of the quality of data in TACAM, and is another essential step to evaluate the potential contribution of fonio and fonio products to nutrient intakes for better nutrition in West Africa.

Nutritional value of fonio

Fonio is reported having good nutritional properties. It is considered as a rich source of starch and glucidic energy, whereas it's content in lipids and proteins are rather low compared to other ordinary cereals such as rice and maize. The TACAM indicates fonio as the cereal with the second highest concentration of protein (7.1 mg/100g dry matter) after sorghum (11 mg/100g dry matter) [57]. The proteins in fonio grains are not easily extractable and their digestibility is better than those of sorghum and millet [58]. The high levels of residue protein in fonio may have important functional properties [59]. Fonio has a high content of essential amino acids like methionine (4.5 mg/100g), cystine (2.5mg/100g) and leucine (10.5mg/100g) [37, 58]. The level of methionine in fonio is twice the level of that in egg's protein, highlighting the potential importance of fonio not only as survival food, but also as a complement for standard diets [60]. Milled fonio contains less saturated fatty acids than millet and rice and less polyunsaturated fatty acids than sorghum [37]. As most cereals, fonio is deficient in linolenic acid and the quantity of total pentosaneses is negligible compared to that present in sorghum or pearl millet [61]. Some species contain less polyphenols than sorghum [62]. In terms of health, fonio is believed to have a healthy nutrition profile because of its high content in fibre, low fat content and easy digestion, but this needs to be confirmed. The glycaemic index is lower than other cereals because of the slow assimilation of sugars [37, 50]. It contains branched chain carbohydrates that release slowly to keep a steady blood sugar level in the body and could therefore be a suitable food for diabetics [50]. The TACAM indicates fonio as the cereal with the second highest concentration of iron (8.5 mg/100g dry matter) and zinc (1.5 mg/100g dry matter) after sorghum (11 mg/100g dry matter for iron and 2.1 mg/100g dry matter for zinc) [57]. The reported iron and zinc content is mostly from decorticated grain of one variety, the Fingoloni variety [37], and analysis based on composite samples coming from retailers in different markets [57], which could also be contaminated by soil iron.

No data on other species, cultivars, landraces or variety names were reported. Recent research has provided data to confirm the micronutrient superiority of some lesser-known cultivars and wild varieties over others, more extensively utilized cultivars. Intake of one variety rather than another could make the difference between micronutrient deficiency and micronutrient adequacy [26]. These data are important for the sectors of health, agriculture, trade and the environment, and can be used in practically every domain of nutrition: nutrition education, community nutrition, nutrition interventions, food emergencies, nutritional labeling, food consumption surveys [25, 26], to name but a few. Information on cultivar specific composition can be used in breeding programs to enhance the nutrient content of more commonly used varieties of the same species, eliminating the need for transgenic modifications and their associated difficulties [63].

In addition, none or little information has been reported concerning the concentrations of iron and zinc absorption inhibitors in fonio, though their content might be significant as in many African cereals [64]. Data on nutrients and anti-nutrients are also lacking for many food species [65, 66], and are even fewer for varieties, cultivars, and breeds within species.

Furthermore, data on traditional preparation methods contribute to understanding how different processing or cooking methods can compromise (or improve) the nutritional quality of under-utilized species [67]. Fonio needs long and complex processing before consumption which could affect the iron and zinc content of the grain as the major part of the outer layers where the micronutrients are concentrated, is removed by milling [68]. No information is available on nutrient composition of fonio derived products such as milled, mid wet (milled fonio, washed and ready to cook), precooked or cooked fonio. Data on iron and zinc losses during processing are needed to support efforts in improving micronutrient supply and intake among vulnerable groups in developing countries.

Knowledge on dietary energy and nutrient intake is essential for evaluating the contribution of fonio to nutrient intake. A valid food composition table is fundamental, but so far the quality of the Mali food composition data base has not been evaluated. Most information on the nutritional value of fonio does not take into account varietal differences nor the effect of processing on nutrient (iron and zinc) content.

Dietary iron and its bioavailability

One of the main challenges in searching for opportunities to improve nutrient quality of fonio is the bioavailability of iron in fonio and fonio products. Iron is a trace element that is involved in numerous biochemical processes that are vital for human body functioning. It is an integral part of proteins and enzymes that maintain good health [69]. Hemoglobin, the principal form of body iron, is the oxygen-carrier pigment of the red blood cells that play a critical role in transferring oxygen from lung to tissues, and to tissue enzymes where it is involved in energy production [2, 69]. Stored iron serves as a reservoir to supply needs, mainly for hemoglobin production. Iron transport in the bloodstream is carried out by the protein transferrin [70].

Haemoglobin and myoglobin form the functional compartment of body iron involved in cellular metabolism while ferritin or hemosiderin is the storage form [71, 72] that can bind ~4500 atoms of iron. Iron is not actively excreted from the body and its balance is predominantly maintained by a regulation of iron absorption from the diet in the proximal small intestine [7, 73]. Iron is found in two basic forms in the diet, either as haem or non-haem iron [2, 73, 74]. A third and fourth type are ferritin iron and the so called contamination iron.

Non haem iron is found primarily in plant based foods such as cereals (fonio), pulses beans, and fruits, and iron- fortified foods [2, 74]. It is largely present in the less soluble ferric (Fe^{3+}) form and must be reduced to ferrous (Fe^{2+}) form or solubilized and chelated in the stomach to be available for absorption in the less acidic proximal small intestine [75, 76]. Haem iron (absorbable form) is found mainly in animal source foods where it accounts for about 30-60% of total iron [77]. It is absorbed intact and inside the enterocyte [74] at a higher percentage than non heme iron: 25-35%. It represent 10% of the total dietary iron, and provides about 40% of the absorbed iron from diet of industrialized countries [78]. Absorption is regulated by hepcidin, a hormone secreted by the liver and is dependent on iron status where iron depletion up regulates absorption as well as infection and inflammation [79].

Contamination iron originated from soil, processing and cooking equipment can be also found in prepared foods, mostly from developing countries [80]. Although the mechanism of absorption of contamination iron is not well known, it has been assumed to be poorly absorbable [81].

Nutritional iron deficiency arises when absorbed iron from the diet cannot meet the physiological requirements, mainly due to a low level of dietary iron combined with a low bioavailability. In most developing countries especially in communities living in urban West African, where the diet is mainly based on non-diversified cereal- and plant-based diets, the main part of the dietary iron is non-haem. Contrary to haem iron, the absorption of non-heme iron from these diets is often less that 10% [7] due to presence of absorption inhibitors such as phytates and polyphenols [82] and the absence of enhancers such as vitamin C or meat [83]. Within these communities, fortification of staple foods with iron has been recommended as a strategic processing option to increase the content of available iron in foods [7, 84].

Phytic acid

Phytate (the salt of phytic acid) is the most recognized and documented antinutritional factor that chelates divalent minerals such as iron and zinc. Phytic acid is the major storage form of phosphorus in cereal grains, legumes and nuts, representing 75% of seed total phosphorus [85]. In cereals, it is mainly located in the aleurone layer, pericarp and germ [86]. Phytic acid, myo-inositol 1,2,3,4,5,6 hexakis [dihydrogen phosphate] forms insoluble complexes with minerals such as iron and trace elements and influences their bioavailability, and its effect is more pronounced with increased concentrations [87]. One mole of phytic acid binds 6 moles of ferric iron so that even relatively small quantities of residual phytic acid are still strongly inhibitory [88]. Inositol hexakis phosphate is the most abundant derivative of myo-inositol and thus exerts significant influence on iron absorption. Other inositol phosphates such as inositol tetraphosphates and pentaphosphates are other less abundant derivatives depending on the extent of phytic acid degradation or removal [89] . In a single meal, phytic acid inhibits iron absorption even in a small amount [90] and may be partly responsible for the widespread mineral deficiencies observed in populations that subsist largely on cereals [91]. However, it has also been suggested that dietary phytic acid might have beneficial health effects as it can act as an anti-cancer agent or antioxidant [92]. The phytic acid-to-iron molar ratio is an important determinant of iron absorption. Phytic acid-to-iron molar ratio of <0.4:1 is preferable for iron absorption [93]. A recent investigation on phytic acid content in fonio reported a concentration of 123 mg/100g dry weight after cooking [94], indicating that the phytic acid-to-iron molar ratio from fonio products is much greater than the optimal level of <0.4:1 needed to achieve a significant increase in iron absorption [93, 95]. The removal or degradation of phytic acid has been reported to increase absorption of iron from the monotonous cereal based diets seen in many developing countries [87, 96].

Low iron intake through habitual intake of diets rich in iron absorption inhibiting compounds such as phytic acid may contribute to iron deficiency. It remains to be shown that increasing both the iron content and bioavailability in fonio or fonio products could potentially improve the nutrient quality and therefore contribute to improve iron intake and status of vulnerable populations.

IMPROVING THE IRON VALUE OF FONIO AND FONIO PRODUCTS

Improving the iron content of fonio and fonio products could be achieved through i) selecting for varieties with natural high iron content and good sensory characteristics, and ii) improved processing to retain iron in the food and to reduce phytic acid using home processing and fortification. These measures could contribute to increased iron and reduced phytic acid levels, thereby improving content of bioavailable iron in endogenous staple foods such as fonio.

Identifying high iron fonio varieties with good sensory characteristics

Increasing the native micronutrient content of plant-based foods has been suggested as a strategy to combat micronutrient deficiencies in vulnerable populations [97]. This could be achieved through traditional plant breeding or genetic engineering which can increase the concentration of total iron levels in the edible part of the plant [98] or select varieties containing enhanced levels of bioavailable iron or breed for new varieties with lower phytic acid–to-iron and phytic acid-to-zinc molar ratios [99, 100]. For this, breeders need existing information on the genetic variation for a given trait in a collection of germplasm to justify selection for that trait [101].

Most breeding activities are focused on commonly used staple foods as rice, maize and sorghum [99], but not on fonio. Existence of genetic variation in the native iron content in the landraces of some staples crops such as fonio may suggest the possibility to select those with higher iron content (for example within local germplasm) or to breed for varieties with lower phytic acid-to-iron molar ratios. Knowing the nutrient content of fonio and fonio products, its variation among landraces and the nutrient losses of especially iron due to processing can help to make choices for specific fonio landraces with high iron levels that can contribute to improve iron supply and intake of West African communities.

Selection of iron rich varieties to improve intake of bioavailable iron can only be effective if these varieties are accepted by the targeted vulnerable group of the community and are compatible with the consumers' food preferences [102]. The sensory properties of several cereals have been widely described through quantitative descriptive analyses [103] or through the description of consumer preferences using hedonic tests [104, 105]. Sensory studies on fonio have mainly concerned bakery products [106] and a traditional beverage "kunun zaki" [107]. The whiteness of fonio grain, absence of sand and impurities have been identified as main criteria when purchasing raw fonio grain [46]. However, to our knowledge, no systematic sensory analysis of cooked fonio grains is done comparably to that reported on cooked rice grains [108, 109]. Furthermore, quality criteria concerning cooked fonio grain as well as the sensory differences between fonio varieties have not yet been established. Knowing the sensory diversity of cooked fonio varieties can further help to make choices for specific fonio landraces with high iron levels that are acceptable for the population.

Home processing

The agricultural and breeding approaches should be combined with appropriate processing technologies that warrant the supply and bioavailability of nutrients to consumers. The process is mainly used to improve retention of iron and to increase bioavailability of iron by reducing the concentration of its absorption inhibitors such as phytic acid [110]. Decortication, particularly for cereals, is the common home processing practice to enhance the nutrient content yield at milling, as well as rice parboiling [111, 112].

Decortication (dehulling or debranning) can significantly reduce the level of phytic acid and phenolic compounds in cereals like sorghum because these antinutrients are mainly concentrated in the bran and the aleuronic layer of the grain [97, 113]. However, decortication will also lead to significant losses of iron, because minerals are also located in the outer layers of the grain. Therefore, decortication does not universally improve the provision of bioavailable iron [114].

Soaking, malting, cooking, germination and fermentation increase bioavailability of iron in cereals [115-117]. Soaking under optimal conditions or germination activate the naturally occurring enzyme phytase in cereals and legumes resulting in varying degrees of phytic acid hydrolysis. The amount of phytic acid remaining after food processing will depend on the species and variety, their initial content of phytic acid and the extent of extraction during processing [118, 119]. During fermentation and germination, phytic acid is degraded by native or microbial phytases from yeasts, moulds, and/or lactic acid bacteria. Depending on fermentation time, type of microorganisms involved and temperature, phytic acid was almost completely degraded in some studies [120-122]. However, several lactic acid bacteria strains have been tried for phytic acid reducing activities in tef-ingera without success [123]. Compared to other processing steps, fermentation or germination seems to be the most effective approach for phytic acid reduction [124, 125].

In addition to food iron, exogenous iron from the soil or originating from cooking or processing equipment can be present in significant quantities on the surface of food and may enter in the dietary pool and therefore will have nutritional importance [80]. Little is known about the mechanism of absorption of contamination iron, but it is assumed that it is rather poorly absorbable [81].

Another relatively new approach is the activation of natural intrinsic phytase of plant-based foods or microbial phytase treatment to reduce phytic acid levels [126, 127]. Addition of microbial phytase immediately before consumption significantly affects the iron bioavailability from an inhibitory test meal as the pure phytase enzyme degrades phytic acid during digestion before it is degraded by peptic enzymes [128]. High apparent phytase activity was found in untreated whole grain rye, wheat, triticale, buckwheat and barley [96]. Adding whole grain rye, wheat or buckwheat (10%) to cereal-legume-based complementary food mixtures, completely degraded phytic acid in a relatively short time (1.5-3 h) when incubated at optimal conditions for cereal phytase [129]. The efficacy of this technique in improving iron absorption from staple cereals such as fonio needs to be demonstrated in humans but up till now, evidence has not been reported.

Fortification

Improving the nutritional value through processing can also be achieved by iron fortification which is the addition of iron to a food vehicle with the goal of increasing its content in the fortified diets [7, 84, 130]. Staple foods such as fonio consumed on a regular basis and in relatively consistent amounts by a large share of vulnerable populations, or products with specific nutrient value for specific groups of the population [130] could serve as food vehicles for fortification. Water soluble ferrous sulphate, is the most suitable and commonly used iron fortificant in developing countries as it is cheaper and shows similar bioavailability as native iron, compared to poorly water soluble and insoluble compounds [7, 84, 131]. However, rancidity and subsequent off-flavours, unacceptable colour changes and metallic taste were reported of foods fortified with ferrous sulphate [132]. These sensory properties alteration of the ferrous sulphate fortified foods can be reduced by using a low fortification level [7, 84, 131].

Zimmermann et al. 2011 reported recently that iron absorption from ferrous sulphate was well up-regulated in iron deficiency, and emphasized its use for low-level fortification in iron-deficient populations [133]. However, iron absorption from highly bioavailable iron compounds can also be inhibited by phytic acid in cereals-based foods [134-136]. Therefore, reducing phytic acid levels is also essential to improve iron absorption from iron-fortified foods [136]. Although iron fortification has been shown to be potentially cost-effective for West African countries [137], very few efficacy trials are being implemented to bring forward evidence of the positive effects of iron fortification [138, 139]. In West Africa, fonio is a traditional staple cereal for some communities in rural and urban areas. Increasing the content of iron using ferrous sulfate as a cheap iron compound after reducing the concentration of phytic acid in fonio products is a strategy to be investigated to improve nutrient quality of fonio and fonio products. An iron absorption study with iron-fortified fonio products is an essential exploratory step towards enhancing the contribution of fonio to iron intake of women living in urban areas.

Screening for high iron varieties, improving home processing and iron fortification are promising strategies to improve the iron content and bioavailability in fonio and fonio products. However, a number of outstanding research questions need to be addressed in order to achieve nutritional-value added fonio products.

Rational of the thesis

Micronutrient deficiencies, especially iron and zinc, are a persisting public health challenge in sub-Saharan Africa especially affecting women. The major cause is the low quantity and quality of foods consumed. Indigenous foods or/and wild edible plants such as fonio, which constitute a major part of the diet during periods of food shortage, are suggested to play a major role in reduction of micronutrient deficiencies, but evidence is lacking. The FONIO project was initiated with the vision to develop high nutritional value fonio products in West Africa with good sensory quality and commercial value. In order to know the potential contribution of fonio to iron intake, knowledge on the dietary patterns and nutrient intake is necessary. A validated food composition data base is essential for assessing this. Improving the nutrient (iron) quality of fonio can be achieved through selection of varieties with natural high iron content and good sensory characteristics, and through improved home processing and fortification to retain iron into the fonio products and increase its bioavailability by reducing phytic acid. A number of outstanding research questions need to be addressed in order to look for opportunities to improve nutrient quality of fonio.

Objective and outline of the thesis

As part of the FONIO project, the main aim of the present research was to improve the nutritional value of fonio (Digitaria exilis) and fonio products through home processing and fortification.

To achieve this aim, four objectives were formulated as follows:

1. To assess the consumption patterns and contribution of fonio to nutrient intakes in urban Malian women of reproductive age;
2. To validate the Mali Food Composition Database for evaluating intakes of energy and nutrients at population level;
3. To assess nutrient and phytic acid content, genetic and sensory diversity, and effect of processing of fonio landraces in Mali;
4. To assess whether dephytinisation with intrinsic wheat phytase and iron fortification significantly increase iron absorption from fonio meals in West African women

The outline of this thesis follows the temporal arrangement of the four specific objectives (linkages shown in **Figure 1.2**) corresponding to the chapters of the thesis.

To evaluate the potential of fonio and fonio products to contribute to improve nutrient intakes, **chapter 2** focuses on the importance of fonio in urban areas, by describing the role of the grain in urban diets pattern of women, as well as the beliefs and attributes related to its consumption. Underlying the assessment of food consumption is the use of a valid food composition database to assess the nutrient intakes of the population of the urban areas as well as the contribution of fonio to nutrient intake. The validation of the food composition database used to assess the nutrient intakes of the population of the urban areas for better nutrition was described in **chapter 3**.

To support efforts in improving nutrient value of fonio especially iron and zinc supply and intake among vulnerable groups in West African communities, we assessed the genetic diversity of West African fonio landraces, the nutrient content of fonio and fonio products, its variation among fonio landraces and the nutrient losses of especially iron during processing to see if some landraces could be selected in their natural iron level (**chapter 4**).

Selection of iron rich varieties to improve intake of bioavailable iron can only be effective if these varieties are accepted by the targeted vulnerable group of the community and are compatible with the consumers' food preferences. Therefore, we assessed the sensory diversity of cooked fonio landraces to be able to make choices for specific fonio landraces with high iron levels that are acceptable for the population (**chapter 5**).

Chapter 6 explores whether we could improve the nutrient values of fonio by fortification and dephytinisation using intrinsic wheat phytase to decrease phytic acid in fonio and increase iron absorption from fonio porridge. A synthesis of the main findings of this thesis, external validity and public health implications of the findings as well as future research is presented in **chapter 7.**

Consumption of fonio	***Chapters 2 and 3***
Food consumption of, and beliefs about fonio (*Digitaria exilis*) in urban areas in Mali	Validity of Food Composition databases in low to middle income countries: evaluating the Mali Food Composition Table

Variation on fonio varieties	***Chapters 4 and 5***
Fonio (*Digitaria exilis)* landraces in Mali: Nutrient and phytate content, genetic diversity and effect of processing	Sensory diversity of fonio landraces from West Africa

Strategy to decrease phytic acid and increase iron absorption from fonio diet ***Chapter 6***
Dephytinisation with intrinsic wheat phytase and iron fortification significantly increase iron absorption from fonio (*Digitaria exilis*) meals in West African women

Figure1.2. *Framework to study potential for improvement of nutrient quality of fonio to contribute to improved micronutrient status among women in West Africa.*

Study area and population

The women involved in our research were randomly selected in Bamako and Cotonou, the largest cities of Mali and Benin (West Africa) respectively (**Figure 1.3**). As of 2011, the population size of the two cities were 1 690 471 (Bamako) and 862 445 inhabitants (Cotonou) of which more than 50% are female [140].

The entry point for selection of study site in the research project described in this thesis related to fonio collection is the interest of the Fonio project in Mali. Tominian in Segou and Bougouni in Sikasso regions (**Figure 1.4**) were selected because both regions out of the 8 regions of Mali were the leading producers of fonio. Within these regions, Tominian and Bougouni districts are leaders in both lands used and fonio production.

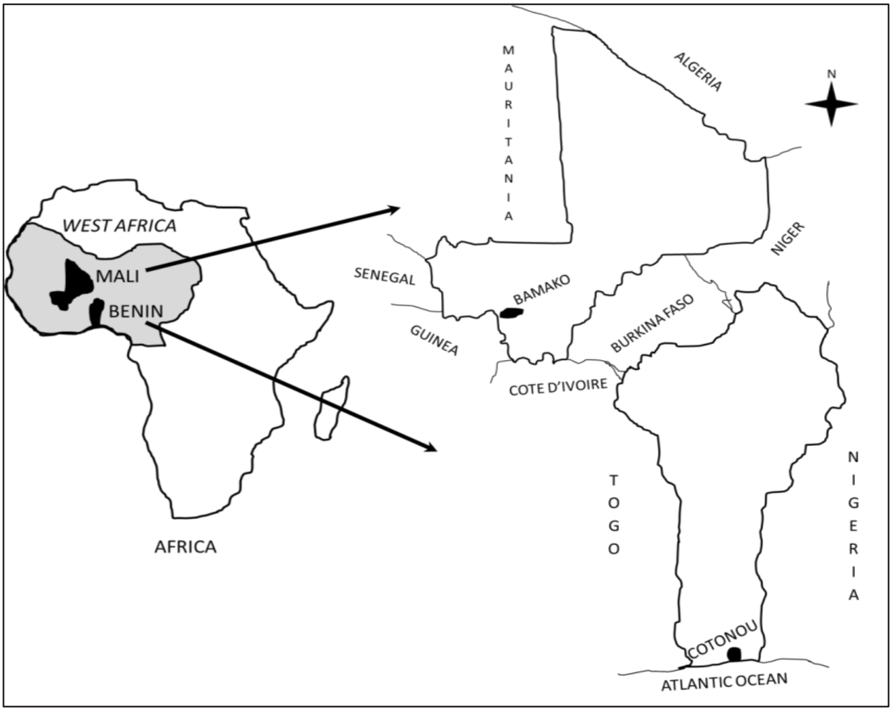


Figure1.3. *Map of Benin and Mali (in West Africa) showing women population study sites*

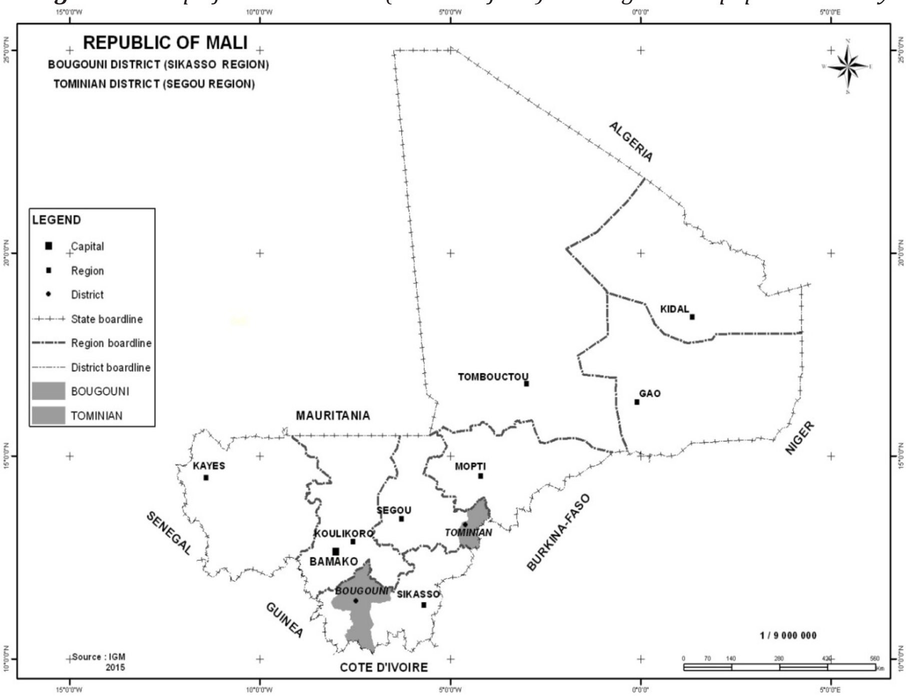


Figure1.4. *Map of Mali showing fonio landraces areas, Tominian and Bougouni situated in dark grey highlighted areas in Segou and Sikasso regions.*

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Chapter two

- Consumption of, and beliefs about fonio (*Digitaria exilis*) in urban areas in Mali

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Published in AJFAND 2009,9(9)1927-44

Abstract

The study sought to determine beliefs and practices about neglected crops in West-Africa, using fonio (Digitaria exilis) as a model to understand how obstacles impede the consumption of this cereal in Bamako, the capital city of Mali. This was a cross-sectional study on food ethnography in three steps: a market survey on availability of fonio, a food consumption survey on utilisation of fonio, and on beliefs and attributes on fonio. The study covered the pre-harvest and post-harvest periods and involved key informants, food vendors, and women of reproductive age in households. Fonio, as all cereals, is available year-long on markets in Bamako, and is abundant from September to May before most of the common cereals mature. More than two-thirds (68%) of the women reported having consumed fonio one to three times a month. Fonio was more consumed as snack (djouka) on working days (62%) than on weekend and special event days, suggesting that encouraging the development of ready-to-serve fonio-based products would help increase the consumption of fonio among women in urban area. The average individual portion size of fonio was 152g/day, and the contribution to daily energy intake was 16%. A large share of the women was convinced that eating fonio was good for them (95%) and their family members (94%). Also, most of them thought that fonio had good cooking, organoleptic and nutritional qualities and could contribute to diet's variation (91% to 100%). Decision by the women to purchase or prepare fonio in the household could be favourably influenced by factors such as media, household members suffering from anaemia, neighbouring people buying fonio and shortage of other cereals; whereas shortage of fonio products (77%), high cost of fonio products (69%), difficult cooking process (51%), and lack of knowledge about processing and cooking fonio (43%) were likely to limit fonio consumption among the women. Also, in the present study, fonio was perceived to be for rich people by more than half (58%) of the women. Improving cooking process and knowledge of the women about fonio cooking, as well as creating a demand for the women with the household's head and others through media, social and health care services would help increase fonio consumption in Bamako.

Introduction

Malnutrition and numerous deficiency diseases continue to persist in the developing world as a result of deficiencies of essential nutrients in the daily diet (1). Women of reproductive age are mostly affected because of increasing needs during pregnancy and lactation. In Mali, a prevalence of 13% has been reported for women of reproductive age suffering from chronic energy deficiency and 50% for anaemic women in Bamako the capital city (2). Household food insecurity is an important underlying cause of malnutrition and how households cope with scarce food are key determinants in maintaining healthy nutrition (3). Promotion of local and traditional food has been recommended to improve household food security and further contribute to reduction of malnutrition (4).

Fonio (*Digitaria exilis),* one of the most ancient indigenous West African cereals is a major part of the diet in some communities in Mali (5-7). Regarded for a long time as a minor crop without economic potential (8), fonio is attracting renewed interest in urban areas of West Africa for its organoleptic and nutritional qualities (9). Recently, the crop has been listed as priority crop for West Africa (10). Because of its short growing cycle, fonio can be harvested in the critical shortage season before major food crops (7) and its contribution to food security has been suggested (11).

The food composition table of Mali indicates fonio as second cereal with higher iron content (8.5 mg/100g dry matter) after sorghum (11 mg/100g dry matter) (12). The grain is also an excellent source of methionine, cysteine and leucine, whose concentrations are slightly higher than those defined for the FAO reference protein (13). Fonio-based products and modern recipes have been developed in urban areas in Mali (14). Promoting fonio consumption in urban areas could help improve household food security not only as income source but also as a transition food that could contribute to energy intake of household members during seasonal food shortage.

However, fonio consumption is still low, particularly in urban areas. Based on a study in three West African cities, Bamako (Mali), Conakry (Guinea) and Ouagadougou (Burkina Faso), a consumption frequency of less than once a month for 54% of fonio consumers in households has been reported (5). Average amount consumed per person was estimated at 4.4 kg/ year for Mali (6) with a range of 650 to 840g/year in Bamako (5). Difficult post- harvest processing, high-quality product demand, time consuming cooking process, and high-cost of fonio products were pointed out to explain the low consumption of fonio in Bamako (5, 7-9, 15). However, social factors and cultural beliefs are also important factors influencing food choice and consumption patterns (3, 4).

The present study sought to determine the intake of fonio in households and shared beliefs about fonio consumption in urban Mali. The research is part of the

FP6/EU/INCO/STREP[1] funded FONIO project, which aimed at upgrading the quality and competitiveness of fonio (*Digitaria exilis*) for improved livelihoods in West Africa.

Methods

A cross-sectional food ethnographic study (3, 4) was performed in Bamako, the capital city of Mali, during the pre-harvest (August-September) and post-harvest (February-July) periods. The study was carried out in three steps: 1) a market survey on availability of fonio, 2) a food consumption survey on utilisation of fonio and 3), a beliefs and attributes study on fonio among women of reproductive age (4).

Informants and respondents

Food vendors

Three categories of food vendors were selected: the street food vendors, the supermarkets food vendors and the markets food vendors. Street food vendors are those who sell ready-to-serve food either at fixed places (restaurants) or at non determined places (road sides). The supermarket food vendors are those who sell food at fixed prices in supermarket. The market food vendors are those who sell foods at bargaining base prices in markets. In total, 40 street food vendors, 15 in restaurants and 25 on road sides were randomly sampled. Five supermarkets were randomly selected, according to geographical position. Three of the most frequented markets of Bamako were visited: Medina coura, Fadjiguila and Sabalibougou. A total of 63 market food vendors were randomly selected according to the type and diversity of foods sold.

Households

A household was considered as any person or group of persons who share the same living accommodation, who pool some or all of their income and wealth and who take food prepared from a common kitchen or cooking pot (16). A total of 30 households were selected by a convenient sampling (17) based on discussion with key informants (agriculture and demography services staff, community leaders). The average size of the households was 11±6.8 members, and 33.7% of the households had more than 11 members. One food preparer was conveniently selected in each household based on discussion with the members. The preparers are the persons in the households who play a key role in the preparation of the food for all household members.

[1] Project number 0015403

Women of reproductive age

A total of 108 women of reproductive age (15-49 year-old) were randomly selected in 12 quarters of Bamako using three-stage cluster sampling (18) and the random walk method (19). The women were involved in the survey based on verbal agreement.

Availability study

Availability study was carried out in the pre-harvest period to identify available and consumed fonio products in Bamako. This included market survey and interviews in households.
The market survey was done by discussion with the selected food vendors in markets, supermarkets, restaurants and at road sides. Data such as fonio product names, attributes, seasonality, units sold, price per unit sold, were collected.
Interviews were carried out with the food preparers in the selected households. They gave information on the size of the household, the meal pattern, the main fonio dishes cooked in the household, and the seasonal availability of fonio products.

Food consumption survey

A food consumption survey including a 24hr-recall and a food frequency focused on fonio products were performed with the 108 women of reproductive age during the post-harvest period. Data were collected by well-trained local assistants through semi-structured interviews based on standard questionnaires.

The 24hr-recall was performed to assess the mean energy intake of the women, the daily portion size of fonio and the contribution of fonio to energy intake. The recall was performed on two non-consecutive days following a standardized format (19). Weekends and special event days were excluded. Amount of ingredients eaten from mixed dishes and snacks with unknown amounts of ingredients (gifts or foods purchased outside the household) were determined using the standard recipe method (19). Amounts consumed were estimated in household utensils and monetary values. Conversion factors from household measures and monetary value to weight equivalent (grams) were determined. Weights were measured using digital dietary scales (Soehnle, Plateau Art, Germany) Nr 65086 (22 lb), maximum range 10 kg, nearest to 2 g (0.1oz).

Food intake was computed by the VBS Food Calculation System version 3[2] using primarily the Mali food composition table TACAM (12), and secondarily the USDA nutrient database release 18 (20) and 20 (21). The International Mini List (IML), version 2.0[3] and the McCance & Widdowson's composition of foods (22) were used

[2] Bas Nutrition Software, Arnhem, The Netherlands, www.bware.nl
[3] Available from: http://www.fao.org/infoods/

when USDA nutrient values were different from TACAM or when no suitable food match was found. USDA Retention factors release 6 was used to determine nutrient losses during cooking (23).

Foods were classified into 13 groups: starchy staples; dairy; legumes and nuts; small fish eaten whole with bones; all other flesh foods and miscellaneous small animal protein; vitamin A-rich dark green leafy vegetables; vitamin A-rich deep yellow; orange and red vegetables; vitamin C-rich fruits; vitamin C-rich vegetables; vitamin A-rich fruits; eggs; all other fruits and vegetables; and all other foods (including sugars, fats, and stimulants such as tea and coffee). The food groups were taken from the standardized analytical research protocol of the Women's Dietary Diversity Project (24).

The frequency questionnaire focused on fonio and fonio products, and was completed to estimate the usual frequency of fonio products consumption in a period of one month. The questionnaire included a list of 15 fonio dishes with associated frequency categories.

Beliefs and attributes study

The beliefs and attributes study consisted of a questionnaire survey with the 108 women of reproductive age. Topics to be included in the questionnaire were identified by a food attribute and pile sort study (4). For this latter study, 26 women different from the 108, were randomly selected from the households involved in the food consumption survey described above. The main selection criteria were to be willing to participate and to have basic knowledge on fonio.

The study on food attributes and pile sort was carried out in three steps: pile setting, food difference and food attribute (4). The questionnaire was structured in topics such as knowledge of iron deficiency; diabetes and fonio; outcomes of fonio consumption; fonio attributes; perceived barriers to fonio consumption; information source; people and factors that could enhance fonio consumption; and subjective beliefs about fonio. To determine the perceptions of the women, the questions were reflected as statements and the women were asked to indicate if they agree, disagree, or are neutral. For each question, each response corresponds to a one-point score.

