Keeping local foods on the menu:

A study on the small-scale processing of cowpea

Yann Eméric E. MADODE

Thesis committee

Promotors
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Professor of Product Design and Quality Management, Wageningen University
Prof. Dr. Ir. D.J. Hounhouigan
Professor of Food Science and Technology, University of Abomey-Calavi, Benin

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Wageningen University

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. M.J. Kropff,
in the presence of the
Thesis committee appointed by the Academic Board
to be defended in public
on Friday 21 December 2012
at 11 a.m. in the Aula.

Yann Eméric E. Madodé

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176 pages.

PhD Thesis, Wageningen University, Wageningen, The Netherlands (2012)

With references, with summaries in Dutch, English and French

ISBN 978-94-6173-435-8

ABSTRACT

Agriculture plays a significant role in the economy of most African countries. Yet malnutrition and micronutrient deficiencies occur regularly. Concomitantly, many carbohydrate rich staple foods and meat products are dumped on the African market and compet strongly with local products. The present thesis studied the potential of indigenous resources and locally developed practices to supply culturally acceptable and nutritious foods to African resource-poor people, using cowpea as model crop. This research is implemented using an interdisciplinary approach, which comprised plant breeding, food science and technology, human nutrition and social sciences. This thesis reports the findings of the research on food science and technology.

This study aimed to (i) characterise cowpea landraces in use in Benin with regard to nutritional, anti-nutritional and functional properties; (ii) determine present cowpea processing methods and eating habits with special reference to the content of cowpea dishes in available iron, zinc and calcium; (iii) assess the effect of the use of alkaline cooking aids on amino acids of cooked cowpea, and (iv) assess the impact of processing techniques on the flatulence generated by the intake of cowpea foods.

The genetic, nutritional and technological characterisation of cowpea landraces in use in Benin showed that a high level of similarity among unpigmented landraces as opposed to pigmented landraces. The cluster of unpigmented landraces significantely differed from the pigmented landraces for their fibre (24 vs. 56 g/kg, d.w.) and phenolics (3 vs. 8 g/kg, d.w.) contents as well as their seed size (200 vs. 139 g/1000 seeds, d.w.) and water absorption capacity (1049 vs. 1184 g/kg, d.w.).

An inventory of 18 cowpea dishes was obtained, which are produced by the combination of the following main unit operations: cooking, dehulling, deep-fat frying, steaming, roasting and soaking. Fermentation and germination are unusual technological practices in West-Africa. Consumers mainly consume *Ata*, *Atassi* and *Abobo*. These dishes contain little available iron because their [phytate] : [iron] molar ratio is above the required thresholds for a good iron uptake by the human body. The incorporation of cowpea leaves in certain dishes resulted in appropriate available iron and calcium potentials.

The constraints to cowpea processing were identified as: their long cooking time, the tediousness of the dehulling process and the perishability of beans and dishes. The local answer to the long cooking time is the use of alkaline cooking aids. These alkaline salts and

the applied cooking conditions did not induce any significant change in the amino acid composition of pigmented landraces. Moreover, the toxicity potentially associated with this practice was not confirmed as no lysinoalanine could be quantified while using up to 0.5 % (w/v) of alkaline cooking aids.

Flatulence was indicated as the main constraint to cowpea consumption. Cowpea hulls are usually pointed as the main responsible for flatulence. In this research, galactose-oligosaccharides that are indigestible for humans and cause flatulence formation were not found in cowpea hulls. Fermentation wih *Rhizopus* or *Bacillus* bacteria reduced significantly the fermentability of cowpea *in vitro* and *in vivo* as compared with traditional processes.

The present study demonstrates the opportunities to improve the quality of cowpea dishes by the incorporation of the leaves and the possibilities to sustain the consumption of cowpea by focusing on soaking and/or fermentation processes.

CONTENTS

1

GENERAL INTRODUCTION AND THESIS OUTLINE

BACKGROUND

Poverty and health concerns in West Africa

In West Africa, agriculture remains the most important employer of the population. Besides, the gross domestic product (GDP) growth induced by agricultural activities is twice as effective as non-agricultural GDP growth in reducing poverty [16]. Nevertheless, numerous people do not live decently on their income and suffer from malnutrition and mineral deficiencies [105]. The income that the poorest live on, is primarily allocated (50-70 %) to staple foods [244] which are increasingly imported rather than locally produced. Indeed, since the shortages of traditional cereals (mainly in Sahelian countries) in the 1970s, non-traditional cereal products such as rice and wheat products have gained importance in the food habits in West Africa [95, 130]. The globalisation of the world economy and the urbanisation have accentuated the integration of imported food items in the food habits, mainly because (i-) local production is less cost effective, (ii-) foreign food surpluses are dumped on the African market [244], and (iii-) imported products are more convenient to use [206]. Therefore, West African people depend highly on external markets for their supply [95], and their diets are changing. In Benin, Sodjinou et al. [228] reported an increasing incorporation of refined cereal products (bread, pasta, white rice, etc.), dairy, fats and sweets in the traditional diet (which is based on local cereals and/or roots and tubers, legumes, vegetables and fruits, fish and meat) as a transitional diet, especially in the urban areas. New public health issues such as obesity, cardiovascular diseases, cancer and diabetes are emerging increasingly from these new diets [162, 184]. In this context, it is worthwhile to explore and improve the quality of traditional foods.

Relevance of traditional foods in addressing nutritional issues

In the European legislation, a traditional food is "a food of a specific feature (s), which distinguish it clearly from other similar products of the same category, in terms of the use of *traditional ingredients* (raw materials or primary products) or *traditional composition* or *traditional type of production* and/or *processing method*, which were established prior to the Second World War" [241]. Kuhnlein and Receveur [125] considered the traditional food system as "all foods identified within a particular culture, available from local natural resources and which are culturally accepted”. In developing countries like most African countries, “traditional foods” could be taken as foods obtained from locally produced raw materials and processed through a specific technique that has been transmitted from generation to generation and withstands temporal changes. All these definitions show the intimate relation between traditional foods, culture, history, available resources and the ecology of the areas where they are produced [29].

According to Behar [29], traditional diets rather than traditional foods should be maintained as they can promote good health. Behar [29] suggested that a good quantitative and qualitative association of traditional foods (cereals, legumes, roots and tubers, fish and meat) and a great emphasis on the integration of fruits and vegetables in the diet would definitely ensure the consumption of a balanced diet. Trichopoulou et al. [240] and Willett [266] ascertained that the traditional Mediterranean diet (high intake of vegetables, pulses, fruits, unrefined cereals, olive oil and moderate to low intake of fish, dairy products and meat; and a moderate but regular consumption of ethanol in the form of wine) provides consumers with a good health. The Mediterranean diet is somehow comparable with the traditional diet of West African people. In Benin, the traditional diet is based on a high consumption of grains (340-790 g/day), vegetables (110-190 g/day), and legumes (70-107 g/day); moderate consumption of fish (31-37 g/day), root and tubers (56-63 g/day and fruits [228]. However, wine is not part of the traditional diet in Benin. In order to substantiate previous statements, West African traditional foods need to be investigated thoroughly in terms of processing techniques and nutritional characteristics [242]. Indeed, national food composition tables in resource–poor countries, when they exist, lack the description of traditional foods and their macro-, micro- and bio-active nutrients as well as antinutrients contents [229]. These types of database are essential for nutritionists, food technologists, dieticians and policymakers as they contain information required to set up strategies to solve food-related health issues.

Cowpea in the food system and social-economic life of West Africa

The diet of West African people is commonly based on the consumption of starchy foods (cereals, roots and tubers and legume grains), vegetables, edible oils and oilseeds, followed by animal proteins when available, and fruits if affordable. During the last decade, in West Africa, 27-65 % of household food expenses was allocated to starchy foods depending upon the living areas (less than 40 % in urban areas, and more than 50 % in rural areas) [209]. In the group of starchy foods, millet and sorghum were predominantly consumed in the Sahelian areas whereas maize, roots and tubers dominated in the coastal areas. However, a considerable increase in the consumption of rice and wheat products was observed in urban areas, and to a lower extent in rural communities. Legume grains, mainly cowpea (*Vigna unguiculata* (L.) Walp.), stand as the third most important starchy food for West African people, despite the recent introduction of soya bean (*Glycine max*) [81].

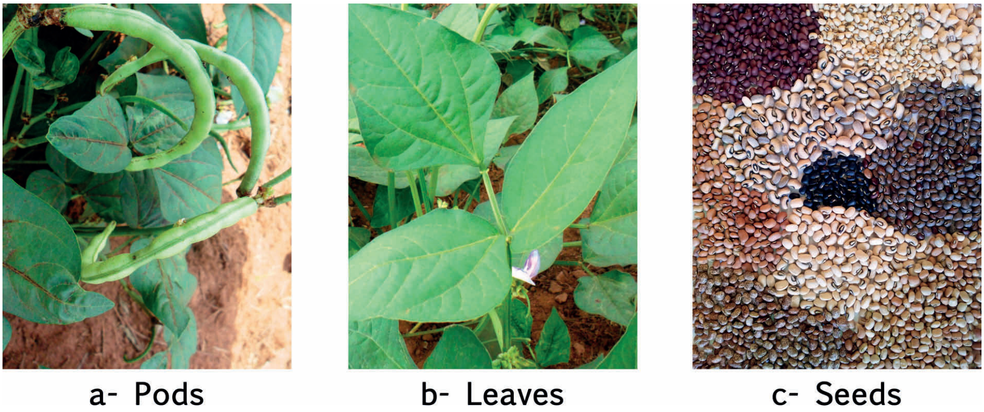


Figure 1

Edible parts of cowpea (*Vigna unguiculata* L. Walp)

Cowpea is an indigenous legume crop originating from Africa [191, 239]. In 2010, the major share (94%) of the world cowpea production was from African farms, in particular West African farms (83 % of the world production) [67]. The 2010 FAO records indicate that 4 618 730 tonnes of cowpea were harvested on 9 273 914 hectares of cultivated land in West Africa. Nigeria, Niger, Burkina Faso, Cameroon and Mali provided most of the West African production. At farm level, cowpea is important because the crop has a short cycle that allows it to be successfully cultivated in semi-arid/arid conditions. Moreover, cowpea does not depend on nitrogen fertilizer as it can obtain its nitrogen from the air by symbiosis with nitrogen-fixing bacteria. Therefore, cowpea is often used as a natural fertiliser and grown as a rotation crop to succeed cereal crops [41]. When harvested, cowpea (dry beans, early maturing pods, leaves; Figure 1) can feed humans, whereas its forage is used as animal feed [4].

In 2012, the mean retail price of cowpea was € 0.80/kg on West African markets [210]. Consequently, the value of cowpea production in 2010 could be estimated at around € 3.7 billion. Cowpea is more than twice as expensive as maize (€ 0.32/kg [210]), but five times cheaper than beef. Consequently, cowpea is an affordable source of protein for the poorest and a cash crop for the growers. For example, Mishili et al. [153] reported that an increase in the income of poor people is usually associated with an increase in cowpea consumption.

Cowpea is strongly embedded in the culture of West African communities. Zannou et al. [272] reported that certain cowpea varieties are used as offerings during local rites related to ancestral spirits, the Vodoun or Fâ oracle. The cowpea landrace called *Atchawe* was mandatorily produced by the farmers to satisfy the local divinities [272].

TELFUN programme to promote local food networks and contribute to food sovereignty

In 2007, the **IN**terdisciplinary **R**esearch and **E**ducation **F**und (INREF) of Wageningen University funded the TELFUN programme meaning "**T**ailoring food sciences to **E**ndogenous patterns of **L**ocal food networks for **FU**ture **N**utrition" for a period of four years. TELFUN aimed at understanding how technological practices developed within food networks can enhance food sovereignty and the nutritional status of people. Two main sociological concepts framed this project: "local food network" and "food sovereignty". The political concept of food sovereignty is considered as the right of people and communities to decide and implement their agricultural food policies and strategies for sustainable production and distribution of food [195].

Globalisation has increased steadily over the past decades, and gradually extended the distance between food production and food consumption. In addition, agro-industrial biotechnology has disconnected (i-) agriculture from its environment (enabling some crops to be produced outside their natural environment), (ii-) agriculture from food production, and (iii-) agricultural products from their intrinsic nutritional quality [214]. The TELFUN programme assumed that the geographical disconnection between production and consumption in global food chains was a potential threat to local food networks. Indeed, local food networks involve various actors who interact within a locality and preserve the meanings associated with local foods. This meaning can be functional (health, taste), ecological (food miles, biodiversity and landscape), aesthetic (diversity vs. standardisation, distinction), ethical (authenticity, identity and solidarity) as well as political (change the power balance in the food chain, orient the production and consumption patterns) [92]. In the context of TELFUN, it was hypothesized that, in the longer term, innovations developed in accordance with the actors of the local food networks can promote food sovereignty. Food sovereignty advocates for a stronger involvement of smallholders in decision making within the agrifood chains. From this perspective, TELFUN was conceived with the principle that its research questions related to the improvement of the local food networks should be initiated and voiced from these networks.

The TELFUN programme was organised as an interdisciplinary research project with a holistic approach to understand the legume food networks of West Africa, India, and Ecuador, to identify and to improve on issues that are relevant for the actors of these networks. Four scientific disciplines were involved in the TELFUN project: Plant breeding, Food technology, Human nutrition and Social sciences. Three cohorts of PhD students investigated legume food networks: the cowpea (*Vigna unguiculata* L. Walp) network in West Africa, the mung bean (*Vigna radiata* (L.) Wilczek) network in India and the lupine (*Lupinus mutabilis* Sweet) network in Ecuador.

In the West African cohort, farmers, processors, consumers and traders were the key actors with whom the four researchers interacted. The present PhD thesis reports on the efforts to understand how cowpea food processors and cowpea consumers utilize the beans and the implications of their processing practices for the quality of cowpeas food products.

COWPEA AT THE FARM GATE

Biotic and abiotic factors usually hamper the production of cowpea. These constraints include insect pests, parasitic weeds and nematodes, which attack different parts of the plant at different development stages. First, the major pests encountered on cowpea in Africa infest the leaves (*Empoasca kraemeri* Ross & Moore, *Amsacta moorei* Botler), the stem (*Ophiomyia* *phaseoli* Trybon), the pods (the pod borer, *Maruca vitrata* Fabricius; *Nezara viridula* Linnaeus; *Riptortus dentipes* Fabricius and the pod sucker, *Clavigralla tomentosicollis* Stål) and perilously the flowers (the bud thrips, *Megalurothrips sjostedti* Trybon; *Maruca vitrata* Fabricius) [108]. These pests drastically affect the yield and the quality of the harvested products [123]. Approaches to reduce the losses induced by these pests include the use of synthetic insecticides on seeds [15], biological control either with plant extracts [189] or with parasites [60], breeding of resistant varieties [69], and environmental management practices [114]. Second, two important parasitic weeds are reported on cowpea in West Africa: *Striga gesnerioides* (Willd.) Vatke and *Alectra vogelii* (Benth.). These parasitic weeds can severely reduce (83-100 %) cowpea yields [22, 40]. Although various measures are used against *Striga*, no single method is entirely controlling the development of the parasite [183]. Resistant varieties have been developed that can withstand the farm colonisation by *Striga* [34]. Lastly, the presence of the root-knot nematodes (*Meloidogyne incognita* and *Meloidogyne javanica*) has also been reported to occur frequently on the farms [178, 218]. Good management of pests, weeds and nematodes during cowpea production is a prerequisite to reach a good cowpea yield (0.8 up to 2 tons per hectare, [57]) and good quality seeds.

In Ghana and Cameroon, cowpeas are evaluated by consumers according to grain size, level of damage to the grains, seed coat colour, and presence of the characteristic black-eye [131]. Cowpea is also appreciated for its nutritional characteristics. Studies carried out worldwide have shown the good nutritional potential of cowpea seeds, leaves and immature pods. The crude protein content of cowpea is relatively high as compared to other grains, namely about 210-360 g/kg dry weight (d.w.) [24, 62, 179]. Lambot [129] reported that cowpea grains contain about 597-716 g/kg of carbohydrates, 29-39 g/kg of ash and 1.1-3.0 g/kg of fat. Dietary fibre is well represented in cowpea (312-340 g/kg d.w.), mainly as insoluble dietary fibre (298-303 g/kg d.w.) [144, 147]. Cowpeas are not a good source of vitamin C as they contain only 5 to 9 mg/kg [174]. Cowpea varieties generally are considered to be a good source of amino acids [107] but they are limited in sulphur containing amino acids (cysteine and methionine). Other amino acids such as leucine, isoleucine, lysine and valine are present in adequate quantities [102]. Calcium and magnesium are the most abundant micronutrients in cowpea [43] followed, at a lower extent, by iron [132, 177]. Phytic acid or phytates and phenolic compounds are frequently mentioned as antinutrients in cowpea. Phytate content ranges from 14 to 29 g/kg d.w. [174, 182, 207]. The phenolic compound and tannin contents range from 7 to 11 g/kg d.w.

THE ROAD FROM HARVEST TO FORK: KEY CONSTRAINTS FOR COWPEA FOOD PRODUCTION

Storage of cowpea

After harvest, cowpea can be stored for a short or long time before processing. During this phase, microorganisms, pests, temperature and humidity can threaten the quality of the beans and lead to losses.

Insect pest control is a major concern in cowpea storage [97]. Mainly *Callosobruchus maculatus* (Fabricius), but also *Bruchidius atrolineatus* (Pic), can severely reduce the harvest within a few months. Usually, beans are infested in the field or get infested later during storage. In the absence of adequate storage conditions, 30-80 % of the beans can be lost because of *Callosobruchus maculatus* infestation [161]. During storage and within the seed, *Callosobruchus maculatus* develops from egg to adult and emerges through a hole, which is the final indicator of the beetle's presence. The occurrence of holes on cowpeas is a quality criterion that decreases its market price and thus the income of producers. Insecticides to combat cowpea beetles, though efficient, were often inappropriately used by farmers leading to health issues. Sand, ash, phyto-pesticides (*Azadirachta indica*, *Hyptis* sp., *Cassia* sp., *Cymbopogon* sp., etc.), solar and/or dry heat treatments, bruchid-resistant varieties and hermetic metal or plastic containers are often used to improve the storage of dry cowpeas [160]. These techniques differ in efficiency, profitability, and health implications for consumers. Nowadays, low cost and chemical-free storage techniques such as solar drying and the use of triple high density polyethylene bags [216] are advocated to drastically reduce storage losses of cowpea. The latter technique is not well spread yet and little is known about its effects on the nutritional and technological properties of stored cowpeas.

Fungi and especially moulds also find in cowpea a favourable growing substrate during storage at high temperature and high moisture [93]. *Aspergillus glaucus* and *Penicillium* spp. [93], *Fusarium* spp. [124], *Aspergillus* spp., *Chaetomium* spp., *Chrysonilia* spp., *Cladosporium* spp., *Monascus* spp., *Phoma* spp., *Neosartorya* spp., *Rhyzopus* spp. and *Mucor* spp. [99] were detected on cowpeas at harvest and during storage. Although cowpea is naturally infested by fungi such as *Aspergillus flavus* and *Fusarium* spp., only very low levels of aflatoxin and fumonisin were detected on cowpeas [99, 124], contrary to maize [27, 35] and sometimes soya beans [109]. Fortunately, cowpeas appear to contain heat-labile (temperature > 60 °C), so far unidentified compounds that inhibit mycotoxin formation [98, 96].

Hard-to–cook (HTC) and hard-shell (HS) defects

In tropical climates, relative humidity and ambient temperature are usually high. Under these conditions (T > 25 °C and RH > 60 %; [31]), legumes develop the HS or the HTC defect. These two phenomena can deteriorate the quality of cowpea with respect to water absorption, protein solubility, cooking properties, colour, texture and nutritive value [221, 243, 271].

The HS defect consists of the failure of beans to absorb sufficient water to soften. The lignification of cell wall components of the beans renders them rigid and hydrophobic [91, 163]. In addition, during seed development soluble phenolics of the hulls can be converted into lignin or dead cells, which accumulate to harden the cell wall [163]. Therefore, it is hypothesized [163] that the presence of lignin induces a low permeability of hulls and reduces the access of water to the cotyledons. The HS defect prevents germination of seeds and reduces the ease of cowpea processing.

Contrary to the HS defect, the HTC defect allows the imbibition of the seed but the seed does not soften under normal cooking conditions (*i.e.*, boiling in water). Several researchers attempted to explain this phenomenon. First, the middle lamellae can be hardened during storage due to the formation of insoluble calcium and magnesium pectinates from phytate – divalent cations and pectin hydrolysis [220]. Second, changes in the properties of protein and starch during the aging of cowpea can induce the insolubility of seed proteins around the starch granules and therefore inhibit their softening [140]. Thirdly, hydrolysis of lipids into fatty acids and further into organic acids and any biological form of acidification can lead to the HTC defect [139, 255]. High pressure-cooking, blanching, irradiation and dry dehulling have proven to shorten the cooking time of legumes. However, these techniques are unaffordable for resource-poor people. The use of alkaline solutions of ash, bicarbonate, potash, rock salts (locally known as *kanwu, kaun* or *kanwa*) has been intensively investigated for its ability to improve water uptake, tenderness, cooking time, and digestibility and reduce the occurrence of antinutrients [247, 249, 250, 252]. However, the diversity of rock salts, their composition and effects on nutritional value (notably amino acid composition) of cowpea have not been investigated.

THE ROAD FROM FORK TO HEALTH AND WELL-BEING: KEY CONSTRAINTS FOR COWPEA CONSUMPTION

Digestibility of macronutrients

Cowpea provides proteins, carbohydrates and dietary fibre as major macronutrients but these are often poorly digestible [165, 199]. The digestibility of cowpea proteins and carbohydrates is compromised by the presence of phytates and tannins, lectins and enzyme (protease and α-amylase) inhibitors [12, 261]. Phytates, polyphenols and tannins are capable of forming complexes with cowpea proteins that render them inaccessible to digestive enzymes [121, 133]. Giami [78] explained that the low digestibility of some cowpea varieties as compared to casein was a consequence of their high phytate content. In addition, phytate and tannins can bind with digestive enzymes as observed during *in vivo* trials [12]. Trypsin inhibitors are important potent inhibitors in soya beans as they are 5-20 times more concentrated in these beans than other legumes [79]. Due to the hyperpancreatic activity that they induce, trypsin inhibitors divert mainly the sulfur-containing amino-acids to the production of to-be-lost enzymes instead of body tissue synthesis [73]. Lectins are sugar-binding proteins. They are capable of agglomerating red blood cells [12]. However, Nnanna and Phillips [169] indicate for cowpeas a low importance of lectins, an intermediate importance of trypsin inhibitors and a high importance of phytates and tannins. The quantitatively less important inhibitors in cowpea are reported to be thermolabile [19, 137] and therefore are highly reduced and sometimes completely eliminated during heat treatments especially when combined with soaking [14, 186, 261].

In the case of carbohydrates, their low digestibility is usually explained by the lack of digestive enzymes in the gastro-intestinal (GI) tract. Galactose – oligosaccharides (GOS, Figure 2), such as stachyose, raffinose and verbascose, resist the digestion through the human digestive track owing to the lack of α- galactosidase secretion to break α (1-6) bond between galactose and other molecules. Dietary fibre is also indigestible along the digestive tract of humans. Mallillin et al. [144] showed *in-vitro* that these dietary fibres from cowpea can produce high amounts of short chain fatty acids, namely butyrate as well as some acetate. Among the carbohydrates, starch can also have a low digestibility as a consequence of the chain length and the amount of amylose [75].

Previous researchers demonstrated the ability of enzymatic treatments [159, 230-232], fermentation [103], germination [168], and soaking followed by cooking [173, 188] to improve the digestibility of GOS. Germination [14, 77, 199], fermentation [44], extrusion [14] and cooking [28, 119, 148] also improved the digestibility of protein and starch in legumes in general. The mechanisms resulting in the enhancement of the digestibility reside in hydrolysis, gelatinisation and removal of antinutrients for starch digestibility and enzymatic breakdown and removal of antinutrients for protein digestibility [148, 207]. Treatments such as roasting, soaking and dehulling, when applied solely, have limited influence on protein and starch digestibility [77, 246].

The poor digestibility of macronutrients in cowpea results in their fermentation by the colonic microflora. This process generates energy, the formation of short chain fatty acids and gas [200]. When the amount of gas generated is excessive and increases the volume of the colon, and also distorts the abdomen and sometimes causes abdominal pain, it is called flatulence. Although this phenomenon is natural, it induces discomfort and inconvenience to consumers. Many people, especially in densely populated areas, may consume less cowpea because of such experiences.

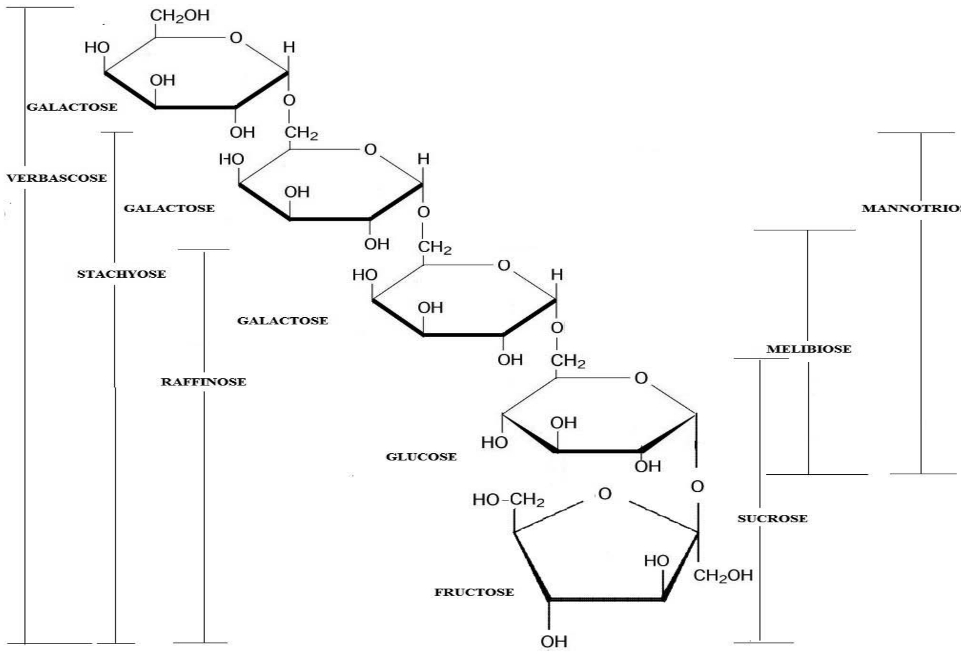


Figure 2

Structures of galactose-oligosaccharides (i.e. stachyose, raffinose and verbascose) and their potential breakdown products

Availability of micronutrients: effect of processing

The limited bioavailability of nutrients is either the result of low levels of those nutrients or, more frequently, high levels of antinutrients that bind or confine them. In the case of cowpea, the latter case applies. Processing is known to influence iron availability. For instance, Latunde-Dada [132] pointed out that cooking improves the dialysable iron content of cowpea significantly whereas dehulling had no effect. Sinha and Kawatra [225] showed a significant impact of cooking, pressure cooking, long time soaking, and germination on phytic acid and polyphenol content of Indian cowpeas. Fermentation decreases the phytic acid content of cowpea as well [58].

Nowadays, many studies consider the benefits and feasibility of biofortification [33, 166, 262], which consist in increasing the bioavailable concentration of an element in edible portions of crop plants through agronomic intervention or genetic selection [262]. Several low phytic acid mutants have been produced by non-transgenic techniques in soya bean [94] with improved mineral levels. However, this kind of modification is costly and has not been realised in cowpea. In addition, the processing properties, nutritional value and acceptance of new genotypes have to be investigated prior successful introduction.

RESEARCH AIM AND THESIS OUTLINE

In the global village that the world has become, the disconnection between food production and food consumption is increasing. Traditional foods are getting neglected. In this context, the TELFUN programme suggests that local food networks, built around traditional food products, can help to strengthen the link between food, culture, and environment. At the long term, enhanced local food networks are assumed to foster food sovereignty.

Cowpea is recognised as the most important legume grain produced and consumed in West Africa. Its embedment into local peoples' practices as a crop originating from the sub-region makes cowpea an ideal vehicle for solving nutritional issues. In the frame of this programme, and from a food technology perspective, the main objective of the present research was to assess the effect of traditional and non-conventional processing on the nutritional quality and the acceptability of the cowpea dishes.

To achieve this goal and help traditional cowpea processors to redesign their processing techniques, the following specific objectives were defined:

I. characterise cowpea landraces in use in Benin with regard to nutritional, anti-nutritional and functional properties;

II. determine present cowpea processing methods and eating habits with special reference to the content of cowpea dishes in available iron, zinc and calcium;

III. assess the effect of the use of alkaline cooking aids on amino acids of cooked cowpea;

IV. assess the impact of processing techniques on the flatulence generated by cowpea foods.

This thesis presents the processing practices related to cowpea dishes as they are produced in Benin as well as the nutritional relevance of these dishes. The quality of cowpea dishes, as they are consumed, depends on the specific quality attributes of the raw materials, the storage conditions before processing and the processing techniques they undergo before consumption. From previous studies a diversity of cowpea landraces is known to be available in different regions of Benin. In chapter 2, we identify and genetically characterise these landraces. The nutritional quality and the functional properties of cowpea landraces in use were assessed. In chapter 3, we present and characterise the main dishes obtained from processing these landraces, the required processing techniques and their effect on the nutrients provided by the raw materials. Moreover, this chapter introduces the long cooking time as a key processing hurdle for the production of cowpea dishes and flatulence as a drawback for cowpea consumption. Therefore, Chapter 4 tackles the long cooking time of cowpea. We investigated how the endogenous solutions to this constraint affect the quantity and quality of the amino acids. Chapter 5 addresses the ability of processing operations to decrease the flatulence potential in cowpea dishes using innovative approaches such as fermentations. Finally, chapter 6 integrates the effects of the processing techniques traditionally used in Benin and suggests new approaches for the production of nutritional and acceptable cowpea foods. Moreover, the added-value and experiences from the interdisciplinary approach of the TELFUN programme are discussed.

2

NUTRIENTS, TECHNOLOGICAL PROPERTIES AND GENETIC RELATIONSHIPS AMONG TWENTY COWPEA LANDRACES CULTIVATED IN WEST AFRICA

ABSTRACT

The genetic relationships among twenty phenotypically different cowpea landraces were unravelled regarding their suitability for preparing West African dishes. Amplified Fragment Length Polymorphism classified unpigmented landraces (UPs) as highly similar (65 %, one cluster), contrary to pigmented landraces (PLs, three clusters). UPs contained, in g/kg dry weight (d.w.), less fibre (24), and phenolics (3) than PLs (56 and 8, respectively), but had bigger seeds (200 g d.w. for 1000 seeds), and lower water absorption capacity at 30ºC (1049 g/kg) than PLs (139 and 1184, respectively). In g/kg d.w., protein (255), ash (39), calcium (0.95), phytate (9.3), iron (0.07), and zinc (0.04) contents were similar. UPs genetic similarities corroborated with their chemical composition and functionality clustered by principal component analysis. Therefore, UPs are well interchangeable regarding chemical composition and suitability for boiled and fried cowpea dishes in contrast to PLs. PLs have potential for innovative product design due to their functional properties.

Keywords: Legumes, Functional properties, Chemical composition, DNA analysis

International Journal of Food Science and Technology, 2012, *47*, 2636-2647.

INTRODUCTION

A landrace is defined by Camacho et al. [39] as 'a dynamic population of a cultivated plant species that has historical origin, distinct identity and lacks formal crop improvement, as well as often being genetically diverse, locally adapted and associated with the traditional farming systems'. Culinary and cultural preferences and secondary uses of the landraces (leaves for sauce, roots for infusions, etc.) are also associated with their preservation and cultivation [13]. Consequently, farmers chiefly cultivate landraces, which have social and economic value for them [115, 273].

