Scuola di Dottorato per il Sistema Agro-alimentare

Doctoral School on the Agro-Food System

cycle XXIX

S.S.D: AGR/19; AGR/15; MED/49

ASSESSMENT AND IMPROVEMENT OF NUTRITIONAL STATUS OF POPULATIONS IN DIFFERENT PEDOCLIMATIC AND SOCIO-ECONOMIC CONDITIONS

Coordinatore: Ch.mo Prof. Marco Trevisan

Candidate : André Ndereyimana

Matriculation n° : 4212130

Tutors: Ch.mo Prof. Paolo Ajmone Marsan

Ch.mo Prof. Giuseppe Bertoni

Academic Year 2015/2016

A mia figlia Rosella Ndereyimana

A mia moglie Christella Munezero

Tor Vergata 19 Ago '00

"VOI...... NON VI RASSEGNERETE AD
UN MONDO IN CUI ALTRI ESSERI
UMANI MUOIONO DI FAME,
RESTANO ANALFABETI, MANCANO
DI LAVORO.
DIFENDERETE LA VITA IN OGNI
MOMENTO DEL SUO SVILUPPO
TERRENO, VI SFORZERETE CON
OGNI VOSTRA ENERGIA DI
RENDERE LA TERRA PIÙ ABITABILE
PER TUTTI"

Giovanni Paolo II

RINGRAZIAMENTI

Nel mattino del 3 settembre 2013 lasciavo mia famiglia neofondata per intraprendere il percorso formativo necessario per raggiungere il traguardo tanto desiderato, ossia quello di dottore di ricerca (Ph.D) per il sistema agroalimentare. È stato un percorso pieno di gioie ma anche di tanti sacrifici. Proprio per questo, pur temendo di omettere il contributo di tante persone importanti, vorrei ringraziare vivamente tutte le persone che hanno contribuito a renderlo possibile.

Desidero ringraziare i professori Giuseppe Bertoni e Paolo Ajmone Marsan per avere accettato la mia richiesta, per avermi guidato e supportato; senza di loro, sarrebbe stato quasi impossibile raggiungere questa meta. Sono grato al Professore Umberto Bernabucci dell'Università degli Studi della Tuscia per avermi aiutato nell'orientamento e per i suoi preziosi consigli ed incoraggiamenti. Ringrazio mia moglie Christella Munezero e mia figlia Rosella Ndereyimana per il costo pisco-sociale che hanno dovuto pagare.

Tantissimi rigranziamenti a tutti i colleghi del progetto "*Produzione di cibo appropriato: sufficiente, sicuro, sosetnibile*" per lo spirito di squadra e per il loro sostegno operativo e morale. In particolare Paolo Grossi, Andrea Minuti, Andrea Minardi, Margherita Fiorani, Valence Ndayisenga, Tancredi Ficano, Andrea Masseroni, Marco Pellizzoni, Cristina Ganimede, Matteo Anaclerio, Riccardo Dioli e Valentina Ficuciello.

Ringrazio tutti i colleghi della scuola di dottorato per l'amicizia e la solidarietà dimostrata, in particolare ringrazio: Elia Viajana, Cavanna Paola, Letizia Treccate, Dora Ines Melo Ortiz e Irene Falasconi.

Vorrei ringraziare anche Dr. Sezibera Annick per la fiducia dimostratomi nelle mie prestazioni professionali presso la CAPAD, spronandomi anche ad intreprendere il percorso di dottorato di ricerca.

Non potrei non ringraziare la FONDAZIONE ROMEO ED ENRICA INVERIZZI per avere finanziato tutto il suddetto progetto in generale e la mia borsa di studio in particolare.

Infine, ma non per ultime, ringrazio tutte le persone che, in un modo od un altro hanno contribuito al raggiungimento di questo traguardo.