Data analysis

Incomplete data of 6 women were dropped. Descriptives were used to determine average portion size of fonio, mean energy and macronutrients intake, and contribution of fonio to the mean energy intake. Descriptives were also performed to determine the most consumed fonio dishes and the consumption frequency. Beliefs about fonio were reported as the proportion of women who agree with the statements of the questionnaire. All analyses were performed using SPSS 14.0.1 (2005) for windows.

Results

Fonio availability and consumption frequency

Overall, 11 food groups were available on Bamako markets: 1) cereals and cereal products, 2) roots and tubers, 3) legumes, nuts and seeds, 4) fruits and sweets, including beverages, 5) vegetables including leafy vegetables; 6) meat and poultry; 7) fish and fisheries; 8) dairy and eggs; 9) oils and fats; 10) spices; 11) stimulants and others (**Table 2.1**).

Table 2.1. Food availability on Bamako (Mali) markets

Food groups	Foods
Cereals	Wheat, maize, rice, millet, sorghum, fonio
Starchy roots and tubers	Potato, sweet potato, cassava, yam (white and yellow), plantain
Legumes/nuts/ seeds	Cashew nuts, groundnut, bambara groundnut (white and red), coconut, cocoa, African locust bean seeds, cowpea (white and red), Hibiscus seed, green peas, baobab seeds, tamarind seeds
Fruits and sweeties	Orange, lemon (yellow and green), tangerine, avocado, Pineapple, melon, Pear, Liana fruit, apple (green, yellow and red), Papaya, plum (yellow and red), nectarine, grape fruit (red and green), dates, banana (yellow and green), Mango, shea fruit, sugar powder, Chocolate, Honey, guinea sorrel juice, orange juice, soft drinks,
Vegetables	Cucumber, tomato (fruit and paste concentrated), okra, onion, shallot, shallot leaves, Hot pepper, sweet pepper green, Eggplant, Bitter tomato, cabbage, lettuce, Parsley leaves, Turnip, Carrot, Beet root, french bean, baobab leaves, Hibiscus leaves, Green leaves,
Meat/poultry	Beef, veal, goat, lamb, pork, chicken, Duck
Fish and fisheries	carp (red and grey), pink trout, grouper, sardine, catfish, threadfin, shrimp, freshwater fish, sea crab, gamba
Dairy/eggs	cow milk, yoghourt, cheese, chicken eggs
Oil and fats	sunflower oil, olive oil, palm oil (white and red), peanut oil, soya oil, butter, Shea butter,
Spices	pepper grain, aniseed, garlic, curry, ginger, clove, laurel leaves, vinegar, maggi cube, mustard
Stimulants	Coffea, tea, colanut

Wheat, rice, millet, maize, sorghum and fonio were the most common cereals available in Bamako. Like all cereals in Bamako, fonio is available year-long but is abundant before all other cereals from September (one of the typical shortage months in Mali) to May. On markets, fonio is sold either as whole grain, husked grain, precooked grain, or washed and dried. Two main fonio dishes were sold at the road sides: foyo (fonio couscous) and djouka (mixture of fonio, vegetables and groundnut). The most common fonio products available in supermarkets were dried precooked fonio, djouka and dèguè (mixture of fonio and curdled milk). In restaurants, fonio is served either as foyo supplemented with various sauces, or as djouka. At home, the most common fonio dish cooked is foyo, supplemented with various sauces.

Of the 15 fonio- based dishes, djouka, foyo and fini zamé (fried fonio) were eaten by 73 out of 102 women (71%). Among those consumers, foyo and djouka were eaten by 41% and 55%, respectively (**Table 2.2**). Among those consuming foyo and djouka, 68% reported a consumption frequency of one to three times per month. Few women (8%) reported consumption of more than 10 times per month (**Table 2.2**). Fonio was more frequently consumed as snack (djouka) on working days (62%) than on weekend (26%) and special event (baptism and wedding) days (13%).

Table 2.2. Frequency of consuming fonio among women in Bamako (n = 73)

	Times / month									**Total**
	1	2	3	4	5	6	8	10	13	
Number of women consuming all fonio dishes	31	12	7	6	3	6	2	4	2	73
	Number of women consuming by dish									**Total**
Djouka fonio	14	5	4	4	3	3	2	3	2	40
Foyo	15	6	3	2	0	3	0	1	0	30
Fini zamé	2	1	0	0	0	0	0	0	0	3
Days of consumption	**Number of women**									
	Working days			**Weekend days**			**Event days**			**Total**
	45			19			9			73

Fonio intake and contribution to energy intake

Starchy staples, vitamin C-rich vegetables, all other fruits and vegetables, other flesh foods and miscellaneous small animal protein, and legumes and nuts were the food groups mostly consumed by the women (**Table 2.3**). Among starchy staples, rice (38%), wheat products (33%) and millet products (20%) were the most commonly consumed, the least consumed being fonio (5%). Tomato (36%), cabbage (19%) and hot pepper (15%) were the most often consumed vitamin C-rich vegetables, used

Table 2.3. Number of women consuming food groups and contribution to mean macro-nutrients intake (n=102)

Food groups	Most consumed foods	Number of women	Contribution to intake		
			Energy	Protein	Fat
All starchy staples	Rice, wheat, millet, fonio, sorghum, yam, cassava, potato, sweet potato, plantain	102	45.7	36.0	12.1
Vitamin C-rich vegetables[a]	Green sweet pepper, tomato, cabbage, dried onion, fried dried shallot, hot pepper	102	1.6	2.4	0.3
All other fruits and vegetables	Cucumber, okra, onion, garlic, tomato paste, bitter tomato, eggplant, pumpkin, courgette, french bean, garlic, ripe banana, pineapple juice unsweetened	101	3.2	4.2	0.3
Flesh foods and miscellaneous small animal protein	Beef, large fish (nile perch, catfish mudfish)	100	6.7	27.3	11.3
All legumes and nuts	African locust bean, groundnut, cowpea	74	10.5	16.8	23.2
All dairy	Milk	49	4.6	9.0	7.6
Vitamin A-rich dark green leafy vegetables[b]	Lettuce, amaranth leaves, green leaves, shallot leaves, sweet potato leaves, fakouhoye leaves, bean leaves, parsley/celery leaves	42	0.4	1.2	0.1
Vitamin A-rich deep yellow, orange and red vegetables[b]	Carrot	17	0.1	0.1	0.0
Vitamin C-rich fruits[a]	Orange, ripe papaya	9	0	0	0
Eggs	Hen egg	8	0.3	0.9	0.6
Vitamin A-rich fruits[b]	Ripe mango, red palm oil	8	0.5	0.0	1.4
Small fish eaten whole with bones	Small fish	6	0.1	0.4	0.0

[a] *Vitamin C-rich fruits and vegetables are defined as those with >18 mg/100g in the form eaten*
[b] *Vitamin A-rich fruits and vegetables are defined as those with >130 RAE/100g in the form eaten.*

mainly as ingredients of sauces (percent not shown in table). Mean daily energy intake of the women ranged from 525 to 4150 kcal, averaging 2054 kcal (**Table 2.4**). With 35% sourced from animal foods, mean protein intake of the women contributed 11% of the daily energy intake, being in the range recommended by WHO (25). Energy intake was mostly provided by starchy staples, with cereal-based dishes being the largest contributors to energy intake, and legumes and nuts groups the largest contributors to fat intake (**Table 2.4**). Protein intake was mostly provided by starchy staples group (36%), other flesh foods and miscellaneous small animal protein group (27%), and legumes and nuts group (17%). Based on the proportion of women consuming fonio products, individual portion size of fonio when consumed ranged from 113 to 208 g/day, averaging 152 g/day. The average energy intake from fonio was 321.6±74.6 kcal/day, giving a contribution of 16% to mean daily energy intake for that portion size.

Table 2.4. Mean daily macronutrient intake of women (n=102)

	Mean nutrient intake			
	Mean	**SD**	**Range**	**% kcal**
Energy (kcal)	2054.0	716.5	525-4150	
Protein (g)	57.7	25.6	12.6-136.0	11
Animal protein source (g)	20.1	20.0	0-8.6	4.6
Plant source (g)	38.7	20.8	9.2-109.9	8.8
Total carbohydrate (g)	310.1	112.3	106.6-605.6	57
Total fat	73.2	34.8	1.7-181	32
Fonio consumption				
Individual portion size of fonio (g)	152.4	35.3	113-208	
Energy intake from fonio (kcal)	321.6	74.6	238.4-438.9	

Beliefs and attributes about fonio

According to their background, more than half of the women reported that fonio contains iron and its consumption could help prevent anemia and treat diabetes (**Table 2.5**). A large share of the women (94%) was convinced that eating fonio is good for them and their family members. Further, most of them thought that fonio had good cooking, organoleptic and nutritional qualities and could contribute to diet's variation. However, some barriers to fonio consumption have been identified. These are: 1) seasonal shortage (or frequent unavailability) in restaurants (77%) and on markets (74%); 2) high cost of fonio products (69%); 3) lack of knowledge about processing (43%); time consuming processing and difficult cooking process (51%); and 5) small size, dirtiness and dark colour (some varieties) of fonio grains (63%). The size of the household (52%) and the apathy of the household head for fonio consumption (45%) were additional barriers reported by the women. Decision to buy or prepare fonio for the household could be favourably influenced by factors such as media (94%), household members suffering from anaemia (91%), neighbouring people buying fonio (85%) and food shortage (82%). The women also reported that they ate fonio mostly in restaurants or when they have guests for dinner at home (74%) and during important celebrations like weddings, baptism (92%). Paramedical staff, friends, husband and family members are persons who could positively or negatively influence fonio consumption. Beliefs such as "fonio is for rich people" were also reported by 58% of the women as factors likely to influence fonio consumption.

Table 2. 5. Beliefs about fonio consumption

Topic	Questions	Percent
Knowledge	Fonio is important to treat diabetes	88
	Fonio can prevent anaemia	70.4
	Fonio contains iron	64.8
Outcomes from fonio consumption	Eating fonio is good for my household members	95.4
	Eating fonio is good for me	94.4
Fonio attributes	Fonio has good taste, swells up well during cooking, is pleasant in mouth	99.6
	Fonio is a traditional food and diversifies meals	94.5
	Fonio stimulates appetite, easy digestible, nutritious, healthy, good for weight loosing	92.8
	Eating fonio helps to treat diseases and to prevent stomach problems	91.2
Perceived barriers	Fonio not available throughout the year	76.9
	Fonio not available on markets and restaurants	73.5
	High cost of fonio products	69.4
	Fonio contains impurities, not white, has small size grain, low quality variety	62.8
	Not knowing how to cook fonio	42.6
	Household size too large to prepare fonio	51.9
	Time consuming, hardness of cooking	51.4
	Head of the household does not like fonio	45.4
Information source, people and factors enhancing fonio consumption	The media favourably affect decision to eat fonio	93.5
	Nurse, social workers, favourably affect decision to eat fonio	93.5
	Fonio consumed mostly during important ceremonies, like weddings, funerals or baptism	92.1
	Household members suffering from anaemia favourably affect decision to buy fonio	90.8
	Friends, members of my association, neighbours favourably affect decision to eat fonio	85.8
	People around me buying fonio makes me want to eat fonio	85.2
	Husband, household members, mother-in-law favourably affect decision to eat fonio	83.9
	A shortage of food favourably affects decision to eat fonio	81.5
	Fonio sellers favourably affect decision to buy fonio	79.7
	Fonio consumed mostly in restaurants and when guests in household	74.1
Subjective beliefs	Fonio is for rich people	58.3
	Fonio is for poor people	35.2

Discussion

Food ethnography was performed to determine the contribution of fonio to the dietary pattern, and to identify shared beliefs about fonio consumption in urban Mali.
Fonio is available in shortage season before the most common cereals are harvested. The usual diet is based on cereal, mainly rice, and fonio was reported as consumed by 5% of the women. The same trend has been earlier described for a rural area in Mali, but the main cereal dishes were based on maize and sorghum, and fonio consumption reported by 12 % of men and women (26). This difference could be linked to the relative abundance of almost all common cereals during the food consumption survey period (post-harvest), and also to the contrast of urban vs. rural areas, where dishes are still mostly based on indigenous cereals (8).

Consumption frequency (one to three times a month) and average daily individual portion size of fonio (152 g) were higher in the study compared to the frequency of less than once a month and the individual amount of 650 to 840 g/person/year reported earlier in the study on fonio consumption in urban areas (5). This suggests an increase in fonio consumption since 2004. However, because of the low proportion of women consuming fonio dishes (5 %) and small size of daily portion consumed (152 g/day) compared with those of rice (38 % and 489 g/day, respectively), the contribution of fonio was about the fifth of the daily energy intake of the women consuming fonio dishes. This small portion size of fonio compared to rice could be explained by the fact that fonio was mostly consumed as snack. No previous studies reported contribution of fonio to energy intake, thus hampering comparisons, but under same conditions (equivalent proportion of consumers and daily portion size), fonio (steam-cooked) would better contribute to energy intake than rice, due to its higher energy content: 223 kcal/100g for cooked fonio as compared to 102 kcal/100g for cooked rice (12).

Fonio has often been reported as a food mostly cooked and consumed during weekends and special events (5, 7-9, 15). A previous study in Bamako also reported that fonio was often consumed outside and cooked in households mostly when there are guests (5). The availability study confirmed that fonio products are mostly served in restaurants and most of the women are likely to consume fonio outside. The food frequency survey revealed that fonio is more often consumed on working days than on event days. This suggests that encouraging development of ready-to-eat fonio-based products would help increase the consumption of fonio among women, especially in urban areas.

Difficult post-harvest processing, time consuming cooking process, and high-cost of fonio products were often reported as common barriers to fonio consumption (5, 8, 9, 15). The results of the present study not only supported previous studies but revealed other factors that were also likely to hamper fonio consumption in Bamako. One of the perceived barriers was the lack of knowledge about processing and cooking fonio. Due to its very small size, fonio is contaminated with sand and dust during post- harvest processing and needs to be carefully washed for several times using a traditional sedimentation method to get a cleaned product, which must be steam-cooked two to three times at least to get a soft-in-mouth and easy-to-digest product (9). This cleaning and cooking process needs some basic ability and knowledge that might not be common among all women, especially in the context of urban areas. Another reported obstacle was the size of the household. This might be linked either to the high cost of fonio products which could not allow purchasing large amounts when the size of the household is large, or to the difficult cleaning and cooking process that might increase cooking time in large size households.

Furthermore, the apathy of the household head for fonio consumption (perceived barrier for 45% of the women) might be related to the low quality of cooked fonio served by the women in the households due to their lack of knowledge and skills. On the other hand, factors like food shortage, media, family members suffering from anaemia, neighbourhood opinion, and paramedical staff, family members and friends are likely to influence fonio consumption among the women. Association between these factors and consumption of fonio needs to be tested in further studies.

Finally, in the present study, fonio was perceived to be for rich people by more than half of the women. This viewpoint contradicts previous reports on fonio which often postulated fonio to be for poor people (7, 9). These discrepancies in results might be due to the fact that this study was carried out in an urban context and women could have linked their opinion to the high cost of fonio products.

Conclusion

The purpose of this study was to determine shared beliefs that could positively or negatively influence fonio consumption. Results showed that fonio is available year-long on markets in Bamako, as all cereals, but is abundant before most of the common cereals. More than two-thirds (68%) of the women reported consuming fonio one to three times per month. Fonio was more consumed as snack on working days than on weekend and special event days, suggesting that encouraging development of ready-to-eat fonio-based products would help increase consumption of fonio among women in urban areas. Energy intake was mostly provided by starchy staples, with cereal-based dishes being the largest contributors to energy intake. The average individual portion size of fonio when consumed was 152 g/day, and its contribution to daily energy intake was 16%. A large share of the women was convinced that eating fonio is good for them and their family members. A large share of the women was convinced that eating fonio is good for them and their family members. Further, most of them thought that fonio had good cooking, organoleptic and nutritional qualities and could contribute to diet's variation (91% to 100%). The decision to buy or prepare fonio by women in households might be favourably influenced by factors such as media, household members suffering from anaemia, neighbouring people purchasing fonio and shortage of other cereals; whereas shortage of fonio products, high cost of fonio products, difficult cooking process, and lack of knowledge about processing and cooking fonio are likely to limit fonio consumption among women in urban area. Further, fonio was perceived to be for rich people by more than half of the women. Improving cooking process and knowledge of women about fonio cooking, as well as creating a demand for the women with the husband and others through media, social and health care services would help increase fonio consumption inside households in Bamako. Further research should be designed to examine the effect of these factors on consumption of fonio in urban areas in Mali.

Acknowledgements

We acknowledge European Union project N° 0015043 for funding the project. We are also grateful to the late Guindo Doré (Institute of Rural Economy, Mali) for giving the opportunity to conduct the field work; Jean-François Cruz (CIRAD, general manager of Fonio project) for institutional support; all the women for their willingness to participate and their important contribution to the study. We thank Bianca Van Dam (Division of Human Nutrition, Wageningen University) for her contribution to study design, data collection and data entering.

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3

Chapter three

- Evaluation of food composition databases in low to middle income countries: the Mali food composition table

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Submitted for publication

Abstract

Food composition databases are essential for estimating nutrient intake in food consumption surveys. This study aims to evaluate the Mali food composition database (TACAM) for assessing intakes of energy and nutrients at population level. Weighed food records and duplicate portions of all foods consumed during one day were collected from apparently healthy non-pregnant, non-lactating women from Bamako, Mali (n=36, age 15-36 y). Intake of energy, protein, fat, available carbohydrates, dietary fiber, calcium, iron, zinc and vitamin A was estimated by calculating nutrient intake based on food records using two distinct food composition databases: 1) an adjusted TACAM (a-TACAM) and 2) chemical analysis of the duplicate portions. Agreement between the 2 methods was determined using Wilcoxon signed-rank test, and Bland-Altman plots. The evaluation of the a-TACAM showed that eighty-eight percent of the nutrient values, mostly macronutrients, were "acceptable" (197 of 225[4] nutrient values); eight percent (17 nutrient values) were considered implausible because of a low confidence score and replaced; and five percent (11 nutrient values) that were missing were updated. Replaced nutrient values are iron (4 nutrient values), β-carotene (5 nutrient values), fat (1 nutrient value), available carbohydrates (1 nutrient value) and dietary fiber (1 nutrient value). Updated nutrient values are mainly for zinc (8 nutrient values). Compared to chemical analysis, nutrient intakes estimated based on the a-TACAM were underestimated (except for fat and vitamin A intakes), while density of all estimated nutrients were overestimated (except available carbohydrate, dietary fiber and iron). Correlations coefficients between estimated and analyzed values ranged from 0.38 to 0.61 and were significant for all intakes and densities except for calcium intakes. At population level, difference between mean estimated and analyzed nutrient intakes was significantly different from zero for carbohydrates (203.0 vs 243.5 g/day), iron (9.9 vs 22.8 mg/day) and vitamin A (356 vs 246 μg RAE). At individual level, we observed significant differences between estimated and analyzed intakes for all the nutrients and these differences tend to increase with higher intakes. Estimated PAs was significantly lower for iron and significantly higher for vitamin A compared to analyzed intakes. The a-TACAM seems sufficiently acceptable for measuring average intake at population level for macronutrients, calcium and zinc in a low intake population, but not for carbohydrate, vitamin A and iron intakes, nor for probability of adequate intake of single micronutrient and nutrient densities.

[4] The 225 nutrients values include the 22 specific nutrients which are not present in selected foods.

Introduction

Food composition databases (FCDB) are fundamental for estimating dietary intake of energy and nutrients based on food consumption surveys (1, 2). High quality, comprehensive, and country-specific nutrient values of foods are required as inadequate food composition data may lead to a failure in understanding relationships between nutrient intake and health or disease (3). When establishing FCDB, laboratory analysis carried out specifically for the database compilation is preferred, but the costs involved go far beyond resources available in many parts of the world (4, 5). Available FCDBs often comprise a combination of original analytical values taken from published or unpublished laboratory reports, imputed values derived from analytical values obtained for a similar food or for another form of the same food, calculated values derived from recipes, copied values from other tables and databases, or presumed values (4).

Assessing the quality of the data in FCDBs is necessary to verify the appropriateness of the compiled nutrient values. This necessitates to look deeply into the origins of nutrient values, and to evaluate not only the laboratory and the analytical methodology, but also the compilation process and sampling protocols, the actual sampling, and handling, preparation and storage of the samples (1). Guidelines for quality assessment of food composition data have been developed (6,7,8) including criteria for food identification and description, component nomenclature, sampling plan, handling and storage, number of analytical samples, analytical methods and analytical quality control and documentation, and mode of expression of results. However, such quality assessment is depending on the availability of thorough documentation of the nutrient values. This documentation is missing for most FCDBs, especially in Africa.

Furthermore, databases are often incomplete for key foods and nutrients which may result in large errors in some key nutrient intake estimations (9). This requires imputation of nutrient values from other sources thereby facing incompatible food and nutrient definitions, analytical methods and modes of expressions across databases, resulting in diverging nutrient values. In addition, nutrient values for similar foods can be different due to agronomic factors and (industrial) food processing (e.g fortification) (10).

The European Union funded FP6 research project FONIO aims to explore quality and competitiveness of fonio as a potential healthy and nutritive food for urban areas in Sub-Saharan Africa and Europe (11). In the framework of this project, energy and nutrient intakes of women of reproductive age in Bamako (Mali) are evaluated in a food consumption study. The current Mali food composition database, TACAM (Table de Composition des Aliments du Mali) (12), provides composition data for 268 food items. For 59 of these food items, the nutrient composition of samples was analyzed in Norway and South Africa (12, 13). For the other 209 food items, composition data were taken from other sources, mostly from FAO food composition table for use in Africa (14). Absence of documentation for many values prohibited a quality assessment of the TACAM.

In addition, as the TACAM does not cover all foods and nutrients of interest, there is a need to review and complete nutrient values to allow the estimation of energy and nutrient intakes. At first, this paper describes the pragmatic approach we used to evaluate the quality of TACAM and to update this FCDB. Secondly, in order to evaluate whether the adjusted TACAM is acceptable for assessing average energy and nutrient intakes and for assessing probability of adequacy of intakes for selected nutrients, we compared the nutrient intake calculated from a food weighed record with that based on chemical analysis of duplicate portions of diets collected on the same day.

Materials and methods

Subjects

The food consumption study conducted in the framework of the FONIO project comprised 108 apparently healthy non-pregnant, non-lactating women in the age range 15-49 years, randomly selected in Bamako, capital city of Mali, using a three-stage cluster sampling protocol. Sampling procedure has been described elsewhere (15, 16). For the present study, a convenient sub-sample of 36 women was selected, based on their willingness to participate in the study. Verbal informed consent was obtained from the participants before the dietary assessment.

Dietary intake data collection

One-day weighed food records (1d-WFR) were obtained in March 2007 (weekends and special event days excluded), which corresponds to the post-harvest season. Data were collected by trained experienced field assistants. The women received verbal instructions about the weighing of foods and ingredients before preparation, and about the collection of duplicate portions for all consumed foods. All foods, ingredients, beverages and snacks consumed by the women were recorded and weighed by the assistants, during one day, from 7.00 am until the last evening meal consumed. Leftovers were also weighed when necessary. Detailed description of the foods/ingredients and recipes were obtained. When the meal was cooked in batch for the family, the total amount of cooked food was also weighed to derive the proportion consumed by the women from the total cooked dish for the family. This proportion was used to determine the amount of ingredients consumed from the total amount of ingredients used in the preparation of the dish. Amounts of ingredients consumed from mixed dishes and snacks with unknown amounts of ingredients (gifts, foods purchased or consumed outside the household) were determined using standard recipes method (17).

To allow duplicate portions collection, the women were requested to cook enough amounts for this purpose, and to serve two identical portions in separate plates for their own consumption before serving the other household members. This was done under close supervision of the field assistants. In case of gifts, food purchased or consumed outside the household, women were asked to collect a duplicate portion identical to what they consumed. Also, if going out, the women were followed by the field assistants who recorded and weighed and collected duplicate portions of all foods consumed. Women were financially compensated for collecting a duplicate portion. Weights were measured using an electronic kitchen scale (Soehnle, Plateau Art, model 65086, 10kg maximum) and recorded to the nearest 2 g.

The duplicate portions of all foods, beverages and snacks consumed (reduced with the same amount of left-over when any) were weighed, collected separately, pooled per day per woman, and packed in airtight polyethylene bag by the field assistants. The packed bags with foods were stored immediately in isotherm containers filled with ice. The filled isotherm boxes were collected by the study supervisor and transported immediately to the Food Technology Laboratory (LTA) of the Institute of Rural Economy (IER) in Bamako, Mali and the duplicate portions stored at -20ºC. They were transported on dry ice to the Division of Human Nutrition, Wageningen University (The Netherlands) and stored at -80 ºC until analysis.
Energy and nutrients of interest include protein, fat, available carbohydrates, dietary fibre, calcium, iron, zinc, and vitamin A (retinol and β-carotene expressed as RAE), considered as relevant for the FONIO project based on their public health relevance and the likely availability of nutrient values in food composition tables.

Phase 1: Food composition database

For the purpose of the FONIO project, a FCDB comprising nutrients values for all 90 foods consumed by the participants was developed specifically for this study, using TACAM (12) as primary source. For the compilation of our FCDB, further referred to as the adjusted TACAM (a-TACAM), a two stages process was used. First, we updated the database with missing data (foods and nutrients). Second we evaluated the a-TACAM in a pragmatic way based on an expert systems approach.

For the 90 foods, 180 nutrient values (20%) out of 900 were not available from TACAM. Micronutrients such as calcium, iron, zinc and vitamin A (RAE) were mainly missing. In addition, eleven consumed foods (110 nutrient values, 12%) were not available in TACAM. For missing nutrient values, values for comparable foods were imputed from the following sources: South African Food Data System (13), East Africa CTA/ECSA (18), United States Department of Agriculture database release 20 (19), International Minilist (20) and Dutch food composition table NEVO (21). Additional sources (22, 23, 24) were used for zinc values when not available in the above mentioned sources. Before imputing values, comparable foods were checked for food description (taxonomic, name, species), moisture and component description (water, fat, and protein content), and composition of brand names especially when borrowed from above mentioned FCDBs. Nutrient values imputed from comparable foods with different moisture content but comparable component description (definition, analytical method, and same expression) were adjusted to account for differences in moisture content[5]. For the staple foods found in TACAM, the nutrient value of the cooked form was preferred when documented. To account for nutrient losses during cooking of other foods for which only raw values are provided in TACAM, such as fish, meat, and vegetables, USDA Retention Factors release 6 (25) were applied (15). RAE values were missing in TACAM for all consumed foods, so β-carotene and retinol values were converted into RAE using the International Vitamin A Consultative Group recommended conversion factors (26). Only β-carotene as pro-vitamin A carotenoid was used since information on other pro-vitamin A carotenoids (β-cryptoxanthin and α-carotene) was not available. The general Atwater factors for available carbohydrates, protein, fat and dietary fibre was used in calculating energy (27)

To value the trustworthiness of the a-TACAM, a pragmatic approach based on the modified expert system, as developed by Holden et al. 1996 (28), was used for 25 frequently consumed foods (29). Compositional values for nine nutrients (protein, fat, available carbohydrates, dietary fibre, calcium, iron, zinc, retinol and β-carotene) were evaluated. When documentation of these nutrient values was available, a confidence scoring system was applied to assess their trustworthiness based on food description, sampling plan and number of samples, sample handling and validity/quality control of analytical methods. If not available, nutrient values from the a-TACAM were compared with values of similar foods provided in other FCDBs. If a-TACAM nutrient values were falling within the range used for comparison, they were accepted. If the values fell out of the range, Dixon's criteria for testing extreme observations in a single sample (30) were used to test whether the a-TACAM value significantly deviated from the value reported in other food composition sources. For statistically significant outliers, values were replaced by averaging nutrient values of similar foods with comparable component description from other sources.

[5] International Mini List tables do not report moisture content. The moisture content for foods taken from the International Mini List was estimated based on the closest food match from the USDA.

Phase 2: Comparing nutrient intake based on a-TACAM and chemical analysis

Nutrient intake

Individual daily nutrient intake was computed using the VBS Food Calculation System, version 4 (BaS Nutrition Software, The Netherlands) based on the newly created FCDB, a-TACAM. The values thus obtained were called "estimated" values.
The contribution of individual foods to energy and nutrient intake was calculated based on the estimated values. Nutrient densities, which identify the proportion of nutrients in foods, with terms such as nutrient rich and micronutrient dense referring to similar properties, were calculated per 1000 kcal.

Chemical analysis

Bones and other non-edible parts such as pits were removed from the duplicate portions. The edible parts were freeze dried, homogenized and analyzed for macro- and micronutrients composition. Moisture was determined by drying the fresh and freeze-dried foods in a vacuum oven at 95 ºC until constant weight (31). Total nitrogen was analyzed using the automated Kjeldahl method (32). Protein was calculated using nitrogen to protein conversion factor of 6.25 (33). Total fat content was measured using acid hydrolysis of the sample and subsequent extraction of the fat with a 1:1 V/V% mixture of diethyl ether and petroleum ether (34). Total dietary fibre was determined using the Prosky procedure (35). Ash was determined by heating the defatted and dried food in a muffle furnace at 550 ºC for one night. Available carbohydrates per 100g fresh food were calculated by difference: 100- (weight in g/100g of protein + fat + water + ash + fibre). Alcohol was not included in the snacks and meals of the women and therefore not included in the calculation of available carbohydrates and energy. Energy content of each duplicate portion was calculated from the amount of protein, fat, carbohydrate and fiber on the basis of energy conversion factors (27) as follows: protein, 16.7 kJ/g; fat, 37.7 kJ/g, carbohydrate, 16.7 kJ/g and dietary fiber, 2 kJ/g. Energy contents of all duplicate portions were calculated in kilocalories (1 kcal = 4.184 kJ).

Element analysis (calcium, iron and zinc) were carried out at the chemical biological laboratory, Wageningen University, after microwave digestion as described by Novozamsky et al. (1996) (36). The analytical procedure is described elsewhere (37). Vitamin A and carotenoids were analyzed by High Performance Liquid Chromatography (HPLC) at the Division of Human Nutrition of Wageningen University. In brief: To two grams of the homogenized sample, an amount of 5 mL de-ionized water and 0.2 g MgCO3 was added. The samples were extracted with 20 mL 1/1 v/v% methanol / tetrahydrofuran (THF) in a 100 mL measuring cylinder, using a Polytron rod mixer (Kinematica PT 31005, Kriens, Luzern, Switzerland). The extract was decanted into a glass funnel fitted with Whatman paper no 541. The residue was reextracted three times with 10 mL each of methanol/tetrahydrofuran until colourless. The filtrate was quantitatively transferred to a 50 mL volumetric flask and made up to volume with methanol/THF (1/1 v/v%). Four mL of the filtrate was transferred to a 10 mL Kimax tube and the filtrate was evaporated to dryness in a heating block of 35 °C using a stream of nitrogen. The dry residue was saponified by adding 1.5 mL potassium hydroxide (5% w/v in 96% v/v ethanol), containing 0.2% w/v pyrogallol. The tubes were purged with nitrogen, firmly closed with teflon lined caps, placed in the dark, and the content mixed for 3 h on a tube shaker at 250 reciprocations per minute (model Edmund Bühler SM 25, Hechingen, Germany). After addition of 1.5mL water, the mixture was extracted three times with 3mL hexane. The hexane layers were combined and evaporated in a dry block heater as described above. The residue was dissolved in 250 µL methanol/THF (3/1 v/v%) and 25 µL was injected into the HPLC system. Separations of lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene and lycopene were performed on a Vydac 201TP52 RP-column (Grace Alltech, Breda, The Netherlands) on a Spectra HPLC system using gradient elution, and monitored at 325 nm (retinol) and 450 nm (carotenoids) using a PDA detector (Thermo Separation Products Inc, San Jose, CA, 95134). Runtime was 25 minutes per sample. Retinol Activity Equivalent (RAE) was calculated as Retinol + 1/12β-carotene + 1/24 α-carotene + 1/24β-cryptoxanthine. All results based on chemical analysis are further referred to as "analyzed" values.

Analytical quality control

For ash, fat and nitrogen, baby food (Humana Milchwerke Westfalen eG, D- 4900 Herford, Germany) was used as in-house control sample to monitor analytical performance. Within- and between-run CVs were 0.9 % and 1.6% for ash; 2.3% and 3.9% for fat; 0.8% and 1.2% for nitrogen, respectively over a more than 10-year period. For fiber, the in-house control sample was bread crumbs; between-day CV: 4.2% (within-day variation: not recorded). For retinol and carotenoids, homogenized baby food (mixed vegetables with egg) from Nutricia (Nutricia Nederland BV, Zoetermeer, The Netherlands) was used as in-house control material. Within- and between-run CVs were: 1.9% and 9.6% for retinol; 6,1% and 13.3% for α-carotene; 3.9% and 7.7% for β-carotene respectively over a three year period. Results from the 2006 FAPAS proficiency testing showed z-scores as a measure for deviation from the consensus value (accuracy) for fiber -0.2; for nitrogen +0.7 and for ash 0.0. Results from proficiency tests organized by the National Food Administration (Uppsala, Sweden) showed average z-scores over a 10 year period for ash +0.52; for dry matter + 0.99; for fat +1.72 and for nitrogen -0.24.

For iron and zinc, grass and strawberry samples were used as in-house control material to monitor analytical performance. Analytical variation was 6% for both elements. The chemical biological laboratory participates in the International Plant-Analytical Exchange program organized by Wageningen Evaluation Programs for

Analytical Laboratories (www.wepal.nl). Results from proficiency tests (average result of 11 ring test samples) showed a mean z-score of 1.7 for iron and 0.4 for zinc over a 1-year period. Analysis on 4 separate days of NIST SRM 2383 with certified values for retinol (80 μg/100g), β-cryptoxanthin (138 μg/100g), α-carotene (83 μg/100g), β-carotene (312 μg/100g) showed average deviations of 2.3% for retinol, 2.3% for β-cryptoxanthin, 0.9% for α-carotene and 1.9% for β-carotene.