In Africa, cowpea (*Vigna unguiculata* (L.) Walp) is considered a 'hunger-season crop' [4]. It is recognised as originating from Africa [191, 239] although a debate is on-going on the story of its domestication. Ng & Marechal [167] identified West Africa as the centre of diversity for cultivated cowpea. A wide range of cultivated cowpea landraces exists in different West African countries as judged on phenotype. Different molecular techniques such as simple sequence repeats molecular markers (SSRs) [20], amplified fragment length polymorphism (AFLP) [65] and random amplified polymorphic DNA markers (RAPD) [25, 273] demonstrated a low genetic diversity in cultivated cowpea. Pasquet [192] showed that the diversity in cultivated cowpea is much lower than the genetic diversity in wild cowpea and postulated its single domestication event. This was confirmed by Ba et al. [25]. Nigeria [254] and North Eastern Africa [46] have also been suggested as domestication centres for cowpea. Timko & Singh [239] suggested the probable domestication of cowpea by different ethnic groups or the contamination of the native gene pool by the cultivated cowpea.

The three most frequently consumed cowpea-based dishes in Benin are as follows: (i) *Atassi* (a mixture of boiled rice and whole cowpea) also called *Waakye*, (ii) *Ata* (deep-fat fried cowpea dumpling from decorticated ground beans), also named *Akara* or *Koose* and (iii) *Abobo* (boiled whole cowpea) [142]. They are also consumed in other West African countries [56]. The behaviour of cowpea during processing of these dishes depends on the content and properties of its proteins, starch, and fibres [83, 217]. Moreover, properties such as fat absorption capacity (FAC) are referred to as critical for the profitability of Ata production as fat is an expensive commodity [171]. Consequently, functional properties are relevant for food processors.

Oluwatosin [179, 181], who studied fifteen varieties of cowpea cultivated in Nigeria, concluded that genotype and environment notably affected their yield and/or physicochemical properties. Zannou et al. [273] identified and genotyped, in Benin, 70 landraces but their nutritional and functional characteristics were not investigated. Nutritional information on such landraces is relevant for enhanced dietary diversification and food security programmes [74]. Up till now, cowpea breeding has mainly focused on crop duration, yield, resistance to major diseases, insect attacks, parasitic weeds, tolerance to severe environmental stresses, protein content and cooking time [223]. Recent studies pointed out the necessity to integrate the needs of the food chain actors (farmers, processors, traders and consumers) early in the procedure leading to the development of new varieties [120, 204]. The satisfaction of these actors can reconnect them to their productive resources, local market and locally processed and culturally accepted dishes. Such a reconnection was identified by Quaye et al. [202] as an essential step towards food sovereignty.

Our study of cowpea landraces was undertaken to assess the relationship between genetics, chemical composition, divalent minerals and complexing compounds, and some functional properties of practical importance to predict the cooking behaviour of cowpea landraces. The ultimate goal is to identify the landraces with the best nutritional and functional properties.

MATERIAL AND METHODS

Seed collection

Seed collection areas

Twenty landraces were collected from farmers and markets in three agro-ecological zones of Benin in 2008, namely the Coastal Sandy Zone (CSZ), the Sudan-guinea Zone with Ferralitic soils (SZF) and the Transitional Sudan-guinea Zone (TSZ) [48] (Figure 1). CSZ and SZF are characterised by a subequatorial climate with four seasons, an annual rainfall of 900–1400 mm and a plant growth period of 240 days. The TSZ is an intermediate area between the Sudan-guinea zone with four seasons and the Sudan-zone with two seasons, which is characterised by 1200–1400 mm rainfall, and a plant growth period of 240 days.

Seed multiplication

After collection, seeds were multiplied in Zogbodomey (7°4’60” N – 2°6’0” E) a district located within the SZF. Seeds were sown in plots at a spacing of 0.80 m between rows and 0.2 m between plants (6.25 plants/m^2) during the major rainy season (April–July 2009). The plots were not fertilised. Weeds were controlled manually by hoeing twice before the end of the season. At maturity, dried pods were harvested and threshed. Foreign material was removed and seeds were sundried until 10–15 % moisture content. Dried seeds were stored in sealed plastic bags at -25 °C prior to the laboratory investigations; the storage lasted more than 6 months prior to the determination of functional properties.

DNA extraction and AFLP analysis

Cowpea seeds were grown in small pots in a greenhouse of the Wageningen UR Plant Breeding group, Wageningen University, till about four leaves’ stage. Two samples of 1 cm^2 leaf material each were harvested from fresh and young leaflets of each landrace. DNA was extracted with the KingFisher (Thermo Fisher Scientific Oy, Vantaa, Finland) using the Agowa Sbeadex Maxi Plant kit according to the manufacturer’s protocol (Agowa genomics, Berlin, Germany).

The DNA concentration was measured using the Nanodrop (NanoDrop Technologies, Inc., Wilmington, DE, USA). Landraces were fingerprinted by AFLP, essentially as described by Vos et al. [258]. About 250 ng DNA was used for a one-step restriction/ligation reaction [18]. DNA was digested with EcoRI and MseI. Pre-amplification was with non-selective primers E01 and M02. Selective amplification was performed with the E44M59, E39M59, E39M60 and E33M60 primers, which were labelled with the 700_dye, while E33M59, E35M59, E35M50, E32M59 and E32M62 primers were labelled with the 800_dye. The polymerised chain reaction (PCR) products were separated on a 6.5 % polyacrylamide gel on a Licor 4200 Global system. About 228 markers were identified. Bands were scored as present (1) or absent (0) for each marker using the Quantar software (Keygene, the Netherlands) and transferred into a binary data matrix.

Crude fat, crude protein, ash and crude fibre

Seeds were milled to flour in a Retsch mill fitted with a 0.5 mm sieve. AOAC methods 27.006, 7.070 and 14.006 [17] were used to determine their crude fat, crude fibre and ash content, respectively.

Crude protein content (N x 6.25) was determined from nitrogen content quantified with Dumas method [112] by using a Flash EA 1112 N analyser (Thermo Electron Corporation, Delft, the Netherlands) and D-methionine (Acros organics, Geel, Belgium) as nitrogen calibration standard.

Iron, zinc and calcium

Cowpea flours were digested using a mixture of hydrofluoric acid and concentrated nitric acid (HNO_3-HF-H_2O_2). An inductively coupled plasma optical emission spectrometer (ICP-OES) (Elan 6000, Perkin-Elmer, Norwalk, CT, USA) was used to determine iron, zinc and calcium content of the digested cowpea flours [238].

Mineral complexing compounds

Phytate content (IP6) was assessed in duplicate according to Bentsink et al. [30] using an IonPac AG11- HC guard column and a Dionex IonPac AS11-HC analytic column. Eluent and elution were set as follows: 0–15 min, 25–100 mM NaOH; 15–20 min, 500 mM NaOH to rinse the column; 20–35 min, 25 mM NaOH to equilibrate the column.

Total phenolic compounds (TPC) were extracted in duplicate with HCl/methanol (1:100) and subsequently measured by the method described by Singleton and Rossi [224] which was modified as follows: 5 mL of water, 1 mL of extract, 1 mL of Folin–Ciocalteu's phenol reagent (Merck, Darmstadt, Germany) and 1 mL of saturated sodium carbonate solution were mixed. Then, the volume of the solution was adjusted to 25 mL with Millipore water (0.22 μm) and the mixture was incubated for 15 min. Absorbance was measured at 725 nm. Freshly prepared blanks and standard (Tannic acid, EC 215-753-2, Sigma Aldrich, Steinheim, Germany) were analysed according to the same procedure.

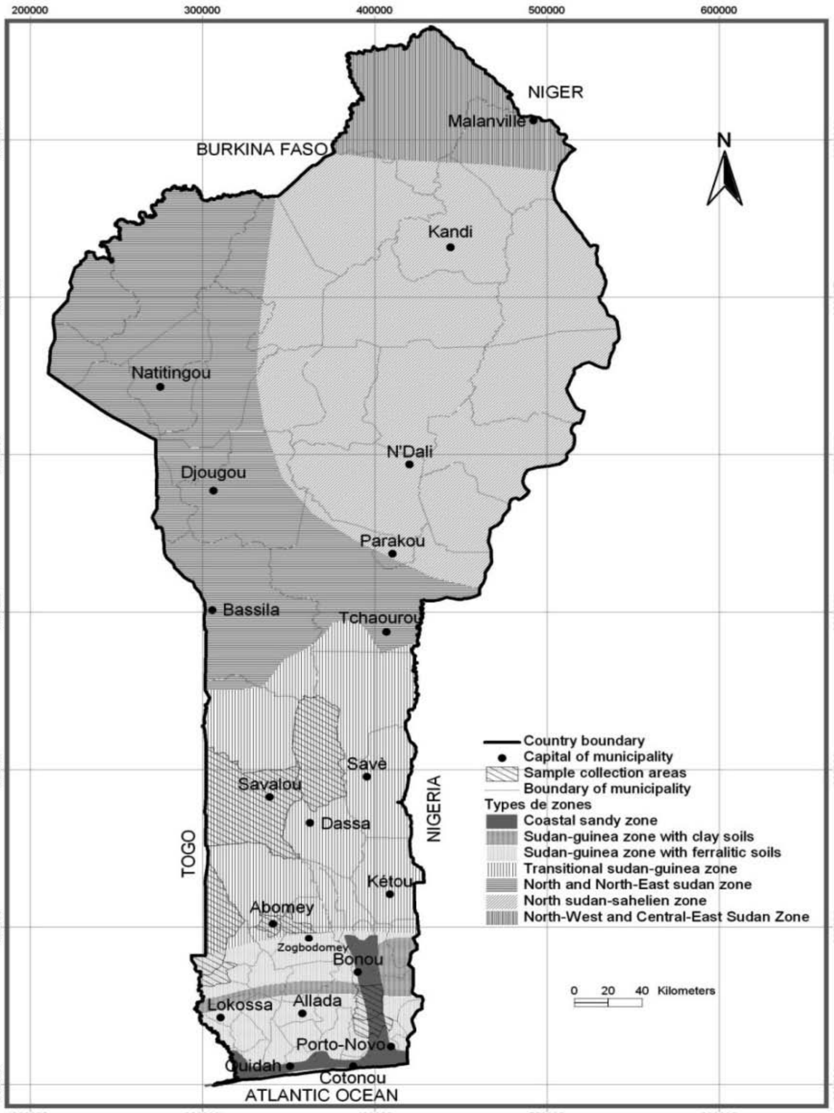


Figure 1

Map of Benin indicating municipalities of landrace collection

1000 Seed weight

Thousand seed weight (1000Sw) was determined in triplicate as described by Kayode et al. [115].

Functional properties

Water and fat absorption capacities (WAC and FAC) were determined as described by Ghavidel and Prakash [76] and expressed in g of water or oil absorbed per kg of cowpea flour dry weight (d.w.).

To determine foaming capacity and stability (FC and FS), 2 g of cowpea flour was mixed with 100 mLof distilled water and blended for 6 min with an Ultra-Turrax emulsifier at 12 000 rpm at ambient temperature (28–30 °C). The contents were immediately transferred quantitatively to a 250 mL measuring cylinder and the volume of the foam recorded. Foaming capacity was expressed as the percentage of volume increase against the initial volume of the suspension. Foam stability was determined by monitoring the fall in the foam volume as a function of time for 120 min at 28–30 °C and was expressed as the foam stability at 120 min (FS120).

Survey of cowpea processors

Beninese food processors from two cowpea production areas (Savalou and Abomey) and two areas where cowpea is not produced (Porto-Novo and Cotonou) were interviewed to assess their perception of the required quality of raw materials for *Atassi* production. Sample size was set according to Dagnelie [49]. In each community, the proportion of *Atassi* processors was assessed through a random check on 175 people, selected at the main market of the locality, during a market day. *Atassi* processors identified that day helped to find the other processors active in the region. In total, fifty two *Atassi* processors (twenty two in production areas and thirty outside production areas) were identified in the neighbourhood of the main market of these localities.

Data analysis

A similarity matrix was generated on the basis of AFLP band scorings using the Jaccard similarity coefficient (JSC) to determine genetic diversity [55]. A dendrogram was obtained using the unweighted pair group method of arithmetic mean (UPGMA) algorithm implemented in the NTSYS package (version 2.1) [212]. The statistical package IBM-SPSS 19.00 was used to process the data on the properties of the landraces. The characteristics of the clusters were compared using the Multivariate ANOVA followed by the Student Newman and Keuls post hoc test. Physicochemical and technological properties of cowpea landraces were subjected to a principal component analysis to cluster these landraces on the basis of the parameters that best characterise them. Five properties (seed weight, TPC, fibre, phytate and WAC) were considered to run the PCA as the determinant of the correlation matrix obtained from these properties (0.046) was greater than 0.00001 as recommended by Field [70]. Moreover, the Kaiser–Meyer–Olkin measure of sampling adequacy (0.7) is higher than the threshold of 0.5 recommended by Field [70]. Kaiser criterion of retaining components with eigenvalues greater than 1 and the average communalities value (greater than 0.6) were used to determine the number of components. The PCA was followed by an oblimin (oblique) rotation as the variables appeared to be correlated. The groups obtained from this data reduction analysis were compared with the clusters obtained from the genetic analysis.

RESULTS AND DISCUSSION

Genetic discrimination of cowpea landraces and relation with the collection areas

The dendrogram depicted in Figure 2 shows two main clusters, namely C1 and C2 with two sub-clusters each: C11, C12 and C21, C22. These sub-clusters contain eight, five, four and three landraces, respectively (Table 1). All the unpigmented landraces in our collection are in cluster C12.

The two major clusters in the dendrogram suggest that the cultivated cowpea landraces collected in Benin probably have two different origins. Wild cowpeas may have been domesticated by different socio-cultural groups [239] following multiple selections according to the production environments and the needs of their users.

Table 1

Genetic clustering and physical characteristics of collected cowpea landraces

Sub-cluster (C)	Landraces (local names)	Agro-ecological Origin	Seeds physical characteristics		
			Testa texture	Colour	1000Sw (g d.w.)
C11	*Adjawan*	CSZ	Smooth	Streaked brown	200.0
	Sowetin	CSZ	Smooth	Streaked light brown	116.7
	Nanwi	CSZ	Smooth	Pure black	105.7
	Adjohoun	CSZ	Smooth	Brown	165.2
	Kpeikoun	FSZ	Smooth	Maroon	107.1
	Sewekoun	FSZ	Smooth	Maroon	131.3
	Kplobe wewe	FSZ	Smooth	Light Brown	110.8
	Laivi	TSZ	Smooth	Creamy	92.6
C12	*Atchawe-tola*	CSZ	Wrinkled	Unpigmented	222.9
	Kodjovi	TSZ	Wrinkled	Unpigmented & black spots at bottom	214.9
	Aiglo	TSZ	Wrinkled	Unpigmented	177.7
	Togovi	TSZ	Wrinkled	Unpigmented	167.1
	Malanville gros grains	TSZ	Wrinkled	Unpigmented	215.3
C21	*Teivigboto*	CSZ	Smooth	Maroon	128.6
	Sokan	CSZ	Smooth	Maroon	124.3
	Kpodjiguegue	CSZ	Smooth	Green grey	112.7
	Deux couleurs	CSZ	Smooth	Light brown and Brown	124.9
C22	*Mahounan*	TSZ	Smooth	Brown	167.5
	Kpletchevi	TSZ	Smooth	Brown	148.8
	Vohounvo	CSZ	Smooth	Brown	144.4

CSZ, Coastal and Sandy zone; FSZ, Ferralitic Soil Zone; TSZ, Transitional Sudan Guinea Zone; 1000Sw, Thousand seed dry weight

The Jaccard genetic similarity coefficient was equal or higher than 85 % for three pairs of landraces: *Teivigboto* and *Sokan* (99%), Kpeikoun and Sewekoun (88 %), *Atchawe-tola* and *Aiglo* (85 %). *Teivigboto* and *Sokan*, collected in Dangbo and Adjohoun, both located in the CSZ, have the same seed colour, testa texture and 1000Sw. It is likely that these landraces are identical as the 1 % dissimilarity (one band difference) shown may result from experimental errors [111, 259]. *Sewekoun* and *Kpeikoun*, from the Abomey region in TSZ, were indicated by farmers as different but have the same seed colour and testa texture. *Atchawe* and *Aiglo*, which were collected in CSZ and TSZ, respectively, are morphologically rather similar. Landraces of these pairs may share common ancestry.

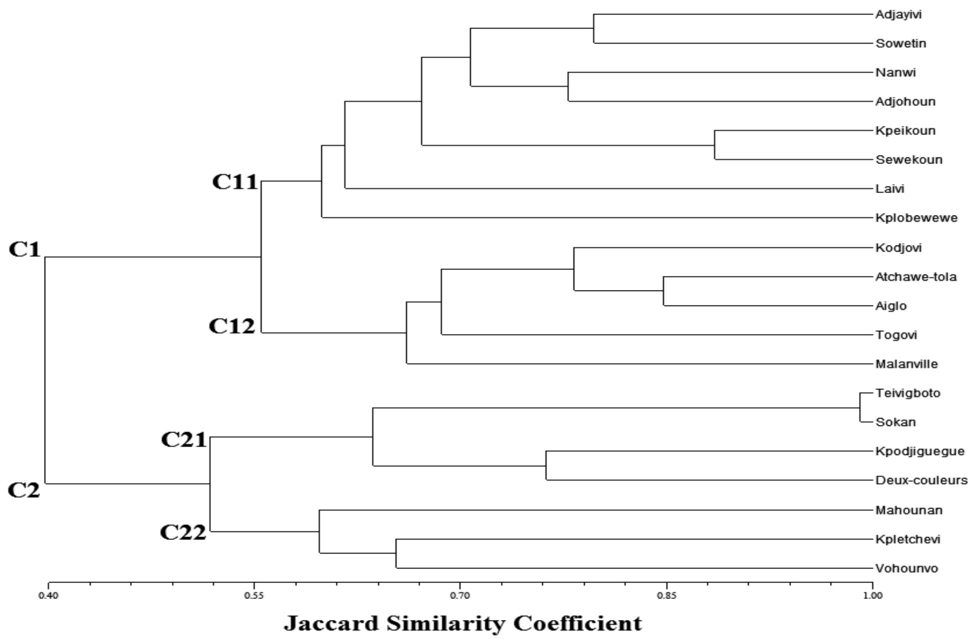


Figure 2

Dendrogram of the genetic relationship among cowpea landraces collected in Benin.

Nutritional composition of cowpea landraces

The protein, fat, ash and fibre contents (g/kg d.w.) range from 218 to 299, 11 to 27, 32 to 43, and 18 to 69, respectively (Table 2) and are in the same range as reported by Longe [141] for varieties obtained from different origins, and grown at a Nigerian research centre, namely 280 ± 45; 19 ± 3; 38 ± 8, 31 ± 6, respectively. Pigmented landraces (C11, C21, C22: 58 g/kg d.w.) contained more than twice as much fibre as unpigmented landraces (C12: 24 g/kg d.w.) (Table 3).

These observations are in line with those made by Morrison et al. [157] who demonstrated that the amount of lignin, a major component of fibre, in pigmented cowpea seed coats was twice the amount found in unpigmented ones. In pulses, tannin content also contributes to seed coat colour and pigment distribution or intensity [37] and tannins are positively correlated to fibre [193]. As fibres are known to be mainly located in the seed coats, seeds with thin coats like unpigmented cowpea are expected to contain less fibre than thicker coated seeds like pigmented cowpeas. In this study (data not presented), we found that when beans are soaked, manually dehulled and dried, the dried seed coat portion represents 4 and 11 % of the weight of the whole white (unpigmented) and brown (pigmented) beans, respectively.

Calcium content varied largely among landraces, namely from 0.7 to 1.4 g/kg d.w. The landraces *Laivi* and *Sowetin* showed the highest content, 1.4 and 1.2 g/kg d.w., respectively. Cowpea landraces contained far less iron (56–104 mg/kg d.w.; *Teivigboto* and *Sokan*, respectively) and zinc (37–54 mg/kg d.w.; *Sokan* and *Sewekoun*, respectively) than calcium. Chinma et al. [43] found higher calcium contents (4.2– 5.8 g/kg d.w.) and lower iron contents (40–48 mg/kg) in cowpea varieties cultivated in Nigeria compared to landraces grown in Benin in our study. Oluwatosin [180] reported similar iron (10–120 mg/kg d.w.) and zinc contents (30–80 mg/kg d.w.) in cowpea landraces commonly used in Nigeria while calcium content was very high (2.5–7.1 mg/kg d.w.). Such differences between studies could be attributed to varietal differences but are more likely to result from differences in soil fertility. Oluwatosin [180] demonstrated that the growing conditions affect the mineral content of the harvested cowpea seeds.

Two antinutrients, namely phenolics and phytate, are known to form complexes with divalent minerals such as calcium, iron and zinc. The contents of these antinutrients are variable from one landrace to the other (Table 2). No statistically significant difference ($P > 0.05$) was observed between the different genetic clusters neither between pigmented and unpigmented landraces (Table 3). Furthermore, TPC occurred in significantly lower amounts ($P < 0.05$) in unpigmented landraces (3.0 g/kg d.w.) than in pigmented ones (8.0 g/kg d.w.). Among pigmented landraces, TPC of genetic sub-clusters C11 and C22 differ from C21. Therefore, although the pigmented landraces are not all alike, they concentrate more TPC than unpigmented ones. Kachare et al. [113] observed the same pattern for Indian cowpea varieties. They proved that the difference in TPC content between unpigmented and pigmented landraces originates from the substantial quantity of phenolics in the seed coats of pigmented landraces; about 50 % of TPC of pigmented landraces comes from seed coats against less that 20 % for unpigmented landraces.

Functional properties

Characteristics of whole cowpea flour and cowpea seeds are presented in Tables 2 and 3. On average, pigmented landraces revealed a better ability to absorb water at room temperature (1458 g/kg d.w.) than unpigmented landraces (1259 g/kg d.w.). Fat absorption capacity at room temperature was similar (928 ± 62 g/kg d.w.) for pigmented and unpigmented landraces (944 ± 63 g/kg d.w.). The foaming capacity was similar for the different landraces and clusters. After 2 h at room temperature, the foam produced from cowpea flours was disaggregated. The remaining foam represented 18–22 % of the initial volumes. High variability of foam stability among landraces was observed. *Aiglo, Sowetin* and *Deux couleurs* showed very low foam stability (Table 2).

Kerr et al. [116] reported about 1560 g of water and 520 g of oil absorbed per kg of cowpea flour d.w. (milled through a 1.0 mm screen). Differences in hardness of seeds from different landraces and particle size distribution of the flours explain the variability in water and oil absorption capacities of our landraces as compared with others [116, 150]. The crude fibre content and WAC, in this study, were highly correlated ($r = 0.66$, $P < 0.01$), confirming that fibre also affects the hydration properties of the flour [83].

As reported previously [142], many cowpea dishes are either fried or deep-fat fried in cooking oils and their storage is problematic. One of the most consumed cowpea dishes is *Ata*. High FAC values have been explained by the high prevalence of nonpolar amino acids in the flour [3] or the high moisture content in the batter prepared for frying [194]. Therefore, if the ideal moisture content in *Ata* paste production is identified, both pigmented and unpigmented landraces could be used to generate low fat containing dumplings. A low fat content is desirable from nutritional and health point of view to prevent obesity [194].

FC values (42.5–44.9 %) measured in this research are comparable with those (40%) reported by as Ghavidel and Prakash [76]. The foaming capacity of the landraces showed no relation with the genetic clusters. Thousand seed weight (1000Sw) varied significantly among landraces (Table 2); the pigmented landraces in our study were smaller (132 ± 28 g d.w.) than the unpigmented ones. A significant Pearson correlation (r) was found between 1000Sw and fibre ($r = -0.762$), TPC ($r = -0.574$) and WAC ($r = -0.621$) at significance level of 1 %.

Table 2

Chemical composition and functional attributes of cowpea landraces

Genetic sub-clusters (C)	Landrace	Crude Protein	Crude Fat	Ash	Fibre	Ca	Fe	Zn	IP6	TPC	WAC	FAC	FC	FS120
		(g/kg d.w.)				(mg/kg d.w.)			(g/kg d.w.)				(%)	
C11	*Adjawan*	291.8	13.0	40.9	61.3	888.6	63.4	42.1	8.6	8.1	1414.7	999.5	43.0	24.0
	Sowetin	262.5	22.2	43.2	55.7	1220.8	66.7	49.9	9.8	8.3	1568.9	867.7	42.0	7.0
	Nanwi	267.5	17.2	36.7	60.1	1157.8	70.7	44.1	6.8	10.5	1569.7	1058.4	41.0	28.0
	Adjohoun	268.2	26.9	39.6	46.3	891.2	64.7	50.0	7.8	9.1	1501.4	945.1	62.0	20.0
	Kpeikoun	298.7	16.5	36.2	57.2	798.0	67.0	46.8	7.5	8.9	1424.9	906.1	44.0	23.0
	Sewekoun	283.6	11.3	38.6	55.8	884.8	69.8	54.1	10.0	7.0	1496.9	820.4	44.0	14.0
	Kplobe wewe	249.9	16.3	40.9	63.0	1006.5	69.4	52.2	8.8	4.6	1533.1	844.8	42.0	21.0
	Laivi	224.2	10.9	42.6	68.8	1444.5	60.1	41.2	15.2	4.3	1356.5	891.1	47.0	29.0
C12	*Atchawe-tola*	244.0	13.8	37.1	24.5	1104.8	64.2	46.5	13.1	2.9	1181.4	943.3	45.0	19.0
	Kodjovi	262.9	15.3	31.8	27.1	880.7	62.8	39.7	7.0	3.4	1212.3	866.6	46.5	33.0
	Aiglo	263.0	17.6	36.5	25.8	1104.0	76.3	44.3	8.9	2.9	1312.5	971.9	42.0	4.0
	Togovi	239.8	16.0	37.1	17.8	987.4	75.8	41.8	9.9	3.6	1237.7	1039.3	42.0	37.0
	Malanville gros grains	218.4	14.1	36.9	25.8	883.0	57.5	39.8	9.9	2.5	1343.8	900.7	37.0	14.0
C21	*Teivigboto*	228.0	14.7	38.2	59.8	1001.2	55.7	40.0	10.2	6.7	1510.8	913.6	46.0	13.0
	Sokan	241.5	16.6	35.9	59.5	1030.3	104.3	36.7	6.7	8.3	1438.6	1007.1	44.0	27.0
	Kpodjiguegue	242.8	14.8	40.3	54.0	1114.4	59.2	45.8	8.4	10.8	1355.5	927.3	44.0	28.0
	Deux couleurs	257.2	16.9	36.9	60.4	689.7	73.7	48.1	6.8	11.7	1520.8	926.7	38.0	5.0
C22	*Mahounan*	259.7	14.7	41.5	48.3	661.6	57.4	39.8	8.5	5.9	1402.4	913.3	46.0	11.0
	Kpletchevi	243.4	14.4	40.7	56.0	998.5	58.9	42.0	11.9	7.2	1335.5	913.0	45.0	24.0
	Vohounvo	273.6	14.3	41.9	56.5	893.3	60.4	40.2	11.0	8.4	1446.5	974.9	39.0	17.0
	Maximum	298.7	26.9	43.2	68.8	1444.5	104.3	54.1	15.2	11.7	1569.7	820.4	37.0	4.0
	Minimum	218.4	10.9	31.8	17.8	661.6	55.7	36.7	6.7	2.5	1181.4	1058.4	62.0	37.0

TPC, Total Phenolic Compounds; WAC, Water Absorption Capacity; FAC, Fat Absorption Capacity; FC, Foaming Capacity; FS120, Foam Stability after 120 mn; 1000Sw, 1000 Seeds dry weight

Table 3

Chemical composition and functional attributes of cowpea landraces clustered based on genetic similarities

Sub-clusters (C)	Colour grouping	Crude Protein	Crude Fat	Ash	Fibre	Ca	Fe	Zn	IP6	TPC	WAC	FAC	FC	FS120	1000Sw
		(g/kg d.w.)				(mg/kg d.w.)			(g/kg d.w.)				(%)		g d.w.
C11 (n=8 landraces)	Pigmented landraces	268 ±24b	16.8 ±5.6a	39.8 ±2.5b,c	58.5 ±6.5b	1037 ±213a	66.5 ±3.5a,b	47.5 ±4.7b	9.3 ±2.7a	7.6 ±2.1b	1483 ±77c	918 ±78a	45.6 ±7.0a	20.75 ±7.1a	129 ±35b,c
C21 (n=4 landraces)		242 ±12a	15.7 ±1.2a	37.8 ±1.8a,b	58.4 ±3.3b	959 ±172a	73.2 ±20.5b	42.6 ±4.9a	8.0 ±1.7a	9.4 ±2.1c	1456 ±71b,c	945 ±40a	43.0 ±4.1a	18.25 ±10.4a	123 ±6.7a
C22 (n=3 landraces)		259 ±14a,b	14.5 ±0.3a	41.3 ±1.7c	53.6 ±5.5b	851 ±154a	58.9 ±1.3a	40.7 ±1.4a	10.5 ±1.7a	7.1 ±1.1b	1397 ±54b	934 ±33a	43.3 ±3.1a	17.3 ±5.9a	154 ±11b
Mean of pigmented landraces ± sd		260 ±22	16.0 ±4.2	39.6 ±2.5	57.5 ±5.8	979 ±200	66.7 ±11.5	44.9 ±5.1	9.2 ±2.4	8.0 ±2.1	1458 ±77	928 ±62	44.5 ±5.8	19.4 ±7.8	132±28
C12 (n= 5 landraces)	Unpigmented landraces	246 ±18a	15.3 ±1.6a	35.9 ±2.2a	24.2 ±3.7a	992 ±105a	67.3 ±7.8a,b	42.4 ±2.8a	9.7 ±2.2a	3.0 ±0.4a	1259 ±66a	944 ±63a	42.5 ±3.7a	21.4 ±12.9a	200 ±24c

Values in this table are indicated as mean ± standard deviation; Measurements are done in duplicate for each parameter. Values with the same letters within a column do not differ significantly ($P > 0.05$)

When WAC was taken into account, our results differ with the ones observed by several authors [23, 267] where a positive correlation between seed weight, water hydration capacity and hydration index was reported for chickpea and faba beans in whole seeds. This discrepancy can be explained by the fact that we measured it on flour instead of entire seed. This could be related to the barrier function of the seeds coat [100]. In addition, seeds with high WAC had also high fibre content.

Suitability of landraces for cowpea food production

According to Patterson et al. [194] FC, particle size of cowpea batter, and hydration properties are important for processing cowpea into *Ata*. Moreover, Nout [171] demonstrated that FAC influences the final fat content of the dumpling. All landraces showed a FC of 42–45 %, except *Adjohoun* (a pigmented landrace), which has the highest foamability values (62 %). The latter landrace also has a high WAC, in the same range as *Sewekoun, Teivigboto, Deux couleurs, Kplobewewe* and *Sowetin*. Although no significant difference was found for FAC, *Sowetin* has one of the lowest FAC as well as *Kplobewewe* and *Sewekoun*. As reported by processors, pigmented landraces are often not used for *Ata* processing owing to the thickness of their seed coat that necessitates tedious manual dehulling as compared to unpigmented ones. The pigmented seed coats and black eyes in the whole pigmented cowpea flours darken the batter and the interior of the resulting *Ata*, diminishing its acceptability by West African consumers, who prefer *Ata* with a light-coloured crumb [194]; dehulling of pigmented seeds could make them attractive for *Ata* making. The hydration and foaming properties of the cotyledons from different landraces should be evaluated for *Ata* processing. Interviewed *Atassi* processors (Table 4) revealed that a brown seed colour (41 % of respondents) and integrity of the beans (indicating the absence or low incidence of weevil infestation in cowpea seeds, 50 % of respondents) were 'very important' quality criteria of cowpea intended for *Atassi* production. In addition, cookability and high swelling ability were considered 'moderately important' (54 and 52 % of respondents). The WAC of the beans is a good indicator of these criteria selected as 'moderately important' by the processors. White and brown cowpeas are the two types of cowpea available to consumers in most markets, especially in urban areas in Benin and in other countries of West Africa [65]. In general, cowpea processors did not emphasise cowpea seed size as a major quality criteria for *Atassi* processing; 62 % of the processors considered it to be irrelevant.