TABLE OF CONTENTS

INDEX OF FIGURES AND TABLES

Figures

Tables

1 INTRODUCTION

1.1 Overview

Nutritional status is the physiological status of an individual that results from the relationship between nutrient's intake requirements and the body's ability to digest, absorb and use these nutrients. The nutritional status of a population is a relevant indicator of national development. Nutrition is in fact, both an input into and an output of the development process (FAO 2004). The World Bank as well, in a recent report, confirmed that a well-nourished and healthy labour force is a precondition to success any socio-economic development worldwide. This is one of the reasons why a multi-sectorial approach to improve human and economic development includes poverty and hunger reduction (The World Bank 2013). Thus, food security, food safety, appropriate nutrition, health and sanitation are a responsibility of all development actors intervening in different sectors (FAO 2004; The World Bank 2013). Undernourishment and poor health are indicators of a failure in the development process to reach some segments of the population. World Food Program (WFP) defines hunger as the condition in which people do not have enough to eat to meet energy requirements (WFP 2016). However, the absence of hunger does not indicate absence of malnutrition. Also recent studies clarified the difference between this two problems pointing out that the term "malnutrition", is broadly used to indicate both undernutrition and overnutrition; it refers in fact, not only to the deficit, compared to the nutritional needs, but also to its excess (Black et al 2015; Bertoni 2015). This distinction is also supported by international organization defining malnutrition as: "an abnormal physiological condition caused by inadequate, deficit or excessive consumption of macronutrients and/or micronutrients" (FAO 2016a).

Globally, the problems caused by hunger and malnutrition exist both in industrialized and developing countries. However, they are more relevant and burdening in poor countries (Bertoni et al. 2016). This is the reason why solving these problems is always the top priority for a person or for a specific population as their regard necessities for human beings. Hunger and malnutrition cause innumerable socio-economic problems and hinder any attempt to develop if they are not solved first. It is universally known that malnutrition compromises not only the physical development (since conception) but also the mental/brain development of individuals thus undermining the potential to grow, to learn and to work due to the insufficiency of physical and mental capacity, fundamental for the integral development of any human being (FAO 2004). This last aspect led some authors to consider that malnutrition detrimentally affects children, in the first few years, in all of domains such as cognitive, motor, and social-emotional child development (Black et al. 2013; Bhutta et al. 2013). In figure 1, it is shown human brain development during the life cycle (from the conception); it is illustrated how some of the fundamental biological functions (seeing, hearing, language), and especially higher cognitive ones are developed over the different ages. This suggests that is very important to have an appropriate nutrition particularly in the tender age (McGregor et al. 2007).

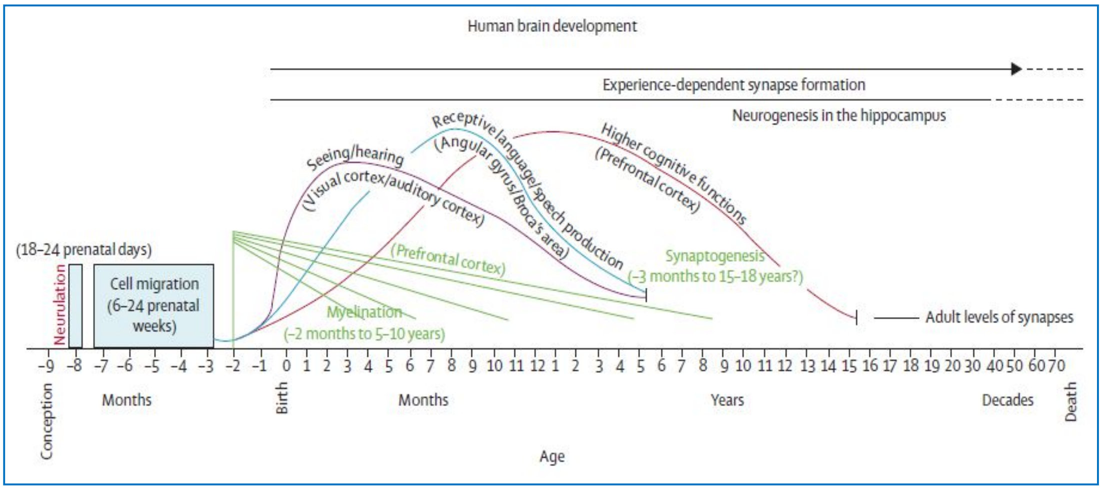


Figure 1.1 Human brain development. Source McGregor et al. 2007

The importance of an adequate infant nutrition has been also recently confirmed by other researchers stating that the more severe problem of children malnutrition in developing countries is not just related to mortality, but is the negative influences on their physical development especially regarding the brain (Black et al. 2013). Thus, the real alarming problem in these early years is not hunger but malnutrition with multiple deficiencies of minerals and vitamins that only Animal Source Foods (ASF) such as meat, fish, eggs, milk and insects could help to solve (Bertoni and Minardi 2015). Before targeting the various (general and specific, global and local) interventions facing poverty, hungry and malnutrition, it is important to give a sufficient emphasis on the child malnutrition in developing countries, that is among the most challenging issues worldwide in the pathways towards a better and just World for all, including all the dimensions of sustainable human and economic development.