Assessing probability of micronutrient adequacy

The probability approach (38) was used to estimate intake adequacy for micronutrients, based on both the estimated and analyzed nutrient intakes. The probability of adequacy (PA) for vitamin A, zinc, and calcium were calculated using their respective estimated average requirements (EARs) and distributions (39, 40). The EAR for zinc was adjusted for moderate (34%) bioavailability[6], determined on the basis of the population level dietary pattern of the study population (41). An adequate intake was used for calcium as the EAR was not available. The probability of adequacy of calcium intake was calculated following the recommendations of Foote et al. (42). Because the distribution of iron requirements is skewed, we used probability of adequacy values derived by Institute of Medicine tables for adult women (39), but adjusted for 10% bioavailability to reflect the partly inhibitory nature of the predominant cereal-based diet in the study area.

Statistical method

Statistical analyses were done with SPSS 19 for Windows (IBM SPSS Statistics Release 19.0.0.1, SPSS inc., Chicago, IL, 2011). During the recording day, one woman was not able to deliver the last meal for personal reasons and was excluded from the analysis, leaving a total sample of 35 women. P-value of < 0.05 was considered statistically significant for all analyses. The distribution of intake data was tested for normality using the Kolmogorov-Smirnov test. Intakes were not normally distributed; thus, summary values are reported as medians and their respective interquartile ranges (IQR).

The Wilcoxon signed-rank test was used to determine whether the differences between estimated and analyzed nutrient intakes were significantly different from zero. Spearman's correlation was computed to assess the relationship between estimated and analyzed nutrient values.

[6] For this sample, we selected a bioavailability of 34% for zinc because the average diet of these women fits the category of moderate zinc bioavailability of 34%. Diets are generally mixed, containing both animal and vegetable sources and not primarily based on unrefined grains.

To assess the level of agreement between estimated and analyzed nutrient values, the method suggested by Bland and Altman (43) was used. Regression analysis on the differences versus average nutrient values was used to see whether the slopes of the difference between estimated and analyzed nutrient intakes were significantly different from zero.

To evaluate energy intakes, the 95% limits Goldberg cut-offs values (44, 45) based on a standard physical activity level for a sedentary lifestyle (46) were used. Basal metabolic rate of the study participants was calculated using the following formula: BMR (kcal/day) = [weight * 8.126] + 845. 6 (46).

Results

Study population characteristics and dietary pattern

Median age was 34 years (IQR 24, 40) years with 11 percent being adolescents (15–18y). Literacy rate, defined as having at least attended primary school or Islamic school, was 61 percent. Body mass index (BMI) ranged from 16.8 to 38.7 with a mean of 24.0±5.9 kg/m^2 with 14 percent of the women having a BMI below 18.5, and 34 percent having BMI above 25 kg/m^2. The women's diet was based on starchy staples, mainly refined white rice, refined wheat flour, millet or sorghum, accompanied by a sauce typically made from vegetables and beef or fish. Eighteen foods in total contributed to more than 50% of energy and nutrient intakes (**Table 3.1**). Energy and carbohydrates were mainly from cereal products (rice) and sugar (used in tea and porridge); protein was mainly coming from animal products, while peanut oil, nuts (groundnut paste) and animal products contributed most to fat intake; fibre was mainly derived from vegetables (mainly dried okra); calcium, iron and zinc were mainly derived from cereals, animal products, and vegetables (mostly dried okra); cow milk, mango, lettuce and red palm oil contributed most to vitamin A (RAE).

Phase 1: Food composition table: the a-TACAM

Eighty-eight percent of the nutrient values, mostly macronutrients, were "acceptable" (197 of 225[7] nutrient values); eight percent (17 nutrient values) were considered implausible because of a low confidence score and replaced; and five percent (11 nutrient values) that were missing were updated. Values mainly replaced were iron (4 nutrient values) and β-carotene (5 nutrient values). Values mainly updated were zinc (8 nutrient values). Only three values for proximate composition (fat, available carbohydrates and dietary fiber) were replaced after comparison. Based on this evaluation we concluded that the a-TACAM is an acceptable FCDB for estimating intakes of energy and nutrients, especially iron and zinc in the study population in Mali.

[7] The 225 nutrients values include the 22 specific nutrients which are not present in selected foods.

Phase 2: Energy and nutrient intakes

Median daily energy intake of the women population was 1447 kcal and 1624 kcal for estimated and analyzed values, respectively. The ratio of energy intake (EI) to calculated basal metabolic rate (BMR) was 1.1 ± 0.4 (IQR 0.4 to 2.2) and 1.2±0.4 (IQR 0.5 to 2.5) in estimated and analyzed intakes, respectively. The ratio fell within the 95% CI of the Goldberg cut-offs only for 9% of the study population in analyzed and 0% in estimated intakes, suggesting that on average the one day food weighed record underestimated energy intake. Fat contributed for 30% and 26%, available carbohydrates 56% and 66 % to estimated and analyzed energy intake respectively. The estimated protein, fat and dietary fibre intakes did not differ, but the estimated intake of available carbohydrates was 17% lower compared to analyzed intakes. Estimated iron was 57% lower and estimated vitamin A intake was 45% higher compared to analyzed intakes (**Table 3.2**), while calcium and zinc intakes did not differ. Protein, fat, and vitamin A density was significantly higher (by 6%, 19% and 71% respectively) and available carbohydrate and iron density was significantly lower (by 14% and 53% respectively) in estimated compared to analyzed intakes (**Table 3.2**).

Micronutrient PA

The probability of adequacy (PA) of micronutrient intakes of the women population ranged from 0.20-0.85 for the estimated intakes and from 0.06-0.91 for the analyzed intakes (**Table 3.2**). Estimated PAs was significantly lower for iron and significantly higher for vitamin A compared to analyzed intakes.

Correlations between estimated and analyzed nutrient intakes, nutrient densities and PA

Correlations coefficients between estimated and analyzed values ranged from 0.38 to 0.61 and were significant for all intakes and densities (**Table 3.2**). Significant correlations were found between the PA of estimated and analyzed iron (r_s=0.49, p=0.003), zinc (r_s=0.48, p=0.004) and vitamin A (r_s=0.46, p=0.005) but not for calcium intakes (r_s=0.09, p=0.618).

Agreement between nutrient intakes from FCT and CA

Bland and Altman plots show absence of agreement between estimated and analyzed values, as the variation in differences between the individual estimated and analyzed values is large with wide limits of agreement (**Figures 3.1 and 3.2**). Regression analysis shows that the differences between estimated and analyzed intakes increase with higher intake levels for most of the nutrients (**Table 3.3**). Calculation of intakes based on a-TACAM underestimates intakes of available carbohydrates and iron, while overestimate intake of fat, calcium and vitamin A compared to analyzed values (**Figures 3.1 and 3.2**). At lower average intakes, differences in iron (<20mg), calcium (<500mg) and vitamin A (<1000µg RAE) intake are clustered around zero (**Figure 3.2**).

Discussion

This study is the first assessing nutrient intakes in Africa from both chemical analysis and food composition table. We compared the nutrient intake estimated from a food weighed record using the adjusted Mali Food Composition Table (a-TACAM) with that based on chemical analysis of duplicate portions of diets collected on the same day. Our study indicates that, at population level, the a-TACAM leads to similar mean energy and nutrient intakes as compared to analyzed intakes, except for available carbohydrates, iron and vitamin A. With respect to nutrient density, only mean estimated and analyzed density of dietary fibre, calcium and zinc were similar. In contrast, at the individual level, the agreement between estimated versus analyzed nutrient intakes, densities and probability of adequate intake is poor.

In general, both estimated and analyzed intakes in our study population are much lower than those reported in previous studies of the FONIO project (15, 16). Intake in these studies was assessed using a non-consecutive repeated 24hr-recall, but limitations related to weaknesses inherent to a 24hr-recall are likely not leading to the magnitude of differences found in this study. Also, the 24hR was carried with approximately hundred women. The sub-sample participating in the weighed food record was not probably representative of the main sample as the women were conveniently selected.

The EI/BMR ratios of 1.10 for estimated and 1.20 for analyzed intakes, as found in our study, are well below the standard of the World Health Organization (1.55) for minimum energy requirements for people having a sedentary lifestyle and also below the cut-off limit (1.45) published by Goldberg et al. (1991) (44). The EI/BMR ratio in our study must be recognized as being incompatible with long-term maintenance of energy balance and therefore with long-term survival (45). Since the women in our study were weight stable, it seems that the weighed record resulted in changing normal dietary intake leading to lower energy intakes during the recording day.
The presence of a field assistant in the household for a whole day and the weighing of all foods and ingredients before preparation may have caused a change of dietary habits by the respondent in order to reduce the burden of weighing and recording (47, 48).

Table 3.1. Contribution of food items to dietary energy and nutrient intakes[1-3]

Food groups	Food items	Energy	Protein	Fat	Carbo-hydrate	Dietary fibre	Calcium	Iron	Zinc	RAE[3]
						(%)				
Cereals and products	Rice (cooked)	13	8		22				10	
	Bread (white)	6			9	4		7		
	Millet (flour)				6	7		7	8	
	Sorghum (flour)				6					
Animal products	Beef (red meat)	6	16	13				8	21	
	Cow milk		11	7			29		13	13
	Fish (small fish)		8	10			6			
Vegetables	Okra (dried powder)					37	20	27	9	
	Fakouhoye leaves					5	6			8
	Amaranth leaves						8			
	Lettuce									9
Legumes	African locust bean seeds (fermented)							7		
	Cowpea					5				
Nuts	Groundnut paste		9	9						
Fruits	Mango									17
Fats and oil	Peanut oil	14		42						
	Red palm oil									19
Sugar and syrups	Sugar (powder)	13			22					

[1] The top five foods contributors to more than 50% dietary energy and nutrient intake are mentioned.
[2]. Empty cells mean no contribution.
[3]. RAE=Retinol Activity Equivalent

Table 3.2. Energy, nutrient intakes and densities of Malian women obtained by estimation using the adjusted TACAM and by chemical analysis of duplicate portions at population level[1-7]

Variables	Estimated nutrient intakes	Analyzed nutrient intakes	P[2]	Ratio[3]	Spearman Correlation Coefficient[4]
n	**35**	**35**			
Energy (kcal)	1447 (1130-1908)	1624 (1186-1963)	0.190	0.11	0.44**
Nutrient intakes					
Macronutrients					
Protein (g)	35.9 (28.0-56.6)	38.9 (29.8-51.6)	0.806	0.08	0.44**
% of energy[5]	10.6 (9.1-12.9)	9.9 (8.6-11.0)	0.033		
Fats (g)	44.9 (29.0-69.2)	41.5 (34.0-61.0)	0.272	-0.08	0.52**
% of energy	30.4 (17.9-40.5)	25.6 (19.5-31.8)	0.004		
Carbohydrate (g)	203.0 (158.0-235.2)	243.5 (210.8-292.7)	0.002	0.17	0.41*
% of energy	56.5 (47.6-65.0)	65.5 (57.5-72.1)	0.000		
Dietary fiber (g)	12.3 (6.9-19.4)	16.2 (11.2-19.2)	0.359	0.24	0.47**
% of energy	1.6 (1.0-2.4)	1.9 (1.7-2.1)	0.492		
Micronutrients					
Calcium (mg)	284.5 (127.1-640.8)	302.2 (151.2-521.8)	0.351	0.06	0.52**
Iron (mg)	9.9 (7.4-23.9)	22.8 (17.2-34.6)	0.001	0.57	0.51**
Zinc (mg)	6.6 (4.4-10.2)	6.7 (5.4-8.1)	0.935	0.01	0.54**
Vitamin A (µg RAE)	356.0 (99.5-703.2)	246.1 (99.7-403.6)	0.012	-0.45	0.57**
Nutrient densities					
Macronutrients					
Protein (g)	26.4 (22.8-32.6)	24.8 (21.5-27.6)	0.028	-0.06	0.49**
Fats (g)	33.8 (19.4-45.0)	28.5 (21.6-35.3)	0.004	-0.19	0.50**
Carbohydrate (g)	141.1 (122.1-162.5)	163.8 (143.8-180.3)	0.000	0.14	0.60**
Dietary fiber (g)	8.1 (5.2-12.2)	9.3 (8.3-10.5)	0.492	0.13	0.38*
Micronutrients					
Calcium (mg)	193.8 (115.5-479.1)	159.6 (100.6-278.7)	0.092	-0.21	0.54**
Iron (mg)	7.3 (6.1-12.5)	15.5 (11.9-19.3)	0.000	0.53	0.41*
Zinc (mg)	4.2 (3.6-5.7)	4.1 (3.5-4.9)	0.064	0.02	0.38*
Vitamin A (µg RAE)	229.9 (77.2-601.0)	134.5 (65.8-275.9)	0.001	-0.71	0.61*
Probability of micronutrient adequacy[6]					
Calcium (mg)	0.20 (0.41)	0.06 (0.24)	0.096		0.09
Iron (mg)	0.40 (0.43)	0.78 (0.29)	0.000		0.49**
Zinc (mg)	0.85 (0.26)	0.91 (0.23)	0.085		0.48**
Vitamin A (µg RAE)	0.59 (0.47)	0.01 (0.01)	0.000		0.46 **

[1] Values are medians (IQR). [2] Estimated by Wilcoxon Signed Ranks Tests, bold nutrients and values are significantly different. [3] Ratio (analyzed – estimated intakes)/divided by analyzed intakes, the sign minus express the overestimation of estimated values. [4] * correlation signifinat at p<0.05 and ** correlation significant at p<0.001 level (two sided),. [5] Contribution of protein, fat, carbohydrates and fibre to energy intakes. [6] Values are mean (standard deviation).

Under-reporting of EI results in serious overestimates of nutrient inadequacies and leads to low probability of nutrient adequacy (49) as was shown in our study for calcium, iron and vitamin A. Although the underestimation is not likely to have influenced the comparison of estimated and analyzed intakes, higher intakes might reduce the observed correlations as for some nutrients the difference between the estimated and analyzed intakes increased with higher intakes.

Table 3.3. Regression analysis of average and difference of estimated and analysed intakes

Model	Slope	95% CI of slope	Significance
Energy	0.387±0.219	(-0.059, 0.834)	0.086
Protein	0.752±0.235	(0.274, 1.231)	0.003
Fat	0.649±0.194	(0.255, 1.043)	0.002
Available carbohydrates	0.059±0.234	(-0.418, 0.536)	0.804
Dietary fibre	1.480±0.138	(1.200, 1.759)	<0.0001
Calcium	1.273±0.206	(0.854, 1.692)	<0.0001
Iron	0.265±0. 272	(-0.289, 0.819)	0.338
Zinc	0.723±0.205	(0.306, 1.141)	0.001
Vitamin A (RAE)	-0.955 ± 0.270	(-1.505, -0.405)	0.001

In general, a discrepancy between estimated and analyzed values may reflect the different methods used to derive nutrient values and the time window related to the composition of the actual foods eaten (short term, analyzed) and documented composition embedded in a FCDB (long term, estimated). In this study, many values were imputed or borrowed when computing a-TACAM. The foods could not be exactly the same. Also, we used retention factors from USDA which could not be totally applicable to the population conditions, as cooking procedure maybe different. The observed differences between available carbohydrates, iron and vitamin A intakes thus may be due to the type of foods, cultivars, genetic, agricultural and environmental factors (growing conditions, geographic location, climate, fertilizer use and plant maturity and color at harvest) and post-harvesting factors such as storage, processing or preparation (4), specific conditions that may prevail in the study locality that are normally not reflected in a FCDB.

The main sources of available carbohydrates in our study were starchy staples such as refined white rice, refined wheat flour, bread or millet/sorghum (15). Variation may occur during the cooking process by modifying the water content and differences in water content will likely account for many of the differences in available carbohydrates content in the food (50). The available carbohydrates estimated based on FCDB were calculated by difference; therefore a small error in the estimation of other nutrients (protein, fat, water, ash and fiber) could affect available carbohydrate values for a specific food. In addition, a definition problem may occur with the use of total carbohydrates instead of available carbohydrates in some FCDBs, such as the South African food composition database, which could have contributed to the observed differences.

Underestimation of iron intakes may be due to iron contamination of drinking water from corroded iron pipes (51) or from an iron pot used for cooking because of iron release into the food cooked (52, 53). The type of grinder, the iron contamination during in-field milling of millet and sorghum, and the extraction rate when milling cereal to flour could also increase iron content in processed cereal foods (54). Soil contamination of flours is very common due to harvesting conditions of cereals, and could increase the iron content in the prepared foods if not cleaned carefully before preparation. Also, the use of water with high iron content may have increased the iron content of the actual foods consumed (55). During rainy seasons, run-off water carries much organic materials and suspended solids in the Niger River, the main pipe water source in Mali (56) and could therefore increase water iron content.

The higher estimated vitamin A intakes may be due to losses during storage (57). Losses of β-carotene related to drying and storage conditions up to 70% have been also reported on dried sweet potatoes after 4 months of storage (58). Another reason of the observed discrepancy between estimated and analyzed values may be related to the preference of some women for specific nutrient dense foods. For example, the difference between estimated and analyzed vitamin A intake could be related to the higher consumption of dried okra, fakouhoye and amaranth leaves, and red palm oil in the sauces during the recording day by some women. In addition, the study was done during the season of mangoes. Previous study has reported a higher contents of vitamin A especially ß-carotene on these foods (12) and most of the women have been found consuming them. Furthermore, the major problem is that the estimated vitamin A intakes based on a-TACAM could overestimate β-carotene (and therefore RAE) because the older analytical methods (from which the nutrients values were derived in the food composition table) do not differentiate between β-carotene and other carotenoids.

Our study was restricted to: 1) one day of food intake recording, excluding weekend days, 2) a unique food season and 3) a relatively limited sample size. With the low diversity of the diet and the restriction in food choice due to low living standards (even when a large range of foods is locally available) (59), there are only a limited number of foods contributing to energy and nutrient intake.

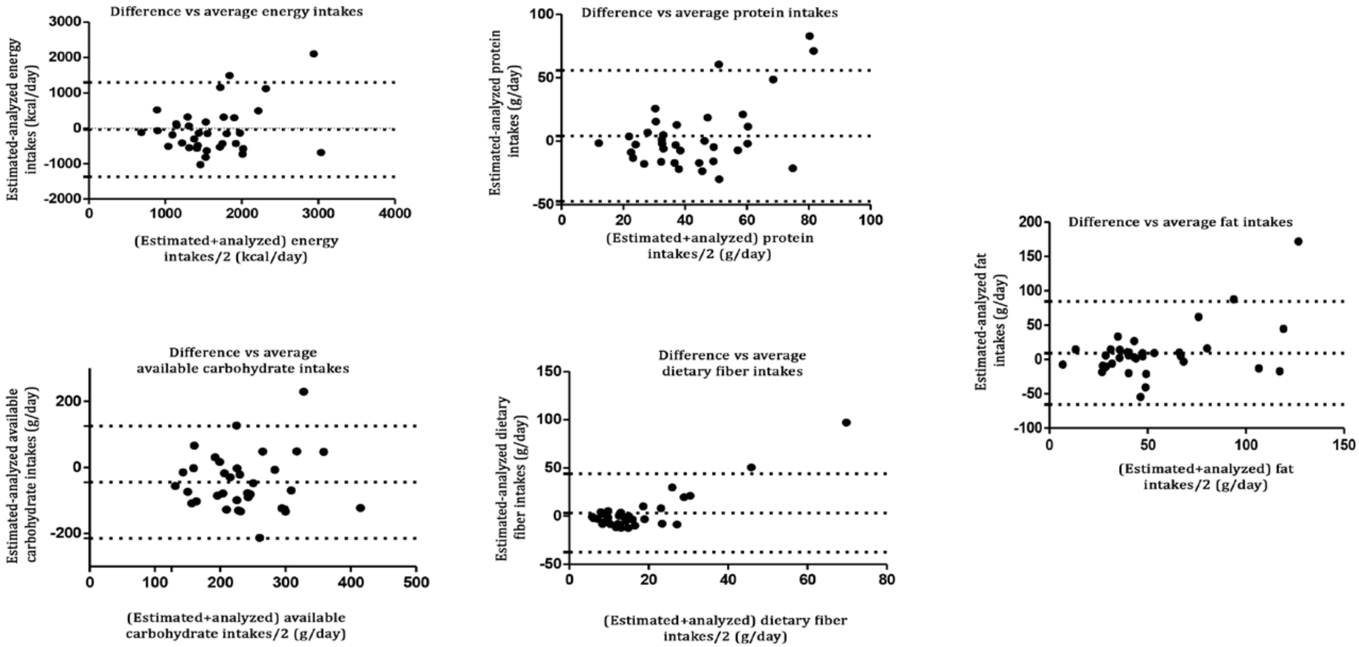

Figure 3.1. *Bland and Altman scatter diagram of macronutrient intakes per day*

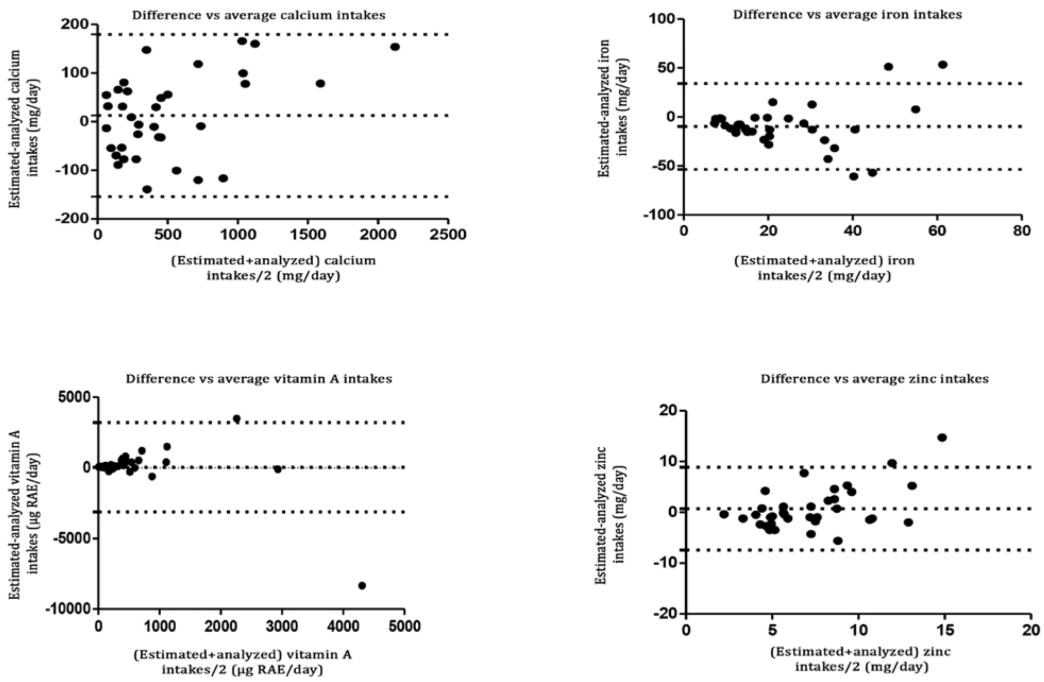

Figure 3.2. *Bland and Altman scatter diagram of micronutrient intakes per day*

Small errors in nutrient values of these foods, especially when eaten frequently may contribute to large bias in intake of that particular nutrient. The small sample size may have limited our power to detect meaningful differences between analyzed and estimated nutrient intakes at population level. For example, if a somewhat arbitrary mean difference of 10% between analyzed and estimated macronutrient content would be considered acceptable, then a sample size of n= 159 would be required to show this difference to be statistically significant.

Despite these limitations, our results are consistent with other (European) studies that documented no significant differences between calculated and analyzed energy, proteins and fat intakes (60), but unreliable retinol and β-carotene intakes when estimated using food composition tables (61). Therefore, we conclude that the adjusted Mali food composition table TACAM seems sufficiently acceptable to be used for assessing average intake at population level of energy, protein, fat, dietary fiber, calcium and zinc for low intake populations. For estimating carbohydrate, vitamin A and iron intakes and for assessing nutrient densities, and probability of adequacy of single micronutrient intakes, using the current Mali food composition table may lead to considerable bias.

Acknowledgements

Financial support by the Netherlands Academic Exchange Service (NUFFIC), the European Union funded project FP6/EU/INCO FONIO project (No. INCO CT-2005-015403) through a PhD scholarship to Yara Koreissi and Nutricia Research Foundation through a supplemental founding is gratefully acknowledged.
We especially thank the enumerators for the food weighed record and the duplicate portion collection, Mrs Traoré Soungalo F. Traoré and Mrs Camara Adam Fané (IER Food Technology Laboratory technicians) for helping with the handling and shipment of duplicate portions to Wageningen University and all the personal of the Wageningen Human Nutrition Division laboratory for handling and analysing the duplicate portions.

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4

Chapter four

- Fonio (*Digitaria exilis*) landraces in Mali: Nutrient and phytate content, genetic diversity and effect of processing

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Published in Journal of Food Composition and Analysis 2013,29(2)134-43

Abstract

The study aimed to assess i) the genetic diversity of fonio landraces in Mali, ii) the nutrient and phytate content in fonio products and iii) the effect of processing on nutrient content of fonio products. Twelve fonio landraces were collected from farmers in central and southern regions of Mali (10kg/farmer/landrace in paddy form), cleaned and processed in laboratory into paddy, midwet, cooked and parboiled fonio. Proximate and nutrient composition was determined using the standard AOAC methods. Three genetic groups were identified and between-individual races variation was observed in one group using the Amplified Fragment Length Polymorphisms method (AFLPs). Mean iron, zinc and phytate concentrations in paddy were 34.6 mg/100g, 3.2 mg/100g and 513.7 mg/100g dry weight. Processing reduced significantly iron, zinc and phytate content to 1.3 mg/100g, 2.2 mg/100g and 129.2 mg/100g dry weight. [Phytate]/[Iron] molar ratio in processed products was above the critical value of 1, suggesting poor iron absorption. Parboiling did not reduce iron and zinc losses due to processing.

Introduction

The considerable variation in nutrient content of single foods originating from different regions within a country forms one of the great challenges in producing food composition databases [1]. Genetic as well as environmental and agronomic factors like temperature, rainfall and access to water, use of fertilizer and nutrient content of soil can significantly affect the mineral levels in cereals [2-5]. Thus, for a single food, nutrient contents can be different between and within varieties [6].

Fonio (*Digitaria exilis*) is a typical and seasonal African cereal [7]. The crop is well adapted to harsh environments, requests low input for its cultivation and is able to grow on very poor soils without fertilization [8]. Fonio is harvested in the critical shortage season before the major food crops (millet, sorghum and maize) due to its short growing cycle. For some communities in Mali, fonio is either the communities' staple food or forms a major part of the diet [7, 9]. In 1974, fonio was included in the US Academy of Sciences' priority list of underexploited African tropical plants with promising economic value [8].

According to FAO statistics in 2009, the average world production of fonio (*digitaria exilis)* and black fonio (*digitaria iburu stapf*) together amount to 460 685 tons per year from 519 846 ha, all in West Africa [10]. For thousands of years, fonio has been cultivated across the dry savannas by West African populations along the Sudanese zone [7] and hundreds of fonio landraces exist and derive from traditional selection [11].

Mali is a land-locked country in West Africa which covers a surface area of 1 241 248 km^2 comprising three ecological zones: the Sahara (56% of the total surface) characterized by less than 200 mm of annual rainfall (regions of Timbuktu, Gao and Kidal); the Sahel zone (19% of the land mass) with an annual rainfall between 200 and 700 mm (region of Mopti and Kayes); and the Sudan zone receiving 700–1400 mm of rain per year and is covered by significant vegetation such as savannah and forests (regions of Ségou, Koulikoro, Bamako and Sikasso) [12]. In addition, Mali has three climatic seasons: the cold season from October to January; the hot season from February to May/June; and finally the rainy season from June/July to September/October. These considerable ecological and climatic variations cause varied soil and growing conditions for plants and animals that may affect the nutrient content of the local food [3]. In Mali, about 21 000 to 28 692 tons of fonio was produced on 46 477 ha from year 1999 to 2007 [8, 13]. Fonio is mostly cultivated in the Sahel and Sudan zones. In these zones Ségou and Sikasso regions together provided 70% of the total production of fonio in the country during the growing season 2006-2007 [14]. However, the nutrient content of the cultivars collected from these regions could be different.

In the past, generic food composition data were considered sufficient for most purposes and limited information has been reported about the varietal differences in nutritional content of under-utilized food such as fonio [6]. Barikmo et al. (2007) reported coefficients of variation ranging from 9% for iron to 61% for zinc content from fonio samples collected in Mali [15]. However, samples were composite samples coming from retailers on different markets and no data on species, cultivars, landraces or variety names was reported. The usefulness of cultivar-specific composition data is increasing for its potential contribution to efficient community health and agriculture programs, nutrition education initiatives, and national health policies [6]. For example, data on toxicants and contaminants of food provide necessary information for nutritional risk factors assessment [16].

Furthermore, data on traditional preparation methods contribute to understanding how different processing or cooking can compromise (or improve) the nutritional quality of under-utilized species [17]. Fliedel et al. (2003) reported iron content of 4 mg/100g and zinc content of 3 mg/100 g dry weight for decorticated grain of fonio Fingoloni variety in Mali [18]. The content of iron was 3 mg/100 g and zinc content was 2 mg/100 g dry weight in both traditionally pounded grain and mechanically dehulled grain of the same variety [18]. However, data referred to the whole grain of only one variety of fonio.

Fonio needs long and complex processing before consumption. This processing probably affects (micro) nutrient content, as the major part of the outer layers of the grain, including germ and pericarp, where the micronutrients are concentrated, is removed by milling [19]. No information is available on nutrient composition of derived products of fonio like milled or mid wet fonio, precooked and cooked fonio. In addition, there is also a knowledge gap on zinc, fiber, and phytate content of these products. Data on nutrient losses during processing, especially those related to public health problems such as iron and zinc deficiencies, is needed to support efforts in improving micronutrients supply and intake among vulnerable groups in developing countries. Knowing the genetic differences, the nutrient content of fonio and fonio products, its variation among landraces and the nutrient losses due to processing, can help to make choices for specific fonio landraces and processing in order to improve iron and zinc supply and intake of West African communities.

This study aimed to examine the genetic differences, the nutrient content, especially iron and zinc and their inhibitors such as phytate, of different fonio landraces collected in Mali and the effect of processing on these nutrient levels.

Materials and methods

Sampling plan and methods

Data used in this study were collected as part of the FP6/EU/INCO FONIO project (No. INCO CT-2005-015403). Twelve (12) fonio landraces were sampled in Mali from the Central and the Southern regions located in the Sahelian and Sudanese zones respectively, based on the sampling frame provided by the FONIO project [20]. Within each region, 2 areas producing most fonio were selected. In each area, 2 villages were randomly selected based on the following criteria: involved in the FONIO project; located in proximity of a large market; accessible by car throughout the year and producing mainly fonio. Selection criteria for farmers were: being fonio producer; consumer and/or retailer of fonio surplus; open for external visitors throughout the year and being receptive; owning or having enough space for fonio cultivation; and having a field of fonio located near a road.

Sample collection

In each village, local farmers' associations were contacted to facilitate the organization of the meetings and the collection of fonio samples. Verbal agreement was obtained from the farmers before starting data and sampling collection. Through focus group discussions, direct observation and individual interviews, farmers were asked to list (using vernacular names) and display the different fonio landraces produced in their village. Detailed traditional morphological descriptions, agronomic and environmental characteristics of the listed landraces were determined by farmers and documented by the Institute of Rural Economy (IER) scientists. Then, farmers were asked to clean and bring samples of each identified fonio landrace. By consensus, three samples of 10kg of each landrace (10kg/farmer/landrace) in paddy form were purchased from 34 farmers. Landraces were wrapped separately in a cloth bag, put in closed polyethylene containers and transported to the IER food technology laboratory in Bamako, Mali.

Fonio sample preparation

Fonio samples collected were processed at IER food technology laboratory using standardized procedures developed based on the traditional processing methods of cleaning and cooking of fonio (**Figures 4.1 and 4.2**).

Fonio paddy

Fonio paddy was cleaned (per batch of 1000 g of grain) using a sieve of 2 mm diameter to remove large particles (other grains, stones, and straw) and of 1 mm diameter to remove fine particles (dust, immature grains). Cleaned *fonio paddy* was simultaneously dehulled and milled for about 5 min using the GMBF dehuller type Engleberg for cereals such as rice, modified and adjusted for fonio dehulling in West Africa [21]. The GMBF dehuller adjusted to fonio was developed with the collaboration of scientific from Guinea, Mali, Burkina Faso and France in the framework of the Fonio project.

Mid wet fonio

The milled grain (similar to polished rice) of fonio obtained after dehulling of the paddy was separated from bran, dust and sand using sieves of 850 and 600 µm diameters, and washed with distilled water in two steps using the traditional sedimentation method based on the principle of density difference [22]. The product obtained from this process is called *mid wet fonio (or* ready-to-cook fonio) (**Figure 4.1**).

Cooked fonio

To prepare *cooked fonio*, the mid wet fonio was steam cooked three times for respectively 10, 12 and 10 min. Each steam cooking was done using 500 g of mid wet fonio (corresponding to 250 g of dry fonio which is a mid-wet fonio dried) for 1.5 L of distilled water in the cooker. Before the second and third steam cooking procedure, 500 ml of distilled water was added to the grain and stirred for about 6 min (**Figure 4.2**).

Parboiled fonio

Parboiling was performed with two types of samples, a sample of single *Peazo* landrace and a composite sample (mixed fonio) from the other landraces. During the whole process, the same procedure was applied for each type of sample.

Ten kg of cleaned fonio paddy of each type was washed in a full container (calabash) of water to remove empty grains, waste and dust using the traditional sedimentation process. Washed fonio paddy was divided into two parts. One part was soaked in distilled water for 24 hours at 20-25ºC to arrive at a moisture content of 32-35%.
The next day, the soaked fonio paddy was put in clean cloth or basket to drain water for about an hour. Fonio was then steam cooked for 10 min and poured after steaming on air dry surface for 24 hours. The product obtained from this process is called *parboiled fonio* paddy (**Figure 4.1**).