Table 4

Importance of cowpea quality criteria for *Atassi* processors

Quality criteria	Modalities	Very	Moderate	Not
Cowpea colour	Brown	14	13	5
	Unpigmented	6	5	4
	Any	0	0	2
	Total (49)	20 *	18	11
Cleanness	Absent	0	0	0
	Few	24	18	0
	Irrelevant	3	3	4
	Total (52)	27	21	4
Cookability	Fast	2	7	4
	Medium	1	13	10
	Slow	0	0	0
	Total (37)	3	20	14
Swelling ability	High	5	23	10
	Low	0	0	5
	Irrelevant	0	0	1
	Total (44)	5	23	16
Seed size	Small	0	1	6
	Medium	3	8	10
	Big	4	2	4
	Irrelevant	0	0	9
	Total (47)	7	11	29

* : number of respondents that allocated a certain importance to a defined quality criterion

All the compositional and functional properties of the unpigmented and pigmented landraces in our study were similar, except for WAC, 1000Sw, TPC and fibre contents (Table 3). The brown landraces collected in this study were *Adjawan, Adjohoun, Sowetin, Kplobewewe, Deux couleurs, Mahounan, Kpletchevi*, and *Vohounvo*. Of these, *Adjohoun, Deux couleurs*, *Kplobewewe* and *Sowetin* have the best WAC. These landraces could be recommended for *Atassi* (and *Abobo*) processing to those who prefer brown-seeded cowpeas for *Atassi* (53 % of processors; Table 4).

Clustering on the basis of functionality

In Figure 3a, we positioned the twenty landraces as a function of two principal components. Only the two-first components displayed eigenvalues higher than 1 (according with the Kaiser criterion), and the scree plot suggested two meaningful components before the inflexion of the plot. Together and after rotation, these two components accounted for 85.3 % of the total variance in the initial data.

Communalities after extraction and component loadings after rotation are presented in Table 5. Communalities were all greater than 0.6. Therefore, enough variance of the initial variables has been extracted from the selected components. In interpreting the rotated component pattern, we considered a parameter as a good contributor to the variation in the dataset when its component loading is greater than 0.6. Therefore, WAC, phenolic and fibre contents contribute positively to component 1 whereas seed weight (1000Sw) related negatively with this component (Figure 3b). Phytate content characterised component 2. An arbitrary threshold of one is assumed to discuss distribution of landraces in the score plot (Figure 3a) as a solution to the implemented data reduction.

Table 5

Pattern matrix and communalities

Variables	Principal Components Component 1	Component 2	Communalities
Fibre	+0.96*	+0.13	0.89
1000Sw	-0.93*	-0.19	0.83
WAC	+0.81*	-0.26	0.80
Phenolics	+0.68*	-0.46	0.80
Phytate	-0.05	+0.98*	0.95

* : Values greater than 0.6; TPC, Total Phenolic Compounds = Phenolics ; WAC, Water Absorption Capacity; 1000Sw, 1000 seeds dry weight

Consequently, landraces with an absolute coordinate greater than one are considered well represented on the targeted component. Moreover, all landraces located near the origin within a ± 1 area are considered not significantly represented on the component. Group 1 (*Kpletchevi, Teivigboto, Vohounvo, Sowetin, Kplobewewe, Mahounan, Kpodjiguegue, Adjawan, Kpeikoun, Sokan*) showed a low correlation with component 1 and component 2. Group 2 (*Atchawe-tola, Togovi, Malanville gros grains, Aiglo* and *Kodjovi*) and group 3 (*Nanwi* and

Deux couleurs) showed respectively negative and positive correlations with component 1. Group 4 as well as *Atchawe-tola* showed a high positive correlation with component 2. In contrast, *Adjohoun*, *Nanwi* and *Deux couleurs* negatively correlated with component 2. From this description, landraces of group 1 possess no particular trait to clearly differentiate them (Figure 3).

Landraces of group 2, owing to their seed weight, separate themselves from the batch of landraces analysed. Landraces of group 3 showed significantly high fibre and phenolic contents as well as greater WAC. Table 2 confirms that their values for these parameters are among the highest. The landrace in group 4 separated itself from the other pigmented landraces because of its very high phytate content. The same also applies to Atchawe-tola which shows also a high phytate content within the landraces of group 1. The integration of all compositional and functional characteristics of the studied landraces revealed that the unpigmented landraces forming G2 are identical to the landraces clustered as C12 on the basis of genetic similarity, proving that the unpigmented landraces are a very homogenous set of landraces. In contrast, the grouping of the pigmented landraces on the basis of their attributes (G1, G3 and G4) cannot be matched with any of the clusters based on genetic similarities (C11, C21 and C22). Landraces in G1, G3 and G4 are scattered in the clusters C11, C21 and C22. Consequently, pigmented landraces showed more heterogeneity regarding genetic make-up, chemical composition and functional properties. Unpigmented landraces could then easily be used interchangeably without a noticeable effect on the properties of the prepared dishes, which certainly is not the case for pigmented landraces.

CONCLUSIONS

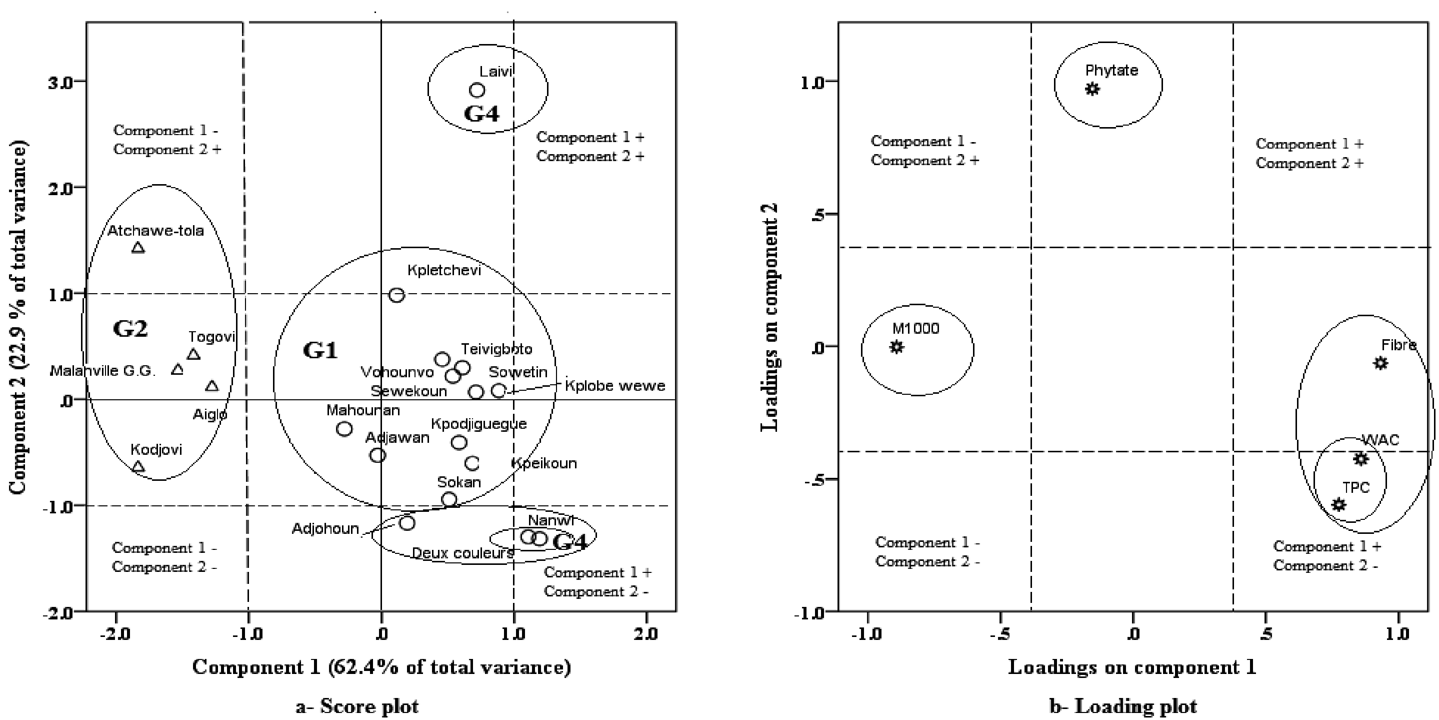
The combined genetic and functional clustering of cowpea landraces showed that unpigmented landraces, although differently named and produced in various zones, have similar genetic, physicochemical and functional properties. These landraces may have the same ancestors. Pigmented landraces, however, were pooled in several clusters that differ with respect to genetic and functional traits. Further studies are required to evaluate the effect of the environment on functional properties of the investigated landraces.

In general, pigmented landraces were as nutritious as unpigmented ones with respect to protein and fat contents. The mineral compositions and phytate content of all the landraces were comparable except for *Sokan*, which showed an outstandingly high iron content (104 mg/kg d.w.) compared with the other landraces. Minerals and phytate contents are known to be affected by environmental conditions; a GxE evaluation may help to identify which landrace to cultivate at which location, for the best result. The analysed landraces revealed different functionalities. However, further research should target parameters such as moisture content, particle size, etc., for a more precise determination of the suitability of a landrace to produce *Atassi*, *Ata* and *Abobo*. Besides, consumers need to be consulted to evaluate the acceptability of the cowpea dishes made from these landraces. This acceptability study is a key step in the selection of the best landraces for each type of food.

ACKNOWLEDGMENTS

This research formed part of a project named TELFUN Tailoring food sciences to Endogenous patterns of Local Food supply for Future Nutrition that was financed by the North–South Interdisciplinary Research and Education Fund (INREF) of Wageningen University.

Financial support for this research was provided by the Netherlands Fellowship Programmes (Grant CF5732/2009) and the International Foundation for Science (Grant E4487-1). We are thankful to Kwadwo Adofo, Fien Meijer and Koen Pelgrom for their contribution to DNA extraction and AFLP analysis.



○ = Unpigmented landraces (Group G2)   △ = Pigmented landraces (Groups G1, G3, G4)

Figure 3

Cowpea landrace grouping based on nutritional and functional characteristics

3

PREPARATION, CONSUMPTION AND NUTRITIONAL COMPOSITION OF WEST AFRICAN COWPEA DISHES

ABSTRACT

In Africa, nutrient intake deficiencies are widespread. We, therefore, investigated the potential contribution of cowpea dishes to the ingestion of several macro- and micronutrients. Processors and consumers were interviewed and cowpea dishes analysed. Energy, protein, iron, zinc, and calcium contents ranged from 1647 to 2570 kJ, 10 to 25 g, 1 to 35 mg, 1.5 to 3.0 mg, 38 to 380 mg per 100 g dry weight (d.w.), respectively. The iron and calcium contents were highest in dishes containing leaves. The consumption of these dishes should be promoted along with research on how to further decrease the associated antinutritional factors of traditional cowpea dishes.

Keywords: *Vigna unguiculata*, Phytate, Polyphenols, Consumption patterns, Benin

Ecology of Food and Nutrition 2011, *50*, 115-136.

INTRODUCTION

The international peasant movement "La Via Campesina" formulated the concept of food sovereignty during the World Food Summit in 1996, as the right of people to healthy and culturally appropriate food produced through ecologically sound and sustainable methods, and their right to define their own food and agriculture systems [127]. The concept of food sovereignty is meant to help overcome or reduce hunger, malnutrition and poverty. From this perspective, Windfuhr and Jonsen [268] reported four core elements for this political concept: (1) the right to adequate food for each person, (2) access to productive resources, (3) mainstream agro-ecological production and (4) trade and local markets development. The right to safe food for all people is linked to the production and consumption of nutritious and culturally acceptable foods. Therefore, it is important to underline the strong linkage between food, culture and locality. Wahlqvist and Lee [260] emphasize that locality matters; local conditions must be considered when a food is to be improved to achieve nutritional targets. Food sovereignty, as a baseline for achieving measurable food security [201] and as a potential solution for the actual food crisis [213], promotes development based on local or indigenous knowledge and practices. Information on such indigenous knowledge and local practices with respect to African food products is lacking in the literature, and may even disappear in actual practice due to increasing globalisation which is largely responsible for the invasion of convenience foods originating from elsewhere into developing country's markets [89]. Indeed, Raschke et al. [205] emphasized the critical need to gather information on traditional African food habits as a starting point to initiate research and education regarding food habits and health implications of food consumption.

In West Africa, traditional food habits of poor populations are associated with limited access to protein from animal sources and diets based on starchy products such as cereals, roots and tubers [184, 228]. Consequently, the population faces protein-energy malnutrition and mineral deficiencies. In 2006, 43% of Beninese people were stunted and 78 % of under-five year old children suffered from anaemia, and predominantly, iron deficiency anaemia [105]. Simultaneously, several indigenous food products exist that have been poorly investigated for their potential to alleviate such deficiencies. This lack of information is also apparent in the fact that no national food composition table exists in Benin [162]. As a result, foreign food composition tables are used. These tables provide data on unprocessed food ingredients, and occasionally, on the effects of single processing operations. Food composition tables for locally processed and consumed foods are urgently needed [229].

This study investigated the diversity in and the nutrient and antinutrient contents of dishes prepared from an indigenous African legume crop, cowpea (*Vigna unguiculata* (L.) Walp). The purpose of this research is to identify cowpea dishes with valuable nutritional properties that can be promoted to improve the nutritional status of local people. Moreover, in this research, compositional data are produced on locally processed and consumed food products that can be used in nutrition research and intervention, public health information campaigns, and academic research.

MATERIAL AND METHODS

Basic Appraisal

A preliminary survey was conducted in six administrative regions of Benin. Fifteen to thirty cowpea households and/or commercial processors per region were individually interviewed using questionnaires with multiple choice and open-ended questions. Socio-demographic data, a listing of cowpea dishes produced locally and the processing techniques used to make these products were recorded.

Quantitative Surveys

The two cosmopolitan and most densely populated cities of Benin, Porto-Novo and Cotonou, hosted the first stage of the surveys as areas where cowpea is not grown. The second stage was held in two rural communities, located in Savalou and Abomey regions, where cowpea is grown. Sample size was set according to Dagnelie [49] as described by Chadare et al. [42]. In each community, the proportion of cowpea-dish consumers or processors was assessed through a random-check on 175 people, selected in the main market of the locality, on a market day. For the in-depth survey, respondents from each group were randomly selected. Consumers' questionnaires included food-consumption frequency of cowpea dishes that ranged from rarely (less than once a week), occasionally (once a week), regularly (2–3 times a week) to often (almost every day). The portion of total food expenses allocated to the regularly- or often-consumed dishes, the reasons for cowpea consumption, and the health disorders associated with cowpea consumption (i.e., bloating, flatulence) were also investigated. Processors' questionnaires included questions on the perception of the quality of raw materials, as well as on end-products, along with descriptions of processing techniques.

Food Composition Analysis

Sampling

For the purposes of this study, a dish was considered to be "a food prepared in a particular way as part of a meal." Approximately 300 g of ten popular cowpea dishes (marked † in Table 1) were collected from five experienced processors throughout South and Central Benin, in regions where the dish is well known. These dishes were collected without the dishes that usually accompany them to make a full meal. Samples were dried in an oven at 60◦C until constant weight was obtained, then blended and kept in a freezer at -20°C prior to analyses.

Dry matter, crude fat, crude protein, ash, crude fibre, and energy

Dry matter, crude fat, crude fibre and ash were determined according to AOAC methods 27.005, 27.006, 7.070 and 14.006, respectively [17]. Crude protein ($N \times 6.25$) was determined from nitrogen content quantified with Dumas method [112] by using a Flash EA 1112 N analyzer (Thermo Electron Corporation, Delft, The Netherlands) and D-methionine (Acros organics, New Jersey, USA) as nitrogen calibration standard. Metabolizable energy (ME) was calculated using the general Atwater factors [269].

Iron, zinc, and calcium

Iron, zinc, and calcium contents were determined by using an inductively coupled plasma optical emission spectrometer (ICP-OES; Elan 6000; Perkin Elmer, Wellesley, Massachusetts, USA) according to Temminghoff [238].

Phytate

Phytate as IP6 (inositol hexaphosphate) was extracted and quantified in duplicate according to Bentsink et al. [30] with minor changes: eluent and elution were set as follows: 0–15 min, 25–100 mM NaOH; 15–20 min, 500 mM NaOH to rinse the column; 20–35 min, 25 mM NaOH to equilibrate the column. DIONEX IonPac AS11-HC analytic column was used in combination with an IonPac AG11-HC Guard column.

Total phenolic compounds

Total phenolic compounds (TPC) were extracted in duplicate with HCl/methanol (1:100), measured following Singleton and Rossi [224] and modified as follows: in 5 mL of water, 1 mL of extract, 1 mL of Folin–Ciocalteu's phenol reagent (Merck, Darmstadt, Germany) and 1 mL of saturated sodium carbonate solution were mixed. Volume was adjusted to 25 mL with Millipore water (0.22 μm) and the mixture was incubated for 15 min. Absorbance was then measured at 725 nm. Freshly prepared blanks and standard (Tannic acid, EC 215-753-2, Sigma Aldrich) were analyzed according to the same procedure.

Data Processing and Statistical Analysis

Survey data were entered in a database with Microsoft Access 2003 (Microsoft Corporation, Redmond, Washington, USA). Correspondence analysis (CA) was performed with SAS v.9 software (SAS Institute, Inc.) to cluster cowpea dishes according to their consumption frequency. SPSS 17.0 (SPSS, Chicago, Illinois, USA) was used to calculate mean values and standard deviations. Analytical data were compared using ANOVA test followed by a Student Newman and Keuls post hoc analysis to generate homogeneous subsets.

RESULTS AND DISCUSSION

Diversity of Cowpea Dishes

Cowpea processing leads to a variety of dishes (Table 1) obtained from beans (90 % of dishes) and/or leaves (10 %). These dishes are mainly produced through a combination of techniques including steeping, dehulling, manual whipping, milling, and cooking. Beans are either prepared alone or in combination with cereals, roots and tubers, and/or cooking oils and seasonings such as salt, pepper, and roasted shrimp, while leaves are prepared by cooking and seasoning.

Cowpea Dishes Solely Based on Beans

Abobo and *Veyi* are boiled beans. Cleaned beans are boiled in a large amount of water and seasoned optionally. While water completely evaporates from *Veyi*, it remains with *Abobo*, resulting in a thick soup. *Abobo* is also known as *Sanzie* or *Tuya* in Northern Ghana [203] and *Ewa* in Nigeria [7]. Cooking of beans is usually time - and fuel - consuming [253]. Therefore, strategies have been developed locally to shorten the cooking time. Cowpea beans can be steeped overnight prior to boiling or boiled in alkaline solutions of sodium bicarbonate or of *kanwu*, a kind of rock salt used as softener. *Kanwu*, also called *Kaun*, *Akaun*, *Kanwa* or *Trona* [152], is mainly a source of carbonate and bicarbonate of sodium and various minerals (Na, Ca, Fe) [63, 247]. The use of *kanwu* as a softener is a common practice in Africa. Uzogara et al. [247] showed that *Kanwu* contributes to a reduction of cooking time during open-pan boiling from 60 min (using tap water) to 35–45 min (in 0.05%–0.1% tenderizing solutions). The combination of pressure-cooking with a tenderizer further reduces the cooking time from 20 to 15 min as compared with pressure-cooking in tap water. In addition, mineral concentrations of boiled beans increase as a consequence of this practice, primarily for Na, but also for P, Ca, Mg, and Fe [247]. Laurena et al. [133] reported more efficient removal of polyphenols by leaching with the addition of dilute alkali during soaking of legumes. *Abobo* or *Veyi* is usually eaten with palm or groundnut oil, or a thick tomato sauce and fermented cassava flour (gari), bread or any type of boiled or fried root or tuber dish.

Table 1

Cowpea dishes from Benin

Food		Plant part used			Main Unit operations												
Names in Benin	Other names	Beans	Leaves	Other ingredients	Roasting	Milling /Grinding	Steeping in water	Wet dehulling	Partial dehulling	Whipping	Deep-frying	Steeping in oil	Slicing	Squeezing	Boiling	Steaming	Description
Abobo†	Sanzie or Tuya (Ghana); Ewa (Nigeria)	X		Salt; *Optional:* Potash, Sodium bicarbonate			O								B		Boiled beans
Ata†	Koose (Ghana); Akara (Nigeria)	X		Salt; *Optional:* Seasonings		M	SW	WD		W	F						Crispy fried-dumplings
Doco or *Ata-doco*†		X		Salt; *Optional:* Seasonings					PD	W	F						Fried-dumplings
Ataclè†		X		Salt		M				W	F	SO					Fritters
Adowè or *Dovlo* or *Adovlo* †		X		Salt, *Optional*: Oil			SW	WD							B		Puree
Fechuada		X		Salt; *Optional:* Seasonings					PD						B		Puree
Magni-magni or *Olèlè* or *Lèlè*†	*Moinmoin* (Nigeria) *Koki* (Cameroon)	X		Shrimps, Salt, Palm oil; *Optional:* Animal protein (fish, meat, etc)		M	SW	WD		W						S	Solid paste in banana leaves or tomato tin
Yoyouè		X		Seasonings	R	M					F						Oily flour
Akpada		X		Seasonings	O	M									B		Thick sauce
Atassi†	*Waakye* (Ghana)	X		Rice grains, Salt; *Optional:* Potash or Sodium bicarbonate or			O								B		Mixture of boiled beans and rice

				Hibiscus sabdariffa' red flowers												
Abla† or *Kowé*		X		Maize flour; Palm oil or Crude fluid palm oil Seasonings	M		WD								S	Steam-cooked dough
Atchonkouin or *Kossibobo* or *Adalou* or *Aibli*		X		Maize grains salt										B		Combined maize and beans
Djongoli or *Zankpiti* or *Gnonmlin*†		X		Maize flour, Palm oil or Crude fluid palm oil, Salt; *Optional:* Seasonings										B		Mixture of boiled beans and maize flour cooked with palm oil
Toubani		X		Salt; *Optional:* Yam or cassava flour, Seasonings, Bicarbonate	M			PD	W						S	Paste packed in tomato tin
Alounganta		X		Grated yam; *Optional:* Seasonings	M				W	F						Fritters
Tchè		X		Yam, Salt										B		Boiled beans and yam
Aiman	Gora (Ghana)		X	Seasonings Palm oil										B		Vegetable sauce
Adjagbé or *Amanmenou*†		X	X	Potash, Crude fluid palm oil, Coarsely milled maize; *Optional:* Cowpea beans								L	Q		S	

Main unit operations are related to beans or leaves processing. O=Optional Operation; R= Roasting; M=Milling; SW=Steeping in Water; WD=Wet Dehulling; PD=Partial Dehulling; W= Whipping; F=Deep-frying; SO= Steeping in Oil; L=Slicing; Q=Squeezing; B=Boiling in plenty of water; S=Steam-cooking; †: Popular cowpea dishes

Ata (Benin), *Akara* (Nigeria), or *Koose* (Ghana) is a cowpea fritter, or snack dish. *Ata* is traditionally made by sorting, steeping, wet-dehulling (hand-rubbing only, or crushing on a millstone or milling in common maize mills followed by hand-rubbing and washing to release hulls), and grinding dehulled cowpea beans into a batter that is whipped to incorporate air. This dough can be seasoned prior to deep-fat frying. The wet-dehulling process is tedious and time consuming (we recorded 35–70 min for 1–1.2 kg of beans). A product similar to *Ata* is *Doco* or *Ata-doco.* Whole or dry-sieved cowpea flour, still containing fragments of hulls, is whipped and deep-fried. *Doco* can be cooled and fried a second time to obtain hard and dry fritters called *Ataclè*, which are immersed in oil (usually palm oil) for preservation. *Ata*, *Doco*, and *Ataclè* are commonly consumed as a side dish with porridges, yam (*Dioscorea* sp.), or sweet potato (*Ipomoea batatas*) fries, *Akassa* or *Lio* (cooked fermented maize dough), and also with mixed gari and oil.

Adowè and *Fechuada* are cowpea purees obtained from dehulled beans. For *Adowè*, beans are steeped and wet-dehulled as for *Ata* processing. Dehulled beans are boiled (with 1–2 g of bicarbonate for 1–1.2 kg of beans). Soft cowpeas are cooked to get a puree, which is salted, and oil is commonly added. For *Fechuada*, beans are parboiled, pounded, and sieved to remove seed coats. The resulting slurry is seasoned and cooked. Usually, white beans are used for *Adowè* and red ones for *Fechuada*. They are consumed after addition of palm, coconut or groundnut oil, or shrimp and thick tomato sauce and served with either gari or bread as desired.

Magni-magni, *Lèlè*, or *Alèlè* (Benin), *Moinmoin* (Nigeria), or *Koki* (Cameroon) is a kind of steam-cooked cowpea dough obtained from steeping, wet-dehulling, and washing of beans. Dehulled beans are ground with seasonings to taste, whipped, mixed with palm oil and salt, and finally wrapped in banana leaves or packed into recycled tins for steaming. Optionally, a source of animal protein (fish, boiled egg, etc.) is added before steaming. *Magni-magni* is usually consumed alone or with *Akassa* in Southern and Central Benin.

Yoyoue is a kind of oily flour. Cleaned beans are roasted, seasoned, ground, and finally deep-fried in cooking oil. *Akpada* is a very thick cowpea sauce. A thick tomato sauce is prepared, to which cowpea flour is added, mixed, and cooked together. *Yoyoue* and *Akpada* are usually consumed with *Akassa* or *Lio*.

Cowpea Dishes Prepared as Mixtures with Cereals

To prepare *Atassi* (Benin) *or Waakye* (Ghana), beans are parboiled in water (51 % of processors), or in *kanwu* or bicarbonate solution (49 % of processors), mixed with cleaned rice (0.2–0.3 as cowpea/rice ratio: 79 % of processors) and cooked together. Another practice of commercial processors is to parboil beans and steep them in the cooking water overnight (38 % of processors) before cleaned rice is added. The alkaline solution serves to soften the beans and, according to users, also darkens the colour of the rice which is a desirable trait. The preferred colour is usually red, but certain types of potash result in a yellowish *Atassi,* which is appreciated in some regions. Red flowers of *Hibiscus sabdariffa* (2 % of processors) are also used to reach the desired red colour. Uzogara et al. [247] explained the change in colour obtained with *kanwu* by a browning process due to the Maillard reaction and oxidation of bean pigments. The type of additive used, according to preferences and localities, can affect iron availability in the end-product. Indeed, *Atassi* prepared with pure sodium bicarbonate will gain no additional mineral content (Fe, Ca, K, etc.), while the use of *kanwu* can improve the iron content. Eyzaguirre et al. [63] showed that two samples of *kanwu* can vary drastically in mineral composition. Therefore, the source, and amount of *kanwu* used, affects the mineral content of the food product. *Atassi* is commonly consumed with a thick tomato sauce, and sometimes combined with spaghetti and animal protein.

Abla is a paste from maize (about 30 %), cowpea (about 30 %) and crude palm fruit extract (CPFE) or refined palm oil (RPO; about 40 %). Beans are wet-dehulled, dried and milled. Fine maize flour is mixed carefully with fine cowpea flour and potash filtrate. This mixture is blended with palm oil to obtain homogeneous dough, which is wrapped in banana (*Musa* spp.) leaves and steam-cooked. Dehulled cowpea flour can be replaced by dehulled beans to obtain *Kowé,* and this is especially done in the Abomey-Bohicon region. *Abla* and *Kowé* are consumed alone or in combination with *Akassa/Lio* or gari.

Atchonkouin, *Kossibobo*, *Adalou* or *Aibli* is a combination of cowpea with maize, mainly prepared in rural areas. Maize is parboiled and, later, boiled together with cowpea. The long cooking time of maize (we recorded 200 min for 1 kg of maize boiled on a charcoal fire) was reported by interviewees to be the main reason for its low consumption.

Djongoli, Gnonmlin or Zankpiti is obtained by preparing *Abobo,* which is mixed with fine maize flour and palm oil (CPFE or RPO) and cooked together. At the household level, the cowpea:maize:oil ratio is usually 3:3:1. However, *Djongoli* may contain far less cowpea at when sold commercially.

Cowpea Dishes Based on Mixtures with Roots or Tubers

Toubani is a kind of *Magni-magni* processed without oil and seasonings. Cowpea is partially dehulled, whipped, moulded in recycled tins or leaves and steam-cooked. Yam (*Dioscorea* sp.) or cassava (*Manihot esculenta*) flour is usually added during the whipping process of *Toubani* production (as a binder) along with sodium bicarbonate. Cowpea can also be used to enhance the acceptability of water yam (*Dioscorea alata),* which is inappropriate for making pounded yam (i.e., a typical meal in Central and Northern Benin). An example is a fritter named *Alounganta*. *Dioscorea alata* tubers are grated, mixed with cowpea flour (5 % of the grated yams' weight), whipped and deep-fried. The addition of cowpea flour is done to improve the nutritional value of the fritters, according to 50 % of the processors interviewed.

Tchè is a kind of *Abobo* obtained by boiling beans together with peeled and sliced yam. *Tchè* is prepared in rural production areas, mainly for traditional ceremonies.

Cowpea Dishes Based on Leaves

Cowpea leaves are commonly boiled and sold in the market as *Aïman* (Benin) or *Gora* (Northern Ghana). Boiled leaves can be seasoned, combined with smoked or fried fish or meat and consumed as a vegetable sauce. Cowpea leaves are also processed into *Adjagbé or Amanmenou* (Benin). Leaves are cleaned, shredded and mixed with *Kanwu* filtrate, CFPE, and coarsely milled maize, and optionally parboiled cowpea beans. The mixture is wrapped in *banana or teak (Tectona grandis) leaves for steam-cooking. The addition of Kanwu filtrate is* done to tenderize the leaves. These leafy products are usually consumed alone, but may also be eaten with *Akassa* or *Lio*.

Importance of Cowpea-Based Dishes in the Beninese Diet

The importance of cowpea dishes in Beninese food consumption habits is analysed on the basis of their consumption frequency and the fraction of total food expenses allocated to their consumption. Figure 1 shows the relationship between cowpea dishes and their consumption frequency through correspondence analysis. The first two axes explain 99.6 % of the collected information, which allows adequate interpretation. All cowpea dishes are well represented on the first axis except *Adjagbé*, which is best represented on the second axis, according to correlations and partial contribution of modalities to each axis. Modalities of consumption frequency are all well represented on axis 1, except for the modality "occasionally," which is best represented on axis 2. *Ata, Atassi*, and *Abobo* are strongly and positively correlated with axis one, as with "often" and "regularly." All the other dishes, except *Adjagbé*, as well as the frequency modality "rarely" are strongly and negatively correlated with axis one. Consequently, *Ata* and *Atassi* can be considered to be consumed "often" by interviewees. *Abobo* is "regularly" consumed and the other dishes, except *Adjagbé*, are consumed rarely. *Adjagbé* and "occasionally" are the modalities best represented on axis 2, and they are positively correlated with this axis. Therefore, *Adjagbé* is considered to be consumed "occasionally" by interviewees.