1.2 The situation of child malnutrition in developing countries

Although prevalence of stunting or linear growth of children younger than 5 years has decreased during the past two decades globally, it is still higher in south Asia and sub-Saharan Africa than elsewhere. Globally, in 2011, stunting affected at least 165 million children; and wasting affected at least 52 million children (Black et al. 2013)

Other authors very recently reported that in 2015, 156 million children under 5 years were still affected by stunting, 50 million were wasted and 42 million were overweight in the World (Unicef/ WHO/Wolrd Bank 2016). To specify the meanings of the different form of child malnutrition, this thesis adopted the definitions given by the lastly abovementioned authors:

- Stunting: Stunting refers to a child who is too short for his or her age. It is the failure to grow physically (but also cognitively) and is the result of chronic or recurrent malnutrition. The devastating effects of stunting can last a lifetime.
- Wasting refers to a child who is too thin for his or her height. Wasting, or acute malnutrition, is the result of a recent rapid weight loss or of the failure to gain weight. A child who is moderately or severely wasted has an increased risk of death, but treatment is possible.
- Overweight: refers to a child who is too heavy for his or her height. This form of malnutrition results from expending too few calories (too few activities) for the amount of food consumed and increases the risk of non-communicable diseases later in life.

According to the same authors, while less than half of all children under 5 live in lower-middle-income countries, two thirds of all stunted children live there. Globally, malnutrition rates remain alarming: stunting is declining too slowly while overweight continues to rise.

Critical levels of the different malnutrition forms within a population are respectively 40 % for stunting and 15% for wasting and overweight. According to this evaluation criteria, the more burdening situations are in Africa and Asia. Among African children in Eastern, Southern and middle Africa the stunting rates are respectively 37.5, 28.4, and 31,2 percent. Wasting also register high values, between 5 to 9 %, and overweight rates is almost at the critical values with 14.5. percent. In Asia, 34.4 percent in southern part are stunted. Futhermore, Asia has the highest record worldwide of children suffering from overweight (14.6%) and wasting (14.1%) (Unicef/ and WHO/Wolrd Bank 2016).

Particularly in India and in DR Congo (as it will be told, our research project operates in those two areas), the child malnutrition is also often critical. Some researchers have reported the undernutrition levels in India remain higher compared to countries of sub-Saharan Africa, even though those countries are currently much poorer than India, have grown much more slowly, and have much higher levels of infant and child mortality (Deaton and Drèze 2009).

As regard the Meghalaya state of India, recent researches have confirmed the alarming child malnutrition in this North East Indian State. In fact, in this area, the prevalence of underweight, stunting and wasting was 19.7%, 35.5% and 8.5% respectively in 2015. Furthermore, it has been observed that the prevalence of underweight, stunting and wasting was significantly higher in boys than girls. Thus, the overall prevalence of underweight (>19%) and stunting (>30%) was high while the prevalence rates of wasting (5–9%) were medium (Duwarah, Bisai, and Barman 2015)

According to WFP, health and nutrition indicators are matters of serious concern even in DR Congo. The average of global acute malnutrition (GAM) rate is 10.7 percent, with some territories having GAM (sum of the moderate and severe acute malnutrition) rates above the emergency threshold of 15 percent. DRC's child mortality rates are among the highest in the world. According to the country's Demographic and Health Survey 2013-14, 8 percent of children under the age of five suffer from acute malnutrition, and about 43 percent are chronically malnourished and show signs of growth retardation (World Food Programme 2016)

In this introductive chapter, it has widely explained in the first paragraph that there are many obstacles to population's development caused by malnutrition especially when it affects subjects in infant age. Moreover, as it has been demonstrated by some researchers, indicators for children younger than two years of age will be critical, particularly for stunting. The two-year-old child's nutritional status is highly determinant for the future (Nicole et al. 2012). At this age, uundernutrition puts children at greater risk of dying from common infections, increases the frequency and severity of such infections, and contributes to delayed recovery. Undernutrition also has long-term effects that include mental illness (Victora et al. 2008), hypertension and diabetes, and impaired working capacity, leading to poor productivity and causing negative consequences on individual health and standard of living of the affected individuals throughout their life (Martins et al. 2011).

Other researchers has also reported the importance of specifics nutrients stating that deficiencies of vitamin A and zinc result in deaths; deficiencies of iodine and iron, together with stunting, can prevent children to reach their developmental potential (Black et al. 2013).The same authors have also explained consequences of maternal malnutrition (during pregnancy) on the children. Maternal undernutrition contributes to foetal growth restriction, which increases the risk of neonatal deaths and, for survivors, the rise of stunting at the critical age of 2 years (Black et al. 2013).