The other part of the sample was not parboiled, it is called normal fonio paddy. The normal and parboiled fonio paddy of each type (peazo and mixed fonio) was processed as previously described using the standardized cleaning and cooking method to get the

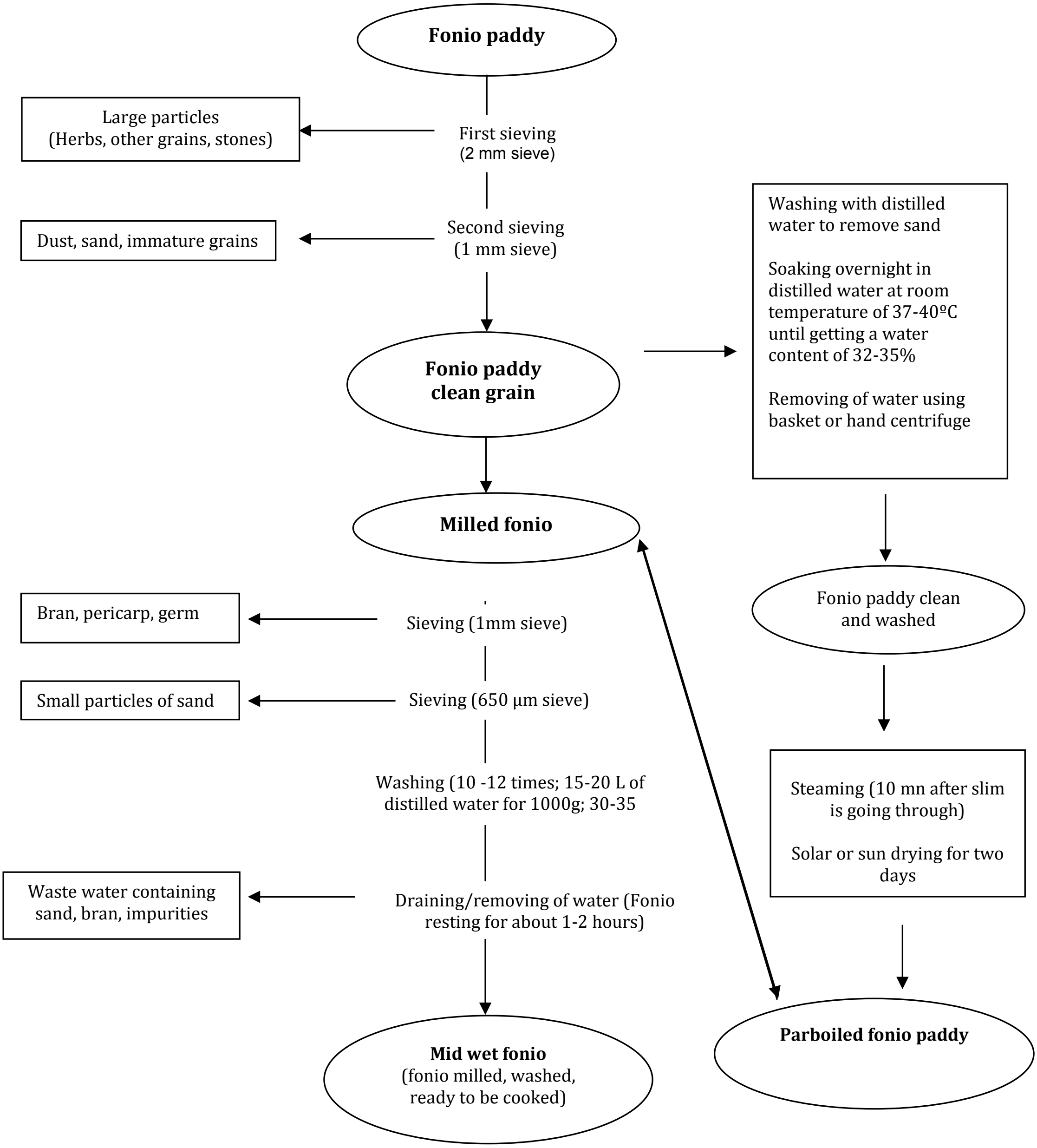

Figure 4.1. *Fonio processing (traditional and parboiling) diagram*

parboiled and non-parboiled form. From each type of sample, four products (normal mid-wet peazo, parboiled mid-wet peazo, cooked normal peazo, and cooked parboiled peazo for peazo; normal mid-wet mixed fonio, parboiled mid-wet mixed fonio, cooked normal mixed fonio and cooked parboiled mixed fonio for mixed fonio) were derived.

For each fonio landrace a sample of 125g was collected from each processed product and stored (12 landraces x 3 farmers = 36 x 5 fonio products (paddy, milled, mid wet, precooked and cooked = 180 samples; parboiled and non-parboiled products: 8 samples). A total of 188 laboratory samples were collected and packed in airtight polyethylene bag and stored in a deep freezer at -20ºC until express shipment for analysis to the Division of Human Nutrition, Wageningen University (The Netherlands). Proximate composition (moisture, ash, protein, fat, fiber) was analyzed in fonio paddy and midwet fonio at the Division of Human Nutrition of Wageningen University. Micronutrients (iron and zinc) content was analyzed at the Analytical Soil Chemical and Biological Laboratory, Wageningen University and phytate content in fonio paddy and mid-wet fonio at the Swiss Federal Institute of Technology in Zurich (ETH). Proximate composition and phytate analyses were performed in duplicate on a composite of 3 samples for each landrace. Each composite was made by mixing equal weights of each of the 3 samples. Micronutrient analysis was performed in duplicate in each single sample. Milled and precooked fonio were not analyzed.

Laboratory analyses

Proximate composition analysis

Proximate composition was determined according to the standard AOAC methods. Moisture content of fonio was determined by drying at 70°C in a vacuum oven until constant weight [23] Total nitrogen was analyzed using the automated Kjeldahl method [24]. Protein was calculated using the nitrogen to protein conversion factor of 6.25 [25]. Total fat content was analyzed using acid hydrolysis (AOAC method 14.019) [26]. Total dietary fiber was determined using Prosky method (AOAC 985.29) [27]. Ash was measured after a dry ashing procedure in a muffle furnace at 550°C for one hour. Available carbohydrates (g/100 g) were calculated by difference as follows:
100 - (water + ash + fat + protein + fiber); all proximate composition data were converted to g per 100 g dry weight basis. Energy content of each fonio product was calculated from the amount of protein, fat and carbohydrate on the basis of energy conversion factors [28] as follows: protein, 16.7 kJ/g; fat, 37.7 kJ/g and carbohydrate, 16.7 kJ/g. Energy contents of all foods were calculated in both kilojoules and kilocalories (1 kcal = 4.184 kJ).

Element analysis

Iron and zinc were determined in the accredited chemical biological laboratory of Wageningen University and Research Center. Microwave digestion were performed as described by Novozamsky et al. (1996) in fonio paddy, mid-wet fonio (milled fonio, washed, ready-to-cook) of each landrace, in (non) parboiled mid-wet and cooked fonio [29]. Approximately 0.4g of dried fonio was weighed in a metal weighing funnel and transferred to a digestion vessel. 5.0 ml of 40% hydrofluoric acid (HF) and 5.0 ml of

65% nitric acid (HNO_3) were added to the samples and mixed. The mixtures were left overnight at room temperature and then heated at 120ºC. Another 5.0 ml of 65% concentrated nitric acid (HNO_3) and three times 1.0 ml of 30% hydrogen peroxide (H_2O_2) was added to the fonio product and put into the microwave for digestion (CEM MDS-2100). After the microwave digestion, vessels were allowed to cool down for 45 minutes and opened in a fume hood. The digests were quantitatively transferred to 50 ml polythene volumetric flasks, made up to mark with Millipore water, mixed and then filtered over fine paper into a polythene bottle. The concentration of iron and zinc in the digests were analysed using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). Values were determined on wet-weight basis and converted to dry weight basis.

Phytic acid analysis

Phytic acid analysis was performed in fonio paddy, mid wet fonio for each landrace, and for (non) parboiled mid-wet and cooked products. Levels of phytic acid, expressed as inositol hexakisphosphate (IP6) was determined according to a modified method of Makower [30] which consists of an extraction and a selective precipitation of phytate in which cerium replaced iron in the precipitation step according to Hurrell et al., (1992) [31]. In brief: phytic acid was extracted from a ~0.5 g sample using 2 x 3 mL 12% trichloroacetic acid (TCA) and next phytic acid in the extract was selectively precipitated using 3 mL 5% cerium sulfate in 10% sulfuric acid solution. Then the sample tubes were centrifuged during 15 min at 3550g. The isolated precipitate was hydrolyzed in concentrated sulfuric acid (H2SO4) for 3 hours at 338 ºC in a Tecator digestion unit (Foss, Hilleroed Denmark).

The inorganic phosphate liberated from the phytic acid digestion was measured according to the van Veldhoven's method [32]: inorganic phosphate forms a complex with ammonium molybdate, which reacts with malachite green to produce a colored compound. Phytic acid content, calculated as inositol hexakisphosphate (IP6), was based on spectrophotometrically measured phosphate concentration at 630 nm.

To predict the inhibiting effect of phytic acid on iron and zinc bioavailability from fonio, [phytate]/ [Fe] and [phytate]/[Zn] molar ratios were calculated as an index for the potential mineral bioavailability [33, 34]. Critical values above 1 for [phytate]/[Fe] and 15 for [phytate]/[Zn] were used as reference for predicting poor bioavailability [33, 35, 36].

Analytical quality control

For ash, fat and nitrogen, baby food (Humana Milchwerke Westfalen eG, D- 4900 Herford, Germany) was used as control sample. Within and between-run CVs over a more than 10 year period are: 0.9 % and 1.6% for ash; 2.3% and 3.9% for fat; 0.8% and

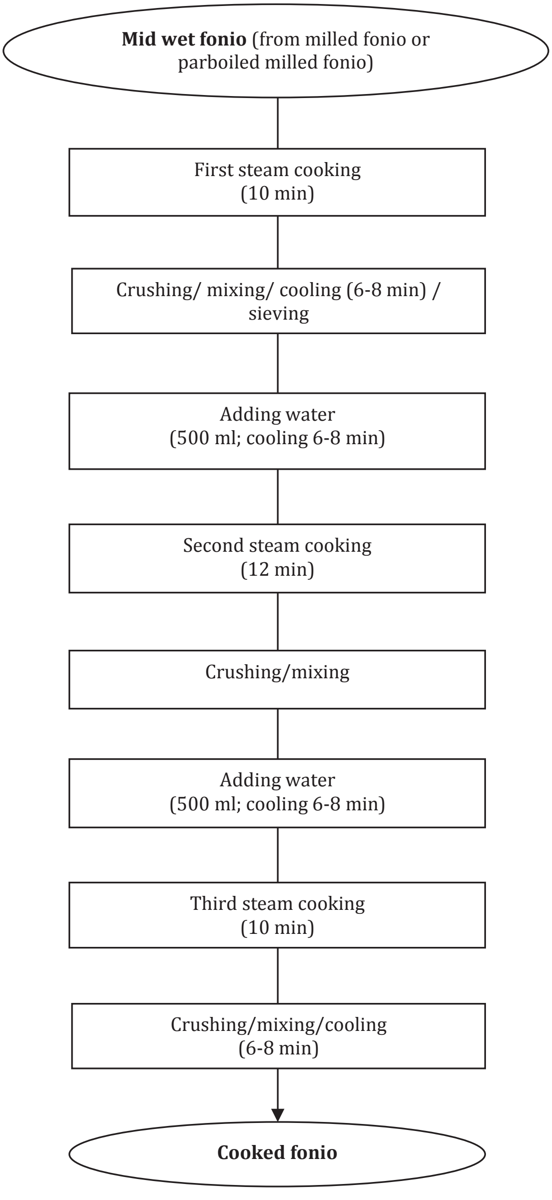

Figure 4. 2. *Fonio cooking diagram*

1.2% for nitrogen. For fiber, the in-house control sample was bread crumbs; with a between-day CV of 4.2% (within- day variation was not recorded). Results from proficiency testing [37] showed z-scores as a measure for deviation from the consensus value (accuracy) for fiber -0.2; for nitrogen +0.7 and for ash 0.0. Results from proficiency tests organised by the Swedish National Food Administration (Uppsala, Sweden) showed average z-scores over a 10 year period for ash +0.52; for dry matter + 0.99; for fat +1.72 and for nitrogen -0.24. For iron and zinc, grass and strawberry samples were used as in-house control material. Analytical variation was ~6% for both elements. The chemical biological laboratory participates in the International Plant-Analytical Exchange program organized by Wageningen Evaluation Programmes for Analytical Laboratories (www.wepal.nl). Results from proficiency tests (average result of 11 ring test samples) showed mean z-scores of 1.7 for iron and 0.4 for zinc over a one year period. To monitor repeatability of phytic acid determinations, hard red wheat bran (Lot 195, 1995, American Assn. of Cereal Chemists, St. Paul, Minn., U.S.A.) was analysed together with each series of samples. Analytical variation of the phytate determinations was 7%.

Assessment of genetic variability

Genetic diversity of the 12 fonio landraces was assessed by Amplified Fragment Length Polymorphisms (AFLP) [38]. To isolate the DNA fonio landraces were grown in the greenhouse (Wageningen UR Plant Breeding Wageningen University, The Netherlands). Total genomic DNA was extracted from bulked young leaves (100-200 mg per landrace) of ten 4 to 5 week-old plants following the automated Kingfisher DNA isolation protocol. In the buffer solution RNAse and Proteinase K were added. The DNA concentration was determined by electrophoresis in 0.8% agarose gel at 100 V using known DNA concentration standards.

Li-Cor AFLP Kit was used according to the recommendations of the manufacturer. 100 ng pure DNA was digested with restriction enzymes *EcoR*I and *Mse*I and enzyme adapters were ligated to the digested DNA. The selective amplification of restriction fragments was done with color labelled primers with three selective nucleotides. After this, 5 µl of the reaction product was mixed with an equal volume of formamide–loading buffer (98% formamide, 10 mM EDTA pH 8.0 and 0.1% Bromo-phenol blue). The total mixture was carefully mixed and heated for 5 minutes at 94^{o}C in a hot-block and then quickly cooled to ice. From the 10 µl, 8 µl was loaded on a 6% denaturing polyacrylamide gel. Li-Cor 4300 S DNA analyser was used to image, analyse and screen amplified fragments.

Statistical analysis

Data were analyzed using SPSS for Windows IBM SPSS Statistics Release 15.0.1.1, SPSS Inc, Chicago, IL, USA [39]. Data were checked for normality using Shapiro-Wilk test. Distributions of iron and phytate value for fonio paddy were (log) transformed to normality. One-way ANOVA was used to compare the mean iron, zinc, and phytate values of the landraces with Tukey's LSD test as post hoc. Student paired t test was used to test differences between fonio products (normal and parboiled mid - wet peazo, normal and parboiled mid–wet mixed fonio, cooked normal and cooked parboiled peazo, cooked normal and cooked parboiled mixed fonio). All statistical tests were two-sided. A *P*-value < 0.05 was considered statistically significant.

Results

Proximate composition of paddy and mid wet fonio

Table 4.1 shows the energy and macronutrients content of 12 fonio landraces frequently consumed in central and southern regions of Mali. Moisture content of the paddy was on average 9.7 g/100g wet weight basis (ranging from 8.2 g/100g for Petama landrace to 11.4 g/100g for Finiba/Kassangara landrace). Available carbohydrate contributed on average 66% to the proximate composition of fonio paddy, with mean values ranging from 62 g/100g (Diéni landrace) to 69 g/100g dry weight (Tamatioi landrace). Mean protein content of fonio paddy was 8.5 g/100g; with minimum value of 7.4 and maximum value of 9.5 g/100g dry weight. Total fat and ash content were on average 4.2 g/100g and 3.1 g/100g dry weight. The average of total dietary fiber was 18.2 g/100g dry weight (range: 15.7-20.7 g/100g). Mean energy provided per 100g of fonio paddy was 1402 kJ (range: 1300-1448 kJ). The landraces did not significantly differ in energy and macronutrient content in paddy form.

Also in the mid wet fonio, the 12 landraces were similar regarding their energy and macronutrients content (**Table 4.1**). The average moisture content of mid wet fonio was 39.8 g/100g and ranged from 37.2 to 43.2 g/100g wet weight. Like for fonio paddy, available carbohydrates were the main component of macronutrient content of mid wet fonio with 88.2 g/100g dry weight. Ash and total dietary fiber content contributed on average 0.4 and 1.7 g/100g dry weight. Mean protein was of 7.8 g/100g and mean fat content was of 1.9 g/100g dry weight. Ash, total dietary fiber, protein and fat content were lower in mid wet fonio as compared to fonio paddy. Mean energy provided per 100 g of mid wet fonio was 1676 kJ (range: 1666-1688 kJ).

In terms of nutrient loss during processing, all macronutrient concentrations reduced when processing paddy into mid wet fonio except for carbohydrate which increased in level. The mean available carbohydrate content was 66 g/100g dry weight in paddy and 89.3 g/100g dry weight in mid wet fonio. The nutrient loss was however high for fiber (18.2 to 1.7 mg/100g dry weight) compared to other macronutrients.

Mineral and phytate content and effect of processing

In fonio paddy, iron content ranged from 14 mg/100g (Tamatioi landrace) to 57 mg/100g dry weight (Tamabé landrace) and averaged 35 mg/100g for the 12 landraces, while the average value of zinc concentration across the 12 landraces was 3 mg/100g (**Table 4.1**). Mean phytate content in fonio paddy was 514 mg/100g with minimum and maximum values of 413 mg/100g and 668 mg/100g dry weight. Coefficient of variation across the 12 landraces was 84% for iron and 15% for zinc but there was no statistically significant difference in iron and zinc content between the 12 landraces. Contrary, the phytate level was significantly different between the 12 landraces (P = 0.000).

In mid wet fonio, mean iron content was 1.3 mg/100g and mean zinc content was 2.1 mg/100g. Variation coefficients were 20% and 40% for iron and zinc. However, the mean was not significantly different between the 12 landraces for the 2 micronutrients. As in fonio paddy, phytate level was significantly different between the 12 landraces (P = 0.000), with an average value of 129 mg/100g, ranging from 31 mg/100g and 212 mg/100g respectively.

There was a statistically significant difference between fonio paddy and midwet fonio for iron (P = 0.000), zinc (P = 0.000) and phytate content (P = 0.000). Processing from paddy to mid wet fonio significantly reduced all micronutrients and phytate concentration. The highest reduction in terms of nutrient loss was observed in iron and phytate. Iron content reduced from 35 mg to 1 mg/100g (96% of reduction) and phytate levels from 514 mg to 129 mg/100g (75% of reduction). The concentration of zinc reduced slightly but significantly during processing. [Phytate]/[iron] molar ratio in paddy, midwet and cooked fonio was 1.3, 8.3 and 4.7 respectively, and [Phytate]/[zinc] molar ratio in the same products was 15.9, 5.9, and 5.7 (**Table 4.2**).

Using traditional processing for both normal mixed fonio and normal peazo landrace, carbohydrate content tended to decrease with parboiling while protein and dietary fiber seemed to increase (**Table 4. 3**). There was no statistically significant difference between mean iron and zinc content when processing from midwet to cooked, for normal mixed fonio (P = 0.104 for iron and P = 0.444 for zinc) as well as for normal peazo (P = 0.885 for iron and P = 0.393). Conversely, phytate levels decreased significantly when processing from midwet (P = 0.000) to cooked fonio (P = 0.000) both in normal mixed fonio and normal peazo.

Iron content was significantly higher in parboiled midwet mixed fonio than in normal midwet mixed fonio (P = 0.038). Conversely, parboiling seemed to slightly reduce zinc content in cooked fonio as compared to midwet fonio but this was significant only for mixed fonio (P = 0.023). Also, phytate levels appeared to be lower in cooked parboiled peazo compared to cooked normal peazo (P = 0.000) and in parboiled midwet mixed fonio compared to normal midwet mixed fonio (P = 0.000). Contrary to this, phytate level was higher in normal midwet peazo compared to parboiled midwet peazo (P = 0.000). No significant difference was observed between parboiled and normal cooked mixed fonio.

Table 4.1. Proximate composition, energy, elements and phytate content of 12 fonio landraces from Mali[1]

Landraces[4]	Moisture	Ash	Total fat	Protein	Total dietary fiber	Available carbohydrate	Energy	Iron[2]	Zinc[2]	Phytate[3]
	g/100g wet weight	g/100g dry weight					KJ (kcal)	mg/100g dry weight (SD)		mg/100g dry weight (SD)
Fonio paddy										
Finiba/ Kassangara	11.4	3.5	4.4	7.7	19.1	65.4	1386 (331)	29.8 (15.1)	3.1 (0.3)	666 (29.1)[b]
Dièni[4]	10.0	7.4	3.6	7.4	19.3	62.2	1300 (311)	-	-	-
Kassangara	10.4	4.3	4.5	7.9	17.3	66.1	1404 (336)	29.6 (15.2)	3.0 (0.6)	668 (8.5)[b]
Finiba	9.5	2.7	4.2	8.2	17.2	67.7	1424 (340)	52.7 (30.4)	2.9 (0.7)	551 (5.3)[a]
Petama	8.2	2.4	3.9	8.8	20.1	64.9	1377 (329)	25.7 (11.1)	3.5 (0.3)	445 (12.6)[c]
Péazo 1	8.9	2.1	3.8	8.9	20.7	64.5	1370 (328)	28.1 (19.6)	3.4 (0.7)	462 (9.0)[c]
Tioi	11.0	2.2	4.4	8.7	19.6	65.1	1399 (334)	17.3 (8.3)	3.1 (0.4)	413 (5.5)[e]
Pêyè	9.0	2.1	4.0	9.5	17.4	671	1429 (342)	54.8 (68.6)	3.6 (0.7)	458 (4.05)[c]
Tama	8.8	2.3	4.1	8.8	17.9	66.9	1418 (339)	29.1 (21.7)	3.0 (0.4)	452 (5.05)[c]
Tamatioi	10.5	2.1	4.0	9.2	16.2	68.7	1448 (346)	14.5 (5.4)	3.3 (0.7)	490 (16.5)[d]
Tamabé	9.1	2.3	4.5	8.7	17.9	66.7	1428 (341)	57.3 (46.1)	3.3 (0.4)	470 (14.4)[cd]
Péazo 2	9.6	3.9	4.5	8.4	15.7	67.7	1438 (344)	41.7 (30.7)	3.2 (0.2)	577 (3.7)[a]
Mean (SD)[5]	9.7 (0.9)	3.1 (1.6)	4.2 (0.3)	8.5 (0.6)	18.2 (1.6)	66.1 (1.8)	1402 (38.6) [335 (9.2)]	34.6 (14.2)	3.2 (0.5)	514 (84.9)
CV(%)								84	15	17
Mid wet fonio										
Finiba/ Kassangara	39.4	0.4	2.0	6. 5	1.8	89.3	1676 (400)	1.2 (0.6)	1.9 (0.2)	190 (5.3)[c]
Dièni	40.8	0.4	1.9	6.5	2.0	89.2	1669 (399)	1.8 (0.6)	1.9 (0.5)	158 (4.0)[a]
Kassangara	38.3	0.5	2.0	7.4	1.4	88. 8	1680 (402)	1.1 (0.2)	2.2 (0.8)	212 (7.8)[d]
Finiba	39.6	0.4	1. 9	7.3	1.5	88.9	1678 (401)	1.7 (0.9)	2.3 (0.1)	146 (3.2)[b]
Petama	39.7	0.5	1.8	8.0	1.1	88.7	1681 (402)	1.2 (0.1)	2.1 (0.2)	31 (0.9)[e]
Péazo 1	40.6	0.6	2.0	8.2	1.4	87.8	1681 (402)	1.7 (0.2)	2.0 (0.1)	164 (6.8)[a]
Tioi	43.2	0.4	1.9	8.0	0.9	88.8	1688 (403)	0.9 (0.2)	2.2 (0.1)	107 (0.8)[i]
Pêyè	39.2	0.5	2.1	9.1	2.4	85.9	1666 (398)	1.9 (0.6)	2.8 (0.9)	185 (2.5)[c]
Tama	37.2	0.3	1.8	8.1	2.2	87.6	1666 (398)	1.3 (0.6)	1.9 (0.2)	89 (1.6)[f]
Tamatioi	38.6	0.3	1.8	8.2	1.7	87.9	1674 (400)	0. 8 (0.2)	2.2 (0.4)	45 (1.0)[h]
Tamabé	39.3	0.4	2.0	8.0	1.8	88.0	1675 (400)	1.2 (0.3)	2.6 (0.1)	80 (0.9)[g]
Péazo 2	42.3	0.4	2.1	8.2	2.0	87.3	1674 (400)	1.2 (0.5)	2.1 (0.3)	144 (2.0)[b]
Mean (SD)[5]	39.8(1.6)	0.4 (0.1)	1.9 (0.1)	7.8 (0.8)	1.7 (0.4)	88.2 (1.0)	1675 (6.3) [400 (1.6)]	1.3 (0.5)	2.2 (0.4)	129 (56.6)
CV(%)								40	20	44

[1] *Values are means of original 3 pooled samples per landrace for all macronutrients, 4 pooled samples per landrace for phytate and mean ± SD of 3 samples per landrace for iron and zinc*

[2] *No statistically significant difference between values; iron in paddy $P = 0.683$, zinc in paddy $P = 0.843$, iron in midwet $P= 0.117$, zinc in midwet $P= 0.25$*

[3] *Phytate is defined as the sum of IP5 and IP6 expressed as IP6 and values with different letter in column are significantly different ($P < 0.001$)*

[4] *No data available for Dièni fonio paddy*

[5] *Mean value for iron, zinc and phytate are significantly different between fonio paddy and midwet fonio ($P = 0.000$).*

Table 4.2. [Phytate]/ [Fe] and [Phytate]/[Zn] molar ratios of fonio landraces grouped by fonio products.

Landraces	Iron	Zinc	Phytate[4]	[Phytate]/[Fe][1]	[Phytate]/[Zn][2]
	mg/100g[3]				
Fonio paddy	34.6	3.2	513.7	1.3	15.9
Mid wet fonio	1.3	2.2	129.2	8.3	5.9
Cooked fonio	2.2	2.1	122.5	4.7	5.7

[1] *Phytate/Fe molar ratio is calculated by (mg of IP6/molecular weight of IP6)/ (mg of iron/ molecular weight of iron)*
[2] *Phytate/Zn molar ratio is calculated by (mg of IP6/molecular weight of IP6)/ (mg of zinc/molecular weight of zinc).*
[3] *Iron and zinc content are expressed on dry weight basis*
[4] *Phytate is defined as the sum of IP5 and IP6 expressed as IP6*

Genetic variability of the 12 fonio landraces

Few polymorphisms were observed in the 12 landraces. Eight primer combinations resulted in 255 AFLP fragments of which only nine (3.5%) were polymorphic and in none of the individual lines unique fragments were seen. Three distinct clusters, representing three groups of landraces can be identified, group one (the landraces 8, 70, 110,138 such as Finiba/Kassangara, Tioi, Tamatioi and Péazo2 landraces), group two (the landraces 22, 62, 94, 50, 86, 18, 126 such as Kassangara, Tama, Pétama, Pêyè, Diéni, Tamabé and Péazo1 landraces) and group three (the landrace 34 the Finiba landrace). Only the second group showed variation between individual races (1-2 fragments) (**Figure 4.3**).

Table 4.3. Nutrients content of fonio products[1,2]

Processed fonio (fonio products)	Total fat	Protein	Total Dietary fiber	Available Carbohydrate	Iron[3]	Zinc[3]	Phytate[3]
	g/100g dry weight				Mean (SD) mg/100g dry weight		Mean (SD) mg/100g dry weight
Normal midwet Peazo	2.0	7.3	0.9	89.5	2.2 (1.6)	2.2(0.5)	98.1 (2.2)a
Parboiled peazo	2.4	8.2	4.5	84.2	2.2(0.3)	2.1(0.1)	242.0 (1.6)b
Normal midwet mixed fonio	2.2	7.8	1.4	87.8	1.4 (0.3)a	2.3 (0.2)	305.9 (2.7)e
Parboiled mixed fonio	2.0	8.3	3.9	85.0	2.1 (0.2)b	2.1 (0.1)	201.7 (3.5)f
Cooked Normal peazo	2.1	7.4	6. 7	83.3	1.9 (0.6)	1.9(0.2)	135.6 (2.5)c
Cooked parboiled peazo	2.5	8.1	7.5	81.3	2.5 (0.9)	2.2(0.3)	119.6 (2.7)d
Cooked Normal mixed fonio	2.4	8.0	7.0	81.9	2.8 (0.9)	2.4 (0.2)a	118.7(1.3)
Cooked parboiled mixed fonio	2.0	8.2	7.5	81.7	1.8 (0.3)	2.0 (0.2)b	114.3(5.7)

[1] *n= 32 samples of fonio products (n = 4 for each fonio product)*
[2] *Fonio products such as normal peazo, parboiled peazo, normal mixed fonio and parboiled mixed fonio are in mid wet form*
[3] *Phytate is defined as the sum of IP5 and IP6 expressed as IP6 and values with different letters within column are significantly different at P <0.05*

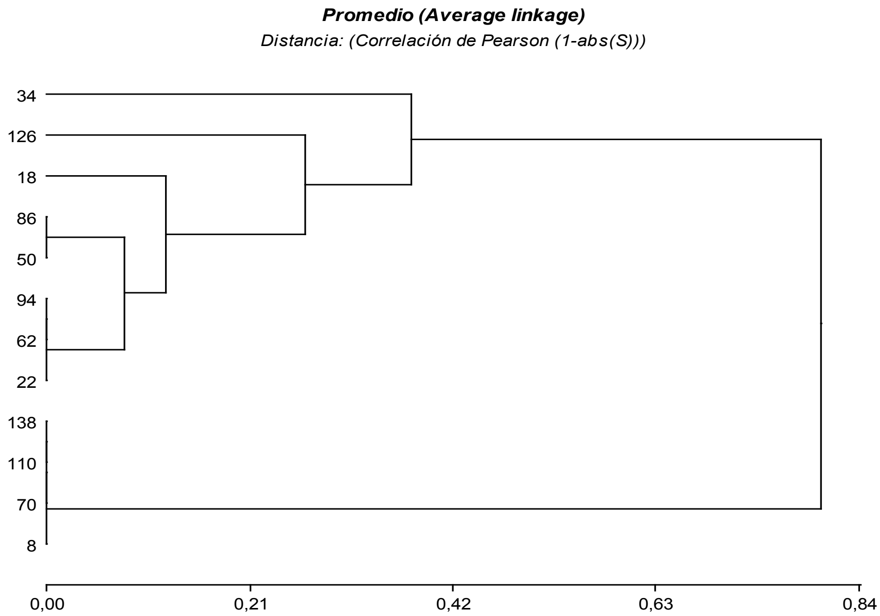

Figure 4.3. *Dendrogram of genetic diversity and population differentiation of 12 fonio landraces from Mali*

Discussion

In this study the genetic difference and nutrient content and the effect of processing from 12 fonio landraces collected in central and Southern regions of Mali were investigated. The main finding of the study was that the genetic diversity from fonio landraces collected in Mali was small and the phenotype represented by the proximate composition, iron and zinc content showed no significant differences between landraces. Traditional processing reduced all the nutrients from paddy to mid wet except for carbohydrate which increased. The reduction was significant in iron and phytate content while zinc concentration was hardly affected even after processing and cooking. Parboiling did not help reduce nutrient losses in fonio during processing and cooking. The apparent reduction in available carbohydrates and apparent increase in dietary fiber are probably due to the formation of resistant starch (retrograded starch) during the drying process after steaming. Phytate content significantly decreased during fonio processing and cooking and varied also significantly between landraces. However, molar ratio [phytate]/[iron] was still above the critical cut-off of > 1 in midwet and cooked fonio, while molar ratio [phytate]/[zinc] for both mid wet and cooked fonio were below the acceptable ratio of 15.

Limitations of this study included the collection of landraces in 2 regions out of 8 in Mali, hampering generalization of the results to the country. However, a sample unit of 30 kg of fonio by landrace from 3 different farmers (10 kg/landrace/farmer) was sufficient for observing any variation between landraces. Also, landraces were identified by farmers based on their phenotypic characteristics. Therefore, it might occur that in different regions, different names were given to landraces with same genetic origin, or same names given to two morphologically and genetically different landraces [11].

Results from this study showed a limited genetic variation in the studied landraces, as indicated by the very low polymorphism level (3.5%) compared to the 63% reported by Adoukonou-Sagbadja et al. (2007) from 118 accessions of *Digitaria exilis* collected in Benin, Burkina Faso, Guinea, Mali and Togo [11]. Also, our 12 landraces were clustered into three groups while other Malian accessions collected by Adoukonou et al*.* (2007) were split into two groups. These discrepancies are probably related to methodology settings: sample size was greater and collection areas wider in this latter study as compared to our study.

In the present study, variation coefficients of 15% to 84% were found for zinc and iron in paddy and midwet fonio between landraces, with zinc content showing the lowest variation. Large variation has also been reported earlier by Barikmo et al*.* (2007) for minerals and vitamins content in fonio grain (as ready to cook) collected from 5 zones in Mali, with the lowest variation (CV of 9%) for zinc content [15]. However, no statistically significant differences were observed in iron and zinc as well as in macronutrient content between landraces from our study. Results of mean difference in nutritive value between varieties or cultivars have not been reported for fonio in previous studies so comparison is hampered. This lack of difference could be related to the low genetic variation observed between our landraces, suggesting that farmers may have misclassified landraces originating from a same gene pool. Secondly, the reduced sample size of the collection (number of landraces) could be a restricting factor for variation. Bland and Altman (2011) demonstrated recently that correlation may be weak in restricted ranges of data [40]. Further research should be designed to investigate relationships between genetic characteristics and nutritional qualities in fonio, taking into account greater size and more widespread sample collection.