Ata, *Atassi*, and *Abobo* are processed and consumed by all the sociocultural groups in our surveys. The other dishes are associated with some regions and certain socio-cultural groups. *Ataclè*, *Zankpiti*, *Yoyoue*, *Akpada*, and *Abla* are generally prepared by Fon and Goun people, whereas *Alounganta* is consumed by Mahi people. *Adjagbé* as well as *Kowé* are restricted to Fon people and the Abomey-Bohicon region. *Magni-magni* is attached to Yoruba and Nago, while *Adowè* is a speciality of Mina people. Fon, Goun, Mahi, Yoruba, Nago and Mina are important socio-cultural groups in Benin. Irrespective of the relationship between consumption frequencies, socio-cultural group and cowpea dishes, consumers state that they eat cowpea dishes because of: (1) the nutritive character of cowpea (44 % of interviewees); (2) the "stomach filling" and "energy provision" properties of cowpea dishes (22 % of interviewees); and (3) the high accessibility of cowpea dishes as street foods (16 % of interviewees). Indeed, Uzogara and Ofuya [253] reported cowpea dishes as high energy foods for peasants and farmers. Around 47 % of the respondents allocate more than 10 % of their food expenses to cowpea consumption (Figure 2). A chi-square analysis revealed that the money spent on cowpea dishes is significantly related to location. Indeed, 60 % of the urban respondents allocated less than 10 % of food expenses to cowpea dishes as opposed to nearly 56 % of rural respondents who spend more than 10 % of food expenses to cowpea dishes.

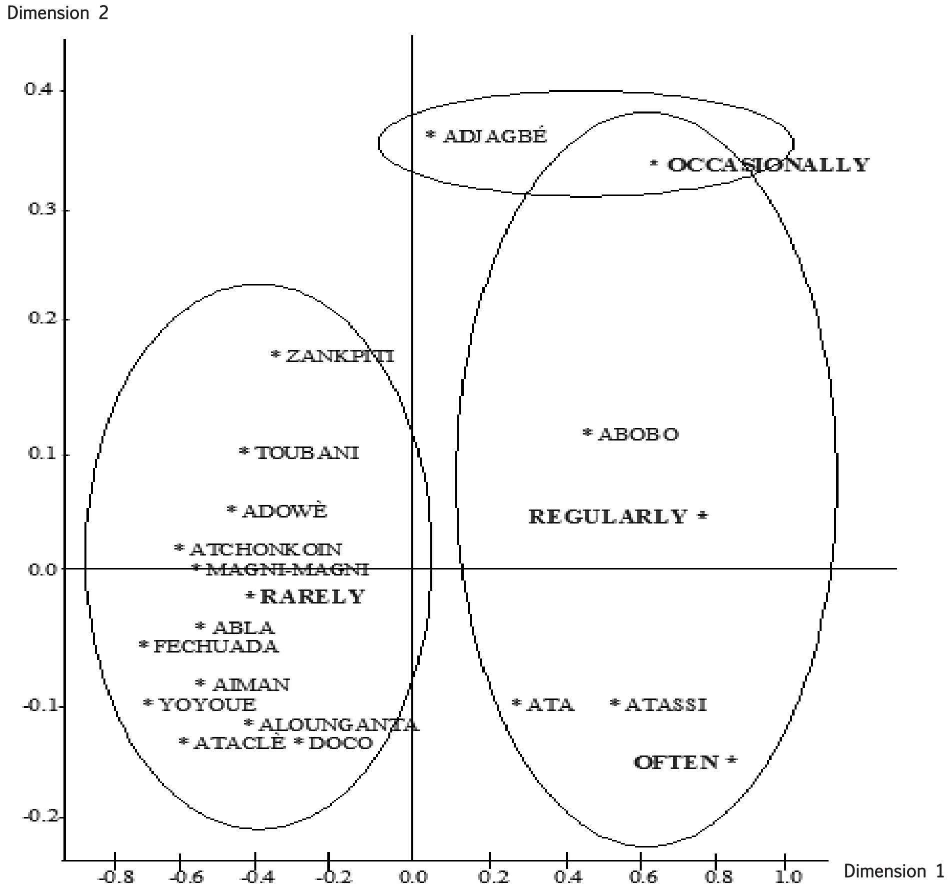


Figure 1

Consumption frequency of cowpea dishes from Benin

This distribution might be influenced by respondents' incomes. At present, about 42 % of the population of Benin lives in urban areas [106], and presumably have higher incomes on average than people living in rural areas. People with higher incomes might consume more meat and less cowpea as a source of protein. In addition, the difference in the consumption of cowpea dishes between urban and rural areas may result from discomforts caused by cowpea consumption (22 % of respondents). Seventy per cent of these complaints, expressed in terms of bloating, constipation, and stomach pain, came from urban areas. Digestion problems also occur in rural areas, but certain effects, such as flatulence, are not perceived as significant problems in rural areas.

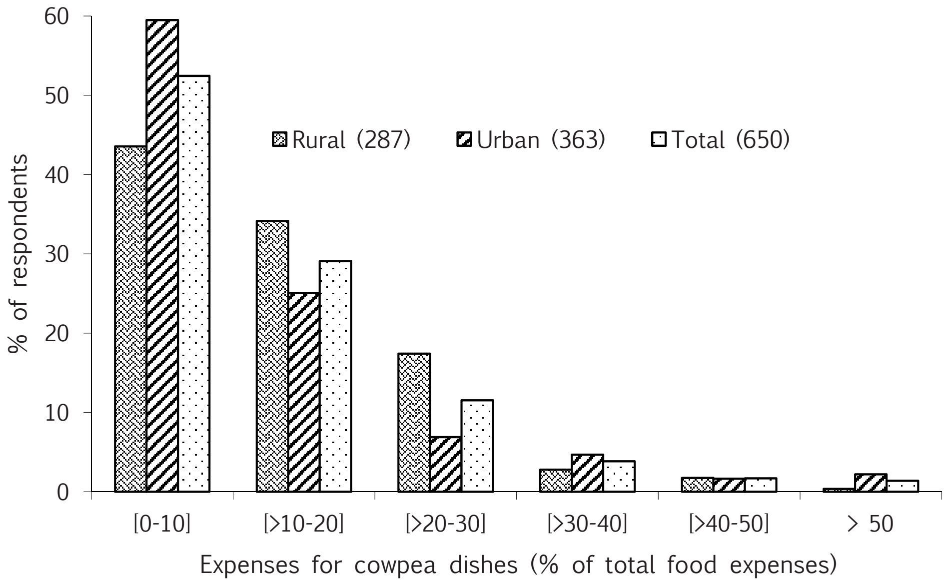


Figure 2

Expenses for cowpea dishes consumption in rural and urban areas of Benin

Bottlenecks in Cowpea Processing and Preservation

Traditional cowpea processing operations were reported to be tedious and time consuming. Processors complain about cleaning, whipping, kneading, and dehulling. To circumvent the constraints related to dehulling, processors in urban areas are accustomed to coarse grinding of cowpea (using cereal or tomato mills) prior to hull removal. Millers often refuse grinding because of the residual and undesired "beany odour" on products milled later. Mills need to be cleaned thoroughly after cowpea grinding, and so the millers who will grind cowpea tend charge extra to cover cleaning costs. Cowpea processors also reported that cowpea dishes have short shelf lives. Most cowpea-based dishes have high moisture (from 18 % in *Ataclè* to 74 % in *Adjagbé*) and fat contents (13 % in *Zankpiti* to 45 % in *Ataclè*).

Therefore, they are difficult to preserve for more than 24 hours at ambient temperature. Most cowpea dishes are either wrapped in local materials (leaves, recycled tomato tins, plastics, etc.) or not packaged at all. They are produced daily for immediate consumption. Multi-purpose intermediate products and/or adequate packaging would facilitate the trade of cowpea dishes enormously.

Proximate, Mineral, and Antinutrient Composition of Cowpea Dishes

Overall, a large variation in proximate composition exists between cowpea dishes and within a particular dish (Table 2), which may be attributed to variations in processing techniques, and to type and amount of varieties and ingredients. Because the analysed samples were not obtained from standardized recipes, they more accurately express the quality of these dishes as they are commonly offered for sale on the market. Crude protein, crude fat and carbohydrate contents, per 100 g d.w., ranged from 9.8 to 25.1, 0.3 to 44.6 and 38.0 to 86.6 g, respectively. Boiled cowpea (*Abobo*) and decorticated cowpea puree (*Adowè*) have high protein contents, comparable with whole cowpea [107, 113] and with *Ewa*, a similar home-made dish from Nigeria [7]. *Atassi*, *Adjagbé*, *Abla* and *Zankpiti* have the lowest protein contents.

Magni-magni and *Ata* are less rich in protein, but have a higher fat content than their Nigerian equivalents (*Moinmoin* and *Akara,* respectively) as reported in Nigeria by Akinyele [7] and Patterson et al. [194]. The average recommended level of protein intake (RLPI) for 6–11 year-old children, as defined by WHO/FAO/UNU [265] is 0.9 g per kg body weight per day. So, a child weighing 26 kg requires 23.4 g of protein daily [264]. Therefore, the main dish of a lunch of 322 g wet basis (w.b.), as given to school children in Eastern Ghana [146], provides 98 and 88 % of the recommended protein intake when it consists of *Abobo* or *Adowè*, respectively. *Adjagbé* (37 % RLPI), *Atassi* or *Abla* (43–44 % RLPI) and *Zankpiti* (49 % RLPI) contribute the least to the daily protein intake as compared with other cowpea dishes.

Furthermore, combinations of cowpea with cereals (as observed in these dishes) compensate for the amino acids deficiencies of both ingredients [122]. However, in this study, we did not investigate the protein quality of these dishes.

Except for *Abobo*, *Adowè* and *Atassi*, all cowpea dishes presented in this study are rich in fat. Indeed, Patterson et al. [194] qualified *Akara* as a high fat food (about 31 % d.w.). Deep-frying and the use of large amounts of fat in combination with the high oil absorption capacity of some varieties add to the high fat content of these dishes. The metabolisable energy of 100 g cowpea dish ranged from 1623 to 2579 kJ. This is relatively high when compared to cereal porridges (1674 – 1704 kJ/100 g) [6, 175]. The crude fibre content per 100 g d.w. ranged from 0.9 to 3.8 g while the inorganic fraction of 100 g d.w. cowpea dish varied from 2.4 to 7.0 g.

Calcium, iron and zinc contents in 100 g d.w. of cowpea dish vary between 38 and 380; 1.3 and 35.3; 1.5 and 3.0 mg, respectively. These products contain more calcium and iron than similar cowpea dishes analyzed in Nigeria. In fact, *Ewa*, *Gbegiri*, *Akara*, and *Moinmoin* were reported to contain 39.7–41.8 of calcium, 1.0–2.4 of iron and 0.9–5.7 of zinc mg/100 g d.w., respectively [7]. The lowest mineral contents were found in *Atassi*, while *Adjagbé* was the best calcium and iron provider among the analyzed dishes. The high mineral content of *Adjagbé* may be explained by the use of cowpea leaves. Greenhouse cowpea leaves boiled in water and drained, contained 1240 mg of calcium, 43.8 mg of iron and 19.7 mg of zinc per 100 g of solids [104]. Moreover, a high amount of rock salt filtrate (known to be rich in minerals and salt) is used in *Adjagbé* processing, thus adding to the amount of minerals.

At a low bioavailability (*i.e.*, 5 % for iron and 15 % for zinc), a 6- to 9- year-old child requires a daily intake of 12.6–17.8 mg of iron and 9.6–11.2 mg of zinc [68]. Therefore, the main dish of a lunch of 322 g w.b. of *Adjagbé* or *Abla* is enough to cover 100 % and 20 % –23 % of the daily requirements for iron and zinc, respectively. Most of the other main dishes, namely *Adowè, Abobo, Magni-magni*, and *Zankpiti*, cover 31 % – 89 % of iron, and 15 % – 31 % of zinc intake requirements. However, the availability of these micronutrients will be less, depending on antinutritional factors present.

Table 2

Proximate (g/100 g d.w.) and mineral (mg/100 g d.w.) composition of 10 cowpea dishes consumed in Benin

Foods***	Ash	Crude Fat	Crude Fibre	Crude Protein	Carbohydrate**	Metabolisable energy†	Calcium	Iron	Zinc
Abla	5.1 ± 1.0 ^{b}	26.2 ± 2.4 ^{c}	2.0 ± 0.3 ^{b,c}	10.0 ± 1.0 ^{a}	58.7 ± 3.2 ^{d}	2163 ± 52.6 ^{c}	94.0 ± 6.0 ^{b,c}	18.0 ± 7.5 ^{b}	2.2 ± 0.1 ^{b}
Abobo	7.0 ± 0.8 ^{c}	1.8 ± 0.2 ^{a}	3.3 ± 0.7 ^{d}	25.1 ± 2.2 ^{c}	65.4 ± 1.7 ^{e}	1623 ± 20.8 ^{a}	112.0 ± 3.7 ^{c}	6.5 ± 1.2 ^{a,b}	2.5 ± 0.7 ^{b,c}
Adjagbé	2.6 ± 0.4 ^{a}	15.2 ± 4.4 ^{b}	3.8 ± 0.8 ^{e}	10.3 ± 1.0 ^{a}	71.9 ± 3.5 ^{f,g}	1974 ± 96.0 ^{b}	380.0 ± 30.3 ^{d}	35.3 ± 12.0 ^{c}	2.6 ± 0.3 ^{b,c}
Adowè	5.5 ± 0.5 ^{b}	1.7 ± 0.3 ^{a}	1.5 ± 0.2 ^{a,b}	24.0 ± 0.6 ^{c}	69.1 ± 0.9 ^{e,f}	1647 ± 10.6 ^{a}	62.0 ± 3.7 ^{a,b,c}	6.4 ± 1.2 ^{a,b}	2.0 ± 0.2 ^{b}
Ata	3.0 ± 0.6 ^{a}	40.3 ± 6.8 ^{e}	1.4 ± 0.2 ^{a}	14.1 ± 1.9 ^{b}	42.7 ± 6.82 ^{b}	2495 ± 141.4 ^{e}	67.5 ± 7.5 ^{a,b,c}	18.4 ± 15.5 ^{b}	2.5 ± 0.3 ^{b,c}
Ataclè	3.4 ± 0.4 ^{a}	44.6 ± 3.8 ^{f}	1.0 ± 0.2 ^{a}	14.0 ± 1.5 ^{b}	38.0 ± 3.5 ^{a}	2579 ± 79.9 ^{e}	62.5 ± 2.5 ^{a,b,c}	13.4 ± 3.8 ^{a,b}	2.5 ± 0.2 ^{b,c}
Atassi	2.4 ± 1.1 ^{a}	0.3 ± 0.1 ^{a}	0.9 ± 0.5 ^{a}	10.8 ± 1.0 ^{a}	86.6 ± 1.5 ^{h}	1670 ± 20.5 ^{a}	38.0 ± 13.6 ^{a}	1.3 ± 1.1 ^{a}	1.5 ± 0.3 ^{a}
Doco	2.9 ± 0.5 ^{a}	32.0 ± 6.1 ^{d}	1.6 ± 0.1 ^{a,b}	15.8 ± 2.0 ^{b}	49.3 ± 4.7 ^{c}	2322 ± 128.3 ^{d}	92.5 ± 16.0 ^{b,c}	8.2 ± 2.4 ^{a,b}	3.0 ± 0.3 ^{c}
Magni-magni	5.1 ± 2.5 ^{b}	24.0 ± 6.2 ^{c}	1.0 ± 0.3 ^{a}	15.5 ± 4.8 ^{b}	55.4 ± 9.0 ^{d}	2119 ± 164.0 ^{c}	57.5 ± 10.3 ^{a,b}	10.9 ± 2.9 ^{a,b}	2.9 ± 0.2 ^{c}
Zankpiti	3.1 ± 1.2 ^{a}	13.4 ± 6.7 ^{b}	2.4 ± 0.8 ^{c}	9.8 ± 2.7 ^{a}	74.4 ± 6.0 ^{g}	1941 ± 132.3 ^{b}	45.0 ± 6.5 ^{a,b}	9.0 ± 3.9 ^{a,b}	2.6 ± 0.3 ^{b,c}

*, Values with the same letter in the same column are not significantly different ($P > 0.05$); **, Carbohydrate by difference (100 d.w. – (protein + fat + ash); ***, For each food, five samples were collected: one sample each from five different processors; †, Calculated metabolisable energy (protein*17 kJ+ fat*38 kJ+ Carbohydrates * 17kJ) expressed in kJ/100g d.w.

Table 3 gives an overview of antinutrients in cowpea dishes. IP6 and TPC in 100 g d.w. of cowpea dish ranged from 0.08 to 0.50 g and 0.07 to 0.91 g, respectively. Most cowpea dishes (9 and 7 over 10 dishes for IP6 and TPC, respectively) were rich in IP6 (0.18–0.50 g/100 g d.w.) and in TPC (0.57–0.91 g/100 g d.w.). *Atassi* contained the lowest amount of these antinutrients. *Doco, Ataclè, Abla* and *Abobo* had a particularly high content of IP6, while *Adjagbé* had the highest TPC concentration.

Table 3

Antinutrients in cowpea dishes from Benin (g/100 g d.w.)

Foods	Polyphenols	Phytate
Abla	0.62 ± 0.2 [c,d]*	0.42 ± 0.1 [c,d,e]
Abobo	0.26 ± 0.1 [b]	0.50 ± 0.1 [e]
Adjagbé	0.91 ± 0.1 [e]	0.18 ± 0.1 [b]
Adowè	0.18 ± 0.1 [ab]	0.36 ± 0.1 [c,d,e]
Ata	0.78 ± 0.1 [c,d]	0.36 ± 0.1 [c,d,e]
Ataclè	0.65 ± 0.1 [c,d]	0.28 ± 0.1 [b,c]
Atassi	0.07 ± 0.0 [a]	0.08 ± 0.0 [a]
Doco	0.57 ± 0.2 [c]	0.47 ± 0.1 [d,e]
Magni-magni	0.67 ± 0.1 [c,d]	0.34 ± 0.2 [c,d]
Zankpiti	0.65 ± 0.1 [c,d]	0.36 ± 0.1 [c,d,e]

*, Values with the same letter in the same column are not significantly different ($P > 0.05$).

The high TPC contents of these dishes are comparable with those reported by Preet and Punia [199] for brown unprocessed cowpea beans in India (0.8–0.9 g/100 g d.w.), and higher than for white cowpea unprocessed beans (0.5 ± 1.4 g/100 g d.w.) and processed ones (0.2–0.5 g/100 g d.w.) [225]. Oluwatosin [181] and Oboh [174] also reported higher IP6 content in beans (1.4–2.9 g/100 g d.w.) as compared to dishes. Akinyele [7] previously demonstrated a reduction of antinutrients in the same type of dish (*Ewa, Akara, Moinmoin*) due to processing. Frequently, an antinutrient reduction due to processing is accompanied by mineral losses [5, 117]. Therefore, the ratio [antinutrient]:[mineral] is a better indicator of the mineral availability. In our study, the [IP6]:[Ca], [IP6]:[Fe] and [IP6]:[Zn] molar ratios range from 0.03 to 0.44; 0.37 to 6.16; and 4.93 to 19.14, respectively (Figure 3).

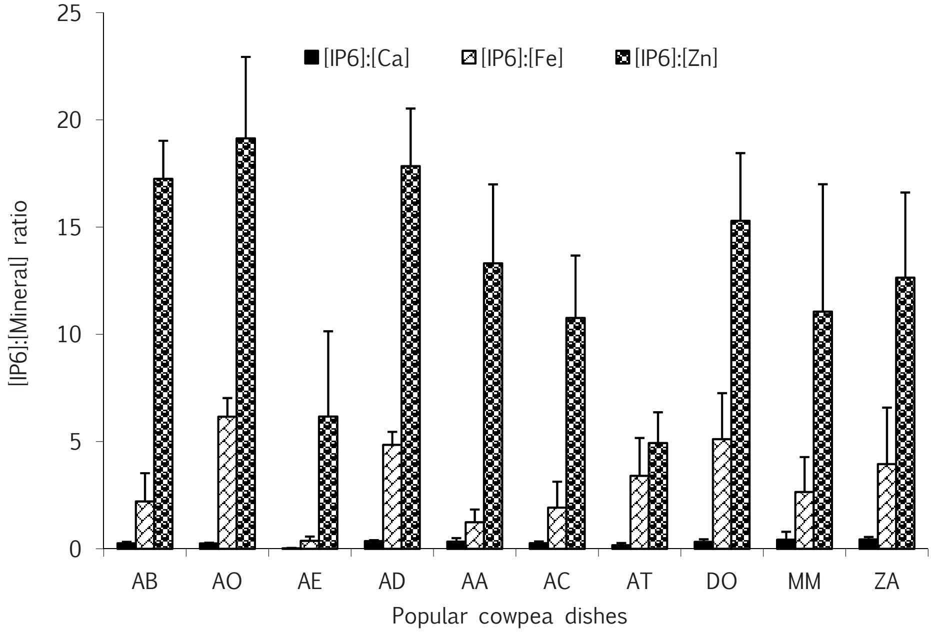


Figure 3

Mineral availability index ([IP6] / [Mineral]) of cowpea dishes

AB: *Abla*; AO: *Abobo*; AE: *Adjagbé*; AD: *Adowè*; AA: *Ata*; AC: *Ataclè*; AT: *Atassi*; DO: *Doco*; MM: *Magni-magni*: ZA: *Zankpiti*

Investigations of Morris and Ellis [156] on adult men recorded an average 13 % – 21 % apparent absorption of calcium at a [Phytate]:[Ca] molar ratio lower than 0.24. *Atassi* (molar ratio 0.17), and *Adjagbé* (molar ratio 0.03) can therefore, be considered dishes that have a moderate to high calcium availability, respectively. Hallberg et al. [86] showed that 10 % available iron could be reached when the [Phytate]:[Fe] molar ratio is equal to 1. Nout [172] reported an adequate (> 70 % of Fe present) iron availability with a ratio ≤ 0.3. Consequently, *Adjagbé* (molar ratio 0.37) is the only cowpea dish with expected adequate iron availability. A [Phytate]:[Zn] molar ratio ≤ 5 indicates a high zinc availability, while a ratio from 5 to 15 predicts moderate availability [263].

Hence, zinc may be highly available in *Atassi* and poorly available in *Doco, Abla*, *Adowè* and *Abobo. Adjagbé* is the cowpea dish in which all minerals were available, probably because cowpea leaves are an important ingredient and phytate levels are reduced. The use of cowpea leaves in cowpea dishes has potential to improve mineral intake. Dephytinisation and removal of phenolic compounds in cowpea dishes warrant additional research to make further improvement possible.

In conclusion, this article contributes to our understanding of indigenous knowledge on cowpea consumption, and also helps to quantify nutritional composition. Moreover, we provided data that can be used in national food composition tables. Our investigations demonstrate that *Ata*, *Atassi* and *Abobo* are the most popular of the 18 cowpea dishes consumed in Benin. They are primarily processed by the following unit operations: steeping, dehulling, milling, whipping, and cooking, whereas fermentation and germination are not applied. Cowpea dishes, processed from beans as well as leaves, have also been established as highly nutritious foods. This is confirmed by analytical data with respect to energy supply and protein content. The mineral contents of dishes prepared with beans are low as compared with dishes that contain leaves as well. A lesser-known dish like *Adjagbé* has been identified as a highly nutritious dish with regard to mineral availability. Mineral binding compounds are present in significant amounts in dishes with beans, while phenolic compounds are the main concern in dishes with leaves. Therefore, strategies should be developed to increase mineral availability in cowpea dishes, especially the ones that contain leaves. Moreover, the consumption of these potentially healthy foods is challenged by the issues of bloating, a long cooking time and dehulling constraints; this issues should also be addressed in future research. Working to improve the nutritional value of cowpea dishes through the methods discussed in this article may help to increase access to adequate food and the development of trade and local market; two essential goals of food sovereignty in Africa.

ACKNOWLEDGEMENTS

The authors thank the North-South Interdisciplinary Research and Education Fund (INREF) of Wageningen University that initiated this research through a project named TELFUN: Tailoring food sciences to Endogenous patterns of Local food supply for FUture Nutrition. We, hereby, gratefully acknowledge the International Foundation for Science (IFS) for its financial support (Grant E4487-1) and Dr. Ir. Romain Kakaï-Glèlè for his help during the statistical data processing.

4

EFFECTS OF ALKALINE COOKING AIDS ON THE COMPOSITION AND THE AVAILABILITY OF AMINO ACID IN COOKED COWPEAS (*Vigna unguiculata*)

Chapter 4

ABSTRACT

In West and Central Africa, legumes are traditionally cooked using alkaline cooking aids (*Kanwu*) to shorten the cooking time. The effect of the mineral composition and concentration (0.05; 0.1 and 0.5 %, w/v) of these cooking aids on the amino acid composition and nutritional quality of pigmented (brown) cowpea was investigated. The composition of *Kanwu* varied significantly according to its origin, though it is mainly a good source of sodium and bicarbonate. Up to 25 % cooking time reduction could be achieved upon boiling cowpeas in normal concentrated *Kanwu* solution (0.1 %). No significant loss of amino acids was observed, neither was the available lysine content affected by the use of cooking aids at a concentration below 0.5 %. The high pH (8-10) induced by the use of cooking aids and the high temperature reached during boiling (100 °C) did not lead to the formation of a detectable amount of lysinoalanine.

Keywords: *Kanwu*, Bicarbonate, Baking soda, Potash, Monovalent cations, Lysinoalanine

Submitted

INTRODUCTION

Cowpea is a legume grain and therefore a plant source of protein containing 200-300 g of protein per kg dry weight (d.w.) [142, 198]. The quality of the proteins is restricted by their poor digestibility, which is a consequence of the presence of several anti-nutritional (phytate and polyphenols) and anti-physiological (trypsin and amylase inhibitors) factors. Moreover, cowpea proteins are limited in some essential amino acids, namely the sulphur-containing amino acids. Therefore, their complementation with cereal proteins improves their biological value, even to a value comparable to that of animal proteins.

Food processing affects the quality of plant proteins [118, 138, 158]. In West Africa, cowpea processing involves various treatments like dehulling, milling, soaking, frying, roasting, boiling, etc. among which cooking in water is the predominant preparation method. At household level, the desire to cook cowpeas within a short period of time has led to the use of cooking aids such as sodium bicarbonate (Na_2CO_3), traditional rock salts (locally known as *Kanwa, Kanwu, Kanmu, Kaun, Akanwa, Trona, Magadi*; [247]) or potash or wood ash [245]. These cooking aids are also used to soften leafy vegetables [59, 142] or reduce the sour-acidic taste of some foods. Cooking beans in solutions rich in monovalent cations accelerates water uptake and softens beans faster [248]. Moreover, the use of cooking aids improves protein digestibility [134, 187], decreases the content of antinutrients and darkens the beans [133, 249]. However, a decrease in B-vitamin content and amino acid content was reported by Uzogara et al. [251] and Minka et al. [152], respectively.

The rock salt *Kanwu* contains bicarbonate, carbonate and minerals. The exact composition of *Kanwu* depends on its source [247], and it forms alkaline solutions in water. These alkaline conditions and the high temperature during cooking can lead to the formation of cross-linked amino acids (mainly lysinoalanine) and the disappearance of amino acids (lysine, cysteine, etc.) from foods [72, 143]. Sternberg et al. [235] reported that lysinoalanine production was induced in proteins at a pH value above 10.5 and a temperature of 25 °C or above pH 8 in boiling water. The toxicity of lysinoalanine is under debate as Woodard and Short [270] indicated that lysinoalanine induce renal disorders in rats whereas Corpet et al. [45] showed recently no colonic cancer in rat after lysinoalanine consumption. However, it is clear that no implication for primates and humans has yet been claimed. Besides, Hayashi [88] showed that lysinoalanine has the ability to chelate metal ions.

To date, limited data are available about the chemical composition of cooking aids in use in West Africa and their possible effect on amino acid composition of cooked cowpeas. The present study aimed at assessing the effect of *Kanwu* and sodium bicarbonate on cooking time, amino acid composition and available lysine content of cowpeas.

MATERIAL AND METHODS

Cowpeas and rock salts collection and preparation

A pigmented (brown) cowpea landrace, locally known as *Adjayivi*, was obtained from retailers on the wholesale market of Dantokpa (Cotonou, Benin). This landrace is known to require a longer cooking time than white landraces. On the same market, two types of *Kanwu* (grey *Kanwu*, Gk, and yellow *Kanwu*, Yk) were purchased, both types from two retailers. The two samples of each type of *Kanwu* were mixed in equal amounts and milled to a fine powder.

Sodium carbonate, sodium bicarbonate, calcium carbonate, potassium carbonate and sodium chloride were obtained from Merck (Darmstadt, Germany).

Chemical composition of *Kanwu*

Dried *Kanwu* powders were digested with hydrofluoric acid and concentrated nitric acid (HNO_3-HF-$H2O_3$). Next, the contents of calcium, iron, potassium, sodium and zinc were determined in duplicate by inductively coupled plasma optical emission spectrophotometry (ICP-OES) (Elan 6000, Perkin-Elmer, Norwalk, CT). The method described by Temminghof [238] was used and data expressed in g/kg d.w.

The concentration of carbonate and bicarbonate in *Kanwu* was determined in triplicate by titrating 200 mL of a 0.02 % (w/v) *Kanwu* solution against 0.1 M HCl. The concentrations of carbonate and bicarbonate in the solutions were calculated with the equation of de Levie [53] for simultaneous titration of bicarbonate and carbonate.

$$\frac{Va}{Vb} = \frac{(2[H+]^2+[H+]Ka)Cb1+([H+]^2-Ka1Ka2)Cb2}{\frac{[H+]^2+[H+]Ka1+Ka1Ka2}{Ca-\Delta}} + \Delta \qquad (1)$$

with V_a and C_a, volume (L) and molarity of the titrant (HCl); V_b, volume of titrated solution (*Kanwu* solution, L); C_{b1}, molarity of sodium carbonate (mol/L); C_{b2}, molarity of sodium bicarbonate (mol/L); Ka_1 and Ka_2, acid dissociation constant of H_2CO_3 (6.34) and HCO_3 (10.33); $[H^+]$ or acidity; Δ, $[H^+] - [OH^-]$

The unknown parameters in this equation are C_{b1} and C_{b2}. They were estimated via nonlinear regression of equation (1) versus the titration data using the Solver in Excel. The standard deviations in the parameters were obtained using the macro Solveraid [54].

Cooking solutions

Cooking solution was prepared with equal concentrations of carbonate + bicarbonate in order to assess the effect of differences in mineral concentration on cooking time. Beans were cooked in duplicate in the different solutions (Table 1). They were put in the cooking solution once it started boiling and that moment was recorded as the start of the cooking time. Beans were considered done when they were soft enough to be easily squeezed between two fingers, which is the method used at household level.

Cooking treatments with different cooking aid concentrations

The use of $NaHCO_3$, Gk and Yk was compared in this study. For each type of *Kanwu*, three concentrations were investigated: low concentration (0.05 %, w/v), the commonly used concentration (0.1 %, w/v) and high concentration (0.5 %, w/v). In each case, 50 g of cowpeas were cooked in 200 mL of demineralised water and freeze-dried after the cooking solution was drained. Each treatment was applied in duplicate for each cooking aid. The amino acid composition, lysinoalanine and available lysine contents of these samples were determined. Two controls were considered: raw cowpeas (control 1) and cowpeas boiled in demineralised water with 0 % cooking aid (control 2). The latter treatment was applied in the same conditions as the other treatments.

Table 1

Concentration of carbonate, bicarbonate and minerals in the cooking solutions

Treatment	Molarity (mol/L)						
	Carbonate + Bicarbonate	Monovalent ions			Divalent ions		
		Na^+	K^+	Total	Ca^{2+}	Mg^{2+}	Total
Na_2CO_3	0.005	0.010		0.010			
$NaHCO_3$	0.005	0.005		0.005			
50% Na_2CO_3 - 50% $NaHCO_3$	0.005	0.007		0.007			
33% Na_2CO_3 - 67% $NaHCO_3$	0.005	0.006		0.006			
67% Na_2CO_3 - 33% $NaHCO_3$	0.005	0.008		0.008			
$CaCO_3$	0.005				0.005		0.005
K_2CO_3	0.005		0.010	0.010			
Grey *Kanwu*	0.005	0.0082	0.0008	0.0090	0.0004	0.0007	0.0011
Yellow *Kanwu*	0.005	0.0100	0.0001	0.0101	0.0000	0.0000	0.0000

Amino acid composition

The method described by Peterson et al. [196], which does not require derivatization for the detection of amino acids, was used to determine the amino acid composition of cowpeas. Cowpeas were milled with a Retsch mill (type KM 1) fitted with a 0.5 mm sieve. Then, 0.1 g of sample was hydrolysed with 5 mL of 6M HCl and 3 % (w/v) phenol at 110 °C for 16 h. The hydrolysate was filtrated with Whatman paper grade 595 ½. One mL of filtrate was evaporated using a speedvac concentrator (ThermoElectron, Asheville, NC, USA) at 60 °C until the sample was completely dry, to remove the HCl. The hydrolysate was suspended in 1 mL of 0.075 % trifluoroacetic acid solution (TFA, Merck, Darmstadt, Germany), filtered through 0.2 µm filter and injected in the HPLC. Separation of amino acids was performed on a Dionex IonPac CS-10 column (250 x 4 mm), preceded by a Dionex IonPac CS-10 guard column (50 x 4 mm). A pH gradient elution at a flow rate of 1.0 mL/min was set as follows: 0.00-12.00 min, 100 % of 0.075 % TFA (pH 2.07); 12.01-25.00 min, 20 % of 0.075 % TFA and 80 % of 0.1 % TFA (pH 3.5); 25.01-35.00 min, 10 % of 0.075% TFA and 90 % of 0.1 % TFA; 35.01-65.00 min, 100 % of 0.1 M ammonium acetate (pH 6.5) to clean-up the column; 65.01-85.00 min, 100 % of 0.075 % TFA to re-equilibrate the column. Amino acids were detected with an Evaporative Light Scattering Detector (PL-ELS 2100, Polymer Laboratories, Middelburg, The Netherlands). The nebulisation and the evaporation temperatures, and the nitrogen flow rate were optimised at 80 °C and 1.5 L/min, respectively. Lysinoalanine (Bachem, Bubendorf, Switzerland) and individual amino acids (lysine, methionine, phenylalanine, threonine, valine, leucine, isoleucine, aspartic acid, serine, glutamic acid, glycine, alanine, proline, arginine, cysteine, methionine) were identified by comparing their retention times with those of the standard solutions.

Available lysine

The method of Hall et al [84] was applied, which is based on the reaction of 2,4,6–trinitrobenzenesulphonic acid (TNBS) with free ε-amino groups from lysine present in proteins. The ε-trinitrophenyllysine (ε-TNP-lysine) was quantified by spectrophotometry (Varian, Cary 50 Bio, New Jersey, USA) at 415 mm after acid hydrolysis and removal of residual free picric acid ($C_6H_3N_3O_7$) by extraction with diethyl ether ($C_4H_{10}O$).

Statistical analysis

Each experiment was performed in duplicate. Mean values of amino acid content and lysine availability were compared to the controls by analysis of variance using the IBM-SPSS 19 followed by the Dunnett posthoc test.

RESULTS AND DISCUSSION

Mineral composition of rock salts

The mineral composition of the two types of *Kanwu* consisted mainly of monovalent ions (Figure 1), sodium (in g/kg, 188.5 ± 9.6 for Gk; 327.7±8.8 for Yk) and potassium (in g/kg, 29.8±0.7 for Gk; 6.3±3.5 for Yk). In addition, grey *Kanwu* contained divalent ions such as calcium (16.7±0.5 g/kg d.w.), magnesium (16.3±0.2 g/kg) and traces of iron (4.8±0.02 g/kg), which were negligible in yellow *Kanwu*. Zinc content of both types of *Kanwu* is very negligible (0.0004 g/kg d.w.). This composition confirms previous findings that *Kanwu* is a good source of minerals, mainly sodium (about 28 – 32 g/kg d.w.) and also trace elements such as K, Ca, Mg, Fe, Zn [63, 247]. Besides, $NaHCO_3$ and Na_2CO_3 concentration in *Kanwu* also varied according to the salt's origin. Gk was found to consist of 0.049 mol/L ± 0.003 Na_2CO_3 and

0.146 mol/L ± 0.008 $NaHCO_3$ while 0.017 mol/L ± 0.008 Na_2CO_3 and 0.22 mol/L ± 0.017 $NaHCO_3$ were found for Yk.

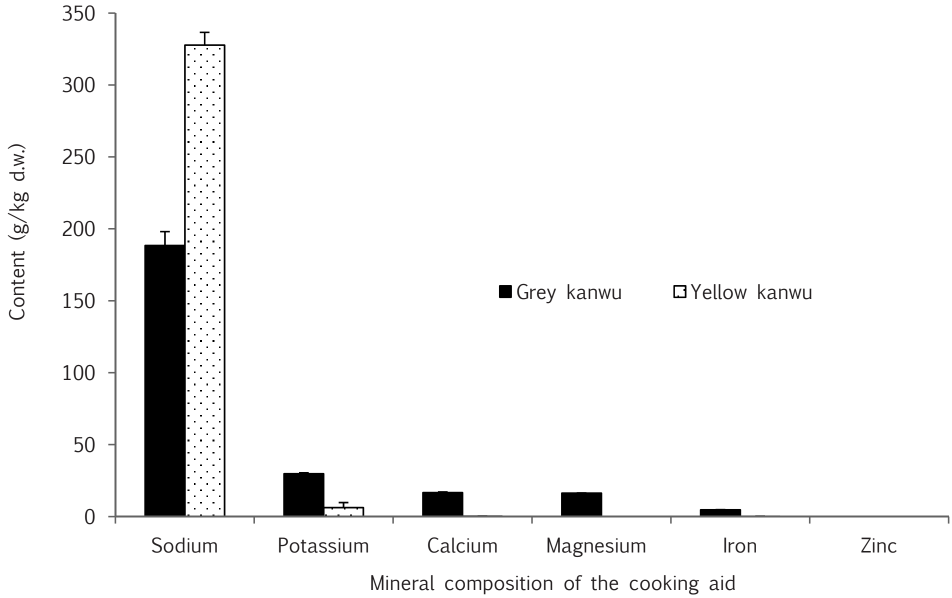


Figure 1

Variation of the composition of rock salts used in Benin

The difference in the composition observed in the analysed *Kanwu* samples can be related to the nature of *Kanwu*: these salts are cropped from sedimentary rock or natural evaporite mines in West and Central Africa [155]. This difference in composition between Gk and Yk can affect the cooking of beans as divalent ions are known to harden water, lower the softness of beans and therefore prolong the cooking time of foods.

Effect of monovalent and divalent cations

Table 2 shows that the predominance of monovalent cations in cooking water is associated with a reduction of bean cooking time of 14-27 % as compared with the control. The predominance of divalent cations hardened the cooking water and increased the cooking time, e.g., by 11 % when calcium carbonate was used. Indeed, de Leon et al. [52] found with pure salt solutions that a monovalent to divalent cation ratio higher than 6.30 reduced significantly the cooking time of *Phaseolus vulgaris* beans. With natural occurring rock salts, our results show that at equal carbonate + bicarbonate concentration, the high concentration of monovalent ions was the driving force to reduce cooking time.

Table 2

Effect of minerals on cooking time

Salts	Cooking time		pH of cooking water	
	(min)	Reduction (%)	Before cooking	After cooking
Control (water and no salt)	70 ± 0		7.2 ± 0	6.0 ± 0.02
Na_2CO_3	53 ± 4	25	10.6 ± 0.1	6.4 ± 0.07
$NaHCO_3$	58 ± 4	18	8.1 ± 0.3	6.4 ± 0.04
50% Na_2CO_3 - 50% $NaHCO_3$	60 ± 0	14	9.6 ± 0	6.3 ± 0.05
33% Na_2CO_3 - 67% $NaHCO_3$	60 ± 0	14	9.3 ± 0	6.3 ± 0.01
67% Na_2CO_3 - 33% $NaHCO_3$	60 ± 7	14	9.9 ± 0	6.3 ± 0.08
Ca_2CO_3	78 ± 4	-11	9.6 ± 0.1	6.4 ± 0.02
K_2CO_3	50 ± 0	27	10.7 ± 0	6.9 ± 0.07
Grey *Kanwu*	53 ± 4	25	10.0 ± 0	6.5 ± 0.06
Yellow *Kanwu*	54 ± 4	24	10.0 ± 0	6.4 ± 0.06

Although the pH of the cooking solutions varied from 7.2 to 10.7 (depending on their composition) before cooking, it decreased to the range 6.0 to 6.9 after cooking. This acidification could be explained by the fact that cowpea is mainly composed of acidic amino acids that may neutralise the hydroxide groups present in the cooking solution resulting into a lower pH after cooking. However, findings of Uzogara et al. [247] reported an increase of pH after soaking and cooking of cowpeas with different concentrations Na_2CO_3 and a decrease with *Kanwu* solutions; this result was explained by the leaching of alkaline cations from the middle lamella of cowpeas to the cooking solution.

Effect of cooking aid concentration on amino acid composition

Table 3 shows the variation in amino acid composition of cooked cowpeas as a function of the concentration of cooking aids. Essential amino acids (lysine, phenylalanine) and non-essential amino acids (glutamic acid, serine, threonine, aspartic acid, glycine, alanine and proline) were detected and quantified in cooked cowpeas. Cysteine, methionine, and tryptophane were not detected. As acid hydrolysis was performed, it was not expected that these amino acids would be detected [197]. Leucine, isoleucine and arginine, which were detected, showed very high variability between measurements and extractions for each sample. Therefore, these data are not reported. Moreover, histidine and valine were not detected, either they were absent or their quantities were below the detection limit.

Regardless of the type and the concentration of cooking aid applied, no significant differences in the amounts of the amino acids in cowpeas were observed, except for glycine. In the latter case, the glycine content was significantly higher at a cooking aid concentration of 0.5 % (w/v) than in the raw cowpeas. Such an increase is, of course, not possible. The most likely explanation for the apparent increase is that a compound, the formation of which is induced by heating, eluted at the same retention time as that of glycine.

In general, the amino acid composition in this research is in accordance with findings of Ene-Obong and Carnavale [61] for threonine, proline and glycine. Lysine and phenylalanine content were higher (up to + 20 %) whereas the contents of serine, aspartic acid, glutamic acid and alanine were lower (up to - 20 %) than the values obtained by Ene-Obong and Carnavale [61]. These differences could be attributed to the use of different analytical methods. Indeed, most authors extract proteins from legumes before assessing the amino acid composition, which is also the reason why the amino acid composition is frequently expressed in g/ 16 N or g/100 g of protein [62, 152]. In the present study, the amino acids were determined directly in the cowpea flours, reducing the number of analytical steps before determination. In some cases, hydrolysis is performed under nitrogen [181]. Besides, the detection procedure was simpler than the usual one: samples were detected by an evaporative light scattering detector (ELSD) whereas complex derivatization was performed by most researchers [196]. The additional derivatization step may induce variations in the final results.

Boiling, autoclaving, and microwaving also had no significant effect ($p > 0.05$) on the amino acid content of chickpeas [9]. Contrary to these results, Minka et al. [152] observed a significant loss of lysine, phenylalanine and threonine when *Phaseolus vulgaris* were cooked in 0.1 % *Kanwu* solution. Besides, lysinoalanine was neither detected in the cooked cowpeas nor in cooking solutions. This result corroborates the fact that the amino acid composition of cowpeas was not affected by cooking in alkaline conditions. Indeed, the mechanism of lysinoalanine formation requires the formation of dehydroalanine as precursor of lysinoalanine by ß-elimination from serine, cysteine [72] or theoretically from threonine [71]. In this study, as the serine and threonine contents were not reduced by processing, no precursor and no lysinoalanine would be expected. Moreover, the pH of the cooking solution decreased to below pH 7. Although the rate of decrease was not investigated in this study, such a decrease could be unfavourable for the formation of lysinoalanine.

The available lysine in raw and cooked cowpeas ranged from 1.29 to 1.54 g/100 g d.w. Despite the apparent increase of the available lysine content from 1.29 to 1.54 g/100 g d.w., no significant change ($p > 0.05$) in available lysine was observed due to the cooking of raw cowpeas in water. Likewise, the use of yellow *Kanwu*, grey *Kanwu* or bicarbonate did not significantly ($p > 0.05$) affect the content of available lysine in cooked cowpeas although the values seem somewhat lower (1.21-1.47 g/100 g d.w.). The low depleting effect of processing on available and total lysine contents was also observed when cowpea was irradiated with doses ranging between 2 and 50 kGy [2]. These authors found 1.56 g/100 g d.w. of available lysine in raw cowpea which is in the same range as the 1.29 ± 0.26 g/100 g d.w. for raw cowpeas and the 1.54 ± 0.18 g/100 g d.w. for cooked cowpeas found in our study. The fact that the content of available lysine is about 60 % of total lysine suggests that 40 % of the lysine residues in the protein are not accessible for the TNBS reagent. In how far that also reflects the bioavailability of lysine remains to be investigated.

Table 3

Effect of the cooking aid concentration of the amino acid composition of cooked cowpeas (g/100 g d.w.)

Treatment	Aspartic acid	Serine	Threonine	Glutamic acid	Glycine	Alanine	Proline	Phenyl-alanine	Lysine	Available lysine
Control 1 (Raw)	2.55 ± 0.24	1.05 ± 0.09	0.96 ± 0.01	3.80 ± 0.88	1.14[a] ± 0.29	0.91 ± 0.08	0.93 ± 0.20	1.69 ± 0.18	2.34 ± 0.23	1.29 ± 0.26
Control 2 (Boiled in water)	2.61 ± 0.40	1.20 ± 0.15	0.99 ± 0.23	4.02 ± 1.14	1.31[a,b] ± 0.13	1.03 ± 0.28	0.92 ± 0.13	1.73 ± 0.12	2.36 ± 0.31	1.54 ± 0.18
0.05% Gk	2.62 ± 0.39	1.22 ± 0.14	1.01 ± 0.22	4.09 ± 1.09	1.55[a,b] ± 0.27	1.09 ± 0.20	1.03 ± 0.31	1.71 ± 0.15	2.45 ± 0.38	1.34 ± 0.21
0.1% Gk	2.67 ± 0.53	0.98 ± 0.22	0.84 ± 0.26	4.32 ± 1.38	1.21[a,b] ± 0.42	0.91 ± 0.29	0.78 ± 0.12	1.59 ± 0.19	2.11 ± 0.30	1.40 ± 0.09
0.5% Gk	2.51 ± 0.45	1.06 ± 0.27	0.98 ± 0.16	4.02 ± 0.76	2.13[b] ± 0.20	0.97 ± 0.12	0.86 ± 0.13	1.73 ± 0.17	2.20 ± 0.92	1.37 ± 0.10
0.05% Yk	2.30 ± 0.33	1.00 ± 0.25	0.90 ± 0.16	3.26 ± 0.95	1.46[a,b] ± 0.03	1.23 ± 0.08	0.76 ± 0.11	1.69 ± 0.01	2.15 ± 0.22	1.25 ± 0.06
0.1% Yk	2.89 ± 0.36	1.27 ± 0.15	1.09 ± 0.07	4.74 ± 0.70	1.68[a,b] ± 0.04	1.05 ± 0.30	0.94 ± 0.07	1.80 ± 0.22	2.67 ± 0.43	1.23 ± 0.14
0.5% Yk	2.65 ± 0.35	1.16 ± 0.12	0.98 ± 0.16	4.17 ± 0.96	2.11[b] ± 0.22	1.06 ± 0.12	0.93 ± 0.22	1.62 ± 0.43	2.30 ± 0.13	1.47 ± 0.19
0.05% BC	2.63 ± 0.46	1.18 ± 0.20	1.01 ± 0.10	4.08 ± 1.09	1.45[a],b ± 0.34	1.01 ± 0.21	0.87 ± 0.08	1.75 ± 0.19	2.26 ± 0.42	1.21 ± 0.19
0.1% BC	2.64 ± 0.34	1.20 ± 0.14	0.97 ± 0.20	3.87 ± 1.12	1.45[a,b] ± 0.30	1.05 ± 0.22	0.82 ± 0.12	1.67 ± 0.19	2.32 ± 0.40	1.43 ± 0.28
0.5% BC	2.63 ± 0.49	1.27 ± 0.08	0.99 ± 0.13	3.96 ± 1.26	1.88[b] ± 0.57	0.81 ± 0.10	0.96 ± 0.11	1.71 ± 0.18	2.21 ± 0.43	1.29 ± 0.12

All values are expressed as mean ± SD from duplicate full experimental repetitions; Values with different letters in the same column are significantly different ($p<0.05$); Gk, Grey *Kanwu* ; Yk, Yellow *Kanwu* ; BC, Sodium bicarbonate

CONCLUSION

The use of alkaline cooking aids up to 0.5 % (w/v) of cooking solution was not detrimental for the amino acid composition and the available lysine in cowpeas, while it did reduce cooking time significantly. The common practice of cooking cowpeas with 0.1 % (w/v) alkaline cooking aids is therefore a good practice. However, the in-vivo bioavailability of lysine in alkali treated cowpeas need to be assessed. Moreover, breeding of easy-to-cook brown cowpea landrace may also be an option which reduces the use of additives while processing cowpeas.

ACKNOWLEDGEMENTS

Financial support for this research was granted by the Nuffic Netherlands Fellowship Programmes (Grant CF5732/2009) and the *Tailoring food sciences to Endogenous patterns of Local Food supply for Future Nutrition* (TELFUN) INREF programme of Wageningen University.

The authors are grateful to Jeff Petterson for his invaluable contribution to the HPLC analysis of amino acid composition, to Nele van Ingen, Rineke Boonen and Jing Xiao for their participation to this study; and to Frans Lettink for his technical assistance.

5

ENHANCING THE DIGESTIBILITY OF COWPEA (*Vigna unguiculata*) BY TRADITIONAL PROCESSING AND FERMENTATIONS

ABSTRACT

Flatulence being an important drawback for the consumption of legumes, chemical, *in-vitro,* and *in-vivo* approaches were used to investigate the reduction effect of processing techniques. Cowpeas were subjected to dehulling, boiling, and soaking at 4 ℃, and bacterial, fungal or yeast fermentation. Galactose-oligosaccharides were quantified in cowpeas. *In-vitro* cowpea digests were used as main carbohydrate substrate for *Clostridium perfringens* to produce gas, as an *in-vitro* fermentability index. In a crossover *in-vivo* study, the alveolar hydrogen concentration of the breath of 18 healthy adults was quantified after the consumption of a porridge breakfast as *in-vivo* fermentability index. Galactose-oligosaccharides could not be detected in cowpea hulls which yielded low *in-vitro* fermentability index as compared with other treatments. Traditional processing induced a limited reduction of raffinose and verbascose content contrary to fermentations. The *in-vitro* fermentability index appeared similarly high for all processed cowpea except for *Rhizopus* and *Bacillus* fermentation. The *in-vivo* fermentability index of fermented cowpeas was significantly lower than that of traditionally processed cowpeas. As the consumption of cowpea-based dishes contributes significantly to the protein intake of many people, new products with a low-flatulence potential and socially acceptable to African consumers should be developed based on soaking and/or fermentation to sustain cowpea consumption.

Keywords: Carbohydrate digestibility, Flatulence, *In-vitro*, *In-vivo*, Stachyose

Submitted

INTRODUCTION

Carbohydrates are the most abundant nutrients in cowpeas (*Vigna unguiculata*). Among carbohydrates, galactose-oligosaccharides (GOS), resistant starch (RS), and non-starch polysaccharides (NSP) withstand the digestion in the human gastrointestinal tract mainly because of the lack of secretion of some enzymes required for their hydrolysis such as α-galactosidases [26, 236]. Therefore, in the caecum, undigested carbohydrates can undergo fermentation by a diverse anaerobic microflora chiefly composed of *Bacteroides*, *Bifidobacterium*, *Fusobacterium*, and *Clostridium* spp. [26, 222]. This anaerobic fermentation results in the formation of several gases (hydrogen, carbon dioxide, methane, etc.) and short chain fatty acids (acetate, butyrate, propionate, etc.) [47, 237]. These gases represent the major fraction of the flatus generated after consumption of legumes whereas hydrogen sulphide and sulphur containing gases account for the fetid flatus [87]. Besides potential digestive disorders that may occur, flatulence caused by cowpea consumption is a natural and harmless process. Moreover, cowpea consumption provides substantial protein and energy to consumers. Indigestible compounds inducing flatus formation are frequently reported to protect humans from colon cancer and cardiovascular diseases [128, 149].

Flatulence is an important limitation to cowpea' acceptance, and thus its consumption, as reported by many consumers, especially city dwellers in West Africa [142]. Enhancing the consumer acceptance and thus, consumption of cowpea dishes requires the reduction of their intestinal fermentability. Several studies demonstrated the efficacy of food processing in improving the digestibility of legumes by investigating the degradation of carbohydrates [10, 58, 64, 82, 188]. In these reports, GOS were focussed on as the main cause of flatus formation. Calloway, Hickey, and Murphy [38], through breath and flatus analysis, reported that a total removal of GOS from soya beans did not eliminate all flatus formation. Their results suggested that undigested carbohydrates other than GOS also play a role in the flatus formation.

Consequently, this study was undertaken to identify food processing techniques that fit with the consumers' low-flatulence quality criterion by associating three research approaches that estimate the fermentability of most indigestible carbohydrates in cowpeas. This paper discussed the potential of food processing to reduce flatulence in cowpeas and the reliability of these approaches in predicting this flatulence reduction potential.

MATERIAL AND METHODS

Cowpeas

Two batches of a pigmented landrace (*Adjayivi*) and one batch of an unpigmented landrace (*Atchawe-tola*) of cowpea were purchased from the International market of Dantokpa, Cotonou, Benin.

Microbial strains

Weissella beninensis LMG25373 was cultured in a sterile de Man Rogosa Sharpe broth tube (Merck, Darmstadt, Germany) at 30 °C for 24 h to obtain 9.0 log cfu of viable cells/ml.

Bacillus subtilis M112 was cultured in a Nutrient broth (Oxoid, Basingstoke, England) at 37 °C for 24 h until a viable count of 9.0 log cfu/mL was obtained.

Rhizopus microsporus LU573 was cultured onto Malt Extract Agar (Oxoid, Basingstoke, England) slants for 6 days at 30 °C. Prior to use, spores were scraped into 9 mL of sterile peptone physiological salt solution.

A spray-dried powder of *Saccharomyces cerevisiae* (Fermipan Red, DSM, The Netherlands) was suspended into sterile peptone physiological salt solution to obtain a viable cell count of 9.0 log cfu/mL.

Cowpea processing

In-vitro study

Figure 1a depicts the processing techniques implemented on cowpeas for the *in-vitro* study.

Fraction 1 of the raw beans (RB) was immersed in water at 30 °C for 15-60 min prior to a manual separation of the hulls (RH) from the cotyledons (RC). Both fractions were dried at 60 °C for 24 h.

Fraction 2 of RB was boiled until beans could be easily squeezed between two fingers (ratio beans/cooking solution was 1:10, w/v). Three media were used for boiling: 1) water, 2) bicarbonate solution (1 g/L; pH 7.9), and 3) *Kanwu* solution (1 g/L; pH 9.8). *Kanwu* is a local rock salt available in West-Africa, rich in bicarbonate, carbonate and minerals and used traditionally to reduce the cooking time of cowpeas. After boiling, the beans were drained to discard the boiling water and to collect the boiled beans (BBW, BBB and BBK, respectively).

Fraction 3 of RB was soaked overnight (16 h) in tap water at 4 °C to avoid acidification through uncontrolled fermentation, and subsequently dehulled by hand. The cotyledons were boiled in tap water (ratio cotyledons/water of 1:10, w/v) for 20 min, and cool down to room temperature. Boiling water was drained and boiled cotyledons (S16BC) were separately inoculated with the different microorganisms. One share of S16BC was inoculated with 7.0 log cfu of *Bacillus subtilis* per gram of cotyledons, and incubated at 37 °C for 24 h (BsFC). The second share was mixed with cooled cooking water (ratio of 1:1, w/v), inoculated with 2 x 7.0 log cfu of *Weissella beninensis* per gram of cotyledons, and incubated at 30 °C for 48 h (WbFC)). The third share was mixed with cooled cooking water (ratio of 1:1, w/v), inoculated with a natural enrichment of lactic acid bacteria (2 x 7.0 log cfu of total lactic acid bacteria per gram of cotyledons) and incubated at 30 °C for 48 h (NLFC). The lactic acid bacteria enrichment was obtained by a backslopping process [170] performed on the raw beans.

Fraction 4 of RB was soaked in tap water mixed with 10 % soaking water from the backslopping process at 30 °C for 24 h. After soaking and draining, cowpeas were dehulled manually and cotyledons boiled (ratio cotyledon: water of 1:10, w/v) for 20 min. Boiling water was discarded and cotyledons were cooled, superficially dried, inoculated (10^4 spores of *R. microsporus* per gram of cotyledons) and incubated at 30 °C for 48 h. This fermentation resulted in a solid cowpea-tempe cake (RmFC).

All the samples of the *in-vitro* study were produced in duplicate experiments, freeze-dried and kept at – 20 °C until use.

In-vivo study

Figure 1b depicts the processing techniques implemented on cowpeas for the *in-vivo* study.

Fraction 1 of pigmented cowpeas was boiled in water (BBW), or *Kanwu* solution (BBK) as described for the *in-vitro* study. Fraction 2 of pigmented cowpeas was immersed in water at

30 °C for 5 min, dried at 60 °C until a moisture content of 10 % and dehulled by the Prairie Research Laboratory (PRL) dehuller [151] for 4 min. We obtained the dry dehulled raw cotyledons (DRC). DRC were soaked at room temperature (28-30 °C) for 24 h allowing a natural acidification from pH 7 to pH 5 to take place. We obtained the soaked/acidified cotyledons (SAC). One share of SAC was minced in a sterile mill, inoculated (with 6.0 log cfu of *Saccharomyces cerevisiae* per gram of SAC) and incubated at 25 °C for 72 h. Two other shares of SAC were fermented with *R. microsporus* and *W. beninensis* as described for the *in-vitro* study.

All the processed samples prepared for the *in-vivo* study were dried at 60 °C for 24 h, and milled in a Retsch type KM1 mill through a 0.5 mm screen to obtain a shelf-stable powder.

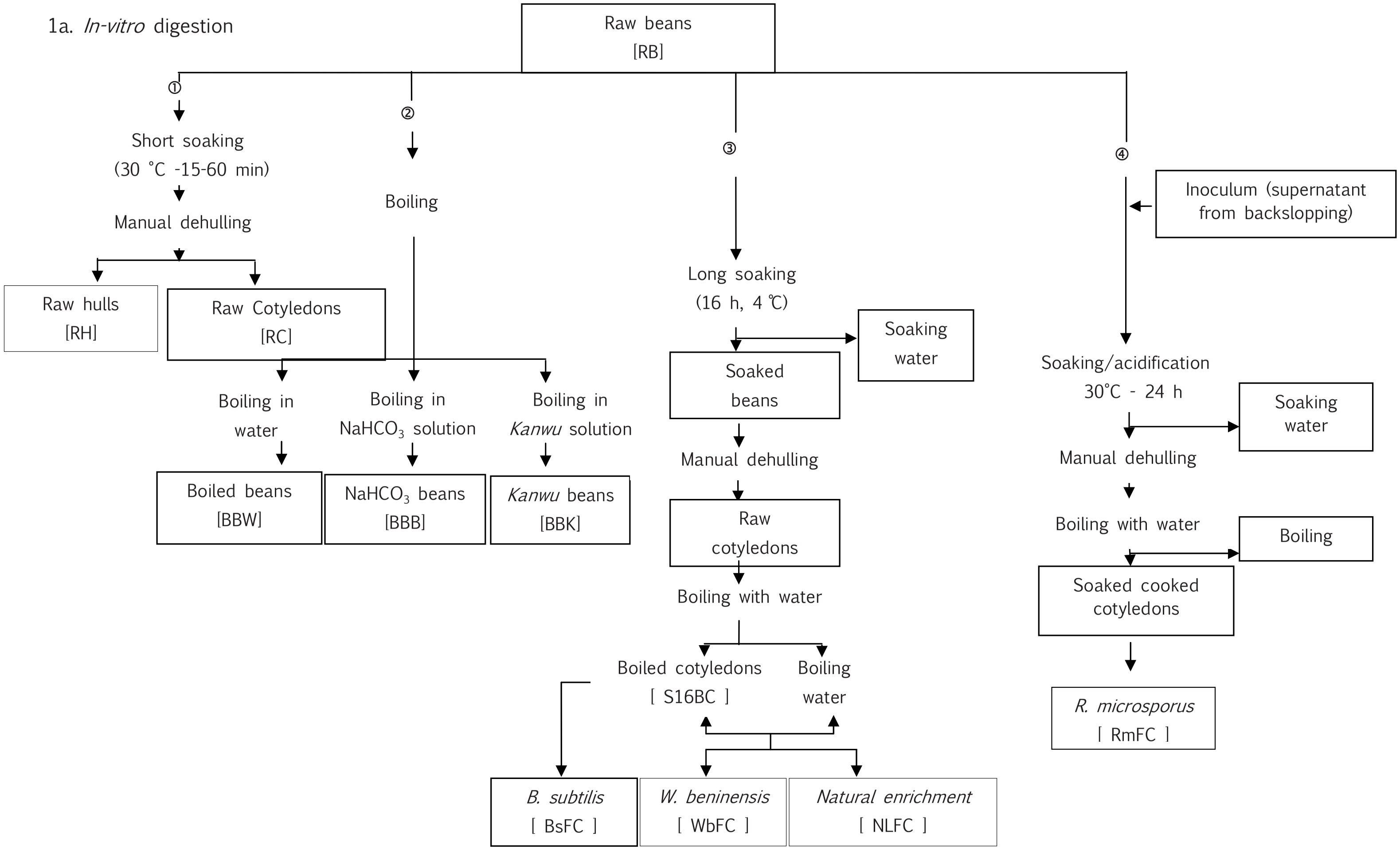
In-vitro digestion and fermentability

The *in-vitro* digestion (enzymatic degradation and dialysis) was performed as described by Kiers, Nout, and Rombouts [119]. The suspension obtained after enzymatic digestion was centrifuged at 3000 x g and 4 °C for 15 min. The pellet was washed twice and constituted the non-dissolved undigested moiety of the beans. The supernatant was dialysed in dialysis tubes against running water to collect the dissolved undigested moiety of the beans. The fermentability of these two moieties was assessed on the basis of the volume of gas released from their fermentation by *Clostridium perfringens* VWA GZ-1005 taken as a reference microorganism as defined by Nche, Nout, and Rombouts [164]. The volume of gas produced by *C. perfringens* was considered as the *in-vitro* fermentability index of the analysed product. In total, 10 cowpea products were digested and randomly fermented by *C. perfringens.* All samples were fermented and analysed in duplicate.

In- vivo digestion and breath sampling

Eighteen healthy volunteers (7 women and 11 men, aged 23 - 41 years with a body mass index ranging 18-25 kg/m^2) were enrolled. They were informed about the study procedures and signed a written informed consent. Three months before and during the study none of these non-smokers took antibiotics nor reported any gastro-intestinal or lung diseases. The subjects of this study were Caucasian (4), Asian (5) and African (9).