Our results revealed high iron concentration in paddy fonio, with average maximum values of 53-57 mg/100g dry weight. A similar observation was reported by Barikmo et al. (2004b) for iron in whole grain fonio [1], comparable to paddy from which only the ball has been removed. This high concentration may be a consequence of the level of iron in West African soils which can lead to increased level of iron in cereals. This probably occurred, as the paddy fonio from our study and probably the husked fonio in Barikmo et al. (2004b) were not washed before analyses and could have been contaminated by soil dust, hence increasing the iron content in fonio paddy. Similarly, Besrat et al. (1980) also showed in samples of white and red tef grain a high iron concentration of 3.6 to 7.8 mg/100 g DM and they suggested that some food samples might have got extra iron from the soil [41]. As other African soils, for example Ugandan soil with a higher soil iron concentration of 14825 mg/kg [42], the topsoils in West Africa are rich in available iron, reaching toxic levels up to 20% of the soils [43]. Soil residues and dust can settle on the surface of cereals during harvesting [44] and could contaminate cereal grains.

Our study found iron content of approximately 2 mg/100g dry weight in midwet (ready-to-cook) and cooked fonio (ready-to-eat), regardless the landraces. Similar values were reported by Barikmo et al. (2004b) from husked fonio (comparable to midwet fonio) in Mali (2.1 mg/100g dry weight) [1]. This value appeared the lowest, as compared to the products of other traditional cereals such as maize, millet and sorghum (5.9 mg/100g in yellow maize; 5.8 mg/100g in pearl millet flour; and 5.8 mg/100g in sorghum flour) [15]. This suggests that a low contribution of fonio to the iron intake from fonio diets and appropriate food-based strategies should be designed to improve iron supply from those diets.

Regarding macronutrients, the mean protein level in paddy approximately 9 g/100g is similar to 9-11g/100 g dry matter for whole grain and 7-9 g/100g dry matter for milled grain, reported earlier [18]. A similar result was obtained for midwet fonio (7.8 g/100g which is comparable to the 7 g/100g edible portion previously reported in the Mali Food Composition Table for husked fonio and white whole grain fonio [1].

As previously stated, results of our study showed that traditional processing considerably reduces macronutrients and mineral content of fonio, the most important losses occurring during processing of paddy product into mid wet product. Significant reduction was observed for iron and zinc content from paddy fonio to mid wet fonio but not from mid wet to cooked fonio. Leaving aside the soil contamination issue discussed above, a non-negligible proportion of this difference could be attributed to nutrient losses during processing, particularly husking/dehulling and milling which significantly affects the nutritional qualities of mid wet fonio [45]. Previous studies on wheat reported that the extent of nutrient loss varies according to severity, type and time of processing, but also upon the sensitivity of the nutrient to various conditions prevailing during the processing, including pH, light and oxygen [46]. Minerals such as iron and zinc are known to be concentrated in the bran and will then be easily leached during processing [47], although in the present study, zinc content was not significantly affected by the traditional processing.

In order to mitigate nutrient losses during processing, we performed a parboiling process with the expectation that like for rice, this will lead to a partial redistribution of nutrients from the surrounding layers into the endosperm, and so reducing nutrient leaching during and after milling [48]. However, our results were mixed and did not help to draw consistent conclusions. Nonetheless, regarding the amount of iron left in the parboiled mid wet fonio after processing (2 mg/100g in mid wet peazo as well as in mid wet mixed fonio) it can be highlighted that parboiling did not help reduce nutrient loss, especially of iron in processed fonio.

Phytate is the most common anti-nutritional factor found in cereals, strongly inhibiting the absorption of minerals such as iron and zinc [49]. Nearly complete degradation of phytate from meals containing few or no enhancers of iron absorption is necessary to obtain a meaningful increase in iron/zinc absorption [49, 50]. The 12 landraces of fonio collected from Mali showed a mean phytate value of 514 mg/100g in paddy, with significant differences between landraces and a significant decrease to 129 mg/100g in mid wet fonio caused by processing such as dehulling/milling and washing. As fonio is dehulled/milled prior to be washed, a large amount of nutrients including phytate is reduced because of its high concentration in the bran layers being removed during dehulling/milling [51-54] and depended on the degree of milling determining the amount of bran removed [52, 55]. During the washing process, fonio is staying longer in water and most of the phytate could be lost by leaching. This is not surprising as phytate loss is common with soaking [49, 56, 57]. Despite this reduction, amounts left are sufficient to inhibit the absorption of iron. Indeed, the phytate/iron molar ratio being above the critical cut-off of > 1 found in cooked fonio suggested a poor bioavailability of iron from fonio as eaten. This, added to the issue of low content of iron from a fonio-diet discussed above, suggests that a food-based approach promoting fonio as valuable staple food should also focus on the complete degradation of phytate from fonio diets in order to assure absorption to at least 18% of the content of iron.

Conclusions

The results of this study show no significant difference in iron and zinc content between fonio (*Digitaria exilis*) landraces in Central and Southern Mali. This is supported by the fact that many landraces have the same genetic origin. The minor differences showed by the genetic analysis suggest that agricultural conditions do not influence nutrient composition between landraces. In addition, for sample description, scientific and local names are not sufficient when studying cultivars/landraces such as fonio that can hardly be distinguished from the outside. The above implies that there is no benefit to select fonio landraces based on their iron and zinc content. Processing significantly reduces the concentration of most of the nutrients in fonio. However, the reduction of zinc was lower compared to others nutrients. The slight difference observed in iron levels in fonio paddy might probably be due to soil contamination which is removed by cleaning and washing. Parboiling did not help reduce nutrient loss, especially for iron in processed fonio. A molar ratio of phytate to iron and phytate to zinc of processed fonio (midwet and cooked fonio) predicts poor iron but adequate zinc bioavailability from fonio based diets. Reducing phytate level in fonio could be a sustainable strategy to improve the iron value of fonio.

Acknowledgements

Financial support by the Netherlands Academic Exchange Service (NUFFIC) and the European Union funded project FP6/EU/INCO FONIO project (No. INCO CT-2005-015403) through a PhD scholarship to Yara Koreissi is gratefully acknowledged. We also thank Fien Meijer Dekens and Sjaak van Heusden (Wageningen UR Plant Breeding, Wageningen University, The Netherlands) for the assessment of genetic variability of fonio landraces. We especially thank Mr Kola Mamadou Tangara, Mrs Traore Soungalo Traore and Mrs Camara Adam Fane (Food Technology Laboratory of IER) for helping with fonio processing.

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Chapter five

- Sensory diversity of fonio landraces from West Africa

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Published in the African Journal of Biotechnology 2013,12(15):1836-44

Abstract

The study aims to establish whether there is some sensory variability among West African fonio landraces. Fonio, the oldest indigenous and very tasty cereal growing in West Africa, is usually consumed as a couscous. Group interviews of consumers were conducted in Bamako, Mali to identify the main quality criteria of a cooked fonio grain. After cooking, the grain must be swollen, not sticky with a soft consistency, pale and containing low impurities. Sensory properties of 20 fonio landraces from Mali, Guinea and Burkina Faso were investigated using a descriptive sensory analysis. Five sensory descriptors were chosen among the quality criteria. Each landrace was tasted and scored in triplicate by a group of 18 trained panellists. Principal component analysis and hierarchical cluster analysis were used. The 20 landraces clustered into four sensory classes. Sensory criteria of variability were first visual characteristics (colour and impurities) and then the consistency of cooked grains. Landraces from Guinea and Mali were variable for both visual and textural characteristics; those from Burkina Faso appeared to be more homogenous. The sensory variability of fonio offers to processors, who intend to promote this tiny cereal both in the sub-region and beyond, the possibility to choose adapted landraces to develop new products.

Introduction

Fonio *(Digitaria exilis)*, also called acha or hungry rice, is the oldest indigenous African cereal. West African populations have cultivated fonio across the dry savannas for thousands of years [1]. Nowadays, fonio grows in non-wooded regions from Cape Verde to Lake Chad. Because of its short growing cycle, fonio supplies food to several millions of people during the critical period of shortage [2]. It is grown on approximately 450,000 ha with yields of 450-1400 kg/ha despite the poor soils and low rainfall [3]. The production of fonio declined sharply in the 1960s but began to recover 20 years later [4]. Since then, it has been increasing regularly and represents approximately 500,000 tons per year [3].

Although considered to be a minor species of millet, fonio is described as one of the best tasting West African cereals with high nutritional potentiality due to its particular richness in methionine and cystine [5]. Fonio is usually consumed as a couscous, after a long and tedious process comprising dehusking, milling, washing and three steam cookings. A precooked fonio is obtained after only one steam cooking followed by drying and packaging [6]. Fonio is also prepared using different traditional or non-traditional recipes [7-9]. Knowledge of sensory variability of fonio landraces is needed for processors who develop new products and intend to promote this tiny cereal both in the sub-region and beyond to Diaspora in Europe and United States.

The sensory properties of several cereals have been widely described through quantitative descriptive analyses [10, 11] or through the description of consumer preferences using hedonic tests [12, 13]. However to our knowledge, no studies have been published on sensory analysis of cooked fonio grain comparable to those reporting on cooked rice grain [14-16]. Sensory studies on fonio have mainly concerned bakery products [17-19]. One sensory study has concerned a traditional beverage "kunun zaki" [20]. The ways fonio is consumed have already been studied through several surveys conducted in the capitals of three West African countries (Guinea, Mali and Burkina Faso) [21]. In these surveys, interviewees were asked to prioritise criteria for the purchase of raw fonio grain. However, quality criteria concerning cooked fonio grain were not included. The overall aim of this study was to establish the sensory variability of fonio landraces.

Materials and methods

Materials

Twenty fonio landraces were collected from farmers in villages situated in different agroecological regions in three West African countries: 11 landraces from Mali, five from Guinea and four from Burkina Faso. All the landraces were cultivated and harvested in September 2006.

Preparation of samples

Fonio landraces were processed in the Laboratory of Food Technology, Institute of Rural Economics (IER), Bamako, Mali (**Figure 5.1**). Paddy grain of each landrace was cleaned then milled with a GMBF dehuller, an Engelberg type huller adapted for fonio [22]. It removes the husks and bran in two stages and produces grain of good technological quality [6]. Milled fonio was washed in two steps according to traditional processing which has been standardized in the laboratory. The first step removes any remaining particles of bran and dust, and the second, particles of sand using the principle of density difference. Water was drained and the grain was dried in a Hoheheim type solar dryer until its moisture content did not exceed 10% (wet basis w.b.). The grain was stored at ambient temperature until sensory analysis.

For each sensory evaluation session, fonio grain was cooked using a standardized protocol developed for this study and based on traditional processing: 250 g of dried milled grain was cooked four times for 10 min in a traditional steam cooker containing 1.5 l of water. Before each steam cooking, the grain was rehydrated with successively 150, 180, 100 and 70 ml of water, a total of 500 ml (water/fonio ratio = 2 volume/weight).

Survey to establish quality criteria for cooked fonio

Qualitative surveys were conducted in Bamako, Mali, prior to sensory descriptive analysis in order to improve our perception of consumer preferences and to identify quality criteria for cooked fonio. Five focus groups were organized. Each focus group contained six people recruited from one type of stakeholder: consumers of precooked fonio, consumers of traditionally milled and cooked fonio, cooks in restaurants, large-scale processors and small-scale processors, making a total of 30 stakeholders. Each interviewee was asked to answer the question: “what are the grain characteristics that make fonio good to eat”.

Descriptive sensory analysis of fonio landraces

A group of 18 trained panellists scored the intensity value of 5 descriptors chosen among those mentioned during qualitative interviews. Four visual descriptors (colour, size, cohesiveness, and presence of impurities) and one “mouth-feel” descriptor (consistency) were used (**Table 5.1**). Each landrace was coded using three random digit numbers, then cooked and rapidly served to the panellists. Three sessions were organized per week. In each session, three landraces were tasted one by one by panellists individually.

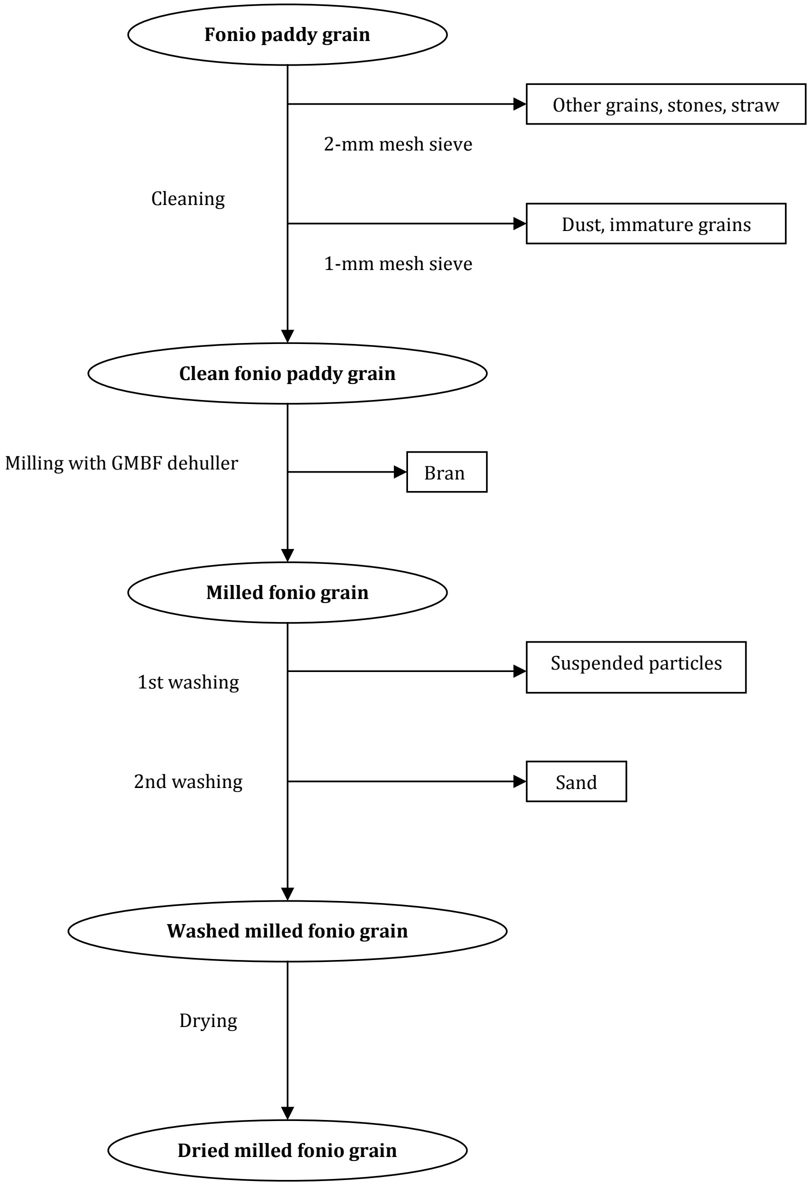

Figure 5.1. *Flow chart of milling, washing and drying of fonio paddy grains*

A glass of water was provided to each of them to rinse their mouth between samples. The 20 fonio landraces were tasted in triplicate. A discontinuous numerical scale from 0 to 10 was used to assess the intensity value of descriptors. Details on the definitions of descriptors, scale of intensity value, order of perception and protocol for scoring each descriptor were fixed (**Table 5.1**).

Table 5.1. Guideline for panellists on how to score cooked fonio landraces

	Descriptor	Definition of descriptor	Protocol
Visual	Grain colour	Colour of cooked grain 10: paler grain colour 0: darker grain colour	Put the white bowl containing a sample of cooked fonio on a dark support (e.g. a black mat). With a spoon, place a small quantity of product on the black mat to judge grain colour more easily if necessary, then give a sensory value (scale from 0 to 10).
Visual	Grain size	Size of cooked grain 10: smaller grain 0: larger grain	Observe the whole sample of fonio in the white bowl, and then if necessary, place a few grains in the spoon for better assessment of grain size.
Visual	Grain cohesiveness	Texture of cooked grain 10: the grains remain separate 0: the grains form a compact mass	Remove some cooked fonio grains with the spoon and let them fall into the bowl. Note if the texture is light with separate grains or, on the contrary, if the grains are sticky and form a compact heavy mass.
In mouth	Grain consistency	Consistency of cooked grain 10: soft 0: firm, stiff	Put a teaspoon of fonio into your mouth. After chewing a few times, estimate the consistency of the product, is it harder rather than softer or the reverse. If necessary, take a small quantity of product between your fingers to confirm the feeling in your mouth and give a sensory mark.
Visual	Presence of impurities	Presence of black foreign seeds and/or fonio paddy grains 8-10: very few impurities 6-8: few impurities 4-6: average number of impurities 2-4: too many impurities 0-2: far too many impurities	With the back of the spoon, spread a sample of fonio over the inside of the white bowl. Observe the product as a whole and estimate the quantity of impurities present (whole or paddy fonio grains and black foreign seeds).

Statistical analysis

All sensory data analyses were performed using XLSTAT Pro system software (version 2009.4.03, Addinsoft™, Paris, France). Two-way variance analysis was used to evaluate the variability among landraces and the performance of the panel. The coefficient of reliability for each descriptor was calculated as the ratio between the mean square of the product (MSp) and the mean square error (MSe). R=1- MSe/MSp [23]. Principal Component Analysis (PCA) was used on the least square mean values across panellists to explore the relationship between descriptors and to explore the variability of the sensory characteristics of fonio landraces [24]. PCA was conducted using a correlation matrix [25].

Hierarchical Cluster Analysis (HCA) was performed to classify fonio landraces. Cluster analysis is a classification method which forms subgroups of similar objects based on assessment of the distance between measured variables [24]. Sample dissimilarities were calculated using the scared Euclidian distance and the Ward hierarchical method was used to form clusters [26].

One way variance analysis was used to determine for which descriptor there were significant differences between classes. If ANOVA detected significant differences, Newman-Keuls mean multiple comparison test was performed to detect significant pairwise differences between landraces per descriptor with a confidence interval of 95%. This test was also used to determine differences between classes [27].

Results and discussion

Quality criteria of cooked fonio

Results of the interviews in five separate groups showed that a good cooked fonio is swollen, not sticky, with a soft consistency, and contains no sand (for 30 stakeholders out of 30, 30/30). The grains must be smooth (28/30), individual (27/30), and not rough (25/30). Besides these textural criteria, some other criteria such as colour and flavour were mentioned. The colour of the cooked grains must be pale (20/30), with a small amount of paddy, herbs and other impurities (26/30). A sweet flavour and an indigenous flavour of cooked fonio are appreciated according to 23 and 10 stakeholders out of 30 respectively, while a dusty and old smell must be avoided in the opinion of nine stakeholders.

The texture of cooked fonio appeared as one of the main quality criteria for Malian consumers. Studies on rice have also underlined that the most discriminatory sensory attributes were appearance and texture characteristics of cooked rice [28, 29].

Relevance of sensory analysis of fonio

Relevance of the five descriptors

The five descriptors were not redundant. Correlations between mean panel scores for each sensory descriptor ranged from -0.5 to 0.7. No correlation close to 1.0 was found, indicating that the five descriptors were not redundant.
The descriptors discriminated the landraces efficiently. Two-way variance analysis of the overall panel data showed a highly significant landrace effect for all the five descriptors.

Reliability and efficiency of the panel

The coefficient of reliability was above 0.80 for all descriptors. It reached 0.99 for colour and impurities. Bianchi et al. (2009) found that a coefficient of reliability above 0.75 indicated good repeatability and discrimination by the panel [23]. The reliability of our panel was thus good for the five descriptors and very good for colour and impurities descriptors.

Variability in sensory characteristics of the 20 fonio landraces

The fonio landraces were described in average as clear (7.4) with relatively small (6.8) and individualized (6.7) grains. Their texture was described as rather soft (6.8) with few impurities (6.9) (**Table 5.2**). We demonstrated significant differences between the 20 landraces. Péazo1, Fungban, Finiba/Kassangara and Dalaman showed darker colour than all the other landraces (score of 4.9, 5.5, 6.0 and 6.3 respectively). The clearest landrace was Fonibagbè (8.8). The differences in grain size were lower and all landraces had rather small grains (score between 6.3 and 7.3). The landraces with smaller grains were Pétama, Tamabè and Tioi, the one with bigger grains was Foniba. All the landraces had a score of cohesiveness showing grains rather individualized after cooking (mean score between 6.0 for Souloukou Mania, the stickier landrace after cooking, and 7.3 for Tamabè, the landrace with better separated cooked grains). Fonibagbè had a softer consistency (8.1) than all of the other landraces. Péazo1 had the firmer cooked grain (5.9). Fonibagbè and Tamatioi grains contained very few impurities (scores of 8.5 and 8.4 respectively) while Péazo1 contained more impurities (4.5). Colour and impurities were the descriptors which better differentiated the 20 fonio landraces. The differences between landraces for the size and cohesiveness were small.

The two first axes (F1 and F2) in the Principal Component Analysis (PCA) of sensory data explained 86% of the variance, the first and second axes accounting for 57 and 29% respectively (**Figure 5.2**). Descriptors contributing mainly to axis 1 were colour, impurities, and size with respectively 31, 27 and 22% of global inertia of the axis. Axis 2 was strongly influenced by cohesiveness which contributed for 60% of variance while consistency contributed for 33%.

A significant and negative correlation between panel mean scores for consistency and cohesiveness (-0.5) may explain the opposite relation between these two sensory characteristics. Most variability was located on the two axes, indicating that sensory data are rather bi-dimensional. Variability between the 20 fonio landraces was based mainly on one physical axis and one textural axis.

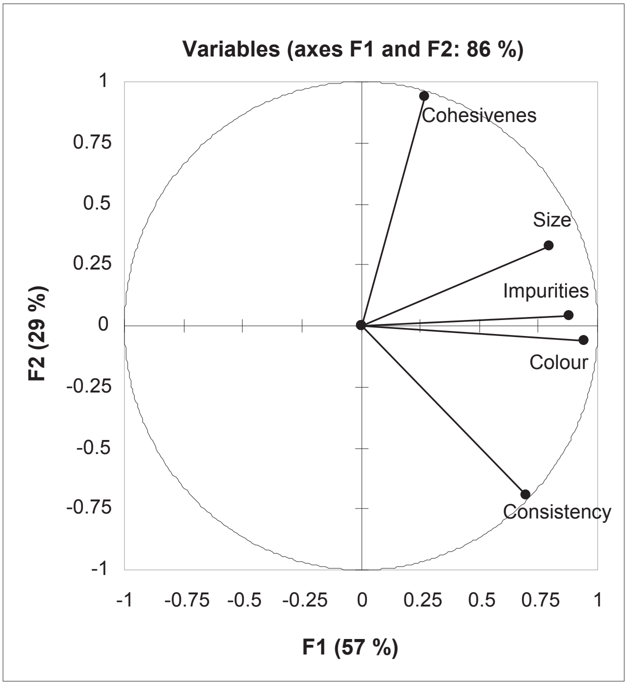

Figure 5.2. *Principal component analysis (PCA) of sensory data: projection of sensory descriptors*

The 20 landraces were distributed over the entire sensory PCA plan (Figure 5. 3) indicating that it is possible to find one fonio landrace with one specific sensory characteristic regarding textural or visual aspect. The right part of the projection plan (F1, F2) grouped all the fonio landraces associated with higher intensity values for the descriptors.

The 20 fonio landraces were clustered in four sensory classes by hierarchical cluster analysis (**Figure 5.4 and Table 5.2**). The classes were relatively homogeneous as shown by the projection of classes on the PCA plan (F1, F2) (**Figure 5.3**). Class 1 was scored lower for visual aspect (larger grains with many impurities and darker colour) and lower for consistency (firmer cooked grains). Class 2 was scored lower for visual aspect and lower for cohesiveness (sticky grains). Class 3 was scored higher for visual aspect (smaller and paler grains containing few impurities) and higher for consistency (soft grains). Class 4 was scored higher for visual aspect and higher for cohesiveness (individualized grains).

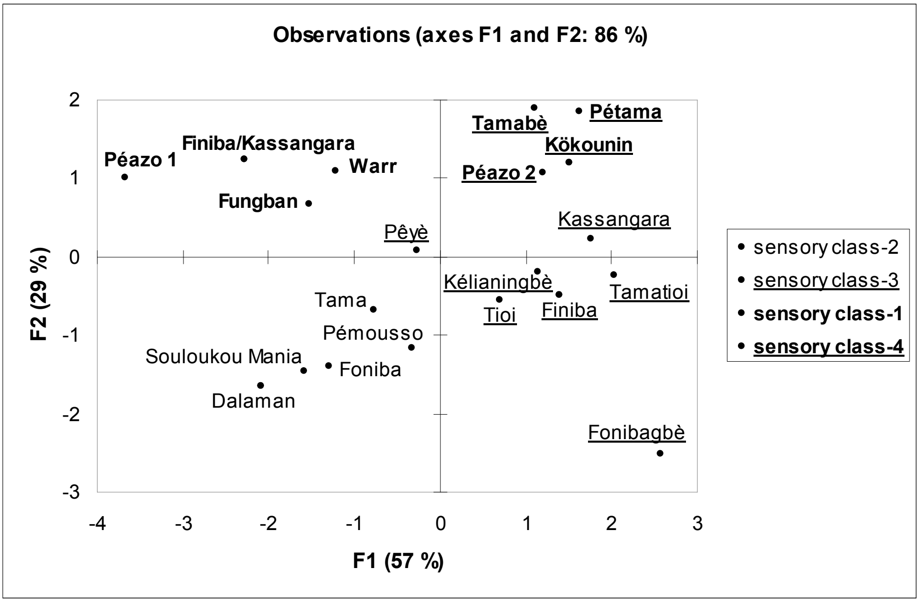

Figure 5.3*. Principal component analysis (PCA) of sensory data: projection of fonio landraces*

Multiple comparison tests showed that the four landraces belonging to class 1 were scored among the lowest for all descriptors except for cohesiveness (**Table 5.3**). Five landraces belonged to class 2 and had bigger and rather stickier grains with more impurities. Seven landraces belonged to class 3 and were among those scored higher for all descriptors, except cohesiveness for which they got a medium score. Four landraces belonged to class 4 and were among those scored higher for all descriptors, except consistency for which they got a medium score. Classes 3 and 4 had smaller and paler grains with fewer impurities. They had softer but less separated grains or the reverse.

No significant differences were observed between the 3 countries. The projection of the countries of origin on the PCA plan (F1, F2) showed that the fonio landraces on the right part of the plan came from Guinea and Mali (**Figure 5.5**). These landraces belonged to class 3 (Kélianingbè and Fonibagbè from Guinea, Tioi, Finiba, Tamatioi, Pêyè and Kassangara from Mali), and to class 4 (Tamabè, Pétama, and Péazo 2 from Mali and Kökounin from Guinea) (**Figure 5.3**).

Table 5.2. Sensory characteristics of 20 fonio landraces from three West African countries

Landrace	Country	Colour	Size	Cohesiveness	Consistency	Impurities	Sensory class
Dalaman	Guinea	6.3 f	6.4 ef	6.1 def	7.0 bcd	5.2 h	2
Finiba	Mali	7.9 c	7.0 abcd	6.6 bcde	7.3 bc	7.8 bc	3
Finiba/Kassangara	Mali	6.0 g	6.5 cdef	6.9 abc	6.1 fg	5.7 g	1
Foniba	Burkina Faso	7.3 d	6.3 f	6.2 def	6.6 cde	6.6 ef	2
Fonibagbè	Guinea	8.8 a	6.9 abcde	6.1 ef	8.1 a	8.5 a	3
Fungban	Burkina Faso	5.5 h	6.6 bcdef	6.8 abcd	6.3 efg	7.3 d	1
Kassangara	Mali	8.1 bc	7.1 abc	6.7 abc	7.1 bcd	8.2 ab	3
Kélianingbè	Guinea	8.3 bc	6.9 abcde	6.6 abcde	7.1 bcd	7.0 de	3
Kökounin	Guinea	8.1 bc	7.1 ab	7.1 ab	6.8 bcde	7.8 bc	4
Péazo1	Mali	4.9 i	6.6 bcdef	6.7 abcde	5.9 g	4.5 i	1
Péazo2	Mali	8.2 bc	6.9 abcdef	7.1 ab	6.7 cde	8.2 ab	4
Pémousso	Burkina Faso	7.4 d	6.5 def	6.4 cdef	7.1 bcd	7.1 de	2
Pétama	Mali	8.6 ab	7.3 a	7.2 ab	6.6 def	7.5 cd	4
Pêyè	Mali	7.3 d	6.8 abcdef	6.7 bcde	6.6 cde	6.7 ef	3
Souloukou Mania	Guinea	6.7 e	6.8 abcdef	6.0 f	6.8 bcde	5.1 h	2
Tama	Mali	7.2 d	6.5 def	6.6 bcde	7.1 bcd	6.3 f	2
Tamabè	Mali	8.3 bc	7.2 a	7.3 a	6.6 def	6.8 ef	4
Tamatioi	Mali	8.3 bc	7.0 abcde	6.8 abc	7.4 b	8.4 a	3
Tioi	Mali	7.9 c	7.2 ab	6.4 cdef	7.1 bcd	6.3 f	3
Warr	Burkina Faso	7.0 de	6.5 def	7.0 abc	6.3 efg	6.6 ef	1
Mean value		7.4	6.8	6.7	6.8	6.9	
Landrace effect		*	*	*	*	*	

[a, b, c, d, e, f] *In a column, means with the same letter were not significantly different at $p<0.05$ level based on Newman-Keuls test.*

** Significant at $p>0.05$ level in a two way variance analysis.*

Table 5.3. Multiple pair-wise comparisons between landraces in sensory classes for each descriptor

Descriptor	Class 1	Class 2	Class 3	Class 4
Colour	5.8 c	7.0 b	8.1 a	8.3 a
Size	6.6 b	6.5 b	7.0 a	7.1 a
Cohesiveness	6.8 b	6.3 c	6.6 b	7.2 a
Consistency	6.2 c	7.0 ab	7.3 a	6.7 b
Impureties	6.0 b	6.1 b	7.6 a	7.6 a

In a line, means with the same letter (a or b or c) were not significantly different at p<0.05 based on Newman-Keuls test.

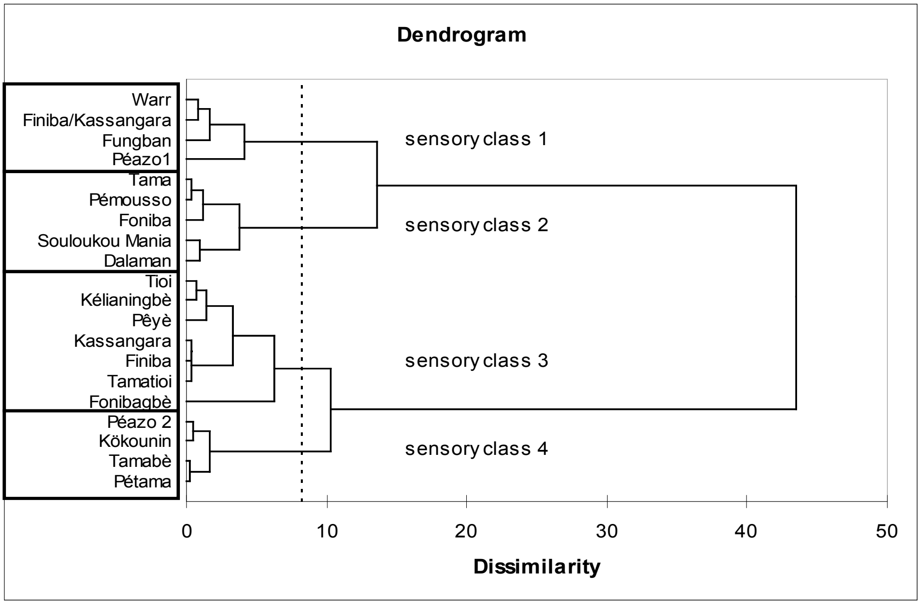

Figure 5.4. *Hierarchical clustering of the 20 fonio landraces*

Fonibagbè differ from others mainly because of the soft consistency of the cooked grain. Pêyè had average scores for all descriptors. Landraces from Guinea and Mali scored higher or lower and scattered over the sensory plan. All landraces from Burkina scored lower for visual characteristics and grouped in the left part of the PCA plan. They appeared more homogeneous compared to that from Mali and Guinea. Our results on the sensory variability of fonio landraces from Mali, Guinea and Burkina Faso showed some similarity with results of other studies on genetic diversity of West Africa fonio landraces [30]. In these studies, 118 accessions of *Digitaria exilis* from five countries (Benin, Burkina Faso, Guinea, Mali and Togo) were analyzed by Amplified Fragment Length Polymorphism (AFLP). They found that all fonio accessions of the same geographic origin clustered in one and the same group, except for the Guinean and Malian accessions, which split into two different groups. These results could explain the differences of variability encountered on sensory properties of Burkinabe landraces compared to that of Malian and Guinean ones. However, the number of landraces we used to establish a sensory analysis of fonio was intentionally limited and not representative of each country.

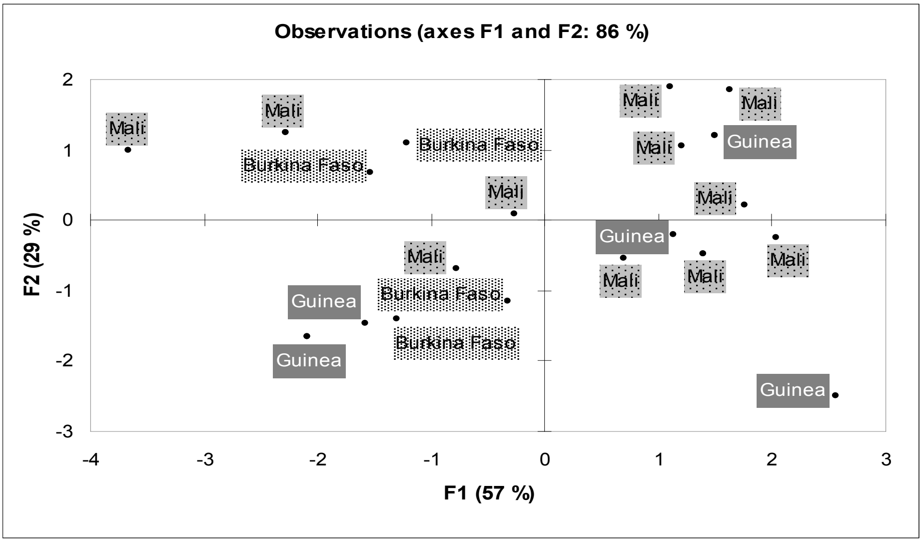

Figure 5.5. *Principal component analysis (PCA) of sensory data: projection of countries of origin of fonio landraces*

Further investigation on landraces collected from farmers in the entire growing area of eachcountry would be necessary to conclude on the effect of geographic origin on the sensory properties of fonio. Other factors of variability should be studied such as farming methods or post-harvest treatment, as already demonstrated in rice grain [14, 15].