1a. *In-vitro* digestion



1b. *In-vivo* digestion

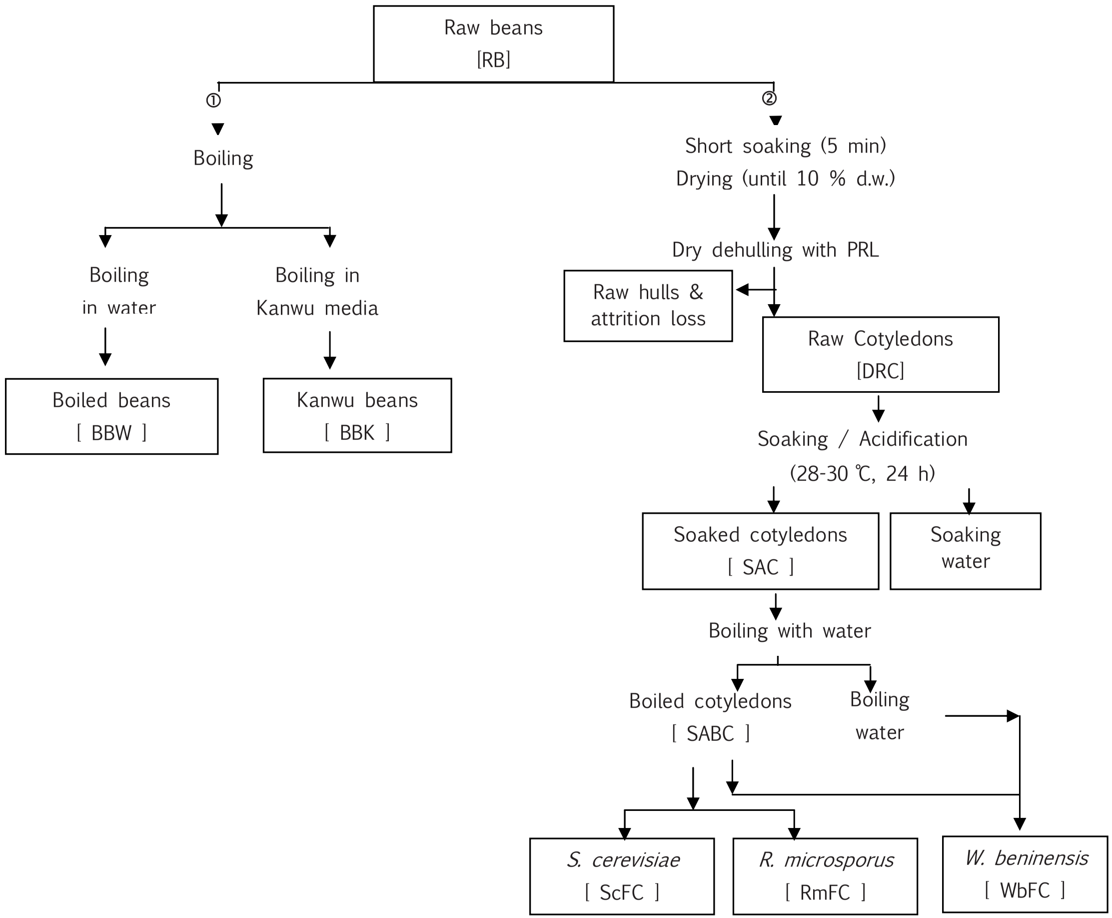


Figure 1

Flow sheet of cowpea processing for a) the *in-vitro* and b) *in-vivo* digestion studies

According to the Medical and Ethical Assessment Committee of Wageningen University, this study did not fall within the scope of the Netherlands' law on medical scientific research on human subjects (WMO). We were advised that written informed consents from subjects would be adequate.

Raw and processed pigmented bean flours, obtained as described in section 2.3.2, were cooked with water (flour/water ratio of 1/10, w/v) for 15 min (starting from the boiling point) to obtain porridges. Water loss due to evaporation during boiling was corrected for. Three portion sizes of porridge (0.40, 0.60 and 0.75 kg) were consumed by the participants depending on the stomach capacity of each subject, after a fasting period of 10 h. The portion consumed by each subject was maintained during the whole study. Each subject was served the portion size that corresponded to his/her daily breakfast habits. Alveolar breath was collected from the mouth with an alveo-sampler bag (QuinTron instrument company, Milwaukee, WI, USA) equipped with a 30 mL sampling syringe, on empty stomach and hourly for 8-12 hours after the consumption of the porridge. The subjects could take any beverage containing no indigestible sugars any time after the consumption of the porridge, but no solid food until 6 hours after the consumption of the porridge as breakfast. The concentration of hydrogen (alveolar hydrogen) was measured in the alveolar air released by each participant using the Breath Tracker DP (QuinTron instrument company, Milwaukee, WI, USA). The alveolar hydrogen concentrations were corrected with the lowest alveolar hydrogen measured after fasting, and plotted against time (min), as shown in Figure 2. The triangular areas under the aveolar hydrogen versus time curves were summed to obtain the area under the curve (AUC, ppm x min):

$$AUC = \Delta t \ x \ \sum_1^n((C_1 + C_2) + (C_2 + C_3) + \cdots + (C_{n-1} + C_n))/2 \qquad (1)$$

where Δt = 60 min as measurements were performed hourly; C1, C2, ..., Cn-1, Cn = corrected H_2 concentration (ppm) at the beginning and the end of each interval; n = number of H_2 concentration measurements.

The experimental AUCs were normalised to normal distribution by natural logarithm (ln (AUC)) and used as *in-vivo* fermentability index. The eighteen subjects received all treatments in a random order. Nine participants received two of the 9 treatments a second time, in order to investigate the within person-treatment variation. The subjects also filled a questionnaire to indicate the type of foods that they consumed before the fasting period and on the sampling day.

Chemical analysis

An aqueous extraction of sucrose and GOS in processed cowpea flours was performed by suspending 0.5 g of flour in 50 mL of demineralised water and incubating the suspension at 80 °C for 20 min [110]. After extraction and centrifugation at 2670 x g for 10 min, the supernatant was collected, mixed (ratio 1:1) with absolute ethanol, stored at – 20 °C for 30 min to precipitate dissolved proteins and centrifuged at 2670 x g for 20 min. The supernatant was evaporated in a speedvac concentrator (ThermoElectron, Asheville, NC, USA) at 60 °C for 1-2 hours. The pellet was re-suspended in water, and filtered through a 0.45 µm filter. Sucrose and GOS were separated on a Prevail carbohydrate ES 5u column (Grace, Breda, The Netherlands) [257] with an acetonitrile/water gradient elution (increased from 80:20 to 50:50) and detected by an Evaporative Light Scattering Detector (PL-ELS20100, Polymer Laboratories, Middelburg, The Netherlands). Extractions and measurements were performed in duplicate.

Statistical analysis

All analyses were performed in duplicate. The residual sucrose and GOS in processed cowpeas were compared using the analysis of variance followed by a Tukey posthoc test. The mean values of the *in-vivo* fermentability index (ln(AUC)) were also compared through the analysis of variance followed by a Tukey posthoc test in a block design setting, with the 18 subjects as blocks. For all these tests, a significance level was reported at 0.05.

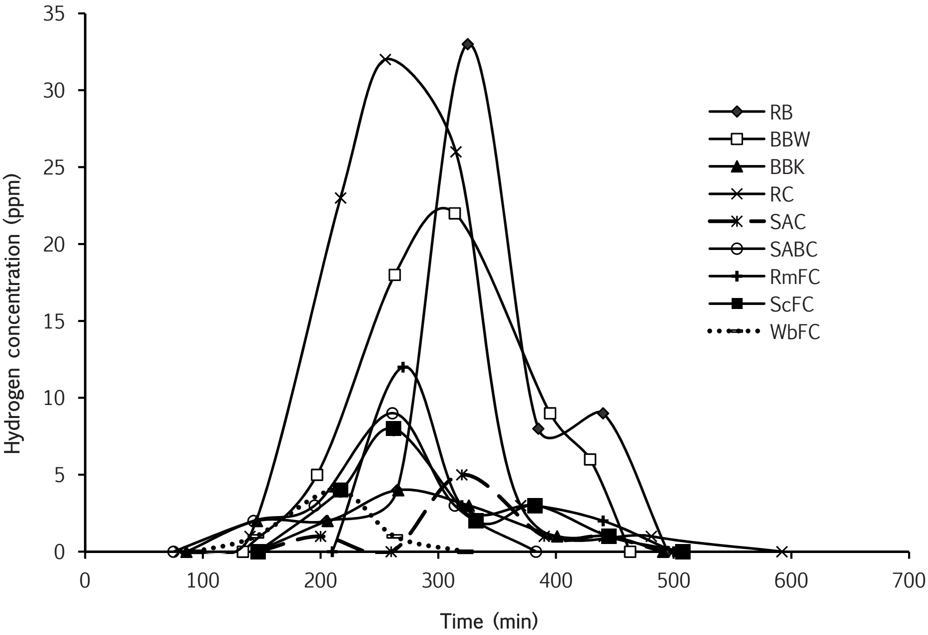


Figure 2

Shape of H_2 concentration curves from one subject during the *in-vivo* study.

RB, Raw beans; RC, Raw cotyledons; BBW, Beans boiled with water; BBK, Beans boiled with *Kanwu* solution; SAC, Cotyledons soaked /acidified at 28-30 ℃ - 24 h; SABC, Boiled SAC; RmFC, *R. microsporus* fermented cotyledons; ScFC, *S. cerevisiae* fermented cotyledons; WbFC, *W. beninensis* fermented cotyledons.

RESULTS

In-vitro assessment of the digestibility of processed cowpea

Effect of traditional processing on the digestibility of carbohydrates

In this study, the pigmented and unpigmented cowpeas contained mainly stachyose (42-52 mg/g dry weight (d.w.)) and sucrose (18-32 mg/g d.w.), and less raffinose and verbascose (7-8 mg/g d.w. in both cases). Figure 3 (a and b) showed the sucrose and GOS profile of cowpeas as affected by dehulling, soaking at 4 °C for 16 h and/or boiling. The trend observed was the same for unpigmented and pigmented cowpea landraces. Raffinose and verbascose were less affected by traditional processes than sucrose and stachyose. The analysis of the hulls revealed that they did not contain significant amounts of sucrose and GOS. Taking the raw cowpeas as a reference, the use of softeners during boiling reduced the content of sucrose (60-80 %) and stachyose (48-70 %) significantly more than boiling in water (30-52 % for sucrose and 16-33 % for stachyose). Soaking at 4 °C for 16 h followed by dehulling and boiling (S16BC) greatly reduced the sucrose and stachyose content of pigmented (69 %) and unpigmented (more than 75 %) cowpeas.

Figure 4a showed the *in-vitro* fermentability index of the dissolved- and the undissolved-fractions of the undigested products as metabolized by *C. perfringens*. In all cases, the undissolved moiety contained four times more fermentable substrate than the dissolved moiety, except for the hulls where these fractions were equally fermentable for pigmented and unpigmented landraces. Overall, the traditional processes implemented did not significantly reduce the fermentability of cowpea assessed *in-vitro*.

Effect of fermentation on the digestibility of carbohydrates

S16BC was the common product leading to all biological processes except the fungal fermentation. The sucrose and GOS profiles depicted in Figure 3 (c and d) indicated a complete removal of sucrose and all GOS of S16BC when fermented either by *B. subtilis* or *W. beninensis,* except verbascose. The natural lactic acid fermentation (NFLC) showed 100 % reduction of sucrose and all GOS of the pigmented landraces whereas it was less efficient to degrade stachyose and verbascose of the unpigmented landrace.

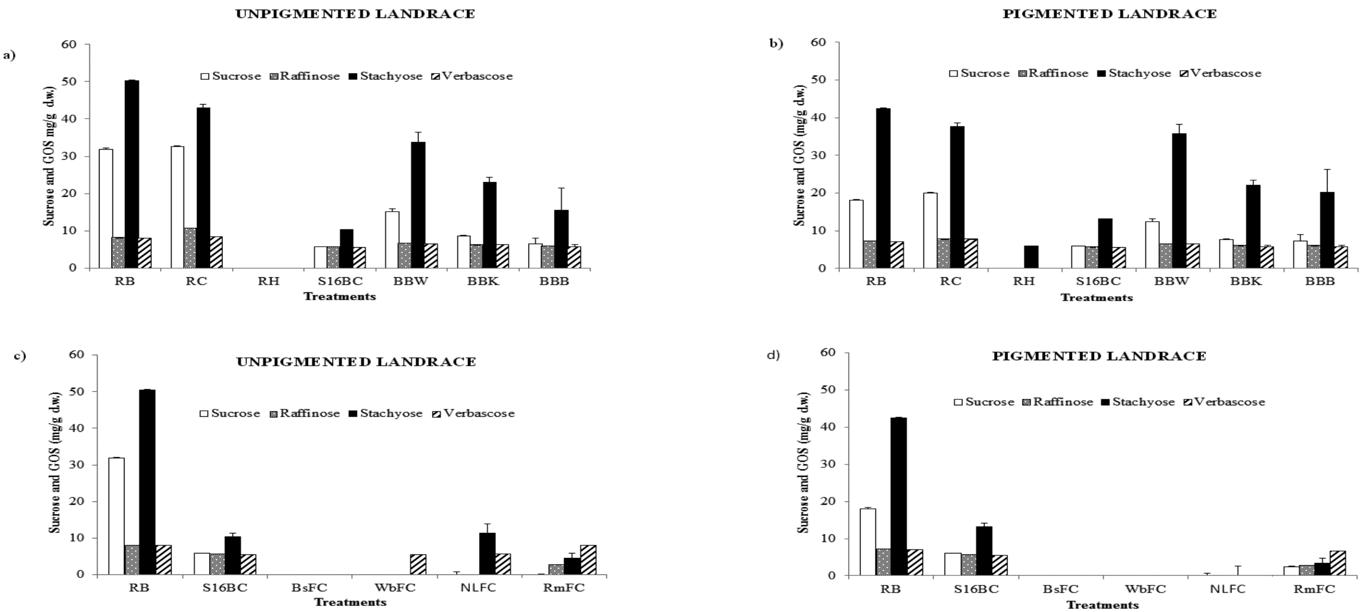


Figure 3

Variation of the sucrose and GOS contents as a function processing treatments and landraces (*in-vitro* study)

RB, Raw beans; RC, Raw cotyledons; RH, Raw hulls; S16BC, Beans soaked (16 h at 4 ℃), dehulled and boiled with water; BBW, Beans boiled with water; BBK, Beans boiled with *Kanwu* solution; BBB, Beans boiled with $NaHCO_3$ solution; BsFC, *B. subtilis* fermented cotyledons; WbFC, *W. beninensis* fermented cotyledons; NLFC, S16DB fermented with a natural lactic acid bacteria inoculum; RmFC, *R. microsporus* fermented cotyledons.

Error bars indicate the standard deviation of the mean of sucrose and GOS contents from two trials.

The whole process of tempe production with *R. microsporus* reduced 87-100 % of sucrose, 60-90 % of raffinose and stachyose in raw cowpea, but hardly degraded verbascose (1-6 %).

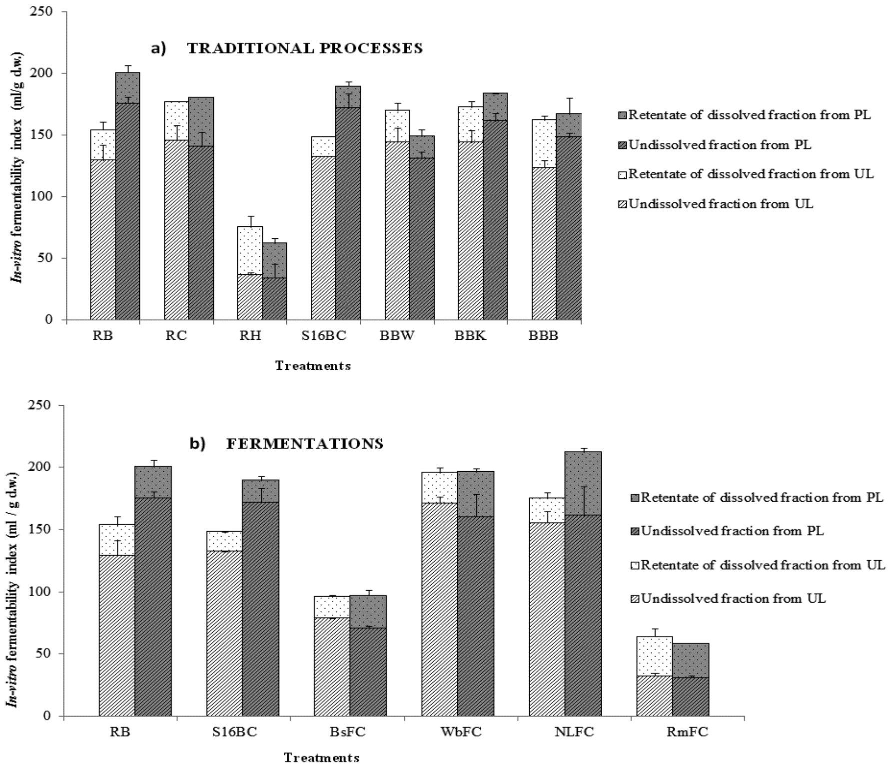


Figure 4

In-vitro fermentability index as affected by processing treatments and landraces.

PL, Pigmented landrace; UL, Unpigmented landrace; RB, Raw beans; RC, Raw cotyledons; RH , Raw hulls; S16BC, Beans soaked (16 h at 4 ℃), dehulled and boiled with water; BBW, Beans boiled with water; BBK, Beans boiled with *Kanwu* solution; BBB, Beans boiled with $NaHCO_3$ solution; BsFC, *B. subtilis* fermented cotyledons; WbFC, *W. beninensis* fermented cotyledons; NLFC, S16DB fermented with a natural lactic acid bacteria inoculum; RmFC, *R. microsporus* fermented cotyledons.

Error bars indicate the standard deviation of the in-vitro fermentability index from two trials.

As observed previously, the undissolved moiety of the undigested residues obtained from the bioprocessing of cowpeas was significantly better fermentable than the dissolved fraction (Figure 4b). Lactic acid fermented cowpeas produced as much or even more gas than S16BC and raw cowpea from both landraces. *B. subtilis* and *R. microsporus* considerably decreased the fermentability of RB and S16BC.

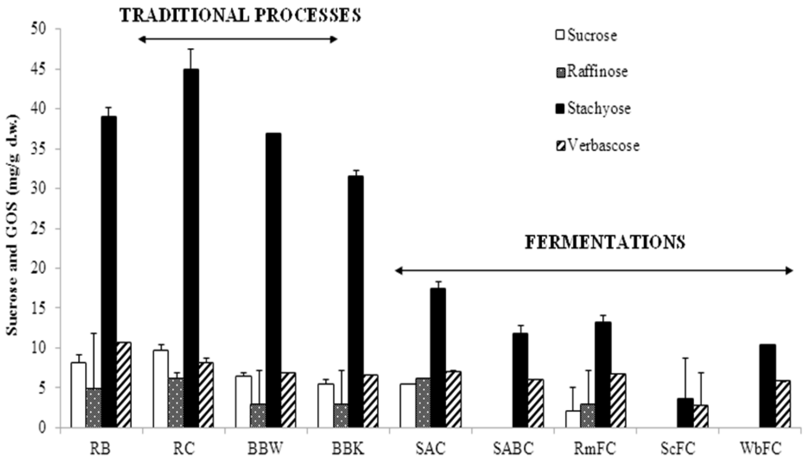


Figure 5

Variation of the sucrose and GOS contents as a function of processing treatments (*in-vivo* study)

RB, Raw beans; RC, Raw cotyledons; BBW, Beans boiled with water; BBK, Beans boiled with *Kanwu* solution; SAC, Cotyledons soaked /acidified at 28-30 ℃ -24 h; SABC, Boiled SAC; RmFC, *R. microsporus* fermented cotyledons; ScFC, *S. cerevisiae* fermented cotyledons; WbFC, *W. beninensis* fermented cotyledons.

Error bars indicate the standard deviation of the *in-vivo* fermentability index from two trials.

In-vivo assessment of the digestibility of processed cowpea

Effect of traditional processing on the digestibility of carbohydrates

The pigmented landrace processed for the *in-vivo* study contained 8, 5, 40 and 10 mg/g of sucrose, raffinose, stachyose and verbascose, respectively (Figure 5). Boiling in water (22, 39, 6 and 35 %, respectively) or boiling in *Kanwu* solution (33, 39, 19 and 38 %, respectively) slightly decreased the sucrose and GOS contents in the pigmented landrace, whereas dehulling slightly increased the raffinose and stachyose content on dry matter basis.

The *in-vivo* fermentability index of the raw cowpea porridge did not significantly differ from the index associated with cowpea cotyledons or cowpeas boiled in water. Compared with soaking of cotyledons for 24 h at 28-30 ºC (SAC), boiling in water did not lower the *in-vivo* fermentability of the cowpeas (Figure 6a). In general, cowpea cotyledons were associated with a high *in-vivo* fermentability index.

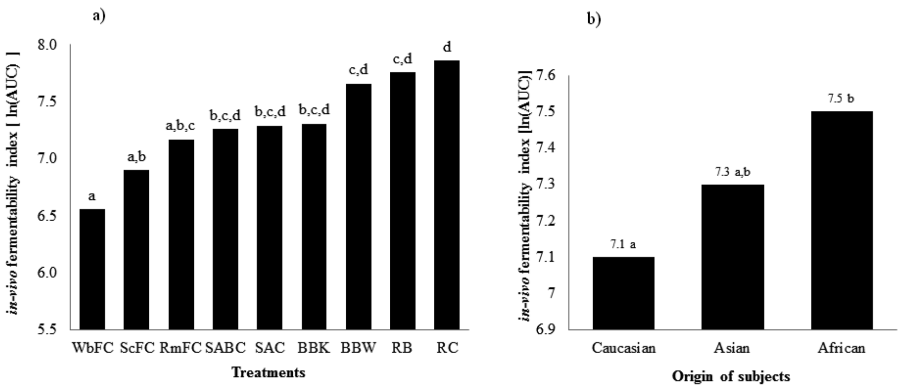


Figure 6

In-vivo fermentability index as affected by a) processing treatments and b) origin of tested subjects.

RB, Raw beans; RC, Raw cotyledons; BBW, Beans boiled with water; BBK, Beans boiled with *Kanwu* solution; SAC, Cotyledons soaked /acidified at 28-30 ℃ -24 h; SABC, Boiled SAC; RmFC, *R. microsporus* fermented cotyledons; ScFC, *S. cerevisiae* fermented cotyledons; WbFC, *W. beninensis* fermented cotyledons.

Effect of fermentation on the digestibility of carbohydrates

Figure 5 shows that SAC reduced the sucrose (43 %), stachyose (61 %) and verbascose (15%) content of the cotyledons. Compared to SAC, boiling leached out all sucrose and raffinose, 32 % of stachyose and 12 % of verbascose. The combined effect of dehulling, soaking/acidification and boiling was the removal of all the sucrose and the raffinose, 83% of the stachyose and 27 % of the verbascose. Compared to SABC, *R. microsporus* did not significantly reduce the sucrose and GOS contents. *S. cerevisiae* and *W. beninensis* also reduced stachyose and verbascose contents, namely 69 % and 53 %, respectively, for the ScFC and 13 % and 4 %, respectively, for the latter treatment (WbFC).

The *in-vivo* fermentability indexes of fermented cowpeas were all low in comparison to the other treatments (Figure 6a). Fermentation by *W. beninensis* and *S. cerevisiae* were associated with the lowest *in-vivo* fermentability index as opposed to raw beans, and traditionally processed beans. The stachyose content of cowpeas showed a high positive correlation with the *in-vivo* fermentability index ($r = 0.88$, $p < 0.01$) and no significant correlation with the *in-vitro* fermentability index ($r = 0.370$, $p > 0.05$).

Figure 6b illustrates that regardless of the processed cowpea consumed, the subjects involved in the *in-vivo* study reacted differently as they originate from Europe, Asia or Africa. Indeed, the mean *in-vivo* fermentability index associated with processed cowpeas increased significantly from Caucasians (7.1) to Asian (7.3) and African (7.5), meaning that Caucasians produced less abdominal gas than Asians who in turn produced less than Africans.

DISCUSSION

Traditional processing treatments (soaking at 4 ºC, dehulling, and boiling) as applied separately had a lower effect on the reduction of GOS content than fermentation (lactic, yeast, fungal). Although hulls of legume seeds are commonly considered as the main causes of the high fermentability of ingested legumes, their removal by wet-manual dehulling preceded by 1 h soaking at room temperature reduced only 17-18 % of stachyose and 30-48 % of raffinose [173, 185]. Akinyele and Akinlosotu [8] reported, however, a higher reduction of verbascose (76 %) and raffinose (56 %) and a similar stachyose reduction (17 %) in cowpeas when beans were soaked for 10 min prior to wet-manual dehulling.

The negligible amount of GOS that we detected in the hulls corroborated the findings of Revilleza, Mendoza, and Raymundo [211] as only traces of GOS were found in the seed coat of *Dolichus lablab* and *Mucuna pruniens*. For as far as cowpea is concerned, no previous study reported the content of GOS in hulls. Onigbinde and Akinyele [185] analysed the raw cowpeas and their cotyledons and concluded, by deduction, that raffinose and stachyose abound in the testa without analysing the GOS content of the testa. The reduction observed subsequently to the dehulling process in the cotyledons as compared with the whole bean could be explained by the solubilisation of GOS in the water during the soaking process preceding the dehulling. In addition, the dehulling process is usually accompanied by the detachment of the embryo or germ which is rich in GOS. Kuo, Lowell, and Smith [126] showed that during seed maturation, soya bean embryo as well as cotyledons are enriched in GOS contrary to hulls. Furthermore, the hulls may constitute a barrier for the diffusion of oligosaccharides into the soaking water. Therefore, the added-value of the dehulling process would be to favour the leaching of GOS or their breakdown products rather than the removal of the GOS from the hulls.

All boiling practices reduced the GOS content of the beans. The use of alkaline solutions (bicarbonate or *Kanwu*) decreased the GOS content more than only water. The GOS might have been leached to the cooking solution. The alkaline conditions caused by the softeners probably tenderized the hulls and favoured a better release of GOS and/or their degradation products from the cotyledons into the boiling water. This leaching mechanism could also happen when cowpeas are soaked at 4 ºC, dehulled and cooked.

The observed reduction of stachyose content when dry cotyledons were soaked at room temperature for 24 h (SAC) could be due to the leaching of GOS simultaneously with their assimilation by the naturally microbiota that acidified the soaking water. *W. beninensis* was recently identified and characterised during cassava fermentation, and its ability to metabolise GOS such as raffinose and melibiose, and produce organic acids as described by Padonou et al. [190], was confirmed by this study. The efficiency of *W. beninensis* is comparable with the enzymatic degradation by α- galactosidase as a reduction of 93.3 % of raffinose was observed by Somiari and Balogh [230]. However, the result of the fermentation by *W. beninensis* should also be considered as the sum of the effects of all the preliminary steps prior to the fermentation, namely soaking, dehulling and boiling.

Although the overall effect of *R. microsporus* fermentation is high, the reduction of GOS due to the activity of *R. microsporus* is rather limited. Such result suggests that the fungal strain used for tempe production is unable to produce a sufficient amount of α-galactosidase. Indeed, Rehms and Barz [208] demonstrated that *R. microsporus var. chinensis*, contrary with *R. oryzae*, can produce ß- fructosidase but not α-galactosidase, enabling the entire assimilation of sucrose but only a partial degradation of raffinose.

The utilization of carbohydrates, especially GOS, varies greatly depending upon the *Clostridium perfringens* strain used. *C. perfringens* type A, which is a usual human gut inhabitant, can metabolise raffinose [164, 215]. In our experiments, the total gas produced by *C. perfringens in-vitro* did not correlate with the amount of GOS present in the processed cowpeas. As processed cowpeas which contain a negligible amount of GOS still induce gas production, it can be suggested that *C. perfringens* can metabolize saccharides other than GOS such as resistant starch or cell wall components or their breakdown products [38, 215]. This hypothesis is consistent with the fact that the undissolved fraction of the analysed samples showed more than 80% of the fermentability of the undigested residues.

The difference in intestinal response of the subjects from Europe, Asia and Africa can possibly be attributed to the differences in dietary habits. These differences could highly influence the population of the gut microflora and its activity [26]. de Fillippo et al. [51] demonstrated that the gut microbiota of infants from rural Africa was dominated by bacteria able to utilise cellulose and xylan contrary to the gut microbiota of European children. The diet of the African children was based on foods rich in starch, fibre and plant polysaccharides whereas European children were accustomed to protein and fat rich foods. Although the non-Caucasian subjects involved in our study live in Europe for a while, most of them have not shifted drastically from their original food consumption patterns. Our study recorded that the subjects still consumed many of the foods they were used to in their country of origin.

The different approaches implemented to estimate the potential of the processed cowpea to generate flatus after consumption yielded various outcomes. The *in-vivo* approach (as implemented in this research) revealed the utilisation of the undigested fraction of the ingested cowpea porridges by a large range of microorganisms [26]. The *in-vitro* approach, as implemented in this research, involved only one bacterial strain: *C. perfringens*. Therefore, the type of substrate and the metabolic ability of the microbiota present with regard to this substrate can explain the observed differences. Moreover, the sampling time scale was 8 - 12 hours for the *in-vivo* test and 24 hours for the *in-vitro* test. It can be hypothesized that the easily fermentable compounds in the undigested bulk, namely GOS and resistant starch, induce the formation of the hydrogen measured *in-vivo* during the first 12 hours after the food is consumed [36]. Such hypothesis is in line with the correlation found between *in-vivo* fermentability index and stachyose content. The slow fermenting polymers, namely pectin, cellulose, lignin, etc. induce a significant gas release. As a matter of fact the total gas produced by *Clostridium perfringens* from the various undigested materials is an integration of the gas generated by the fermentation of all the types of fermentable sugars. The 24 hours quantification of gas production seems more appropriate to obtain a complete view of the gas production potential of processed cowpea.

CONCLUSIONS

The present research suggests that soaking and fermentation are the most efficient techniques to leach out and/or degrade flatus-inducing factors present in cowpea. In the context of West Africa, soaking should be promoted as a pre-treatment in any cowpea processing procedure. Moreover lactic acid fermentation, especially with *Weissella beninensis*, could be optimised for low flatus induction and good taste.

The various approaches used to assess flatus production appear to express different phenomena, each of which has its own merit.

ACKNOWLEDGEMENTS

This research formed part of the TELFUN programme, *Tailoring food sciences to Endogenous patterns of Local Food supply for Future Nutrition,* of Wageningen University. Financial support was also obtained from the Netherlands Fellowship Programmes (Grant CF5732/2009). The authors are grateful to Zhaoying Li, Vinnie de Wilde and Judith Wolkers-Rooijackers for their skillful technical contribution and to all participants in the *in-vivo* study for their commitment.

6

GENERAL DISCUSSION AND PERSPECTIVES

INTRODUCTION

In the framework of reconnecting food production with food consumption by strengthening local food networks, this thesis explored the traditional processing of cowpea in West Africa and studied its effects on the nutritional quality and the acceptability of the derived foods. This thesis aimed specifically at:

i. characterising cowpea landraces in use in Benin with regard to nutritional, anti-nutritional and functional properties;
ii. determining present cowpea processing methods and eating habits with special reference to their content of available iron, zinc and calcium;
iii. assessing the effect of the use of alkaline cooking aids on the amino acids of cooked cowpea;
iv. assessing the impact of processing techniques on the flatulence generated by cowpea foods.

The approach taken in studying cowpea production and consumption was to investigate the food chain from raw materials to consumed dishes, the nutritional quality of cowpeas, the processing techniques in use, the processing and consumption constraints, and the sustainability of local practices and solutions suggested overcoming these constraints.

The present chapter reflects on the extent to which the objectives of this thesis have been reached. In addition, the contribution of this technological study to the goals of the TELFUN programme is discussed. Finally, at the end of this chapter, recommendations for further research are given.

CONSIDERATIONS ABOUT THE STUDY AREA AND SAMPLING METHODS

This study formed part of the research by the TELFUN Ghana/Benin team on the production and consumption of cowpea in West Africa in local food networks. First, a coordinated network survey was performed in the Northern region of Ghana. This region supplies about half of the total cowpea production of Ghana [154]. On the basis of the importance of cowpea production, the presence of school feeding programmes and the accessibility of the area, the district of Tolon-Kumbungu was selected with the help of extension services of the ministry in charge of agriculture of Ghana. The majority of the farmers of this district produce cowpea, at small scale and all the actors of the cowpea food network could be found, namely farmers, processors, consumers and traders. Very often, actors played more than one role for example, simultaneously being farmer, processor and consumer. The presence of primary schools involved in the Ghana school feeding programmes was essential for the human nutritionist of the team to allow the investigation, in a controlled environment, of the health implications of the consumption of specific cowpea dishes. Certain processors were selected after identification of the different cowpea dishes known by informants during focus group discussions. The sample size per type of food was determined in relation to the population of cowpea processors in the surveyed communities. Street food vendors and housewives were considered to be the small scale processors in this thesis. No medium scale or industrial form of processing was encountered. This survey, the results of which are not reported in the present thesis, made the team aware of the production, processing, consumption and trade of cowpea at the rural level.

Surveys were also performed in Benin in which urban communities were interviewed. As a follow-up, the processing and consumption aspects of the survey were also investigated in Central and Southern parts of Benin. The statistics and advices from the extensions services of the ministry in charge of agriculture directed us to the Ouémé and Collines regions as areas with a high production of cowpea. In order to increase the possibilities to encounter a greater diversity of cowpea foods and landraces, other regions were also surveyed (Mono and Zou), all in Central and Southern Benin.

The surveys showed that processing techniques, used for the production of cowpea dishes in both Ghana and Benin, were very similar. Therefore, the study was not pursued in both countries but only in Benin to reduce research costs. This thesis on cowpea processing in West Africa did not involve Nigeria or Niger, the biggest cowpea producers in the sub-continent. Nevertheless, we assume that our findings apply to all West African countries because of the fundamental nature of our investigations. Indeed, Dovlo et al. [56] and Akinrele [7] reported a large diversity of cowpea products in West Africa, which are prepared with the same unit operations applied in Benin.

COWPEA AS A VEHICLE FOR THE ALLEVIATION OF NUTRITIONAL DEFICIENCIES

Protein and energy malnutrition as well as micronutrient (in particular of iodine, vitamin A, iron, zinc and, folic acid) deficiencies are major health challenges encountered in West Africa.

Cowpea has always been considered as a good supplier of protein and energy. The supply of proteins by cowpea could be hindered by the presence of lectins, trypsin inhibitors, phytate and phenolic compounds whereas starch could be unavailable for digestion due to its physical inaccessibility, its presence in native form in foods or its presence in indigestible form as a consequence of cooling after hydrothermal processing. Apart from phytate and phenolic compounds, that have been addressed in this thesis, lectins, trypsin inhibitors and resistant starch were not quantified in cowpea landraces nor in processed cowpea foods. Indeed, previous studies already reported on the prevalence of lectins and trypsin inhibitors in cowpea and the efficacy of heating treatments to inhibit these anti-nutrients [19, 62, 169, 181, 261]. Soaking followed by cooking for about 80 min) is recommended [19]. This kind of process is commonly practiced in West Africa, which justifies the lack of focus on these parameters. However, further investigations could tackle the effectiveness of cooking with cooking aids, and the combinaison of wet dehulling with cooking on the presence of components inhibiting protein digestibility.

The supply of sufficient metabolisable micronutrients is reported to depend on four factors: 1) the amount of food consumed 2) the amount of micronutrient precursor present in the food, 3) the proportion of nutrient available for metabolism (bioavailability) and, 4) the proportion effectively metabolised or converted in an active form (bioefficacy) [226]. In the case of iron deficiency, an effective supply of available iron can be ensured by adding available iron as supplements to the diet (supplementation) or as ingredient to specific foods (fortification), by breeding for a required micronutrient content or functional compounds in staple crops (biofortification), by diversifying the diet to gather enough iron from different foods (dietary diversification), by reducing the presence of chelating agents in foods or by increasing the consumption of iron absorption enhancers. Apart from these strategies, a food chain approach can be applied. This approach consists of studying the effect of all events along the food chain, from seed production to food consumption, on iron availability to identify applicable solutions [66].

The Beninese situation, which also applies to West Africa in general, is characterised by:

- the predominance of crops that have a low iron content and/or are rich in compounds that inhibit iron absorption (cereals, pulses, root and tubers);
- the low affordability and seasonality of iron-rich foods (*e.g.* red meats) and foods that enhance iron absorption (vegetables and fruits containing vitamin C);
- limited access of vulnerable populations to health care services;
- food habits consisting of household or small scale food production rather than large-scale industrial production.

These facts are known to strongly affect the implementation of any strategy to solve deficiencies in micronutrients. The food chain approach is advocated by Slingerland et al. [227] as it offers the opportunity to achieve a more sustainable production of nutritious foods. As this approach constructs solutions based on the use of local resources, it is assumed to insure better appropriation of the outcomes. The "From Natural Resources to Healthy People" INREF programme on sorghum foods in Benin and Burkina Faso showed that the combination of breeding for high iron - low phytate varieties, efficient fertilisation, organic amendment and improved dephytinisation of food helped to provide food rich in available iron to the local population [227].

In the frame of our project, the potential of legume crops and legume-based dishes was evaluated (Chapter 2 and 3). For consumption purposes, food crops are often processed or consumed in combination with other crops or ingredients. Cowpea leaves, cereals (maize, rice), roots and tubers (cassava, yam), fat sources (palm oil, refined vegetable oil), fruits (plantain), seasonings (salt, shrimps, and tomatoes), cooking aids (sodium bicarbonate, *Kanwu*, potash) were identified in the case of cowpea. Whereas some ingredients are mandatorily used in certain dishes, others are optional. Most of these ingredients increase the phytate and polyphenols content of the dishes. In order to encompass the contributions from ingredient other than cowpeas, we determined of iron and inhibitors per dish (Chapter 3). However, side-dishes like tomato stew (prepared with tomatoes, smoked and grounded shrimps, and salt), plantain and yam were not analysed. Shrimps are important ingredients as they provide heme iron [32, 234] to cowpea side-dishes. However, it is reported that high-fat diets adversely affects the absorption of iron [233]. Therefore, the frequent consumption of dishes like *Magni-magni* (steam-cooked cowpea dough), *Doco* (deep-fat fried dumpling made with whole cowpea), *Ata* (deep-fat fried dumpling made with dehulled cowpea), *Ataclè* (twice fried *Doco*) and *Abla* (steam-cooked maize and cowpea dough) which contain 24-45 % of fat d.w. could lead to low iron absorption. In dishes like *Adjagbé* (with 15% of fat) and *Ata* (40 % of fat) care should be taking to reduce the fat content to ensure a better absorption as the [phytate]:[iron] molar ratio is rather low (around 0.3). Thus, the study of the cowpea food chain to improve the intake of available iron should also take into account ingredients of the diet other than cowpeas.

Common cowpea processing techniques or combinations of techniques utilised in West Africa (namely dehulling, cooking, frying, steaming, roasting) are unable to substantially lower the [phytate]:[iron] molar ratio of cowpeas under the threshold ratio of 0.3 [172] for a high iron availability that would allow more than 70 % iron availability. Most combination of treatments showed a [phytate]:[iron] molar ratio of 2-6, resulting in less than 10 % availability. Moreover, some treatments were reported previously to induce losses of minerals [136, 135]. Only the incorporation of a large amount of cowpea leaves in cowpea dishes showed a high iron availability on the basis of [phytate]:[iron] molar ratio (0.37), so is the case of the food called *Adjagbé* (Chapter 3). Indeed, cowpea leaves contain more iron (190-244 mg/kg d.w.) and less phytate (0.17-0.19 g/kg d.w.) than cowpeas (56-76 and 7-15 g/kg d.w., respectively). Therefore, the promotion of the consumption of cowpea leaves is an option. This recommendation derives from the fact that many vegetables are already produced in home gardens, in intercropping systems, in low-lying flood lands or year-round on intensive urban and peri-urban market-gardening systems [21]. Moreover, the consumption of sauces based on leaves with cereal or root or tuber paste side-dishes is a common food habit in Benin [50]. The effect of the diversity in cowpea landraces on the availability of iron in cowpea leaves deserves further investigation. Furthermore, although the [phytate]:[iron] molar ratio is favourable dishes containing cowpea leaves, phenolic compounds, citrate and fibres can act as barriers for the absorption of iron [80, 135] whereas the inhibitory effect of oxalate is not proven [85]. All these compounds are present in cowpea leaves. The *in-vivo* bioavailability of iron in dishes based on cowpea leaves also deserves assessment. Besides, beans are the most often consumed part of the cowpea plant and options are needed to upgrade the availability of iron in cowpea based dishes. Therefore, we tried to lower the concentration of iron absorption inhibitors. Fermentation has long been advocated as a process that favours the degradation of phytate in cereals and legumes and consequently renders iron more available. Moreover, bacterial, fungal and yeast fermentations have a positive effect on the reduction of the most important constraint reported for cowpea consumption: flatulence (Chapter 4). The spontaneous sour fermentation of cowpea flours and cowpea leaves was investigated. A few preliminary results of cowpea flour fermentation are depicted by Figure 1.

The sour fermentation of cowpea leaves, when inoculated with a fermented maize dough and incubated for up to 7 days at room temperature (28-30 °C), caused no significant reduction of the phytate and phenolic compounds in cowpea leaves. After 24 hours, a sour fermentation of cowpeas reduced around 50 % of the phytate in cowpeas whereas no significant effect was observed on total phenolic content. Consequently, optimisation of the fermentation of cowpeas needs to be investigated to increase the availability of minerals in these cowpeas. Besides, the preliminary results of Martens [145] show, during the fermentation of cowpea flour at 30 °C, that a flour: water ratio of 1:1 or 1:3 reduced more flatulence factors than a ratio of 3:1. The variation of the duration of the fermentation and the flour: water ratio affects functional properties and, organoleptic properties, which are important factors for the acceptance of fermented cowpea products.

Another option was to screen the variability in the iron content of existing cowpea landraces. From the joint screening of landraces in use in Benin and in Ghana, the average iron content ranged from 0.05 to 0.10 g/kg. This low variability did not allow breeding for high iron containing landraces from the available local varieties. Biofortification of cowpea landraces via the crossing of promising landraces to achieve a higher iron content in cowpea landraces was therefore not considered to be a viable option.

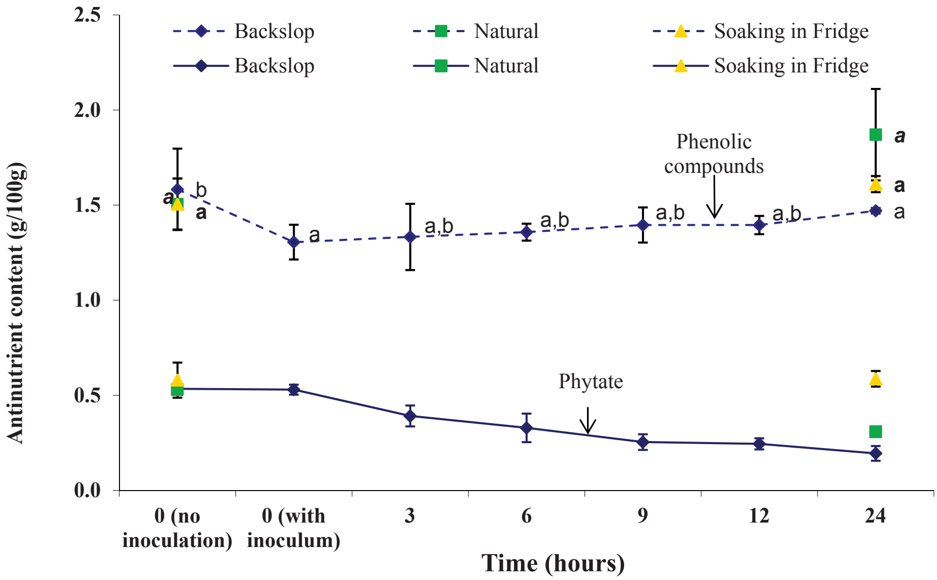


Figure 1

Effect of the fermentation of cowpea flours on the content of phenolics and phytate

All these trials pointed towards food fortification as an option to increase iron availability in cowpea dishes though local people consume few foods processed at a central location. This fortification could be performed in weaning foods, which are now more and more available in West African markets or in food processed for school feeding programmes. Several small-scale enterprises specialized in the production of weaning food are spreading in West African cities and these can serve as a location for the central production of fortified foods. Organoleptic changes, especially colour changes are reported to constrain the acceptance of fortified foods. With respect to this matter, two aspects are in favour of cowpea flours or cowpea dishes as a vehicle for iron fortification. First, several cowpea landraces are pigmented and their pigments can easily mask the colour of the fortificant. Second, many cowpea dishes are prepared with palm oil, which has a dark red colour. The fortification of this palm oil, which is very common cooking oil in West Africa, also seems to deserve further research. In the TELFUN programme, the school feeding programmes were used as channel for fortification. Abizari et al. [1] showed that NaFeEDTA fortification of white cowpea flour can successfully improve the iron and haemoglobin status of 5-12 y-old school children. However, the presence of infections (malaria, helminthic worms, intestinal worms, etc) sometimes hampers the enhancement of the haemoglobin status [101]. Moreover, experiences with iron supplementation indicate that iron supply to malaria infected subjects exacerbate the infection and might lead to death [176, 219, 256]. Therefore, until evidence is collected on the effect of iron fortification on malaria and worm infections, these infections might have priority over iron deficiencies.

REDESIGNING COWPEA FOOD PROCESSING

Consumer preferences and the use of cooking aids

Traditional food processing is an expression of the knowledge inherited and transmitted from generation to generation by local populations. These processes are often modelled according to the organoleptic properties sought for and characterising the prepared dish. The nutritional implications of food processing are often not the primary focus of food processors (*i.e.*, housewives and street vendors) in West Africa. We observed a preference of cowpea consumers for unpigmented landraces. This behaviour is governed by the demand for convenient-to-process cowpea landraces (short cooking time and easy dehulling) and the perception that unpigmented cowpeas are healthier legume grains than pigmented cowpeas. During our surveys, we observed a shift towards preference for unpigmented landraces, even for dishes like *Atassi* where pigmented landraces are originally used for their colouring properties. In this case, rock salts are used to extract the colouring compounds from unpigmented beans and colour the rice. Such practices increase the use of cooking aids and may decline the use of the diversity present in pigmented cowpea landraces. Whole flour of pigmented and unpigmented cowpeas could be interchangeably used for processing because their technological properties are comparable for many parameters. The dehulling of pigmented landraces deserves to be promoted especially for dishes that only require cowpea cotyledons. A successful trial was performed with the PRL dehuller [151]. Dry dehulled cowpeas from pigmented and unpigmented landraces are currently being assessed in Benin for their suitability to process *Ata*. In West Africa, several enterprises produce dehulled cowpea flour for *Ata* (Benin) or *Koose* (Ghana) or *Akara* (Nigeria) and *Adowè* processing but the texture of the end-product is often of a lower quality than when freshly wet dehulled cowpeas are used. An optimisation of the dehulling procedure is therefore required to satisfy processor and consumer expectations.

Legume grains take long to cook as a consequence of seed coat hardening. This hardening process can be mitigated by storing the beans at low temperature but this is unaffordable for most street vendors and small-scale processors [90]. Locally applied measures, efforts to reduce time and fuel consumption during cooking of legumes in West Africa involved soaking, dehulling or more commonly the use of cooking aids during soaking and/or cooking. Softeners effectively shorten the cooking time and may change the flavour or other organoleptic properties of the processed foods. Different approaches based on the hypothesis that the divalent cations of the middle lamella of legume seeds are responsible for the hard-to-cook defect were implemented to study the effect of softeners on legumes. The effect of processing with different softeners at equal concentration (*Kanwu* and pure salts; [249]), at different concentrations (*Kanwu* and pure salts; [247]), or at various monovalent:divalent cation ratios in pure salt solutions [52] on the properties of legumes were compared. In this thesis, the concentration of carbonate and bicarbonate was made equal for all treatments in order to study the effect of the variation of mineral content. Overall, the efficacy of monovalent ions to lower the cooking time of legumes is confirmed. It is assumed that the presence of monovalent ions counteracts the formation of insoluble Ca or Mg pectinates. During these tests, cooking time was determined by manual assessment of the softness of the cooked beans, namely by squeezing them between fingers and thumb. In a random sample of ten beans, when 8 out of 10 beans can be easily squeezed, the batch is declared cooked. This method is subjective as it depends on the judgement of the operator. Therefore, the modified Mattson cooking device [255] might be a better method because it allows for an objective determination. This cooking device was developed for rather big seeds like those of common bean (*Phaseolus vulgaris*). The device requires the positioning of 25 beans on a rack where each bean is vertically surmounted by a rod of a specific weight (82 g) which will pierce the bean when it is cooked. We observed that small-sized grains do not to fit into the holes of the device as it is being used at present. Besides, the weight of the rod might not be appropriate for smaller seeds like cowpeas.

Consequently, we recommend developing a modified version of this device to get an objective method to determine the softness of cowpeas.

This thesis showed that cowpea could be cooked with up to 0.5 % (w/v) of *Kanwu* solution without major damage to the amino acid composition and lysine availability. Nevertheless, the high concentration of sodium in the *Kanwu* solution especially the yellow *Kanwu* might be detrimental to human health. Present nutritional insights recommend lowering the intake of sodium as a daily consumption of 100-200 mmol of sodium was associated with damageable increase of blood pressure [11]. The option to commercialise dehulled precooked or parboiled cowpea should be investigated.

SOAKING AND FERMENTATION OF COWPEAS: CONSUMER PREFERENCE PERSPECTIVES

In West Africa, consumption of fermented leguminous seeds is limited to alkaline condiments such as *Afitin, Netetou* or *Dawadawa*, obtained from African locust beans, melon seeds, and similar protein-rich seeds. Contrary to this, the acidic taste of fermented cereal, root and milk products is widely spread and appreciated. Our surveys in Ghana and Benin did not reveal any fermented cowpea dish. This situation is in contrast to that in Asia where fermented legumes are common. They are produced from various crops and well appreciated by consumers of all ages. However, we noticed recently that soya beans and sometimes cowpea were used to process the local cheese called Wagashi (personal observations). This is a sign that traditional practices can evolve.

Soaking shortened the cooking time. In addition, soaking at room temperature (28-30 °C) reduced flatulence (Chapter 4). Lactic acid fermentation, either induced by backslopping, or by inoculation with *Weissella beninensis,* and fermentation with *Saccharomyces cerevisiae* diminished the flatulence produced from cowpea consumption (Chapter 4). Besides, the Togolese, Beninese and Nigerian participants of the flatulence experiments appreciated the taste of the *Weissella* fermented cowpea porridge and emphasised that they liked the absence of the beany flavour in the cowpea porridges. The acidic fermentation of cowpea seems to have potential for the production of consumer products. However, this requires further investigations and product development. Especially, the fermentation conditions need to be optimised and the form in which the products are sold to the consumers deserves better attention.

THE INTERDISCIPLINARY APPROACH TO IMPROVE FOOD SOVEREIGNTY

Our research programme was designed to investigate how technological improvements and promotion of local crops and indigenous foods can enhance food sovereignty and the nutritional status of local populations. Food sovereignty is supposed to flower from four roots: the right to safe food for all people, the access to productive resources, the ecologically-friendly production and, the development of trade and local markets [201]. TELFUN implemented a collaborative and integrative approach for four scientific disciplines to feed these roots. Attempts were made to study and evaluate the production, processing, consumption and trade of a local crop for better health.

On the basis of this conceptual framework, food science and technology research would contribute to the selection of the most appropriate raw material and processing techniques to provide nutritious and culturally acceptable foods to local people. In West Africa, iron deficiency anaemia was reported to be a public health issue to tackle and cowpea a local crop well embedded in people' traditions and food habits. On the one hand, our research showed that landraces available at the local level contain low amounts of poorly available iron. Traditional processing techniques are unable to raise the availability of iron in cowpea dishes to an adequate level. The attempt to find an affordable processing technique that improves on this matter has not been successful: fermentation did not reduce significantly the level of phytate and phenolic compounds in the bean. Enzymatic dephytinisation (with an artificial enzyme or an efficient plant phytase like rye-phytase) would be inapplicable in our case. However, dishes based on cowpea leaves showed acceptable [phytate]:[calcium] and [phytate]:[iron] molar ratios. On the other hand, improving the acceptability of cowpea dishes requires the reduction of flatulence induced by these dishes. We came up with approaches that need to be further investigated with respect to consumer wishes.

The interdisciplinary approach of our project allowed a better appraisal of the needs of local populations and challenges related to the local network of cowpea. This approach served the purpose of a more holistic evaluation of the case to be studied. This case was characterised by a focus on a local crop (cowpea) to solve a health issue (iron deficiency anaemia). These characteristics could both be seen as strengths and weaknesses for the project. The strengths originated from the fact that these characteristics kept the team focussed. Iron deficiency anaemia is a relevant problem for the targeted population though no link was made by local people between cowpea and anaemia. Cowpea foods are well known and culturally accepted by surveyed consumers. However, few options were available to resolve the targeted problem of iron deficiency anaemia. From the breeding and food science point of view, we established the limitations of the crop selected for this study and avoided utopic recommendations. Though all researchers maintained information exchange along the course of the programme, time constraints limited the interdisciplinary activities to the early stage of the programme. The achievements of all disciplines should be integrated and discussed with local communities.

The interdisciplinary approach gave opportunities to the team to study issues that are relevant to the actors of the cowpea network and for some of the researchers, to intervene in this network (*e.g.* the intervention in the Ghana school feeding programme by the nutritionist who fortified cowpea foods to improve the iron status of children [1]). The fact that certain disciplines (breeding and food technology) were supposed to provide innovative technologies to the team or the interdependence of the researchers limited the whole interdisciplinary approach. In the framework of our PhD studies for which disciplinary depth is also a requirement, interdisciplinary activities can only go to a certain level. Nevertheless, the approach used in this thesis shows that an interdisciplinary research project may lead to different choices in comparison with a strictly disciplinary approach.

IMPLICATIONS FOR FUTURE RESEARCH AND RECOMMENDATIONS

The findings of this thesis allow various implications and recommendations.

Cowpea dishes made from leaves should be investigated for their in-vitro and/or in-vivo iron availability. Sauces made from leafy vegetables are popular in West African countries. Our investigations only tackled the potential of these dishes not their actual ability to release available iron. Moreover, ingredients other than cowpea leaves such as meat, shrimps and palm oil can affect the uptake of the iron provided by these dishes.

The production of cowpea as a leafy vegetable in the suburban small-scale agricultural systems deserves further investigation. The actual status of the consumption of cowpea leaves in a leafy vegetable sauce is rather seasonal and coincides with the cowpea production period in production areas. In collaboration with a plant physiologist, the optimal harvesting period and the most appropriate landrace could be identified to obtain iron-rich and edible cowpea leaves that fit with the processing practices used in West Africa. Moreover, other traditional leafy vegetables could also be investigated for their potential to provide available iron to consumers.

A dehuller needs to be developed for an efficient decortication of cowpeas. The necessity to dehull cowpeas is an important constraint for processing many cowpea dishes. The concept of the abrasive dehullers (*e.g.* Prairie Research Laboratories dehullers) can be used as a starting point. Two options could be offered to processors: a specific dehuller for the production of dehulled cowpea flours for different uses or the installation of multi-purpose dehullers like the cereal mills which are very popular in West African communities. When cowpea flour production is the preferred option, the optimal characteristics of the flours that are needed to produce cowpea dishes comparable with the traditional foods should be determined.

Rock salts, which are used to reduce the cooking time of cowpeas and leaves, were shown to provide a high amount of sodium and other minerals but had little effect on the amino acid profile and lysine availability. *Extension services should raise awareness of street vendors and consumers on the probable impact of use of large amount of Kanwu on sodium intake*. Besides, technologies should be developed to supply precooked or parboiled cowpeas to consumers.

In Academia, conditions for the implementation of interdisciplinary research (in the frame of PhD studies) need to be further examined to achieve better productivity. The experience of the TELFUN programme showed that the disciplinary character and time set for PhD programmes limit interactions and integration of scientific activities between different disciplines.

CONCLUDING REMARK

Overall, no food processing practice or technique was developed that could provide high amounts of available iron to consumers. However, this study uncovered options for improvement by the diversification and promotion of the consumption of dishes made from cowpea leaves. Moreover, new options for product development to keep cowpea foods on the menu have been presented. Consequently, our objectives were not fully achieved. The interdisciplinary approach that was implemented, favoured reflection on society-relevant matters whereas its setting required adjustments to fit into the academic frame. Thus, this study illustrated the possibility to perform meaningful technological research within an interdisciplinary research project.

7

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8

SUMMARIES IN ENGLISH, DUTCH AND FRENCH

SUMMARY

In Africa, where the economy is commonly based on agriculture, malnutrition and micronutrient deficiencies are widespread. Moreover, the globalisation of the world economy favoured and increased the dependency of African people on staple crops and foods produced outside the continent and sold at low prices on the local market. Therefore, we hypothesised that restoring the geographical connection between food production and food consumption in local food networks by improving traditional foods can reduce poverty and nutritional deficiencies prevailing in Africa. The present thesis studied the potential of indigenous resources and locally developed practices to supply culturally acceptable and nutritious foods to African resource-poor people, using cowpea as model crop.

An interdisciplinary approach involving a plant breeder, a food technologist, a human nutritionist and a social scientist was implemented in Benin and Ghana (through the TELFUN programme) to capture the potential of the cowpea food network in these countries. In this context, the food science and technology research explored the opportunities for improvement and redesigning cowpea foods by addressing four specific objectives: (i) characterise cowpea landraces in use in Benin with regard to nutritional, anti-nutritional and functional properties; (ii) determine present cowpea processing methods and eating habits with special reference to the content of cowpea dishes in available iron, zinc and calcium; (iii) assess the effect of the use of alkaline cooking aids on amino acids of cooked cowpea, and (iv) assess the impact of processing techniques on the flatulence generated by the intake of cowpea foods.

In chapter 2, the opportunities to improve cowpea at the farm gate were explored. The diversity of cowpea landraces in use in Benin was investigated with regard to the genetic interrelationships, the nutritional properties, and the suitability of cowpea landraces for the preparation of West African dishes. Unpigmented landraces and pigmented landraces were the two major types of cowpea identified by stakeholders. The small-scale cowpea processors (*e.g.* street vendors) prefer to process unpigmented landraces, which are fast-to-cook and fast-to-dehull using condiment mills. Moreover, consumers believe that unpigmented landraces are healthier than pigmented ones (Chapter 3). Our analyses disclosed the high similarity between all unpigmented landraces (65 %, one cluster), as opposed to pigmented landraces (three clusters), irrespective of the parameters used for the comparison. On average (g/kg dry weight (d.w.)), protein (255), ash (39), calcium (0.95), phytate (9.3), iron

(0.07), and zinc (0.04) contents of all cowpea landraces were similar. Nevertheless, unpigmented landraces contained, in g/kg (d.w.), less fibre (24), and phenolics (3) than pigmented ones (56 and 8, respectively). The technological properties showed significant differences between landraces. Unpigmented landraces had bigger seeds (200 g d.w. for 1000 seeds), and lower water absorption capacity at 30 ºC (1049 g/kg) than pigmented ones (139 g d.w. and 1184 g/kg, respectively). The integration of genetic, compositional and technological properties of unpigmented landraces unravelled the interchangeability of unpigmented landraces as opposed to pigmented landraces. Implications of these findings for innovations in cowpea processing are discussed.

From farm-to-fork, the diversity of cowpea dishes and processing techniques in use in Benin was evaluated with special reference to their potential contribution to the mitigation of iron and zinc deficiencies. The implications of the food processing techniques in use in Benin for the nutritional quality of cowpea dishes were assessed. Cowpea processors identified long cooking time, tedious dehulling and, the perishability of beans during storage and processed foods after production, as key constraints (Chapter 3). The composition of the cowpea dishes, as they are traditionally produced, was as follows: energy, protein, iron, zinc, and calcium contents ranged from 1647 to 2570 kJ, 100 to 250 g, 10 to 350 mg, 15 to 30 mg, 0.4 to 3.8 g per kg (d.w.), respectively. The iron and calcium contents were higher in dishes containing leaves than in dishes made from beans. Cooking, dehulling, deep-fat frying and soaking were identified as the main processing unit operations in cowpea processing while fermentation and germination were unusual practices. Present cowpea processing habits lead to low [phytate] : [mineral] molar ratio but some opportunities exist that might contribute to the supply of available iron to consumers, namely the use of cowpea leaves.

The local solution to deal with long cooking time of cowpea is the use of cooking aids (*i.e.* rock salts and sodium bicarbonate). This practice raised concern because the high pH resulting from such salts may impair the nutritional quality of proteins. Therefore, the potential damage of softeners to cowpea proteins was investigated. In chapter 4, the effect of the mineral composition and concentration of alkaline cooking aids on the amino acids of pigmented (brown) cowpea was determined. A significant cooking time reduction could be achieved (up to 25 %) by boiling cowpeas in a rock salt solution (0.1 %, w/v). Fortunately no significant change in the amino acid and available lysine contents was detected at cooking aid concentrations lower than 0.5 % (w/v). The high pH (8-10) induced by the use of cooking aids and the temperature reached during boiling (100°C) did not lead to the formation of a detectable amount of lysinoalanine, a cross-linked amino acid known to be carcinogenic for rats.

From the table to well-being, the constraints related to the consumption of cowpea were investigated. Field surveys revealed that flatulence was the main reason that hinders the consumption of cowpeas. In chapter 5, information is provided about the effectiveness of the contribution of traditional processing practices to a better digestibility of cowpeas. Innovative processing techniques were also studied, involving fermentations. Galactose-oligosaccharides were not detected in cowpea hulls and these hulls yielded a low *in-vitro* fermentability index as compared with other treatments. Traditional processing induced a limited reduction of raffinose and verbascose contents contrary to fermentations. The *in-vitro* fermentability index appeared similarly high for all processed cowpea except for *Rhizopus* and *Bacillus* fermentations. The *in-vivo* fermentability index of fermented cowpeas was significantly lower than that of traditionally processed cowpeas. As the consumption of cowpea-based dishes contributes significantly to the protein intake of many people, new products with a low-flatulence potential that are sensory acceptable to African consumers should be developed based on soaking and/or fermentation to sustain cowpea consumption.

Finally, all findings were integrated in chapter 6 and the applicability of our results was discussed, as well as the opportunities to alleviate iron deficiency and to innovate in cowpea processing. Moreover, the food technology contribution to the interdisciplinary approach of the TELFUN programme and the implications of its findings for future research were assessed.

SAMENVATTING

Ondervoeding en tekorten aan micronutriënten komen algemeen voor in Afrika, waar de economie grotendeels op landbouw is gebaseerd. Daarbij versterkt de globalisering van de wereldeconomie de afhankelijkheid van de Afrikaanse bevolking van geïmporteerd basisvoedsel, dat op de lokale markt tegen bodemprijzen wordt verkocht. Vandaar de hypothese dat herstel van de geografische koppeling tussen voedselproductie en –consumptie in lokale voedselnetwerken armoede en voedingstekorten kan verminderen; dit zou kunnen worden gerealiseerd door traditionele voedselproducten te verbeteren en aantrekkelijker te maken. In dit promotieonderzoek werden de mogelijkheden onderzocht om met plaatselijke grondstoffen en lokale methoden, arme Afrikaanse consumenten van cultureel acceptabele en voedzame producten te voorzien; hierbij werd cowpea (Nederlandse naam: zwarte ogenboon) als modelgewas gebruikt.