Conclusions

Variability of sensory characteristics of fonio landraces was demonstrated. The first sensory criteria of variability of cooked fonio grain were the visual characteristics (mainly colour and impurities). They appeared as important criteria of selection of fonio grain to develop new products. The second criterion of sensory variability was a textural characteristic, the consistency of cooked grain.

Precooked fonio should be developed from a landrace selected for its whiter and softer grains after cooking. The visual and textural variability of fonio offers to processors the possibility to choose landraces adapted to their new processes.

The variability in sensory characteristics of fonio landraces in relation to the country of origin should be confirmed. Some link between sensory variability and genetic diversity seemed possible and should be investigated. The relationship between sensory characteristics of cooked fonio grain and its chemical and physical characteristics needs to be investigated.

Acknowledgement

The research activities presented in this paper were funded by the European INCO-DEV-2 FONIO project "Upgrading quality and competitiveness of fonio for improved livelihoods in West Africa" within the sixth framework programme FP6 of the European Commission (2006-2009). We are grateful to Sandrine Dury, UMR MOISA CIRAD, for her assistance in implementing the group interviews in Bamako, and Christian Mestres, UMR Qualisud CIRAD, for his constructive criticism of the manuscript. We would like also to thank Sandy Blancher, student at CIRAD (2006-2007), for her active participation in the group interviews.

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Chapter six

- Dephytinisation with intrinsic wheat phytase and iron fortification significantly increase iron absorption from fonio (*Digitaria exilis*) meals in West African women

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Published in PLoS ONE 2013.8(10):e70613. Doi 10.1371/journal.pone.0070613

Abstract

Low iron and high phytic acid content make fonio based meals a poor source of bioavailable iron. Phytic acid degradation in fonio porridge using whole grain cereals as phytase source and effect on iron bioavailability when added to iron fortified fonio meals were investigated. Grains, nuts and seeds collected in Mali markets were screened for phytic acid and phytase activity. We performed an iron absorption study in Beninese women (n=16), using non-dephytinised fonio porridge (FFP) and dephytinised fonio porridge (FWFP; 75% fonio-25% wheat), each fortified with ^{57}Fe or ^{58}Fe labeled $FeSO_4$. Iron absorption was quantified by measuring the erythrocyte incorporation of stable iron isotopes. Phytic acid varied from 0.39 (Bambara nut) to 4.26 g /100 g DM (pumpkin seed), with oilseeds values higher than grains and nuts. Phytase activity ranged from 0.17±1.61 (fonio) to 2.9 ±1.3 phytase unit (PU) per g (whole wheat). Phytic acid was almost completely degraded in FWFP after 60 min of incubation (pH≈5.0, 50ºC). Phytate: iron molar ratios decreased from 23.7:1 in FFP to 2.7:1 in FWFP. Iron fortification further reduced phytate: iron molar ratio to 1.9:1 in FFP and 0.3:1 in FWFP, respectively. Geometric mean (95% CI) iron absorption significantly increased from 2.6% (0.8-7.8) in FFP to 8.3% (3.8-17.9) in FWFP ($P < 0.0001$). Dephytinisation of fonio porridge with intrinsic wheat phytase increased fractional iron absorption 3.2 times, suggesting it could be a possible strategy to decrease PA in cereal-based porridges.

Introduction

Iron deficiency anemia is a prevalent micronutrient deficiency in Africa. Low iron intake and low bioavailability contribute to its high prevalence [1]. Fonio (*Digitaria exilis*) is an indigenous West African cereal, contributing to household food security, due to the early maturing growth of some varieties [2]. In semi-arid areas, fonio represents a major part of the diet. In sub-humid zones, fonio is stored for long periods, to be used as staple food during a food shortage season [3]. In consumption areas, fonio is mainly consumed as couscous or as porridge made from grain or flour [4]. In urban areas in Mali, fonio has been estimated to be consumed at least once per week by one-third of the women [5].

Iron concentration in processed fonio products is low, being 0.8 to 1.8 mg/100g on dry weight basis [6]. In addition, as most plant-based foods, fonio grains contain phytic acid (myo-inositol 1, 2, 3, 4, 5, 6 hexakis [dihydrogen phosphate]) which forms complexes with iron, thus reducing its bioavailability [7, 8]. Both iron content and bioavailability in fonio-based meals would need to be increased to contribute meaningfully to the iron requirements. Iron fortification of staple foods has been recommended as a strategy to increase the content of available iron in the foods [9]. Ferrous (Fe^{II}) sulfate has been the standard iron compound used in iron absorption studies and the bioavailability of other iron compounds are expressed relatively to its bioavailability [10]. Because iron absorption is strongly inhibited by phytic acid (PA) in cereals-based foods, reducing PA levels appears to be essential for improved iron absorption from iron-fortified plant-based diets [11].

Phytic acid can be degraded by adding microbial phytases or by enhancing the activity of intrinsic phytase present in a large range of plant-derived foods [12, 13]. Addition of microbial phytase immediately before consumption significantly affects the iron bioavailability from an inhibitory test meal as the pure phytase enzyme degrades PA during digestion before it is degraded by peptic enzymes [14]. High apparent phytase activity (PAC) was found in untreated whole grain rye, wheat, triticale, buckwheat and barley [13]. Previous studies have shown that adding wheat to cereal or legume-based complementary food mixtures completely degraded phytate at optimal incubation conditions [15]. However, when using grains as phytase source, an incubation step is likely to be needed as the permanence time of the free phytase in wheat may be too short in the gastro-intestinal tract to meaningfully degrade phytic acid [16, 17]. This study aimed at assessing the effect of PA degradation in fonio through a food-based approach and to quantify its effect on iron bioavailability in humans. We first investigated PA content and PAC of commonly used grains, seeds and nuts. Secondly, we characterised PA degradation in fonio-based porridges using whole grain wheat flour as native phytase source. Thirdly, we measured the effect of phytic acid degradation on iron bioavailability in iron-fortified fonio porridge in human subjects.

Methods

Three sets of experiments were conducted: 1) analysis of PA content and PAC of grains, seeds and nuts commonly consumed in Mali, 2) PA degradation in fonio porridge using whole wheat flour, and 3) measuring the effect of PA degradation on iron bioavailability from iron-fortified fonio porridge in young women. The experiments were carried out in Mali and Benin, two West African countries. The iron absorption study was registered at http://www.clinicaltrials.gov; ID: NCT01443832

Grains, Nuts and Seeds

An amount of 500g of grains, seeds or nuts was purchased in March 2008, each from three different commercial suppliers located at the three main local markets in Bamako (Mali). Fonio paddy grain (*Tamatioi* landrace) was bought from farmers in Tominian village, Ségou, Mali. All grains, seeds or nuts were of whole grain quality without any thermal or chemical treatment, and were dry cleaned to remove impurities, dusts, sands and immature grains.

At the laboratory of food technology (LTA) of the Institute of Rural Economy of Mali (IER), all samples were pooled per grain, nut or seed respectively and homogenously mixed. A sample of 100g of the pooled Bambara nut, baobab seeds, hibiscus seeds, pumpkin seeds, African locust bean seeds and whole wheat grain was washed with distilled water, dried overnight at room temperature (30ºC) under ventilation and packed in a sealed polyethylene plastic bag. Fonio paddy grains were dehulled to have the whole grain fonio, and washed using the standardized traditional method described elsewhere [6]. All samples were stored at -18°C until shipment by courier express to ETH Zurich for analyses. Prior to analysis all grains, nuts or seeds (untreated) were frozen in liquid nitrogen and milled with a centrifugal mill (0.5 mm mesh; Retsch ZM1, Retsch GmbH, Haan, Germany). The dry matter (DM) content was determined gravimetrically after drying about 1 g milled grains, nuts or seeds for 24 h with forced-air drying oven at 105 ± 3°C.

Phytase Activity

About 1 g of milled grains, nuts or seeds was added to 20 mL buffer (pH 5.0: 0.2 M acetate buffer; pH 8.0: 0.2 M Tris buffer) containing 7.5 mM PA prepared from PA dodecasodium salt (Sigma- Aldrich Chemie GmbH, Steinheim, Germany). The measurement was performed for 1 h at 45°C under constant stirring with aliquots taken every 20 min. The reaction was terminated by adding 0.5 mL 0.9 M trichloroacetic acid to each 0.5 mL aliquot. The determination of PAC was based on the measurement of liberated inorganic phosphate (IP) from PA in a certain time interval. IP was determined according to the van Veldhoven's and Mannaerts method [18] at 4 time points (0, 20, 40, 60 min). IP was liberated at a constant rate and apparent PAC was calculated by linear regression of the IP determined for each time point. Apparent phytase activity is expressed in phytase units (PU) per g DM of grains, nuts or seeds. One PU is equivalent to the enzymatic activity that liberates 1 μmol inorganic phosphate per min.

Phytic Acid

Phytic acid was determined according to a modified method of Makower [19] consisting of an extraction and a selective precipitation of phytate in which cerium replaced iron in the precipitation step according to Hurrell et al. [16]. The inorganic phosphate liberated from the phytate digestion was measured according to the van Veldhoven's and Mannaerts method [18]. The difference of duplicate sample relative to the mean value was <10%. PA content in mg /100 g DM refers to 100 g freeze-dried porridge sample.

Phytic Acid Degradation in Fonio Porridge

The conditions for PA degradation were optimized for the grains with the highest PAC which was wheat. Degradation of PA was tested with fonio porridge by adding 25% of whole wheat flour as previously demonstrated in complementary foods [15].

Porridge Preparation

A standard fonio porridge recipe was developed at LTA Mali based on the traditional recipe collected during a short food consumption survey performed among a sample of households selected in rural areas in Mali. The standardized traditional recipe was adapted to a previously developed process [15]. For the experiment, 50 g of flour in the proportion of 75% whole fonio flour (37.5 g) and 25% whole wheat flour (12.5 g) was used. To prepare the porridge, fonio flour was mixed into 200 mL of water; the mixture was added to 800 mL of boiling water and cooked for 22 minutes under constant stirring. The porridge was removed from the heat source and the measured pH was around 6. The pH was adjusted to ≈5.0 (optimal condition for PAC) with 1 M citric acid (1.4 mL). No other ingredients were added. The water used for the experiment was purified by reverse osmosis (Nanopure Cartridge System, Skan AG, Basel, Switzerland).

Phytic Acid Degradation

The fonio flour porridge prepared as described above was left at room temperature for cooling down to ~50°C (optimal incubation temperature for PAC). Whole wheat flour was added to the porridge, which was mixed with a blender for about 5 min. The temperature of the mixed porridge reduced to 47 - 48°C after mixing, so the porridge was heated again to reach an incubation temperature of 50°C. Six aliquots of 10 g were weighed into 100 mL covered polyethylene containers. One aliquot was quickly cooled on ice to restrain wheat intrinsic enzymatic activity, and frozen immediately at -18ºC corresponding to time zero. The other five aliquots were put into incubator at 50°C under constant stirring (600 rpm, MEMMERT incubator shaker series, MEMMERT GmbH Co.KG, Schwabach FRG, Germany). Thirty minutes after incubation, and then every 60 min, one aliquot was withdrawn from the incubator, quickly cooled on ice to restrain enzymatic activity, and immediately frozen at -18ºC. Temperature and pH were monitored continuously. All samples were kept at -18°C until transport to Wageningen University, Division of Human Nutrition, for PA content analysis as described above.

Iron Absorption

The iron absorption study was carried out at the Department of Nutrition and Food Sciences, University of Abomey-Calavi in Benin.

Subjects

A group of 16 apparently healthy young Beninese women aged 18 - 30 years (confirmed with birth certificate or other official documents) were recruited between September 2010 and January 2011, from neighborhood communities. Inclusion criteria were: *i*) body weight < 65 kg (measured according to standard procedures [20]), *ii*) no pregnancy (confirmed by rapid pregnancy test using Nancy HCG kit, 3H Medical Products, China) and no breastfeeding, *iii*) no reported chronic medical illnesses, *iv*) no reported symptoms of malaria in the last two months (fever, headache, stomach ache, diarrhoea, nausea, vomiting), *v*) no recent malaria (negative blood smear response based on Giemsa stained microscopy following standard guidelines [21]), *vi*) no intake of vitamin and mineral supplements two weeks preceding the study, *vii*) no blood donation in the last six months, *vii*) no iron medication or supplementation two weeks before and during the study, *ix*) no reported allergy to gluten. The subjects represented a population who potentially would use fonio based meals for a certain time period of the year (2-3 months before the harvesting of other cereals), and who are expected to be mildly or moderately iron deficient/anemic.

A sample size of 16 subjects was estimated to be adequate to detect an intra-individual variation in log (iron absorption) of 0.35 [22] and a 30% increase in iron bioavailability [16] with 95% power and a significance level of 5%, accounting for a conservative drop out of 2 participants. Before enrolment, study participants received a full explanation of the study in written form and orally during group discussion sessions. Written informed consent was obtained from all subjects before starting the study. The study protocol was approved by the National Provisional Ethical Committee for Public Health Research in Benin (Comité National Provisoire d’Ethique pour la

Recherche en Santé Publique, CNPERS) and by the Medical Ethical Committee of the University of Wageningen (Medisch Ethische Toetsings Commissie, METC-WU).

Study Design

Using a crossover design, subjects were given two iron-fortified fonio porridges labeled with ^{57}Fe or ^{58}Fe, on two consecutive days (**Figure 6.1**). Two weeks before the test, participants were dewormed with anthelminth (Zentel, 400 mg Albendazole tablets, laboratoire Glaxosmithkline, France).

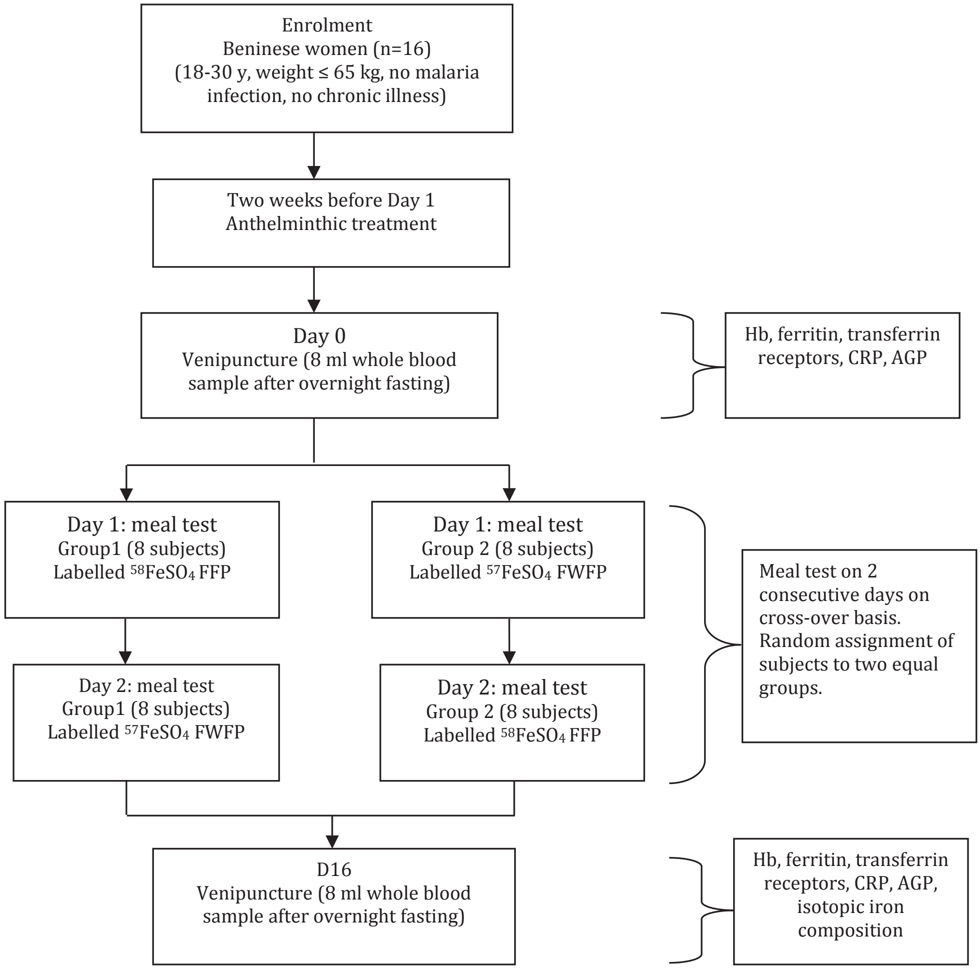

Figure 6.1. *Experimental design of iron absorption test with fonio porridge using stable iron isotope. FFP, Fonio flour porridge; FWFP, fonio + wheat flour porridge; 57FeSO4 and 58 FeSO4, ferrous sulfate isotope 57 and 58.*

On day 0, participants were invited to the study location, and weight and height were measured [20]. On day 1 and day 2, the two test meals were randomly received by the participants. On d 0 and fourteen days later, on d 16, a venous blood sample (8 mL) was collected in a K_2EDTA tubes for Hemoglobin (Hb) concentration measurement, and serum tubes between 7:00 and 8:00 a.m. after overnight fasting (no food after 8:00 p.m. and no drink after 12:00 a.m. on the evening before day 1). During the test period, to ensure that participants were fasted, the evenings before days 1, 2, and 16, participants were lodged in a centre in the neighbourhood of University of Abomey Calavi, and dinner was served from 6:30 to 7:45 p.m. The test meals were served to participants from 7:00 to 9:00 a.m. and consumed under standardized conditions and close supervision. No food and drink was allowed to the participants within 3 hours after the meal was consumed. After this period each participant received a breakfast package which she was allowed to consume ad libitum.

Stable Isotope Labels Preparation and Test Meals

The isotopically enriched $^{57}FeSO_4$ and $^{58}FeSO_4$ were prepared from isotopically enriched ^{57}Fe and ^{58}Fe (enrichment 97.5 and 99.6% respectively, Chemigas Boulogne, France) respectively, by dissolution in diluted sulphuric acid (0.1 mol H_2SO_4/L). The test meals consisted of two fonio porridges of 240 g: single fonio flour porridge (FFP) and mixed fonio-wheat porridge (FWFP, ratio 3:1, weight-to-weight). Fonio and wheat flours were made from whole grains. The porridges were prepared in bulk at the Division of Human Nutrition of Wageningen University, following the recipe described above. Each bulk of porridge (FFP and FWFP) was divided into 16 portions of 240 g. FFP portions were immediately cooled on ice. FWFP portions were placed in incubator for 3h at 50°C under constant stirring at 115 rpm (Innova 44, incubator shaker series, New Brunswick Scientific Co., Inc, Eppendorf Company, USA) after which they were immediately cooled on ice. The porridge portions were stored at -18°C and sent on dry ice by express courier to Benin one week before the study. Each evening before test day 1 and day 2, 16 portions were thawed overnight in a fridge at 4 to 5°C. On the test days, thawed portions were quickly warmed up for 1 minute in a microwave oven (400-1200 W) and 4 mg of iron isotope solutions were added quantitatively about 5 minutes before consumption. Isotope labels were carefully spread on the surface of the test meals. After consuming the entire meal, the participants consumed 50 mL of drinking water used to rinse the bowl.

Phytate and Iron Content of the Test Meals

Iron concentration in the test meals was analysed using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), after microwave digestion with hydrofluoric acid (40%), HNO_3 (65%) and H_2O_2 (30%). Phytate content was determined as described above [18,19]. Phytate-to-iron molar ratio was estimated as [mg phytate/molecular weight of phytate] / [mg Fe/ Fe molecular weight].

Blood Analysis and Iron Isotope Measurements

Hemoglobin (Hb) concentration in whole blood was measured immediately after blood collection, using an automated Sysmex counter (KX 21-N, Sysmex, cyanide-free reagent used for Hb detection, normal values range 11.50-15 g/dL). The 3-level Eightcheck-3WP control material provided by the manufacturer was used for quality check. Overall anemia was defined as Hb < 12 g/dL, moderate anemia as Hb < 10 g/dL and severe anemia as Hb < 9.0 g/dL [23]. Serum ferritin (SF), soluble transferrin receptors (sTfR), C-reactive protein (CRP) and α_1-acid glycoprotein (AGP) were measured simultaneously using an in-house sandwich Enzyme-linked immunosorbent assay (ELISA) technique [24]. All measurements were done in duplicates and measurements showing CVs ≥10% were repeated. The CVs (inter-essay) for the various indicators were: SF, 2.6%; sTfR, 2.4%; CRP, 7.2% and AGP, 2.9%. Certified quality control samples from the CDC/Atlanta and BioRad Liquicheck (BioRad, Munich, Germany) were used. Iron deficiency was defined as SF <12 µg/L or sTfR > 8.5 mg/L, and iron deficiency anemia as Hb < 12 g/dL and SF <12 µg/L. CRP > 10 mg/L indicated ongoing inflammation. AGP values > 1 g/L were used to identify existing inflammation.

Isotopic Composition in Blood Samples

Whole blood samples were mineralised and separated as described by Schoenberg and von Blanckenburg [25]. Iron isotopic analyses were performed using a high-resolution multicollector-inductively coupled plasma-mass spectrometer (Thermo-Fisher Neptune, University of Bonn, Germany) [26]. Copper was added (1ppm Alfa-Aesar Specpure) to the solution immediately prior to analysis to correct for mass bias [25,27]. Each isotopically enriched solution was measured in triplicate using the standard-sample bracketing technique [25,27,28]. One third of the samples were re-measured as external duplicates. Blank solutions and ^{57}Fe or ^{58}Fe indicator solutions were used as an external quality control during the measurements.

Calculation of Fe Absorption

Calculation of iron absorption was based on the shift in the isotopic ratios after a 14-d incorporation period as described by Walczyk et al. [29]. Circulating iron was calculated on the basis of the blood volume, which was estimated from the participant's height and weight [30]. Isotopic ratios were calculated according to the principle of isotope dilution [29] and taking into account that isotopic labels are not monoisotopic [31]. An incorporation rate of 80% of the absorbed iron into red blood cells was assumed for our group of young women [32].

Statistical Analysis

Data analysis was performed in Excel (Microsoft Office 2007, Microsoft, Seattle, USA), GraphPad Prism version 6.01 for Windows (GraphPad Software, San Diego, CA, USA) and PASW software (version 18.0; IBM SPSS, Chicago). *P* values < 0.05 were considered significant. Visual check and Shapiro-Wilk test were used to check for normality of the distribution. Normally distributed data were expressed as mean ± SD, non-normally distributed as median (interquartile range) and as geometric mean ± SD. Fractional iron absorption distribution was log-transformed to normality. Log-transformed fractional iron absorption from FFP and FWFP meals were compared using paired *t* tests. Pearson's correlation was used to test for association between (log) SF and log fractional iron absorption from different meals.

Results

Apparent Phytase Activity and Phytic Acid in Grains, Nuts and Seeds

Apparent PAC ranged from 0.2 PU/g DM for fonio to 2.9 PU/g DM for whole wheat (**Table 6.1**). The optimal conditions for PAC of grains, nuts and seeds were 50°C and pH ≈ 5. Whole wheat grain (cereal) showed the highest PAC compared to the legume (Bambara nut) and oilseeds (Hibiscus, Baobab, African locust bean and pumpkin seeds), having PAC lower than 1 PU/g DM. Phytic acid in all the untreated grains, nuts and seeds ranged from 0.4 g/100g DM in Bambara nut to 4.3 g/100g DM in pumpkin seed, with higher levels in oilseeds (except African locust bean) compared to cereals and legumes (**Table 6.1**). The PA content in cereal grains was in the same range while apparent differences were observed among the oilseeds.

Phytic Acid Degradation in Fonio Porridge

Figure 6.2 shows PA degradation in porridge with the addition of 25% of whole grain wheat. Phytic acid decreased from 87.5 g/100g DM at 0 min of incubation to 27.5 g/100g DM at 30 min of incubation, corresponding to 30% of reduction. After 1, 2 and 4 hours of incubation, PA concentration was 0.18 g, 0.12 g and 0.11 g, respectively, indicating that removal of PA was almost complete after 1 hour of incubation (50°C, pH ≈5).

Table 61. Apparent phytase activity and phytic acid content of untreated grains, nuts and seeds

Grains, nuts and seeds common name	Botanical name	Phytase activity[1] (PU[2]/g dry matter)[3]	Phytic acid (g/100 g dry matter)[4]
Cereals			
Fonio	*Digitaria exilis*	0.17±1.61	0.89
Wheat	*Triticum aestivum*	2.88±1.27	0.85
Legumes			
Bambara nut	*Vigna subterranea*	0.65±7.25	0.39
Oilseeds			
African locust bean	*Parkia biglobosa*	0.54±0.95	0.46
Baobab	*Adansonia digitata*	0.28±3.46	1.80
Hibiscus	*Hibiscus sabdariffa*	0.86±7.13	1.16
Pumpkin	*Cucurbita pepo*	0.21±5.08	4.26

[1] *Measured at pH 5.0, 45°C*
[2] *1 phytase unit (PU) is equivalent to the enzymatic activity that liberates 1µmol inorganic phosphate per min under specified conditions.*
[3] *Values represent mean ± SD of triplicate analysis in dry products.*
[4]*Values are mean of duplicate analysis in wet products*

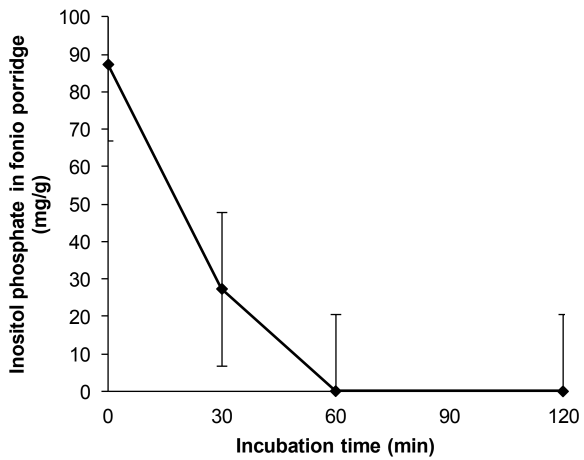

Figure 6.2. *Phytic acid (IP6) content in fonio (75%) - wheat (25%) flour porridge at different incubation time (0 to 240 min)*

Participants in Iron Absorption Study

Sixteen participants were recruited, but one did not complete the test because suffering from malaria on day 16. Participants were aged 24 years-old on average, with a mean (±SD) body mass index (BMI) of 21.2±3.3. Mean (±SD) hemoglobin and body iron stores concentration was 11.9±1.4 g/L and 3.5±4.9 mg/kg, respectively. Median

(25^{th} - 75^{th}) ferritin and transferrin receptor was 22.9 (12.7 – 74.8) µg/L and 6.0 (5.2 - 7.6) mg/L, respectively. Seven subjects had moderate anaemia. Three subjects had iron deficiency anemia and one subject was iron deficient but not anemic. Mean (±SD) CRP and AGP concentration was 3.4±7.8 mg/L and 0.8±0.2 mg/L, respectively. The CRP and AGP concentration was elevated for two (14.7 and 28.2 mg/L) and three participants (1.05, 1.04 and 1.06 g/L) respectively.

Test Meals

Iron concentration in the portion (240 g) of fonio flour porridge and fonio-wheat flour porridge was respectively 0.34 mg and 0.51 mg. Mean (±SD) phytic acid concentration in FFP and FWFP was respectively 96.0 ±16.2 mg and 16.2 ±4.4 mg. The phytate:iron molar ratio decreased from 23.7: 1 in FFP to 2.7:1 in FWFP. The non dephytinised fonio porridge meal with iron fortification showed a phytate:iron ratio of 1.9:1, which decreased to 0.3:1 in FWFP meal (**Table 6.2**).

Table 6.2. Phytic acid, phytate:iron molar ratio and iron absorption from high phytic acid (without wheat) and low phytic acid (with wheat) iron-fortified fonio porridges

Fonio porridges	**Phytic acid content in portion (mg, wet weight basis)**	**Phytate-to-iron molar ratio of fonio prorridges**	**Phytate-to-iron molar ratio of iron fortified fonio prorridges**	**Iron absorption (%)[1]**
Without wheat	96±16.2	23.7:1	1.9:1	2.6 (0.8, 7.8)[2]
With wheat	16.2±4.4	2.7:1	0.3:1	8.3 (3.8, 17.9)[2]

[1] *Geometric mean (-SD, +SD)*
[2] *Values are significantly different (P < 0.0001)*

Iron Absorption

Geometric mean (95% CI) iron absorption from FFP and FWFP meal was 2.6 % (0.8-7.8) and 8.3% (3.8-17.9) respectively (**Figure 6.3**). Fractional iron absorption from FWFP was 3.2 times higher compared to FFP (paired *t* test, $P < 0.0001$).

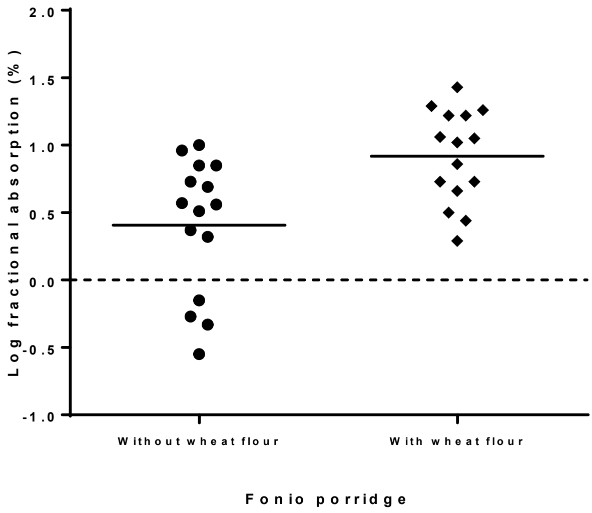

Figure 6.3. *Comparison of (log) fractional iron absorption between non-dephytinised (without wheat flour, FFP) and dephytinised (with wheat flour, FWFP) fonio porridge (n=15).*
Black lines show mean log fractional iron absorption from each meal. Geometric mean (95% CI) of iron absorption ratio from FFP and FWFP is 2.6 (0.8 – 7.8) and 8.3 (3.8 – 17.9) respectively. Log fractional iron absorption between meals significantly different (paired t test, P < 0.0001).

Discussion

Phytic Acid Content and Apparent Phytase Activity of Grains, Nuts and Seeds

Phytic acid was found at different concentrations in all analyzed grains, nuts and seeds. Previous studies also showed PA in grains and legumes to vary depending on the type of plant-based food, growing conditions, harvesting techniques, processing methods, analytical methods and the harvesting year [33, 34]. The PA concentration of 0.85 and 0.89 g/100g DM reported on cereals (wheat and fonio) in our study is slightly lower compared to 0.98 on whole wheat [35] and 1.03 reported in untreated wheat grain and in the range of 0.79 to 1.63 g/100g DM reported for cereals grains [13]. Our results confirm previous studies showing high PA in oilseeds (1.00 to 2.22 g/100g DM) compared to legumes (0.48 to 1.40 g/100g DM) [13, 35], although we report a high value of 4.26 g/100g DM in pumpkin seeds. The 0.46 g/100g DM of African locust bean and 1.80 g/100g DM of baobab seed reported in our study were higher than the 163 and 6.6 mg/100g DM respectively reported earlier [36,37]. These differences could be due to differences in the analytical methods used for PA determination as there is no international agreed upon standardized method for its determination. Our PA analytical method is best suited to quantify IP6 and IP5. Other inositol phosphates like IP4, IP3 and IP2 were not detected, however they are considered to have less negative influence on iron bioavailability as they do not form strong complexes with minerals and trace elements [38, 39].

Apparent PAC was very low in our untreated seeds and nuts compared to grains, especially wheat. These results confirm previous studies showing high PAC (1.8 to 6.9 PU/g DM) in some cereals such as rye, triticale, wheat and barley and PAC comparable to fonio in others such as maize, millet, oat, rice, sweet maize, and sorghum (0.1 to 0.4 PU/g DM) (13, 40). The 2.9 PU/ g DM of wheat reported in our study is similar to 3.1 PU/ g DM reported earlier [13]. The PAC in our grains, seeds and nuts was relatively low (except for wheat grain) maybe because our samples were not soaked, germinated or fermented before being analysed. These traditional food processing methods are known to activate phytase in grains, legumes and seeds, and therefore decrease PA content in plant foods [40, 41]. The high PAC of whole wheat grain could provide sufficient enzymatic activity to degrade PA in mixtures of cereals or legumes [13]. Phytases from different sources are likely to differ in sensitivity to substrate inhibition and temperature and pH optima [13]. In most studies, phytases are isolated from grains, nuts and seeds and sometimes purified before determining the enzymatic activity [42, 43].

In our study, no relationship was observed between PAC and PA content of the untreated grains, nuts and seeds, indicating that the high PAC of cereals, such as wheat, is not associated with high PA content. This was already reported in a study on rye and wheat [13]. Our results confirm that whole wheat flour could be used as a source of natural phytase to produce low PA containing fonio porridge, and suggest its use in other more micronutrient rich legume and cereal mixtures.

Phytic Acid Degradation

The degradation of PA in fonio porridge by using 25% whole grain wheat flour as a natural phytase source was almost complete after 1 hour of incubation at 50ºC with pH of 5.0. This result is in agreement with a previous study showing that adding whole wheat grain to cereal-based complementary foods almost completely degraded PA in a relatively short time [15]. Deviations from the temperature and pH resulted in much less effective PA degradation (data not shown). Most phytases have an optimal pH in the range of 4.5-6.0 and a temperature range of 45-60 °C. Outside the optimal range of pH and temperatures the action of phytase is reduced [44].

Application of the method for PA degradation in cereal/legume based weaning foods using phytase naturally occurring in whole wheat grain flour would include the adaptation to household level or to small scale industries producing cost effective weaning foods, especially in developing countries where commercial weaning foods are not affordable or not available, and infants are often fed porridges based specifically on cereals or mixed with legumes containing high amounts of PA. Wheat is readily available worldwide and could be added to porridges as phytase source. After cooking the porridge for example, 25% of whole grain wheat flour could be added, the pH adjusted to ≈5 by adding acidic fruits juices (as usually done at household level) and kept warm during two hours. The porridge would likely require an additional heating step before consumption to infants, or the maintenance of strict hygiene practices. At household level, the addition of wheat would not substantially change the taste of the porridges, indicating that acceptability would not be a major problem. However, the optimal conditions for complete phytic acid degradation in cereal/legume mixtures and the microbiological quality of the products would need to be further investigated in field studies.

Effect of Phytic Acid Degradation on Iron Bioavailability in Iron-Fortified Fonio Porridge

Dephytinisation with intrinsic wheat phytase reduced phytate-to-iron molar ratio from 24:1 to 3:1, while iron fortification decreased the molar ratio to 0.3:1, and dephytinisation plus fortification increased iron absorption from 2.6% to 8.3% in fonio porridge.

Inhibition of iron absorption by phytic acid in plant-based meals is dose-dependent at very low concentrations of 2-10 mg per meal [9]. Preferably, the phytate:iron molar ratio should be lower than 0.4:1 to achieve meaningful iron absorption when no enhancers of iron absorption are added to the meal [45]. The ratios of 23.7:1 reported in this study was higher than the range of 12-21:1 previously estimated for sorghum flour porridges consumed in Benin [46], and much higher than the 5:1 and 4:1 reported respectively for wheat flour porridge and rice flour porridges in developing countries [47]. These high values above 0.4 highlight the importance of the inhibitory effect of phytate on iron absorption. This is emphasized in our study by the fact that 4 mg Fe sulfate of fortification of fonio meals without dephytinisation resulted in phytate-to-iron ratio of 1.9:1, and a mean iron absorption ratio of 2.6%. This would provide only 7.6% of the 1.46 mg/day median absolute requirement for iron absorbed among menstruating non-pregnant non-lactating women [48]. This also confirms that fortification alone is not sufficient to overcome the negative effect of phytic acid in cereal-based foods [11].

Fortification combined with an almost complete reduction of PA by wheat phytase induced a 3.2 fold iron absorption increase from fortified fonio in humans. It has been previouly shown that dephytinization causes a 2 fold increase in absorption from low-tannin Sudan sorghum porridges, but phytate degradation was achieved with commercial phytase [49]. Also, significant increases in fractional absorption of iron (1.7 fold) and zinc (1.5 fold) in adults have been previously reported for both dephytinized cereal-based foods using purified phytase and wheat intrinsic phytase respectively, but without fortification [14,50]. Previous studies on the inhibitory effect of phytate have also reported a 2-12 fold increase of iron absorption from different dephytinised meals [16, 51, 52]. Together with our results, this adresses the double issue of the low level of bioavailable iron in cereal-based meals caused by the negative effect of phytate on absorption, and the low content of native iron particularly in fonio meals due to losses during processing.

A high level of fortification (4 mg ferrous sulfate / 0.5 mg native iron in 24 g dry weight fonio flour) has been used to modify phytate-to-iron ratio in fonio porridges. A practical issue in the context of low income countries is that such a fortification level can be very costly and difficult to apply. Particularly in mass fortification, this level may be too high in view of the amount of fonio consumed. However, this high level may be relatively close to other applications such as home fortification of specific foods which has the advantage to be cost-effective, while adressing the needs of a clearly defined group of the population [10, 52]. In addition, products are usually fortified in their final form, immediately before consumption, limiting the risk of changes in sensory properties [10]. Such fortification form may be suitable for increasing iron content in fonio meal, and should be further explored for assessing cost and technological feasability. While implementing the incubation of fonio porridge and wheat may be feasible in principle at the household level, we did not investigate the effect of direct addition of wheat flour to a fonio meal at the time of consumption as was done by Troesch et al. [14] with purified free phytase. Intrinsic wheat phytase would need to be released from its food matrix during digestion and would not interact with food PA if not for a short time period during digestion, likely having little effect on phytic acid content. Germinating and pre-digesting the wheat grains may reduce the need for incubation and may provide a source of readily available natural phytase, which would potentially be active in the gastro-intestinal tract. Such approaches need to be further investigated.

The stable isotope study was based on a single meal design, which could have overemphasized the effect of PA reduction on iron absorption [9]. Cook et al. [53] previously reported significantly lower increase in iron absorption from total diet (2.5 fold) compared to increase from a single meal (5.9 fold). In addition, we cannot exclude that other unknown components of wheat flour might have caused the increased iron absorption. However, PA is the main inhibitor of iron absorption [9] and the significantly reduced level of phytic acid means that the natural phytase in wheat is most likely responsible for the improvement in iron absorption.

Conclusion

Reducing PA content in cereal-based foods is challenging in low-income countries because typical home processing practices often do not achieve a sufficient PA degradation to significantly improve iron absorption. In addition, exogenous phytases are often costly and their availability limited in rural communities. Therefore, the use of locally available and widely used cereals as wheat for dephytinisation needs to be further explored in developing countries with other traditional cereal-based porridges and legume-cereal mixtures. Our results also demonstrate that iron bioavailability from fortified fonio meals was significantly improved by an almost complete reduction of phytic acid by wheat phytase. Dephytinisation using intrinsic wheat phytase could be a promising processing practice to improve iron bioavailability and fortification is required to increase the amount of absorbed iron from fonio meals. The feasability of this processing technique with regard to users' compliance needs to be further explored in developing countries household conditions.

Aknowledgements

We thank all women participating in the study; staff members and technicians of *Laboratoire Santé Plus* (Benin) for facilitating anthropometric measurements, blood samples collection and handling, and part of biochemical analysis (hemoglobin and CRP) in Benin; technicians of IER Laboratoire de technologie Alimentaire for fonio processing; Genevieve Fliedel, CIRAD UMR Qualisud, Integrated Food Processing Research Unit, Department PERSYST for contribution to porridge preparation; Ms Diane Djossinou for assisting in meals test supervision; Ms Lucy Elburg for facilitating test meals sending to Benin; Dr Lionelle Fanou for supervision during blood sample collection; Dr Barbara Troesch of ETH-Zurich for assisting in refining research proposal; Prof. Carsten Münker from University of Cologne for providing analytical facilities (Multicollector-ICP-MS) for iron isotope ratio measurements; Erhardt Jurgen for iron blood indicators analysis; Ms Anja Feierabend and Colin Cercamondi for phytic acid and phytase activity analysis at ETH Zurich and in Wageningen University, respectively.

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Chapter seven

- General discussion

Iron deficiency among women of reproductive age still remains a public health problem in many regions of the world including sub-Saharan Africa where more than 40% are afflicted. A key cause is the inadequacy of the diet due to insufficient quality of foods consumed. Indigenous foods and/or traditional grains such as fonio may play a critical role in reduction of the problem for resource poor African populations. However, the potential contribution of these traditional and indigenous foods to improving nutrition especially micronutrient deficiencies in West Africa is not yet fully understood. In order to strengthen the potential of fonio and fonio products to contribute to increased nutrient intakes, this study aimed to improve the nutritional value of fonio and fonio products through home processing and fortification.

In five related studies conducted in Bamako (Mali) and Cotonou (Benin), (i) the consumption patterns and contribution of fonio to nutrient intakes of Malian women of reproductive age were assessed; (ii) the nutrient and phytic acid content, genetic and sensory diversity, and effect of processing of fonio landraces in Mali were analysed, and (iii) the effect of dephytinisation with intrinsic wheat phytase and iron fortification on iron absorption from fonio meals in Beninese women was studied. In the next sections, the main findings of this thesis are summarized and methodological issues are discussed. Furthermore, the main results are put in a broader perspective and implications of the findings for public health as well as suggestions for future research are presented.

MAIN FINDINGS

The main findings of this thesis are summarized in **Figure 7.1**. Fonio is consumed one to three times/month by 68% of the urban Malian women mainly as snack on working days, and to a lesser extent as main dish on weekend days. Average daily portion size consumed (152 g/day) was small compared to rice, the main staple cereal of the diet, contributing 16% to daily energy intake (**Chapter 2**).

The main findings in **chapter 3** showed that the use of the adjusted Mali food composition table seems acceptable for estimating average intake at population level for macronutrients, calcium and zinc in a low intake population, but not for carbohydrate and iron intakes (being underestimated), and vitamin A (being overestimated), nor for probabilities of single micronutrients and nutrient densities.

Consumption of fonio and its contribution to nutrient intakes	*Chapters 2 and 3*
- 68% of women consumed fonio one to three times/ month; - Average daily portion size: 152g. - Fonio contribution: 16% to daily energy intake.	- Estimated available carbohydrates and iron intakes 17% and 57% lower; and vitamin A intakes 45% higher than analysed intakes: - Adjusted TACAM acceptable for estimation population level intake for all other nutrients.

Variation in nutrient content and sensory characteristics of fonio landraces	*Chapters 4 and 5*
- Small genetic diversity of fonio landraces; - No significant difference in iron and zinc content (p < 0.05) between landraces. - Iron decreased from 35.0 to ~ 2 mg/100 g, zinc from 3.0 to 2.1 mg/100 g, and phytic acid from 514.0 to 129 mg/100 g through processing from paddy to mid wet fonio	- Landraces differed in visual (colour, presence of impurity) and textual (consistency of cooked grain) characteristics - Landraces from Burkina Faso more homogenous

Strategy to decrease phytic acid and increase iron absorption from fonio diet	*Chapter 6*
- Phytate to-iron molar ratio decreased from 24:1 to 3:1 with dephytinisation and to 0.3:1 with iron fortification. - Iron absorption increased from 2.6 to 8.3% in fonio porridge through fortification combined with dephytinisation.	

Figure 7.1. *Summary of main findings*

We found no meaningful genetic diversity and differences in iron and zinc content in 12 fonio landraces in Mali (**chapter 4**). However, fonio landraces collected in Mali, Guinea and Burkina Faso showed variation in visual (colour and presence of impurity) and textural characteristics (consistency of the cooked grain), determining the preference of consumers (**chapter 5**). These results indicate that there is probably no benefit in selecting landraces for high natural iron content, but selecting landraces for preferred sensory properties may offer an entry point for processors who intend to promote the consumption of fonio.

We also found that processing fonio from paddy to the mid-wet product significantly reduced the nutrient (iron) content (**chapter 4**). Improving the nutrient (iron) content of fonio could therefore only be reached by reducing phytic acid and increasing the iron content by fortification. Results of the study described in **chapter 6** showed that dephytinisation of fonio porridges with native wheat phytase decreased phytate-to-iron molar ratio from 24:1 to 3:1 and iron fortification further reduced this ratio to 0.3:1. Results also showed that dephytinisation and fortification significantly improved iron absorption from fonio porridges from 2.6% to 8.3%.

INTERNAL VALIDITY

The main methodological issues of the research described in this thesis have been discussed in **chapters 2-6.** Those relevant for the internal validity of the findings and which may potentially influence the conclusions are mainly related to bias in selection of the study population, food sampling errors, information bias and generalizability.

Selection of the study population

The study population described in chapters 2 and 3 was selected using a two-stage cluster sampling design developed by UNICEF for multiple indicator cluster surveys in developing countries [1]. Design effect, non-random selection and non-response are the main limitations which could likely increase the risk of selection bias.

Design effect

The design effect in a cluster sampling is the factor accounting for the variance between different clusters [2, 3]. It is the ratio between the variance of the outcome variable under cluster sampling design and the variance under simple random sampling [4]. It represents the amount by which the sample size needs to be multiplied to account for the design of the study. Contrary to simple random sampling, data are usually dependent in cluster sampling design because individuals within clusters are often linked by environmental, social or genetic factors [5]. Thus, overlooking the design effect in sample size calculation or data analysis leads to an underestimation of the variance of the outcome variable; this needs to be corrected by increasing the sample size. Estimated design effects ranging from 0.8 to 2.7 have been reported from previous studies using 3-stage cluster sampling [6, 7], though with larger cluster sizes and higher number of clusters than in our study (**chapters 2 and 3**). This suggested that a sample size 1 to 2 times larger than estimated was required for our study. In chapter 2 of this thesis, we did not take into account the design effect, and although this would not have affected the estimated mean for nutrient intake and micronutrient adequacy it may have affected the variation around the mean. In **chapter 3** we took a sub sample from all women selected in **chapter 2** with cluster sampling, and not correcting for cluster sampling may have resulted in a reduced variance and that could have led to finding inflated differences. In addition, the influence of the design effect has been reported to be reduced for regression coefficients [8], thus the probability of overestimation of the confidence interval of the correlation and regression coefficients reported in **chapter 3** was assumed to below.

Non-random selection bias

Selection bias is a bias that arises from the way subjects are included in or excluded from the study [8]. This may include non - response bias. Selection bias may lead to a study population that does not represent the target population. The sample selection (study subjects) was performed using a three-stage cluster sampling design (**chapter 2 and 3**) and non-random selection bias may have occurred within each step [1, 9, 10]. During the first stage of the recruitment process, we intentionally excluded 9 over a total of 72 clusters (1% Bamako population) to ensure a good representativeness of the Malian population. This exclusion was related to an over representation of international experts and industries in the 9 excluded clusters. For cluster selection, we applied a probability proportional to size procedure to take into account the variation in clusters size, where large clusters had a greater chance to be selected.

For households' selection in the second stage, we used the random walk method, which is assumed to be a proxy for the random sampling method where no household lists are available [1, 9-11]. This involves identification of the geographical center of the cluster, counting all households from this center to the edge of the cluster in a direction determined by spinning a bottle, randomly selecting one household from these as start household, and continuing selecting every third household, until the required number of households was reached [11]. Although the method does not produce strict probabilities samples, it was chosen as we did not have a comprehensive map of the study area nor complete household lists which could be used as a sampling frame [1]. Wrong identification of the central point or cluster boundaries may lead to selection bias. However, key informants (the head of the quarter or representatives) were consulted when necessary and/or a rapid tour of the clusters was done before the field work began [9], to have a good approximation of the geographical central point of the selected clusters and to minimize the risk of bias that might arise when starting points for random walk recruitment are chosen. In addition, an equal probability of selection for each household might have been jeopardized because when the number of desired respondents was reached, we stopped the selection [1]. This may have led to oversampling of households next to the starting point while underrepresenting those located far away from the starting point. If this occurred and underrepresented households would have had different characteristics related to the outcomes of our study, this could have influenced our findings, for example, on fonio consumption. However, households in our study area were rather homogenous.

In the third stage when selecting a woman, another source of selection bias could arise because in the selected households, often more than one woman met the eligibility criteria. For example, in polygamous households, the systematic selection of the first wife of the head of household could introduce selection bias. To minimize the potential for this type of bias and also the risk of self-selection, a random selection of one woman per household was done from a list of all eligible women in the selected households. Hence, through a carefully implemented systematic approach, the risk of non-random selection bias along the sampling procedure in our research was minimized.

Non-response bias

Another source of selection bias that can lead to spurious conclusions arises from refusal to participate, leaving the study before the end, or exclusion from data analysis due to incomplete or implausible results. All the women who were selected consented and did participate in the studies, so there was no refusal to participate. Data of 6 women out of 108 (6%) were incomplete and these women were intentionally dropped from the analysis (chapter 2). Also, in chapter 3, only one woman out of 35 did not deliver the last meal for social reasons during the recoding day representing only 3% of the study population, and was therefore removed from the analysis. As the percentage of ”non-responders” in our studies is rather low, it is not likely that excluding these women would have led to a selection bias influencing the current findings of our study. Also, the socio-economic characteristics (median age, illiteracy rate, average number of children and matrimonial status of the women) of respondents and non-responders were not different. Therefore, we assume that non-response bias did not occur in our studies.

Information bias

Information bias occurs when there are either random or systematic differences in the measurements or when some respondents provide different information from the rest of the sample [8]. This may include common sources of information bias originating from the respondent (such as recall bias), from the interviewer (interviewer bias), and from the measurements (measurement bias) [8]. The potential of information bias discussed in this section is largely related to the dietary assessment studies (chapters 2, and 3), the nutritional and sensory studies of fonio landraces (**chapters 4, and 5**), and the dephytinisation study (**chapters 6**).

Bias in assessment of diet

Bias due to misreporting might have occurred in assessment of the diet described in **chapters 2 and 3**. Accurate and precise estimation of dietary intake of individuals and groups remains a major challenge in dietary assessment. Systematic bias and random errors are the main limitations for the validity of dietary assessment methods [11]. Systematic errors reduce the accuracy of dietary intake. It appears usually when the respondents misreport the foods consumed, and/or under- or over-estimate the amounts consumed. Random errors contribute to reduction of precision, it appears when amounts consumed are incorrectly estimated from a non-systematic procedure [12].

One of the main sources of error in dietary assessment is misreporting, comprising both under- and over-reporting [13]. Underreporting of energy intake is well known and treated in literature. Also our study, using the 95% limits Goldberg cut-offs [14] to evaluate energy intake assessed by both calculation using food composition table and chemical analyses of duplicate portions, indicated that reporting bias in energy intake was significant at group level, and the one day food weighed record has underestimated on average our energy intake (chapter 3). Besides energy intake, misreporting can also introduce severe error in the estimation of intake other nutrients.

Misreporting can be intentional or unintentional [13]. With intentional misreporting, people tend to report food behaviors that are in accordance with their perceived cultural norms [15] and/or try to convey a better image of themselves [16]. This is difficult to avoid during a recall (**chapter 2**), but especially when an enumerator is present in the household for a whole day and recording all foods and ingredients before preparation, respondents may change their dietary habits in order to reduce the burden of weighing and recording (**chapter 3**).

Systematic errors might likely occur during the weighing of the foods or amounts consumed. Women may be hesitant to show the real portions or interviewers may over- or underestimate the weight during the weighing process, especially when subtracting leftovers from amount of food consumed (chapter 3). To minimize these errors, a systematic procedure [17] was carefully used at each step during data and duplicate portion collection and handling. Precautions have been taken through training of interviewers, impromptu supervision to minimize reporting errors, proper calibration of instruments and random assignment of interviewers. Nevertheless some errors may have inevitably occurred in recalling the dietary intake.

Bias in food composition table

The use of food composition tables to convert food intake into nutrients could introduce bias into nutrient intake estimates [18] related to the variability of values obtained from laboratory analysis or estimated based on conversion factors [11, 19, 20]. Systematic errors are more likely to occur particularly when food composition data are outdated, or when they do not provide the appropriate nutrient information for the foods as consumed [11, 21].

In chapter 2 and 3, the national food composition table of Mali (TACAM) [22] was used as first source of information for our research, based on the assumption that it would provide a good representation of locally available and traditional foods. This table was complemented with values from other databases for food and nutrients not available in the primary source following international standards for food database development and compilation [23]. This was a systematic procedure used to improve the reliability of the food composition database used for the study, including: an internal evaluation of the quality of the TACAM [24]; a substitution of missing nutrient values with values from secondary tables; the use of retention factors to account for nutrient loss during cooking. This resulted in an adjusted TACAM, used in chapter 2 and 3.

In chapter 3 we evaluated whether the adjusted TACAM is acceptable for assessing average energy and nutrient intakes and for assessing probability of adequacy of intakes for selected nutrients at population level, by comparing the nutrient intake calculated from a food weighed record with that based on chemical analysis of duplicate portions of diets collected on the same day. Our results indicated that the adjusted TACAM leads to similar mean energy and nutrient intakes when comparing estimated to analyzed intakes at population level, except for available carbohydrates and iron which are underestimated and vitamin A which is overestimated. At individual level, significant differences were observed between estimated and analyzed intakes for all the nutrients and these tend to increase with higher intakes.

To our knowledge, our study is the first to look at the assessment of nutrient intakes at population level in Africa using food composition table and direct chemical analysis. Our results were concordant with Bedogni et al. (1999) study who reported that their food composition database can be reliably used to assess energy, fats and proteins intakes in an European community [25]. Findanza and Periello (2002) also found that the European Institute of Oncology FCDB cannot be used to reliably determine Vitamin A, particularly for retinol and beta carotene intakes in Italian population [26], confirming the challenge in conducting Vitamin A dietary assessments, consistently with what we found using TACAM in our study. However, these studies are related to European populations with different food composition databases but also different intakes and dietary assessment methods.

Limitations of the evaluation of a food composition data base might be related to quality control and sample size. As every nutrient analysis has its own variability and validity, quality control is therefore an important aspect to reduce this kind of bias. Based on empirical evidence, a coefficient of analytical variation (CV) of less than 10% is considered as an acceptable variability [27] . For our study, within and between-run CVs over a more than 10-year period have been determined. It was 0.9 % and 1.6% for ash; 2.3% and 3.9% for fat; 0.8% and 1.2% for nitrogen, respectively and 1.9% and 9.6% for retinol; 6,1% and 13.3% for α-carotene; 3.9% and 7.7% for β-carotene respectively over a three year period. Between-day CV of 4.2% (within-day variation: not recorded) was observed for dietary fiber. Based on these results, we can conclude that our laboratory analyses can be considered as acceptable with high quality minimizing information bias.

However, the lack of significant differences observed between our analyzed and estimated nutrient intakes could be due to the small sample size of our study which may have limited our power to detect meaningful differences between analyzed and estimated nutrient intakes at population level. This is also consistent with a previous study reporting that a relatively small size of the sample may not be adequate to detect significant differences for some micronutrients [28].

Bias in fonio samples collection

The collection of fonio landraces analyzed in chapter 4 and 5 was based on the information given by farmers through focus group discussions in Ségou and Sikasso regions. Farmers were selected based on criteria such as being fonio producer; consumer and/or retailer of fonio surplus; open for external visitors throughout the year and being receptive; owning or having enough space for fonio cultivation; and having a field of fonio located near a road. Before the focus group discussion with farmers, key informants (agricultural extension services agents and NGOs) were asked to provide names and characteristics of each landrace per village.

During the focus group discussion, farmers were asked to list the names of the landraces available in the community. Next, presence of all landraces from the key-informant list not mentioned by the farmers were discussed and confirmed. For all landraces listed and confirmed to be available in the village, samples were selected based on the "first arrived and first served" principle. However, selection of the landraces by farmers was mainly based on phenotypic characteristics, related to for example the color of peduncle or leaves, or the form of the grains. Differences in landraces could therefore be due to differences in soil or agronomic practices between villages. As a consequence, landraces with the same genetic origin could have been given different names by farmers resulting in two comparable samples. Or, two morphologically and genetically different landraces could have received the same names by farmers resulting in only one of these landraces being sampled [29].

As we did not have information about varieties obtained through breeding lines, our way of selection based on farmer's information could probably have reduced the variation we have found in landraces analyzed in our study. However, the collected landraces were cultivated and harvested the same year in all of the three countries and processed under similar conditions using the same standardized processing method.

Bias in sensory evaluation

Bias in sensory evaluation is related to the selection of panelists or sensory descriptors of the food tested (chapter 5). Our panel is a group of twenty two young people (not smoking) selected among IER scientists and trained to describe the sensory characteristics of cooked fonio grains. Among the group, we purposely selected 18 panelists who did accomplish the sensory evaluation of all the landraces. Four panelists started the evaluation of landraces but were not able to evaluate all the landraces, therefore were excluded from the analyses. By excluding those panelists who did not evaluate all the landraces, we might have missed information on evaluated landraces as panelists were the ones who identified and defined all the sensory descriptors used to characterize the landraces during qualitative interview as well as during assessing the intensity values of descriptors. Only 18% of the panelist did not achieve the sensory evaluation criteria, and they were excluded from the panel. The excluded panelists were not different from those maintained in the panel in terms of educational level and so probably this effect was negligible.

Also, systematic differences in the panelist's ability in the scoring of the intensity value of the five chosen descriptors are another potential source of information bias. Bianchi et al. (2009) found that a reliability coefficient (overall consistency of a measurement) above 0.75 indicated good repeatability and discrimination of descriptors by the panel [30]. In our study the reliability coefficient was above 0.80 for all the descriptors and reached 0.99 for colour and impurities. This indicates that the reliability of our panel was good for all the five descriptors and very good for colour and impurities descriptors.

Generalizability

One of the most common mistakes by researchers seems to make statements about a large population on the basis of a small sample. Generalizability depends on the degree to which the particular sample in question can be said to be representative of the population. Random sampling does not guarantee generalizability. If the targeted population is a small subpopulation within a larger population, the results may not be generalizable to the larger population because it may not be adequately represented in the random sample. Other information such as socio economic status or educational level is needed to establish generalizability [31]. In our study, generalizability issues are related to the selection of the study population (chapter 2 and 3) and the selection of the fonio samples (chapter 4 and 5).

The study population originated from urban areas of Bamako, Mali (chapter 2 and 3). Socioeconomic status and living conditions of these women are probably different from those of rural areas [32] and other West African countries and this could hamper generalization of the results to the entire region. However, most of the West African countries (including Mali) are in the lowest human development category [33] and the diet is still influenced by traditional dietary practices [34], therefore, our results may be generalizable to West African urban areas.

For the 12 fonio Malian landraces, the collection was done in 2 out of 8 regions (chapter 4). We purposively selected target regions (Segou and Sikasso regions), districts (Bougouni and Tominian) and villages (Tanhala, Hanekuy, Sanékuy and

Banco-Tiémala) based on the sampling frame provided by the larger FONIO project. Villages were randomly selected based on criteria like: involved in the FONIO project; located in proximity of a large market; accessible by car throughout the year and producing mainly fonio. Selecting villages who are accessible by road for example introduces the "tarmac" bias [11] as they might be systematically different from those that are difficult to reach. In addition, Mopti and Kayes regions also produce fonio but they were not involved in the Fonio project. By sampling only landraces from the specific areas of Segou and Sikasso regions, we might have missed out landraces from Mopti and Kayes that do not grow in the selected areas. Thus, this would probably restrict generalization of the results to the entire country of Mali.

The 20 West African fonio landraces were collected under the same conditions in different agro ecological regions (Mali, Burkina Faso and Guinea) (chapter 5). By sampling only landraces from the specific countries of the Upper Niger basin, we might have missed out information of landraces from other countries like the Atacora Mountain (Nigeria, Togo and Benin) also being producers of fonio. Furthermore, the unintentionally limited number of landraces per country (eleven from Mali, five from Guinea and four from Burkina Faso) could also hamper generalization to entire West African.

EXTERNAL VALIDITY

This section discusses the main findings in this thesis as outlined in **Figure 6.1** in the context of existing literature. The research described in this thesis was conducted to explore and evaluate the potential contribution of traditional crops such as fonio to iron intake of women in reproductive age, and how to improve its iron content for better nutrition in West Africa. First, we investigated the role of fonio in the diet and then we step wise study how to increase the contribution of fonio to intake of the women by *i)* looking at opportunities to select varieties with a better nutritional (especially iron content) and sensory profile, and *ii)* improving home processing through dephytinization and iron fortification.

Consumption pattern of fonio and its contribution to nutrient intakes

A consumption frequency of one to three times/ month by 68% of our study population was found. The average daily portion size was 152g (chapter 2). Only 5% of the study population consumed fonio dishes contributing to 16% of daily energy intake of the portion size. This to a large extent reflects the low consumption of fonio. Previous studies have also highlighted a low consumption of fonio in urban areas accounting for less than 1% of the cereals eaten [35]. The consumption frequency and the average daily individual portion size found in our study were higher compared to the frequency of less than once a month and the individual amount of 650 to 840 g/person/year found in urban areas, reported earlier [35]. Other studies found an average consumption of 34 and 41 kg per person per year in semi-arid and sub humid production zones respectively. Based on an estimated need of 200 kg of cereal grain per person per year (FAO recommendation) [36], these fonio consumption levels would cover respectively 17 and 21 % of needs in food grains in the two areas [37]. However, these results are based on anecdotal information reported in descriptive socio-economic studies in West Africa [35] but not on systematic food consumption studies as in our study. The small portion size of fonio found in our study as compared to cooked rice could be explained by the fact that fonio was mostly consumed as snack. Significant barriers such as availability of cooked fonio in urban markets, lack of consistent supply throughout the year, difficult post- harvest processing, high-quality product demand, hard texture coupled with time consuming cooking process, and high cost of fonio products, were pointed out to explain the low consumption of fonio in Bamako [35, 38-42]. Other factors such as the lack of knowledge about processing and cooking and also the complicated processing and cooking, social factors and cultural beliefs have also been reported to influence food choice and consumption patterns [17, 42], and these could have limited fonio consumption in Bamako [42] as well as its contribution to nutrient intake especially iron.

Hence, for fonio to have a meaningful contribution to energy and nutrient intake, it is necessary to increase its consumption. As sensory characteristics are important for consumption we looked into the possibility of selecting varieties on better sensory characteristics.

Sensory diversity of fonio landraces

In chapter 5, the sensory diversity of 20 West African farmers landraces collected in Mali, Guinea and Burkina Faso was investigated. Sensory descriptors of food like color, presence of impurities, size, cohesiveness and consistency of the grain are reliable predictors of its acceptance and could play a critical role in its selection for consumption [43] or for processors who intend to promote its consumption and increase its role in diet. The results of our study showed that the visual (colour and presence of impurity) and textural characteristics (consistency of the cooked grain determined by the firmness or the softness of the cooked fonio grain) determine the preference of consumers. Previous studies have also identified the whiteness of fonio grain and absence of impurities as main criteria when purchasing fonio grain [35, 44]. However, these findings are related to consumer's preference for raw fonio grain but not for cooked fonio grain as in our study. No information on sensory differences between fonio varieties and no systematic sensory analysis of cooked fonio grains have been reported like for other cereals as cooked rice grains [45, 46]. Sensory studies on fonio have mainly concerned bakery products [47, 48], and traditional beverage like "kunun zaki" [49]. For other cereals such as sorghum, maize and rice quantitative descriptive analysis and consumer preferences using hedonic test have been reported [50-53]. In these studies, whiteness, as determined by the amount of chalkiness in rice grain, has been demonstrated as a guarantee against contamination (absence of impurity) [54, 55]. For example in Mali, fonio from Guinea is rather preferred than that from other countries because of the whiteness of the cooked grain. Beside whiteness, also consistency of the cooked grain determines preference of consumers as earlier reported in cooked rice that is tender when the gel consistency is softer [54, 56].

Both the visual and textural characteristics of cooked grain were found to be different between landraces. The variation in visual and textural characteristics of fonio may offer to consumers and processors the possibility to choose landraces most suitable for their products and processes. Selecting landraces for preferred sensory properties may offer an entry point for processors who intend to promote the consumption of fonio. We also found that landraces from Burkina Faso are more homogenous than those from Guinea and Mali which split into different groups for visual and textural characteristics. Adoukonou-Sagbadja et al. 2007, also demonstrated that fonio accessions from Burkina Faso, Togo and Benin clustered in one same group while Malian and Guinean accessions split into two different groups [29]. The differences of variability encountered in sensory properties of Burkinabe landraces compared to that of Mali and Guinea could probably be explained by their low genetic diversity. However, the number of landraces we used to establish the sensory analysis was intentionally limited and may not be representative of each country.

Another important issue is to know whether landraces different for their visual and textural characteristics could also be different for their nutritive value especially the iron content. This can help to make choices for specific fonio landraces with high iron levels that can contribute to improved iron supply and intake of West African communities. Therefore selection of iron rich varieties to improve intake of bioavailable iron can only be effective if there is information about the nutrient content of landraces, its variation among landraces and the effect of processing in terms of nutrient losses (especially iron). This can help to make choices for specific fonio landraces with high iron levels that can contribute to improve iron supply and intake of West African communities.

Nutrient and phytate content, genetic diversity and effect of processing on fonio landraces

In chapter 4, we found that the genetic diversity of the 12 fonio landraces collected in Mali was small compared to that reported by Adoukonou-Sagbadja et al. (2007) from the 118 *Digitaria exilis* accessions collected in West Africa [29]. The results of our research also showed that our 12 Malian landraces clustered into three groups, while other Malian accessions collected by Adoukonou-Sagbadja et al .(2007) were split into two groups. These discrepancies are probably related to methodology issues: sample size was greater and collection areas wider in this latter study as compared to ours.

The phenotype represented by the proximate composition and micronutrients contents, especially iron and zinc, showed no significant differences between fonio landraces, implying that there is no benefit in selecting varieties based on their elevated native nutrient content (chapter 4). Available information in literature on chemical and nutritional composition of fonio grains is limited. Differences in nutritive value especially iron and zinc between fonio varieties or cultivars have not been reported as was done for other cereals in previous studies [57]. However, previous studies have shown significant differences in iron content in other cereals [29, 58, 59]. These studies argue that these differences in iron levels may be due to the origin of the sample as soil type, climatic conditions during growth and duration of the growth period influence inorganic constituents in foods. The absence of differences between landraces in our study could be related to the low genetic variation of our landraces, suggesting that the farmers selected the different landraces probably originating from a same gene pool based on phenotype [29].

The native iron content was high in the paddy. A concentration of 8.5 mg/100g dry weight for iron in whole grain fonio has been reported in another study [22], being lower than the range of 14 to 57 mg/100 g dry weight observed for fonio paddy in our study. These discrepancies could probably be due to the high concentration of iron in the pericarp and outer layers which are removed by cleaning, husking/dehulling and washing. A higher concentration of iron has also been reported in other traditional cereals such as millet and sorghum [58, 60, 61]. The high concentration of iron in cereals could be a consequence of the iron level in West African soils which is rich in available iron [62].