In een interdisciplinaire aanpak werden door een plantenveredelaar, een levensmiddelentechnoloog, een voedingsdeskundige en een sociaal wetenschapper in Benin en Ghana de mogelijkheden van het plaatselijke cowpea voedselnetwerk onderzocht in het kader van het TELFUN programma. In deze context richtte het technologisch onderzoek zich op de verbetering en innovatie van cowpeaproducten met vier specifieke doelstellingen: (i) beschrijving van nutriënten, anti-nutriënten en functionele eigenschappen van in Benin gebruikte cowpea landrassen; (ii) inventarisatie van gangbare cowpeaverwerkingsmethoden en -eetgewoonten met speciale aandacht voor hun invloed op de gehalten aan beschikbaar ijzer, zink en calcium in eindproducten; (iii) beoordeling van de invloed van lokale, alkalische kookverbeteraars op aminozuren in gekookte cowpeas, en (iv) het effect van verwerkingsmethoden op flatulentie veroorzaakt door de consumptie van cowpeaproducten.

In hoofdstuk 2 werd de mogelijkheid bestudeerd om in de primaire productie betere cowpeas te verkrijgen. De in Benin gangbare cowpea landrassen werden onderzocht op hun genetische samenhang, voedingswaarde, en geschiktheid voor het bereiden van West-Afrikaanse gerechten. Belanghebbenden onderscheiden twee hoofdsoorten cowpeas, namelijk ongepigmenteerde en gepigmenteerde landrassen. Kleinschalige cowpeaverwerkers (o.a. voor de straatverkoop) geven de voorkeur aan ongepigmenteerde cowpeas, die sneller gekookt en mechanisch ontvliesd kunnen worden. Bovendien denken consumenten dat ongepigmenteerde cowpeas gezonder zijn (hoofdstuk 3). Het vergelijkend onderzoek toonde aan dat, terwijl ongepigmenteerde landrassen onderling weinig verschillen, in de gepigmenteerde landrassen 3

groepen konden worden onderscheiden. De gemiddelde gehalten (g/kg drogestof (ds)) aan eiwit (255), as (39), calcium (0,95), fytaat (9,3), ijzer (0,07) en zink (0,04) waren voor alle landrassen gelijk. Echter, ongepigmenteerde landrassen bevatten minder vezel (24 g/kg ds) en fenolen (3) dan gepigmenteerde landrassen (respectievelijk 56 en 8 g/kg). De technologische eigenschappen van landrassen vertoonden significante verschillen. Ongepigmenteerde hadden grotere zaden (200 g ds per 1000 zaden) en een lagere waterabsorptiecapaciteit bij 30 °C (1049 g/kg) dan gepigmenteerde landrassen (resp. 139 g ds en 1184 g/kg). De combinatie van de samenstelling, en de genetische en technologische eigenschappen laat zien dat ongepigmenteerde en gepigmenteerde landrassen elkaar zouden kunnen vervangen. De consequenties hiervan voor cowpeaproductinnovatie worden besproken.

In de keten van primaire productie naar de consument werd de diversiteit van cowpeagerechten en -bereidingsmethoden onderzocht, speciaal met het oog op hun mogelijke bijdrage aan de vermindering van ijzer- en zinktekorten in de voeding. Verder werd aandacht besteed aan de invloed van in Benin gangbare verwerkingsmethoden op de voedingswaarde van cowpeagerechten. Cowpea verwerkers beschouwden lange kooktijd, bewerkelijke ontvliezing en bederfelijkheid van cowpeas tijdens bewaring en als bereid product als de belangrijkste knelpunten (hoofdstuk 3). Van traditioneel geproduceerde cowpeagerechten liepen de energie, eiwit, ijzer, zink en calcium gehalten uiteen van respectievelijk 1647 – 2570 kJ, 100 – 250 g, 10 – 350 mg, 15 – 30 mg, en 0,4 – 3,8 g per kg ds. De ijzer- en calciumgehalten waren hoger in gerechten met cowpeabladeren dan zonder. De belangrijkste bereidingsmethoden waren koken, ontvliezen, frituren in olie, en weken in water; daarentegen was het ongebruikelijk om cowpeas te fermenteren of te ontkiemen. De gangbare bereidingsgewoonten resulteren in een lage (gunstige) molaire verhouding [fytaat:ijzer], terwijl er mogelijkheden zijn de ijzerinname te vergroten door de toepassing van cowpeabladeren.

Het probleem van de lange kooktijd van cowpeas wordt lokaal bestreden door toepassing van kookverbeteraars (zoals steenzout en soda). Aangezien de hierdoor veroorzaakte hoge pH de voedingswaarde van de eiwitten zou kunnen schaden, werd dit in hoofdstuk 4 bestudeerd. De invloed van de mineralensamenstelling en concentratie van alkalische kookverbeteraars op de aminozuren van gepigmenteerde (bruine) cowpeas werd onderzocht. Koken van cowpeas in een 0,1 % w/v steenzoutoplossing leverde een 25 % kortere kooktijd op. Tot een concentratie van 0,5 % w/v steenzout gaf dit geen nadelige effecten op de gehalten van aminozuren en beschikbaar lysine, terwijl de hoge pH (8-10) in combinatie met de kooktemperatuur (100 °C) geen aantoonbare vorming te zien gaf van lysinoalanine, een aminozuurreactieproduct dat carcinogeen is voor ratten.

In het traject van consumptie naar welbevinden werden de beperkingen van cowpea beschouwd. Uit veldonderzoek bleek dat de consument het eten van cowpea voornamelijk matigt vanwege de veroorzaakte flatulentie. In hoofdstuk 5 wordt ingegaan op de effectiviteit van traditionele bereidingsmethoden op het verbeteren van de verteerbaarheid van cowpeas. Hierbij werd ook de voor cowpea ongebruikelijke methode van fermentatie onderzocht. Vergeleken met andere cowpeabestanddelen bevatten vliezen geen meetbare galacto-oligosaccharide en hadden een lage in-vitro fermentatie (een index voor flatulentie). In tegenstelling tot fermentatie leidden traditionele bereidingsmethoden tot een beperkte afname van raffinose en verbascosegehalten. De in-vitro fermentatie was sterk voor alle cowpea bereidingsmethoden behalve voor fermentaties met *Rhizopus* en *Bacillus* soorten. De in-vivo fermentatie van gefermenteerde cowpeas was significant minder dan van traditioneel bereide cowpeas. Gezien de belangrijke rol van cowpeas als eiwitbron en de wenselijkheid een duurzame cowpeaconsumptie te bewerkstelligen, dienen nieuwe, voor Afrikaanse consumenten acceptabele producten met een laag flatulentiepotentieel te worden ontwikkeld, gebruikmakend van week- en fermentatiemethoden.

Uiteindelijk worden in hoofdstuk 6 alle resultaten gecombineerd en hun toepasbaarheid in cowpeaproductinnovatie besproken, in het licht van de beoogde vermindering van ijzer- en zinktekorten. Tevens worden de rol van de levensmiddelentechnologie in het interdisciplinair karakter van het TELFUN programma beschouwd, en mogelijkheden voor toekomstig onderzoek afgewogen.

RESUME

L'agriculture pourvoyeuse de produits alimentaires représente une part prépondérante de l'économie des pays Ouest-africains. Malgré la forte part prise par les actifs agricoles dans la population active en Afrique de l'Ouest, les problèmes nutritionnels et les déficiences en micronutriments y sont encore préoccupants. Les populations sont encore fortement dépendantes des aliments de base importés et vendus à prix compétitifs sur les marchés locaux. On note ainsi une déconnection entre la production et la consommation de plusieurs produits alimentaires. L'hypothèse est donc émise que la restauration de la connexion géographique entre la production et la consommation des aliments dans les réseaux alimentaires locaux par l'amélioration de la qualité des aliments traditionnels pourraient contribuer à la réduction de la pauvreté et des déficiences nutritionnelles. La présente thèse a étudié l'aptitude des ressources et des pratiques technologiques endogènes à fournir des aliments nutritifs et culturellement acceptables aux populations africaines pauvres en utilisant le niébé comme spéculation modèle.

Une approche interdisciplinaire impliquant la génétique végétale, la technologie alimentaire, la nutrition humaine et les sciences sociales a été mise en œuvre au Bénin et au Ghana (à travers le programme de recherche TELFUN) pour appréhender le potentiel du réseau local du niébé dans ces pays. Le volet sciences alimentaires de cette recherche s'est attelé à explorer les opportunités d'amélioration des aliments à base de niébé (i.) en évaluant les propriétés nutritionnelles, anti-nutritionnelles et fonctionnelles des variétés de niébé en usage au Bénin ; (ii.) en déterminant Les habitudes alimentaires et les méthodes de transformation du niébé telles que pratiquées localement avec un accent particulier sur la disponibilité du fer, du zinc et du calcium dans les produits issus de ces transformations ; (iii.) en évaluant l'effet de l'utilisation des agents de ramollissement sur les acides aminés du niébé bouilli ; et (iv.) en évaluant l'effet des techniques de transformation sur la flatulence résultant de la consommation des aliments á base de niébé.

Dans le chapitre 2, la diversité des variétés de niébé utilisées au Bénin a été déterminée en tenant compte de leurs similitudes génétiques, de leurs propriétés nutritionnelles et de leur aptitude á la production d'aliments dérivés. Deux principaux types de variétés de niébé ont été identifiés par les utilisateurs á savoir : les variétés colorées et les variétés non colorées. Les transformatrices locales préfèrent utiliser les variétés non colorées qui cuisent vite et se décortiquent facilement. Aussi, les consommateurs estiment que les variétés non colorées

sont plus nutritives que celles qui sont colorées (Chapitre 3). En intégrant les divers paramètres de caractérisation, nos études ont révélé, la grande similitude entre les variétés non colorées (groupe homogène á 65% de similitude), contrairement aux variétés colorées (trois groupes différents). En moyenne, les teneurs (base matière sèche (b.s.)) en protéines (255 g/kg), minéraux totaux (39 g/kg), calcium (0.95 g/kg), phytate (9.3 g/kg) fer (0.07 g/kg) et zinc (0.04 g/kg) des variétés en usage au Benin sont similaires. Cependant, les variétés non colorées contiennent significativement ($P < 0.05$) moins de fibre (24 g/kg b.s.) et de composés phénoliques totaux (3g/kg b.s.) que les variétés colorées (56 g/kg b.s. et 8g/kg b.s., respectivement). Les propriétés technologiques des farines de grains entiers montrent des différences significatives entre les types de variétés en usage au Bénin. Les variétés non colorées ont des grains plus gros (200 g b.s. pour 1000 grains) et une capacité d'absorption d'eau á température ambiante (30°C) significativement ($P < 0.05$) plus faible (1069 g/kg) que celle des variétés colorées (139 g b.s. et 1184 g/kg, respectivement). L'intégration des caractéristiques génétiques, nutritionnelles et technologiques des variétés en usage au Bénin montre que les variétés non colorées peuvent être utilisées l'une à la place de l'autre contrairement aux variétés colorées. Les implications de ces résultats pour les innovations technologiques sont été discutées.

De la fourche à la fourchette, les apports nutritionnels liés à la diversité des aliments à base de niébé au Bénin ont été évalués et un accent particulier porté à leur contribution à la fourniture de fer et de zinc biodisponible. Aussi, les implications des pratiques technologiques traditionnelles pour la qualité nutritive du niébé ont été quantifiées. Les transformatrices de niébé ont identifié le long temps de cuisson, le décorticage fastidieux, les difficultés de stockage du niébé et de conservation des aliments dérivés comme les principales contraintes liées à la transformation du niébé (Chapitre 3). Les aliments à base de niébé, tels qu'ils sont produits traditionnellement, ont une composition nutritionnelle en terme d'apport énergétique, de teneurs en protéine, en fer, en zinc et en calcium de l'ordre de 1647-2570 kJ, 100-250 g, 10-350 mg, 15-30 mg, 0.4-3.8 g par kg (b.s.), respectivement. Les teneurs en fer et en calcium sont les plus élevées dans les aliments à base de niébé contenant les feuilles de niébé que dans ceux produits à base de grains de niébé. La cuisson, le décorticage, la friture et le trempage ont été identifiés comme les opérations technologiques principales de la transformation du niébé. La fermentation et la germination ne sont pas pratiquées dans les pays enquêtés. Les pratiques actuelles de transformation et

de consommation du niébé induisent de faibles ratios molaires [Phytate] : [Fer, Zinc ou Calcium]. Cependant, des opportunités d'amélioration de la disponibilité du fer dans les aliments à base de niébé existent notamment par l'utilisation des feuilles de niébé.

Pour réduire le long temps de cuisson des grains de niébé, les transformatrices traditionnelles utilisent les agents de ramollissement (potasse ou *Kanwu* et bicarbonate de sodium, par exemple). L'utilisation de ces agents de ramollissement augmente le pH de la solution de cuisson et pourrait altérer la qualité protéique du niébé. Dans le chapitre 4, l'effet de la composition en minéraux et de la concentration en agents de ramollissement de la solution de cuisson sur les acides aminés de variétés de niébé colorées a été déterminé. L'utilisation des agents de ramollissement (0.1 %, poids/volume) pendant la cuisson du niébé induit une réduction significative du temps de cuisson (jusqu'à 25 % de réduction). Toutefois, aucun changement significatif de la composition en acides aminés et en lysine disponible n'a été détecté pour des concentrations en agents de ramollissement de la solution de cuisson de moins de 0.5 % (poids/volume). Le pH élevé (8-10) issu de l'utilisation des agents de ramollissement et la température de cuisson atteinte dans le système traditionnel de transformation (100 °C) n'ont pas conduit à la formation d'une quantité dosable de lysinoalanine, un acide aminé connu comme étant cancérigène pour les rats.

De la table au bien-être, on peut tirer les conclusions suivantes : les contraintes relatives à la consommation du niébé ont été identifiées. Les enquêtes auprès des consommateurs ont révélé que la flatulence est la principale raison qui limite la consommation du niébé. Dans le chapitre 5, l'aptitude des procédés traditionnels de transformation à améliorer la digestibilité du niébé a été examinée. Des procédés novateurs comme la fermentation ont aussi été testés. Les oligosaccharides n'ont pas été détectés dans l'enveloppe externe des grains de niébé souvent incriminée comme responsable de la production de flatulence. Ces enveloppes ont un faible indice de fermentabilité *in-vitro* comparé aux autres traitements. Les procédés de transformation traditionnelle du niébé entrainent une faible diminution de la teneur en raffinose et en verbascose des grains contrairement aux fermentations. L'indice de fermentabilité *in-vitro* des produits issus de tous les procédés de transformation sont élevés sauf ceux issus de la fermentation avec *Bacillus* sp. et *Rhizopus* sp. L'indice de fermentabilité *in-vivo* des produits issus de la fermentation du niébé est significativement plus faible que celui de produits issus des transformations traditionnelles. Etant donné que la consommation des aliments à base de niébé améliore significativement les apports en protéine du régime

alimentaire de nombre de populations pauvres, le développement d'aliments acceptés par les consommateurs africains et induisant une faible flatulence devrait prendre en compte les avantages qu'offrent des opérations unitaires telles que le trempage et/ou la fermentation pour l'élimination de la flatulence résultant de la consommation du niébé.

Enfin, le chapitre 6 intègre les résultats obtenus lors de cette recherche, discute leur applicabilité, et les opportunités de réduction des déficiences en fer et d'innovation dans la transformation du niébé. De plus, la contribution des sciences alimentaires à l'approche interdisciplinaire du programme TELFUN ainsi que les implications des résultats pour les recherches futures ont été appréciés.

ACKNOWLEDGEMENTS

ACKNOWLEDGEMENTS

Time has come to look back at the journey that led to this thesis and its public defence and give thanks to those who have made it possible, one way or another.

This exciting and instructive journey started when my academic guide, prof. Joseph Hounhouigan, introduced me to Anita Linnemann at the occasion of one of her supervision visits to Polycarpe Kayodé. Later on, I applied for the TELFUN programme and was selected as PhD fellow. Herewith, I'm grateful to you, dear Prof. Hounhouigan. Since you mentored my engineer graduation, you have always been confident in my scientific skills and abilities. You never give me a full "laisser-passer", forcing me to keep moving up and always challenged. Thanks for your rigorous guidance before and during this journey. May our common journey be reinforced.

After my selection, the team composed of prof. M.A.J.S van Boekel, dr.ir. Anita Linnemann, and dr.ir. Rob Nout engaged itself in accompanying me on the Phd acquisition path. With the expectation that you have enjoyed this journey, I'd like to thank each of you for instructing me that being a scientist requires to be critical, open-minded, alert, but also to balance work with leisure and sports. Dear Tiny, your deep thoughts, critical questions on my thesis, your invaluable explanations and inputs to my papers but also your proximity and "open-door" attitude have helped me to feel confident and always challenged to improve myself. Dear Anita, you have been the heart and the stick of this team, always encouraging me as you trust that I was able to do better, taking care of all organisational matters very professionally, and available to review all my scientific inputs and outputs at all stages. Dear Rob, during this journey, I have never lacked your experience and pedagogic manners as well as your critical evaluation of my plans and outcomes. I very much appreciated your personal involvement in the flatcow study. As a coaching team, your intellectual and professional guidance, your moral and social support have created a nice atmosphere to work in. This gratitude is extended to your relatives (Corrie, Ragnar, Frances) who have offered me solidarity and hospitality, in one word "a family away from home".

I want to acknowledge the contribution of the MSc (Nele van Ingen, Zhaoying Li, Jing Xiao) and BSc (Rineke Boonen) students who participated in this research. Your commitment was appreciated.

This thesis made me member of a research programme that is also considered as a community: the TELFUN community. I would like to thanks all the Ecuadorian, Indian, Ghanaian and Dutch members of this community for what we shared during these years. You made each yearly meeting an enjoyable scientific meeting and an opportunity to discover new cultures. Razak, Kwadwo, Mina, Renu, Pradeep, Varsha, Shweta, Francisco, Maria-Elisa, Cesar and Alexandra, may this starting collaboration continue and flourish in the future.

During this research, data were collected with the help of the local population from Benin (Districts of Savalou, Adjohoun, Comè, Abomey, Porto-novo, and Cotonou) and Ghana (District of Tolon-Kumbungu) as well as the international students participating in the flatcow study (Wageningen). I acknowledge here your valuable input to this work. I'm thankful to Alphonse Koukè, Benjamin Datinon and Basile Dato who were of great support during the multiplication of cowpea landraces.

In the Netherlands, this research was performed in the Product Design and Quality Management group and the Laboratory of Food Microbiology of Wageningen University. I'm grateful to all my colleagues from PDQ, FPH and FHM, especially Dieuwerke Bolhuis, Van-Anh Phan, and Maimunah Sanny with whom I shared the office during the most stressful period of this thesis; our discussions were very nice, and open as we shared many similar experiences. I would like to thank the "glucosinolate ladies" (Radhika Bongoni, Kristin Hennig, Teresa Oliviero and Irmela Kruse) for our nice discussions and friendship. Sachin Kadam, Jinghua Xiao, Lidia Rebelo-Lima, and Elissavet Gkogka, thanks for your moral support during this journey. I will not forget my colleagues with whom I shared the experiences of the PhD tour in Canada and the PhD tour in England. You made these experiences unforgettable. Special thanks is also given to those who technically supported my research and have many times been of social support, namely Charlotte van Twisk, Geert Meijer, Frans Lettink, Xandra Bakker-de Haan, and Judith Wolkers-Rooijackers, but also Harry Baptist, Ingrid Maas, and Peter de Gijsel. I would like to thank the staff members of these chair groups for their support and the nice discussion that we had at coffee breaks and during lab trips, especially Ruud Verkerk, Tjakko Abee, Pieternel Luning, and Hein van Valenberg. I have bothered the office managers of these departments several times. Therefore, I would like to acknowledge their patience: Ina Dombeek, Lysanne Hoksbergen, Els Jansen, and Gerda van Laar-Engelen.

In Benin, the Department of Nutrition and Food Sciences of the University of Abomey-Calavi hosted my research. The pleasant working atmosphere, and the good collaboration with my PhD fellow and the staff were appreciated. I'm grateful to Amadou Issa, Mathias Hounsou, Carole Vihotogbe-Sossa, Pélagie Agbobatinkpo-Vodouhè, Janvier Kindossi, Laurent Adinsi, dr.ir. Franck Hongbété, dr.ir. Wilfried Padonou, dr. ir. Paul Houssou, dr. Générose Vieira-Dalodé, dr.ir. Waliou Amoussa, dr. Victor Anihouvi, dr. Evariste Mitchikpè, late prof. Romain Dossa, prof. Polycarpe Kayodé, prof. Paulin Azokpota, prof. Noel Akissoé.

I would like to thank Tiny who introduced me to his volley-ball club. One hour a week I could distress with very nice people. I'm thankful for the nice company that you offered me.

I would like to give thanks to Thilda Chevouline who introduced me to the International Catholic Choir of the St John the Baptist catholic church of Wageningen. With this choir led by Hady Klaassen, I could glorify my Lord and share my experiences with very good friends always willing to help. Anne-Hendrike Schuurman, Caucasella Diaz, Anna Zulu, Ivo Jansen, Jorge Merino, Erika Roth, Rosio Miranda, and Damian Selemobri: I appreciated your company.

Since 2007, I shared my joy and sorrows with several country fellows who joined Wageningen for their MSc or PhD studies. Flora Chadare-Assogbadjo, Florent Okry, Eugène Agbicodo, Alphonse Singbo, Latifou Idrissou Aboubacary, Nadia Fanou-Fogny, Rose Fagbemissi, Augustin Kouevi, Guirguissou Maboudou Alidou, Ozias Hounkpatin, Gerard Zoundji, Nicodème Fassinou Hotègni, Djalal Arinloye, Menouwesso Hounhouigan, Fernande Honfo-Zannou, Soule Adékambi, Sylvain Dabadé, Romaric Vihotogbé, Essègbèmon Akpo, Euloge Togbé, Nathalie Kpéra-Mama Sika, Rolland Yemadjè, Edmond Totin and Folachoadé Akogou. Tough he is Togolese, I would like to add Leo Lamboni to this group and thank him for his friendship and support. I'll not forget our soccer times, barbecues, political and scientific debates.

For the nice job that you did preparing and arranging everything for my defence, my paranymphs (Daylan Tsompa-Sosa and Menouwesso Hounhouigan) but also Nicodème Fassinou-Hotegni and Razak Abizari, please accept my reconnaissance.

Ils ont orienté leur énergie, leur temps, leurs moyens et leurs prières vers le bébé que j'étais et m'ont suivi jusqu'ici, m'inculquant la rigueur à la l'œuvre, la sociabilité et la probité. Chère maman (Charlotte Gauthé-Madodé), cher papa (Louis Bernardin Madodé), recevez ici la reconnaissance d'un fils qui essayera toujours de porter haut votre nom. Permettez que j'étende ma gratitude à mes oncles et tantes qui me portent dans leurs prières et m'ont toujours soutenu. Emilie Venault et Géraud Hounyo, merci infiniment pour votre présence constante sur le bas-côté de la route pour accompagner ma course.

Michelle, you have accepted all the sacrifices, even to face the birth of our second child alone while I was struggling to reach my objectives. Please accept my gratitude. Be aware that I will be your support to realise your dreams. I've frequently been away from you my sons, Freddy and Maël, and was sometimes blamed for that but always allowed to return home. May this adventure spark off in you the energy to perform in the field that you'll engage in with courage, rigour and the desire to approach perfection.

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PUBLICATIONS

Full papers

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2. Quaye W., Adofo K., **Madode Y.E.** and Abizari A.-R., Exploratory and multidisciplinary survey of the cowpea network in Tolon-Kumbungu district of Ghana: A food sovereignty perspective. *African journal of Agricultural Research*, 2009, *4* (4), 311-320.
3. **Madodé Y.E.**, Houssou P.A., Linnemann A.R., Hounhouigan D.J., Nout M.J.R. and van Boekel M.A.J.S., Preparation, consumption, and nutritional composition of West African cowpea dishes. *Ecology of Food and Nutrition*, 2011, *52* (2), 115-136.
4. **Madodé Y.E.**, Linnemann A.R., Nout M.J.R., Vosman B., Hounhouigan D.J., and van Boekel M.A.J.S., Nutrients, technological properties and genetic relationsgips among twenty cowpea landraces cultivated in West Africa. *International Journal of Food Science and Technology*, 2012, *47*, 2636-2647.

Submitted papers

1. **Madodé Y.E.**, Nout, M.J.R., Linnemann A.R., Hounhouigan D.J., and van Boekel M.A.J.S., Enhancing the digestibility of cowpea (*Vigna unguiculata*) by traditional processing and fermentations. Submitted.
2. **Madodé Y.E.**, van Twisk C., van Boekel M.A.J.S., Linnemann A.R., Nout, M.J.R., and Hounhouigan D.J., Effects of alkaline cooking aids on the composition and the availability of amino acid in cooked cowpeas (*Vigna unguiculata*). Submitted.

Proceedings

1. **Madodé Y.E.**, Nout, M.J.R., Linnemann A.R., Hounhouigan D.J., and van Boekel M.A.J.S., Bioprocessing of legumes: *Weissella beninensis* improves the digestibility of cowpea (*Vigna unguiculata*). Proceedings of EFFoST annual meeting “A lunch box for tomorrow: An

interactive combination of integrated analysis and specialized knowledge of food", 20-23 November 2012, Montpellier, France.

Book of abstracts

1. **Madodé Y.E.**, Hounhouigan D.J., Linnemann A.R., Nout, M.J.R., and van Boekel M.A.J.S., Transformation et consommation du niébé au Bénin: Un aperçu de la diversité technologique et des habitudes de consommation. Actes du deuxième colloque des sciences, cultures et technologies de l'Université d'Abomey-calavi, 26-29 May, 2009, Abomey-calavi, Bénin, pp. 166.

Posters

1. **Madodé Y.E.**, Houssou P.A., Linnemann A.R., Nout M.J.R., Hounhouigan D.J., and van Boekel M.A.J.S., Inhibition of iron absorption in legume-based foods: case study of cowpea dishes from Benin. *EFFoST Annual Meeting "New challenges in food preservation: Processing-Safety-Sustainability"*, 10-13 November 2009, Budapest, Hungary.
2. **Madode Y.E.**, Li Z., Wolkers-Rooijackers J., Linnemann A.R., and M.J.R. Nout, *Bacillus* and *Rhizopus* fermentation reduce in-vitro flatus formation from *Vigna unguiculata*. *Food Denmark PhD congress* "*How to obtain high quality healthy foods in different settings*", 22-23 November 2011, Copenhagen, Denmark.

CURRICULUM VITAE

Yann Eméric Elingnan Madodé was born on April 25th, 1981 in Treichville, Ivory Coast. The National University of Benin granted him the "Baccalauréat Mathematiques et Sciences de la Nature", in 1998. At the University of Abomey-Calavi, he studied agricultural sciences, and graduated as Agricultural Engineer in 2003, majoring in Nutrition and Food Science and Technology. His engineer dissertation was entitled "Upgrading of traditional food processes: the case of *Akpan*, a Beninese fermented maize-based beverage". In January 2004, the African Biotechnology Network awarded him a scholarship to study Biochemistry Microbiology - Plant Biotechnology at the University of Ouagadougou, Burkina Faso. In 2005 he obtained his master degree (Diplôme d'Etudes Approfondies), which comprised a thesis on the "Utilization of bacteria and yeast to improve the fermentation of *Gowé*, a sorghum gruel from Benin". From 2005 to 2007, he worked as research assistant in the Laboratory of Food Microbiology and Biotechnologies, University of Abomey-Calavi where he was involved in different research projects dealing with the characterisation of traditional food products. In February 2007, he was selected to join the INREF programme called TELFUN as PhD fellow. His PhD project entitled "Keeping local foods on the menu: A study on the small-scale processing of cowpea" was carried out at the Product Design and Quality Management group and the Laboratory of Food Microbiology of Wageningen University, in collaboration with the Laboratory of Food Microbiology and Biotechnologies of the University of Abomey-Calavi. The current dissertation reports the results of this research, which have also been published in peer-reviewed journals and presented at national and international scientific meetings.

Yann Eméric E. Madodé is interested in investigating, characterising and upgrading traditional food products using modern approaches. He is concerned with the development of new and modern food products from traditional products.

OVERVIEW OF COMPLETED TRAINING ACTIVITIES

DISCIPLINE SPECIFIC ACTIVITIES		
Courses		
Food fermentation	VLAG, Wageningen	2008
Food risk analysis : An integrated approach combining insights from natural and social sciences	Graduate school Mansholt, Wageningen	2009
Reaction Kinetics in food science	VLAG, Wageningen	2009
Advanced food analysis	VLAG, Wageningen	2010
Food perception and food preference	VLAG, Wageningen	2011
Workshop/Symposia/Colloquia		
Symposium on food sovereignty Promoting or undermining food security?	VLAG & ICCO Wageningen	2007
A multi-stakeholder workshop Improving the position of smallholders through knowledge exchange between tropical food chains and research	WGS, Wageningen	2007
Excursion/Experience exchange Sovereign wheat production by Zeeuwse Vlegel, Zeeland, The Netherlands. Integrated and environmental friendly production of wheat processed locally to supply bread and bakeries locally.	TELFUN, Wageningen	2008
Debate/Lecture Food sovereignty : origins, meaning and relation with other discourses	TELFUN, Wageningen	2008
Debate/Lecture Technologies for sustainable food networks: do locally matters?	TELFUN, Wageningen	2008
Workshop Molecular biotechnologies and plant biotechnologies	AFD and UAC, Cotonou, Benin	2008
Consumer behaviour seminars	Wageningen	
PDQ colloquia	PDQ, Wageningen	2007-2012
FHM colloquia	FHM, Wageningen	
TELFUN annual workshops & conferences (with oral presentations)		
Launching workshop	TELFUN, Wageningen	2007
First workshop	TELFUN, Quito	2008
Second workshop	TELFUN, Hisar	2009
Third workshop	TELFUN, Tamalé	2010
International conference How can food and agriculture research contribute to food sovereignty?	TELFUN, Cotonou	2011
International conference Tackling the development challenges: the struggle for interdisciplinarity	INREF, Wageningen	2012
Conference and meetings		
Second colloquium of Université d'Abomey-calavi Oral presentation	UAC, Abomey-Calavi	2009

EFFoST conference **Poster presentation**	EFFoST, Budapest	2009
Food Denmark PhD congress **Poster presentation (Award of the best poster)**	Research school Food Denmark, Copenhagen	2011
EFFoST Conference **Oral presentation**	EFFoST, Montpelllier	2012
GENERAL COURSES AND WORKSHOPS		
Courses Philosophy and ethics in food science and technology	VLAG, Wageningen	2007
Information literacy	WGS, Wageningen	2007
Scientific publishing	WGS, Wageningen	2007
Techniques for writing and presenting a scientific paper	WGS, Wageningen	2007
Communication in interdisciplinary research	WGS, Wageningen	2008
Presentation skills	WGS, Wageningen	2008
Workshop Introduction to PhD trajectory: VLAG week	VLAG, Wageningen	2007
OPTIONAL COURSES AND ACTIVITIES		
Preparation, submission and approval of research proposals	VLAG, Wageningen IFS, Stockolm Nuffic, The Netherlands	2007-2008
Participating in PhD excursion to Canada	FHM, Wageningen	2008
Multivariate analysis, Regression and Data clustering Statistic course	UAC, Abomey-Calavi	2009
Participating in PhD excursion to England	PDQ, Wageningen	2012
Group colloquiums	PDQ and FHM, Wageningen	2007-2012
Supervision of BSc and MSc students	PDQ and FHM, Wageningen	2009-2012

Cover, design and layout

On the basis of an idea of Rob Nout, the cover was designed by Yann Madodé and the painting realised by Kouassi Quenum Alaba

Printing

GVO drukkers & vormgevers B.V., Ponsen & Looijen