Traditional processing considerably reduces nutrient content (iron), with the most important losses occurring during processing of the paddy product into the mid wet product. As for the chemical and nutritional composition, available information on the effect of processing on iron content of fonio grains is limited in literature. However, a previous study found that mechanical dehulling decreased macronutrients and mineral content of fonio and the decrease was more important in fonio than other cereals like millet, sorghum, maize and rice [44] probably attributed to the small size of fonio grain. With processing, iron content was approximately reduced to 2 mg/100 g dry weight in mid wet (ready-to-cook) fonio, regardless of the landraces while zinc concentration was hardly affected by processing and cooking. A similar reduction of iron has been demonstrated on husked fonio (comparable to mid wet fonio) in a previous study [22], however, this content of 2 mg/100 g dry in mid wet fonio appeared to be the lowest [59] compared to the whole grain of other traditional cereals such as maize, millet and sorghum (5.9 mg/100 g in yellow maize, 5.8 mg/100 g in pearl millet flour, and 5.8 mg/100 g in sorghum flour), suggesting a limited potential contribution of fonio to the iron intake from fonio diets.

Phytic acid content decreases from 514 mg/100 g for fonio paddy to 129 mg/100 g for mid wet fonio, regardless of landraces. To our knowledge, our study is the first to look at phytic acid content and effect of processing in fonio landraces, so comparison is hampered. However, similar results were obtained with other cereals like sorghum, millets, maize and rice [58, 63-67], but the amount of phytic acid found in fonio paddy in our study is higher than that reported in whole grain of other cereals [58, 68]. A non-negligible proportion of the difference in iron and phytic acid content between fonio paddy and mid wet fonio could be attributed to nutrient losses during processing, particularly husking/dehulling, milling and washing, which significantly affects the nutritional qualities of mid wet fonio [69]. Despite this reduction of phytic acid content, the amounts left are still sufficient to inhibit the absorption of iron [58, 63, 66, 67]. Indeed, as phytate/iron molar ratio found in cooked fonio was above the critical cut-off of >1, poor bioavailability of iron from fonio as eaten may be suggested. These highlighted the need to adress the double issue of the low level of bioavailable iron in cereal-based meals due to adverse effects of phytate on iron absorption, and the low content of native iron, in fonio meals for example due particularly to losses during processing. This suggests that food-based approach promoting fonio as available staple food should focus on an increase in iron content and on the complete degradation of phytic acid from fonio diets in order to assure absorption of at least 18% of the iron content. Therefore, improved food processing such as dephytinisation combined with iron fortification could be a way to improve bioavailable iron supply from fonio diets.

Improved food processing: dephytinization and iron fortification to increase iron absorption from fonio diet

We investigated the effect of phytic acid degradation with intrinsic wheat phytase and fortification on iron absorption from fonio based test meal using stable isotopes as absorption tracers. We found that dephytinisation of fonio porridges with native wheat phytase decreased phytate-to-iron molar ratio from 24:1 to 3:1 and iron fortification further reduced this ratio to 0.3:1. Results also showed that dephytinisation and fortification significantly improved iron absorption from fonio porridges from 2.6% to 8.3%. Other studies demonstrated that phytic acid can strongly inhibit iron absorption at very low concentration in cereal-based meals and that the molar ratio phytate-to-iron should be preferably lower than 0.4:1 to achieve a significant iron absorption [70]. In our unprocessed fonio flour porridges, the found phytate-to-iron molar ratio of 24:1 is higher than the range of 12:1, 21:1, 9:1, 5:1 and 4:1 estimated previously for sorghum, maize, wheat and rice flour porridges respectively in low income countries [57, 71]. After adding 25% of whole wheat flour to fonio flour porridges, the ratio significantly decreased to 3:1. This result confirmed previous findings demonstrating that adding whole wheat grain to cereal-based complementary foods completely degraded phytate in a relatively short time [72].

However with dephytinisation only, the phytate-to-iron ratio reduction reached in our study did not appear sufficient for achieving significant iron absorption from fonio meals. Therefore, after dephytinisation, we increased the iron content by fortification. The results showed that fortification with 4 mg ferrous sulfate of dephytinised fonio porridges significantly increased iron absorption by 3.2-fold. A previous study demonstrated that dephytinization significantly increased absorption by 2-fold from low-tannin Sudan sorghum porridges, but the degradation of phytic acid was achieved by an exogenous phytase microbiase [73].

Furthermore, 1.7-fold and 1.5-fold significant increases in fractional absorption of iron and zinc respectively in adults have been previously reported for cereal-based foods dephytinized using wheat flour, but without fortification [74]. These substantial achievements from the previous and this research emphasized the need to adress the double issue of the low level of bioavailable iron in cereal-based meals caused by the negative effect of phytic acid on iron absorption, and the low content of native iron, particularly in fonio meals due to losses during processing.

GENERAL CONCLUSIONS

Micronutrient deficiencies are highly prevalent and mainly caused by low quantity and quality of food consumed. Traditional foods or under-utilized crops, such as the native sub-Saharan cereal fonio, may play a major role in reduction of malnutrition among resource poor African populations. Through the INCO FONIO project, efforts have been made recently to increase the market value of fonio by enhancing its productivity, improving the post-harvest technologies and the quality of the end fonio products, and promoting small and medium fonio processing enterprises at individual level or/and women association groups in urban areas. In this thesis we explored and evaluated the potential contribution of fonio to iron intake, and how to improve its iron content for better nutrition in West Africa. We conclude that:

- The current contribution of fonio to daily bioavailable iron intake is low due to 1) small portion sizes being consumed with low frequency, 2) to considerable losses during processing to mid-wet fonio, and 3) a high phytate-iron molar ratio.
- Fonio landraces from Mali, Guinea and Burkina Faso show variation in visual (colour and presence of impurity) and textural characteristics (consistency of the cooked grain), determining the preference of consumers. Selecting landraces for preferred sensory properties may offer an entry point for processors who intend to promote the consumption of fonio and increase its role in diet.
- In absence of meaningful genetic diversity and variation in iron content in fonio landraces in Mali, there is little benefit in selecting landraces for breeding varieties for natural high iron content.

- Dephytinisation using intrinsic wheat phytase could be a promising processing practice to improve iron bioavailability and fortification is required to increase the amount of absorbed iron from fonio meals.

FUTURE RESEARCH

In view of our findings, the following suggestions for future research can be made:

- The use of whole grain fonio instead of milled fonio could reduce the losses considerably. Future research should therefore focus on investigating the nutrient content (especially iron) of the whole grain fonio, as well as its acceptability for "*djouka",* the main form of fonio consumption from this study among urban women population in Bamako. In our research, we did not determine the nutrient content of the whole grain fonio which is expected to contain more iron than the mid wet fonio used for "*djouka"* preparation. Using the whole fonio grain for preparation of "*djouka*' will have a double benefit in terms of more iron content and less time for decortication. However, the djouka made from whole grain fonio will be dark brown colored; this could play a role in acceptability of whole grain "*djouka"*.
- The sensory differences found in our study must have originated from environmental factors as we did not find meaningful genetic variation in the fonio landraces used in our studies. Therefore there is need to investigate which environmental factors (such as geographic origin or locality, farming practices, post-harvest treatment), influence the sensory characteristics. This would help farmers to select and produce landraces under preferred environmental conditions with less impurities and contamination for example. These "clean" landraces will be acceptable by fonio consumers and processors because of the reduction of the processing time and the quality of the end product. Consequently, as other cereals, fonio demand and purchase may be boosted, and producers might be encouraged to increase the lands located for fonio cultivation and production, thereby increasing their income [75, 76] while buyers will be willing to pay a premium to get the higher quality grain [75].
- The large losses of iron during processing from paddy to the mid-wet fonio suggest that the iron present in paddy and/or in the unwashed fonio may originate from soil or from iron containing processing and cooking equipment [77]. There is substantial evidence that contaminated iron contributes significantly to iron intake in developing countries, but the significance of this intake for iron status is clearly dependent on the extent to which the contamination iron is absorbed [77-79]. The intake can be high and even with low bioavailability, this could have a substantial contribution, potentially but there is limited or no evidence for this. Clearly this is an area where more research would be needed. In addition, although the mechanism of absorption of contamination iron is not well known, it has been assumed to be poorly

absorbable [78, 79] but this is still not fully confirmed [80]. Therefore further studies are needed to confirm the poor level of absorption of contamination iron, in cereals in general but also in fonio.

- Significant decrease of phytic acid was achieved from fonio meal using whole wheat flour phytase, but under tight experimental and controlled conditions. A substantial removal of phytic acid from cereal-based foods is challenging in low-income countries because typical home processing practices such as fermentation often do not achieve a sufficient phytic acid degradation to significantly improve iron absorption [70]. Wheat is a locally available and widely used cereal and its potential for dephytinisation has been proven to be higher than most other cereals. As wheat phytase can reach its optimal activity in a relatively short time (1.5 to 3 h) when incubated at optimal conditions (with limited range of temperature), future research should focus on investigating the practical feasibility of this technology in the living conditions of West African populations. Furthermore, although the high cost of exogenous phytases and the restriction of some countries for their used because some microbiases derived from genetically modified species of *Aspergillus Niger* [71], it has been shown that aspergillus phytases addition at time of consumption increase iron bioavailability. This may be worth investigating further.

PUBLIC HEALTH IMPLICATION

In most developing countries especially in West Africa where the diet is mainly based on plant foods, meeting micronutrient requirements such as iron is difficult. Therefore, increasing the native micronutrient content of plant-based foods has been suggested as a strategy to combat micronutrient deficiencies in vulnerable populations [55, 81]. Indigenous foods and/ or traditional grains such as fonio were expected to play a critical role in reduction of the problem for resource poor African populations. From this research, we evidently show that, from a public health point of view, fonio is not a preferred food to be promoted to improve nutrition (iron) of women in West Africa because of its low contribution to daily bioavailable iron intake. However, from poverty alleviation point of view, adding value to fonio may increase fonio demand and purchase, encouraging producers to increase areas for fonio production and, as such, increase their incomes.

From the results of our research, suggestions could be: *i)* to encourage consumption of fonio, it is preferred emphasizing more on sensory characteristics than nutrient (iron) quality; *ii)* to improve nutrient quality, dephytinisation and fortification provide entry points to improve iron content of fonio. Dephytinisation using intrinsic wheat phytase could be a promising processing practice to improve iron bioavailability. This has been shown to be useful in industrial production especially with a complementary food based on wheat and soybean [74]. However, in Mali fonio is mainly produced at household level and all the related activities are manually made, there is also a need for wheat and standardized processing line. Alternatively exogenous phytases could be used for phytic acid degradation, but this is not commonly used because of the costs and availability. Although food fortification is considered to be the best long-term strategy to overcome micronutrient deficiency, there is a need for central processing [82] and as said previously, fonio is mainly home processed, for its application in the Malian context.

Based on the above discussion added to the fact that we cannot improve the nutrient (iron) quality of fonio through selection of varieties with natural high iron content, improving iron nutrition in Mali using fonio is not preferred. More care must be taken when claiming the potential contribution of traditional and indigenous foods such as fonio to improving nutrition especially iron intake of women in reproductive age in West Africa.

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Et cetera

- Summary,
Acknowledgements,
About the author

Summary

Micronutrient deficiencies, especially iron deficiency and iron deficiency anemia, are highly prevalent among women in many low- and middle-income countries and mainly caused by low quantity and quality of food consumed. The potential of biodiversity for improving dietary diversity and quality and for increasing and sustaining food and nutrition security is increasingly recognized by the international community. Traditional foods such as the native sub-Saharan cereal fonio (Digitaria exilis) are potential sources of species for domestication and provide valuable genetic traits for developing new crops through breeding and selection. As such, they may play a major role in reduction of malnutrition among resource poor African populations. During the past decades, efforts have been made to improve genetic potential and post-harvest processing techniques of fonio. Nowadays, large gaps exist in our knowledge about the nutritional composition and the dietary contribution of fonio and its products and the potential of fonio to contribute to improving nutrition and health in West Africa regions. In this context, this thesis investigates whether the nutrient quality (especially iron) of fonio could be improved through home processing and fortification for increasing nutrient (iron) intake of West African women. This research was done as part of an interdisciplinary EU funded FP6 project FONIO involving breeders, food technologists, nutritionists, economics and social scientists from European (France, Netherlands, Belgium) and West African countries (Mali, Guinea, Burkina Faso and Senegal).

In order to understand the dietary pattern, the contribution of fonio to nutrient intakes, and the beliefs around fonio, a food consumption study (24hr-recall) was carried out among women of reproductive age (15-49 years old) randomly selected in Bamako, the largest city of Mali. The results described in **chapter 2** indicate that fonio is consumed one to three times/ month by 68% of the urban Malian women mainly as snack on working days, and to a lesser extent as main dish on weekend days. Average daily portion size consumed (152 g/day) was small compared to rice, the main staple cereal of the diet, contributing 16% to daily energy intake. Potential barriers identified influencing fonio consumption were seasonal shortage, the high cost of fonio products in urban markets, the lack of women's skills in processing fonio, the difficult and time consuming cooking process.

To convert food intake into nutrients, we made use of the national food composition table of Mali (TACAM), adjusted for the purpose of our research. In **chapter 3,** we evaluated whether the adjusted TACAM is acceptable for assessing average energy and nutrient intakes and for assessing probability of adequacy of intakes for selected micronutrients at population level. Among a sub-sample of the women selected from the study population described in chapter 2, the nutrient intake calculated from a one day food weighed record was compared with that based on chemical analysis of duplicate portions of diets of the same day. The results indicated that the use of the adjusted TACAM seems acceptable for estimating average intake at population level for macronutrients, calcium and zinc in a low intake population, but not for

carbohydrate and iron intakes (being underestimated), and vitamin A (being overestimated), nor for probability of adequate intakes and nutrient densities. In contrast, at individual level, significant differences were observed between estimated and analyzed intakes for all the nutrients increasing with higher intakes and use of the adjusted TACAM for estimating individual intakes may lead to considerable bias.

The current consumption patterns as described in chapter 2, made clear that for fonio to have a meaningful contribution to nutrient intake, it is necessary to increase its consumption. As sensory characteristics are important for consumption, we looked into the possibility of selecting varieties on better sensory characteristics in **chapter 5**. The sensory diversity was investigated on 20 fonio landraces in cooked form. The results showed variation between fonio landraces collected in Mali, Guinea and Burkina Faso in visual (colour and presence of impurity) and textural characteristics (consistency of the cooked grain), determining the preference of consumers. This variation may offer to consumers and processors the possibility to select landraces suitable for their products and processes for preferred sensory properties and this may offer an entry point for processors who intend to promote the consumption of fonio.

Another important issue is to know whether landraces different for their visual and textural characteristics could also be different for their nutritive value especially concerning the iron content. This can help to make choices for specific fonio landraces with high iron levels that can contribute to improved iron supply and intake in West African communities. In **chapter 4,** we assessed the genetic diversity of fonio landraces in Mali, the nutrient and phytate content in fonio products and the effect of processing on nutrient content of fonio products. We found no meaningful genetic diversity among the 12 landraces in Mali (indicated by the very low polymorphism level of 3.5%) and the proximate composition, iron and zinc content showed no significant differences among landraces. Traditional processing reduced iron (96% reduction) and phytate (75% reduction) content, however, the molar ratio of phytate to iron remained above the critical cut-off of >1 indicating poor iron bioavailability. Zinc concentration was hardly affected by processing and cooking.

The above highlighted the need to address the double issue of the low level of bioavailable iron due to high levels of phytate in fonio meals, and the low content of native iron particularly due to losses during processing. In **chapter 6**, we investigated the effect of phytic acid degradation with intrinsic wheat phytase and fortification on iron absorption in a study using stable isotopes performed among young Beninese women. The results showed that dephytinisation of fonio porridges with native wheat phytase decreased phytate-to-iron molar ratio from 24:1 to 3:1 and iron fortification further reduced this ratio to 0.3:1. Results also showed that dephytinisation and fortification significantly improved iron absorption from fonio porridges from 2.6% to 8.3%.

Finally, in **Chapter 7**, based on a discussion of our main findings, we conclude that *i)* the current contribution of fonio to daily bioavailable iron intake is low due to small portion sizes being consumed in low frequency, to considerable losses during processing to mid-wet fonio, and to a high phytate-iron molar ratio; *ii)* fonio landraces from Mali, Guinea and Burkina Faso show variation in visual (colour and presence of impurity) and textural characteristics (consistency of the cooked grain), determining the preference of consumers. Selecting landraces for preferred sensory properties may offer an entry point for processors who intend to promote the consumption of fonio and increase its role in diet; *iii)* in absence of meaningful genetic diversity and variation in iron content in fonio landraces in Mali, there is little benefit in selecting landraces for natural high iron content; and *iv)* dephytinisation using intrinsic wheat phytase could be a promising processing practice to improve iron bioavailability and fortification is required to increase the amount of absorbed iron from fonio meals. In addition, the possibility of using whole grain fonio in preparation of "*djouka",* the main form of fonio, should be further studied, as well as the key environmental factors determining the variation in sensory characteristics in fonio. The assumed low bioavailability of contamination iron should be further confirmed. Lastly, the practical feasibility of the used technology of dephytinization and fortification should be studied in the living conditions of West African populations.

Acknowledgments

It gives me great pleasure to thank some nice people who have motivated and supported me making the successful completion of this thesis possible. At risk of inadvertently leaving out some people, I would like to sincerely thank everyone who has contributed in diverse ways and shared this journey with me over the past few years.

Je commencerais par remercier très sincèrement ma chère mère et ma famille qui ont beaucoup souffert de mes multiples absences. J'étais toujours loin quand vous aviez besoin de ma présence. Merci infiniment pour tant d'années de souffrance et de sacrifice. Mes sentiments de gratitude vont aussi à l'endroit de mes frères et sœurs pour leur présence et leur soutien constants, surtout pendant les périodes de dure épreuve. Que vous en soyez remercié. Grand merci à Kadissa Fofana, qui a su garder et s'occuper de la maison pendant tout le temps que j'étais à Wageningen. « *Aw sara ntébolo ».*

There is no doubt that the biggest debt of gratitude is due to my husband Amadou Dembélé and my three lovely children Sidi Ahmed ElBekaye dit Boudama, Hambarké Amadou and Bella Djounou. It was not easy to let me goes abroad frequently, but you have been very wise and kind to make it. Thank you for your love, patience which has seen me through, for your unflinching support and understanding, and for keeping home stable anytime I was away. Thanks to you all for being there, for trusting in me, for being so patient and for your continuous support.

Several colleagues at Institut d'Economie Rural (IER) provided leadership and direction and were the impetus for the beginning of my PhD. In particular, I would like to thank Djibril Dramé for his commitment and guidance facilitating the approval of my candidature by IER. Although, your current position at FAO, Rome, our discussions have not been broken, I greatly appreciate. I am grateful to Dr. Bino Témé, General Director of IER when I started my PhD, for motivating and encouraging me to pursue firstly a master study in US and a PhD thereafter in Netherlands. Thanks for approving my candidature, and for facilitating the administrative facilities. Dr. Oumar Niangado is acknowleged for being my mentor for my Engineer degree graduation, and for motivating and encouraging me to pursue a scientist career.

The collection and analysis of data in this thesis would not have been possible without the support and involvement of different people. My fieldwork in Mali was facilitated and supported by the staff members of IER, colleagues and technicians of Laboratoire de Technologie Alimentaire (LTA), and the FONIO project team members working in Mali, which I thank sincerely. I especially thank Mr Kola Mamadou Tangara, Sidi Fagnan Koné, Mrs Traoré Soungalo F. Traore and Mrs Camara Adam Fane (Food Technology Laboratory) for helping with fonio processing. I was lucky to have good and dynamic team of interviewers and panelists, thank you for all.. Thank you to Dr Oumou Traoré Cissé and Salimata Sidibé Coulibaly, respectively head of the laboratoire de Technologie Alimentaire of IER, for encouragement along the way and to Mme Boré Fanta Guindo for participating to the implementation of the sensory study. I really appreciated the support of Colleagues at Ecofil/IER (Youssouf Cissé, Alpha Oumar Kergnan, Moumini Sidibé, Lamissa Diakité), all the team of programme sorgho, programme coton and laboratoire soil-eau-plante of IER Sotuba.

I owe my deepest gratitude to Dr. Inge Brower, my supervisor and co-promotor who walked with me the way in this research. Inge, thank you for the deep scientific insights you have given me enabling me to develop an understanding of the subject. Not to forget those moments that you just were there and encouraged me to not give up and to keep going on!!!.Thank you very much for being with me and supporting me in the hospitals many times to visit doctor and translation, even in night time, I will never forget and the rest of my life along I will be obliged on you. I enjoyed working with you, I learned a lot from you, particularly how to address challenging scientific issues with a more critical, methodological and open approach. I also admire your strength and your power to create ideas. Your contribution to my research competency, academic and professional development is invaluable. Thank you for the patience and kindness you provided throughout this process. I hope we continue to collaborate for future projects, "*Niéléni*".

Diego Moretti, thank you for being my co-promoter. I am grateful for all your critical comments and scientific inputs to the thesis. I am gratefully acknowledged Michael B. Zimmermann for being my promoter and for your contribution to improve my thesis.

Prof. Dr. Ir. C de Graaf, Dr. C. Mouquet-Rivier, Dr. C. Cercamondi and Prof. B.R. Hamaker, thank you for accepting to be member of my examination committee and for the critical comments during the review of the thesis.

At the Division of Human Nutrition in Wageningen, my stays were made pleasant with the happiness and kindness of a number of people who have contributed in diverse way to arrange my travels, accommodation and permit, and others have helped me immensely in the laboratory during my numerous analyses. Thank you to Jasmijn Mater, Lous Duym, and the strong team of Biotechnion and Agrotechnion secretariat and other administrative staff members of the Division for the discreet but intensive "behind the scenes" work to support the students. To Frans Kok, I say "*Dank u wel"* for your warmness and your great senses of humor that make me feel familiar with the Division. I am particularly grateful to Paul Hulshof, Tineke van Roekel and Betty for all your dedication with the fonio and food samples analyses. Paul, your personal engagement, support and critical comments contributed to the success of this thesis, I really appreciated working with you. Lucy Elburg, thanks for the support, always asking about my health conditions, and for your willingness to reach out a helping

hand, you are grateful aknowledged. To Lucy Okma, Joke Barendse, thanks for your multiple invitations and for allowing me into your home and sharing your meals with me. To Alida Melse and Fré Pepping, thank you for your kindness and the interesting talks we use to. Lucy Elburg and Esmee Doets, thank you to be my my friends and also my paranymphs. I really appreciated your organization and efficient managing that make everything run smoothly for me when I am away. I am grateful to *late* Lidwien van der Heijden for her help in solving personal issues, her contribution to my capacity building, her presence and support during the fieldwork in Mali especially for the interviewer's training. May her soul rest in peace!

To the big team of international PhD students community; Razack Abizaria, Caterine Macharia Mutie, Sophie Ngala, Qing Yu, Rina Agustina, Varsha Rani, Maria-Elisa, Elise Talsma, Sueppong Gowachirapant, Wanjiku Gichohi, Akwilina Mwanri, Nidhi Jaiswal, Eric and Aregash Hafebo, thank you for your support and company through the years. To my country fellow, Bouba Traoré, your presence makes me feel at home in the Netherlands especially during my illness periods. Thank you to my dear colleague, friend and thesis co-fellow, Nadia M.L. Fanou-Fogny. You have devoted your time working closely with me and chairing ideas, pain and sufferance in Wageningen and also in Benin during the field work. Our many long discussions on the topic helped me to shape and refine many ideas and concepts particularly related to our twice thesis. Thank you very much, and also for your recent visit to Mali where we were able to work again together, particularly on the design of the cover page of my thesis. I hope we will have many future opportunities for collaboration for the emergence of nutritional science in major development issues of the developing world.

My project was part of a large program called INCO Fonio project. Thank you to *late* Doré Guindo, former director of CRRA Sotuba (Centre Regional de Recherche Agronomique) and IER coordinator of FONIO project for facilitating the fieldwork of the thesis, and Jean-François Cruz, former coordinator of the FONIO project. I want to especially express my deepest gratitude to Genevieve Fliedel for her personal commitment and support about my thesis work. We had a great moment together in Mali, also at CIRAD/ Montpellier during the field work. Thank you very much for your support, for helping identify funding organizations to pursue part of the discussions at CIRAD/ Montpellier and for organizing my trip to France for the dephytinisation study. I hope we continue to collaborate on future researches and publications to promote fonio consumption in West Africa.

At the point I thought my research was not going to take off because there was no money in my project kitty and also the political situation of my country which was on embargo by the international community. Between doubt and disappointment, I finally got support from the Nutritia Research Foundation under guidance of my wonderful supervisor and co-promoter. Thank you for believing that my research was relevant and for the financial support.

I am grateful to the Dr. Judith Zwartz Foundation for the financial support in printing this thesis.

Finally, I thank all the farmers, car drivers, women and their families, and opinion leaders of respective quarters in Bamako for their participation and cooperation throughout the different phases of the field work.

Thank you all.
Merci.
Aw ni ntié.

Curriculum Vitae

Yara Koréissi was born on November 17th, 1957 in Dia c/ Mopti, Republic of Mali (West Africa). After completing secondary school in 1976, she studied biology at high school (*Lycee de jeunes filles de Bamako)* from 1976 to 1979 where she obtained her certificate/diploma *(Baccalauréat Malian Series Science Biologique Terminale)* in June 1979. She undertook 4 years programs from 1979 to 1984 at IPR *(Institut Polytechnique Rural de Katibougou)* in Mali, where she obtained the degree of Engineer of Applied Sciences *(Ingénieur des Sciences Appliquées, Spécialité Agriculture)*. In 1984, she worked as volunteer at IER in the Food Technology Laboratory (LTA) for two years. From 1986 to 1993, she was recruited by the Canadian Micro-Realisation Project and CMDT *(Compagnie Malienne pour le Développement des Textiles)*, respectively to develop and promote women's activities for generating incomes in Bougouni and Massigui districts. In 1994, she came back to IER as research assistant where she was enrolled in 2003 in Master program in USA through USAID scholarship after completing 5 months intensive English course. After having received her Master's degree in Public Health in 2005, she has undertaken a rich career at IER-CRRA Sotuba as senior researcher in food science and nutrition group and was involved in many research project activities as associate researcher. She obtained the PhD fellow program through NUFFIC the Netherlands fellowship program in 2006 and gets enrolled in January 2007 under the auspices of the Graduate School VLAG at the Division of Human Nutrition of Wageningen University for the Fonio Project (European Union/ INCO No 0015403) presented in this thesis. During her PhD project, Yara attended several (international) conferences and courses, and was involved in many other activities at IER. Furthermore, she attended the PhD study tour to Denmark, Sweden and Finland in 2009. In 2010, she was nominated as junior researcher and Head of the unit of Fruits and Vegetables and wild plant foods at LTA, respectively, while coordinating the processing component of the INTSORMIL collaborative research program aiming to develop women entrepreneurship in urban and rural areas. In 2011, she was selected for the African Nutrition Leadership Programme (ANLP). Koréissi likes associative life and community development programs. She militates in many women promotion associations (Malian scientist's women association, Malian engineer women association to encourage young girls in selecting scientific educational domain, and Cité Ifa Baco women association for community based development programs).

List of publications

Publications in peer-reviewed journals

Koréissi-Dembélé, Y., Fanou-Fogny, N., Hulshof, P.J.M., Brouwer, I.D. Fonio (Digitaria exilis) landraces in Mali: Nutrient and phytate content, genetic diversity and effect of processing. *Journal of Food Composition and Analysis,* 2013. **29**(2): 134-143.

Koréissi-Dembélé, Y., Fanou-Fogny, N., Moretti, D., Schuth, S., Dossa, R.A.M., Egli, I., Zimmermann, M.B., Brouwer, I.D. Dephytinisation with intrinsic wheat phytase and iron fortification significantly increase iron absorption from fonio (Digitaria exilis) meals in West African women. *Journal PloS one*, **8**(10), e70613

Fliedel, G., **Koréissi, Y.**, Boré-Guindo, F., Dramé, D., Brouwer, I., Ribeyre, F. Sensory diversity of fonio landraces from West Africa. *African Journal of Biotechnology*, 2013, **12**(15): 1836-1844

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Fanou-Fogny, N., Saronga, N.J., **Koréissi, Y.**, Dossa, R.A.M., Melse-Boonstra, A., Brouwer, I.D. Weight status and iron deficiency among urban Malian women of reproductive age. *British Journal of Nutrition,* 2011, **105**(04): 574-579.

Kennedy, G.L., Fanou-Fogny, N., Seghieri, C., Arimond, M., **Koréissi, Y.**, Dossa, R.A.M., Kok, F.J., Brouwer, I.D. Food groups associated with a composite measure of probability of adequate intake of 11 micronutrients in the diets of women in urban Mali. *Journal of Nutrition,* 2010, **140**(11): 2070S-2078S.

Fogny-Fanou, N., **Koréissi, Y**., Dossa, R.A.M., and Brouwer, I.D. Consumption of, and beliefs about fonio (Digitaria exilis) in urban area in Mali. *African Journal of Food Agriculture Nutrition and Development*, 2009, **9** (9), 1927-1944.

Submitted papers

Koréissi, **Y.**, Doets, E.L., Fanou-Fogny, N., Hulshof, P.J.M., Moretti, D., Brouwer, I.D. Evaluation of food composition databases in low to middle income countries: the Mali food composition table. Submitted.

Other publications

Koréissi-Dembélé, Y. Identification et capitalisation de la technologie alimentaire du fonio adaptée et appliquée par les transformateurs membres des OPF partenaires de Helvetas Swiss inter coopération pôle de San. Rapport d'Etude, Mars 2014. Helvetas Mali, Pole de San.

Koréissi-Dembélé, Y., Mamadou, D., Hamaker, B. Transfert de Technologie: Un Centre d'Incubation à Sotuba au Service des Transformateurs de Céréales. Journal Essor Quotidien National d'Information. Magazine Agriculture. 19 Juin 2013, 52ème année, No 17458, page 7

Koréissi, Y. Capitalisation d'informations sur la filière fonio dans la région de Koulikoro. Rapport d'Etude, Septembre 2008. Programme de Compétitivité et de diversification Agricole. PCDA C1 Koulikoro-Bamako.

Conference Papers and Posters

Koréissi, Y., Fanou-Fogny, N., Hulshof, P.J.M., Brouwer, I.D., Zimmermann, M.B. Nutritive values of fonio and fonio products. Poster presented at the International Food Data Conference, 8th IFDC, October 2009. Institute of Nutrition. Mahidol University. IUNS. Bangkok, Thailand.

Koréissi, Y., Fanou-Fogny, N., Hulshof, P.J.M., Brouwer, I.D. Nutritive values of fonio and fonio products: Micronutrients content of fonio from Central and Southern Mali. Poster presented at the International Congress of Nutrition, 19th ICN, October 2009. Bangkok, Thailand.

Koréissi, Y., Fanou-Fogny, N., Dossa, R.A.M., Brouwer, I.D. Qualité nutritionnelle du fonio. Poster presented to the Salon International de l'Agriculture (SIAGRI), 24-30 Avril 2008. Palais de la Culture, Bamako, Mali.

Koréissi, Y. La filière fonio au Mali: Enjeux et perspectives. Communication écrite et orale. GRET-CIRAD, 5-6 Novembre 2008, Conakry, Guinea.

Overview of completed training activities

Discipline specific courses, conferences and activities	**Institution and Country**	**Year**
Courses		
Basic statistics and advanced statistics for nutritionists	Wageningen, the Netherlands	2006
Food consumption survey (training)	Wageningen, the Netherlands	2006
Production and use of food composition data in nutrition (HNE/VLAG)	Wageningen, the Netherlands	2007
Food perception and preference	Wageningen, the Netherlands	2007
Conferences and meetings		
Quality from soil to Healthy people (VLAG/PERC/EPS)	Wageningen, the Netherlands	2006
Fortification with iron	Wageningen, the Netherlands	2007
Workshop EU Fonio Project meeting	Wageningen, the Netherlands	2007
Workshop, Fonio SIAGRI, APCAM	Bamako, Mali	2008
Restitution of research results about fonio in West Africa	Conakry, Guinea	2008
Workshop EU Fonio Project meeting	Bamako, Mali	2006-2008
Forum of Nutritional Sciences	Wageningen, the Netherlands	2009
19th International congress of Nutrition	Bangkok, Thailand	2009
8th International Food Data Conference	Bangkok, Thailand	2009
African Nutrition Leadership Programme (ANLP)	North-West University South Africa	2011
Post-harvest processing and value addition of food grains	CIPHET, India	2011
Workshop Carousel	Wageningen, the Netherlands	2014
General courses	**Institute and Country**	**Year**
Introduction and advanced endnote course,	VLAG	2006
PhD introduction week 16th edition	VLAG	2006
Basic statistics	Wageningen, the Netherlands	2006
Introduction to statistics	VLAG/HNE	2006
Scientific publishing/ Literature research	VLAG	2009
Scientific Writing and presentation	Wageningen, the Netherlands	2009
Optional courses and activities	**Institute and Country**	**Year**
Research proposal writing and presentations	Wageningen, the Netherlands	2006
Development PhD proposal	HNE	2006
Advanced statistics for nutritionists	Wageningen, the Netherlands	2007
PhD retreats and meetings	Wageningen, the Netherlands	2009
NIHES Summer Course	Erasmus University-NIHES	2009
Training on use of XLStat	CIRAD, France	2011
PhD excursion	HNE	2009

The research described in this thesis was financially supported by the Dutch Embassy of Mali through the Netherlands Fellowships Programmes (NFP) for PhD studies of the Netherlands Organization for International Cooperation in Higher Education (NUFFIC).

Fieldwork research for the thesis was partly financed by the European Union funded Fonio Project FP6/EU/INCO CT-2005-015403, and Egide France.

Financial support from the Nutricia Research Foundation for achievement of the thesis work (writing the last paper and chapter of the thesis and thesis defense activities) is gratefully acknowledged.

Financial support from Dr. Judith Zwartz Foundation for printing this thesis is gratefully acknowledged.

Cover design and layout
Yara Koréissi and Nadia Fanou-Fogny

Printing
Printed by GVO drukkers & vormgevers B.V. Wageningen