Although significant progress has been made in reducing undernutrition worldwide in the last decades, more effort is needed, especially in south and Northeast India, in Sub-Saharan Africa, and in East and Central Africa and, rethinking agro-food systems is one of the way that can significantly contribute to the reduction of malnutrition in all its forms.

1.3 State of art of the interventions to overcome poverty, hunger and malnutrition worldwide

Several international and national institutions are coordinating efforts to overcome poverty, hunger and malnutrition worldwide since many decades. Seventy-one years ago, on 16 October 1945, 42 countries gathered in Quebec, Canada, to create the Food and Agriculture Organization of the United Nations (FAO). The goal was, and still is, to liberate humanity from hunger and malnutrition, and to successfully manage the global food system (FAOSTAT 2011). In 1960, the United States President Dwight Eisenhower proposed to the UN General Assembly to create a workable scheme for providing food aid through the UN system. In September 1962, an earthquake hit Iran, followed by a tempest in Thailand in October; meanwhile, newly independent Algeria was relocating 5 million refugees. In these conditions food was urgently needed. The World Food Program (WFP) that was scheduled to begin with an experimental period of 3 years (from 1960 to 1963) collected and supplied the food aid, and it has never stopped since then (WFP 2014).

Nevertheless, the success of food delivery - coming from abroad - in cases of emergency situations, did not solve the problem of hunger and malnutrition. Therefore, twenty-nine years after the creation of FAO, during the World Food Conference held in Rome from 5th to 16th November 1974, the international Fund for Agriculture (IFAD) was created in response to the droughts and famines that killed many millions of people in Africa and Asia in the early 1970s. On one of the hand, the World leaders decided to form a global alliance to fight rural poverty, considered to be the primary cause of hunger and malnutrition, on the other hand, it was discussed the requirement to develop agricultural (food) production in developing countries. To reach this last aim, it was recommended an immediate set up of a specific fund (Commission of European Communities 1974). The IFAD governing body would include representatives from developed and developing countries contributing to the recipient countries, and the Fund would finance specific projects meant to increase food production including livestock and fisheries. Moreover, it was decided to adopt the resolution XVII as an "*improved policy for food aid*" , and it was recommended that WFP to become its governing body (Commission of European Communities 1974). In 1996 during the World Food Summit (WFS) held in Rome, the United Nations, for the first time, decided to halve world hunger by 2015 (Nicole et al. 2012) in the Agenda 2000. In this Agenda of Millennium Development Goals (MDGs), poverty, agriculture, food production and nutrition have been considered to be linked and all put in the first goal (Nicole et al. 2012).

Especially in the African continent, there are different regional and continental initiatives and program to face the challenge of hunger and malnutrition. For example, in 2005 the Comprehensive Africa Agriculture Development Program (CAADP) was created as an Africa's policy framework for agricultural transformation for food security & nutrition, economic growth & prosperity. In 2012, the African Union Commission (AUC) and the New Partnership for Africa's Development (NEPAD) Agency decided to examine the main drivers that had a direct bearing on the implementation of the CAADP and its capacity to deliver results and impact on the ground. In July 2013, Heads of State and Government of African Union Members 'States, together with Representatives of international organizations, civil society organizations, private sector, cooperatives, farmers, youths, academia and other partners facing hunger challenge in Africa, met in Addis Ababa. The objective was to explore innovative and actionable measures for putting an end to hunger in Africa under the Framework of the CAAD (African Union 2013). In this high-level meeting, it has been made a declaration adopted as a set of concrete objectives for the transformation of agriculture through the second decade of CAADP (2015–2025). Among the other statements of the declaration, it has been taken the commitment of complimenting measures for increased agricultural productivity with social protection and giving more attention to nutrition while ensuring environmental sustainability (African Union 2013).

Furthermore, it has been recently report that in SSA there are efforts to strengthen the contribution of the agriculture sector in reducing poverty and malnutrition. One example is through the development of the CAADP. National Agriculture and Food Security Investment Plans (NAFSIPs), which provide the much-needed impetus for linking agriculture and food systems to counter hunger and malnutrition in Africa. Nevertheless, most initiatives lack the concrete actions necessary to ensure food and nutrition security (Madzivhandila et al. 2016). Therefore, some contradictory results in the last 15 years within the MDGs have been reached:

- population living under the absolute line of poverty in developing countries dropped from 50 per cent of 1990 to 14 per cent only in 2015 (United Nations 2015);
- the proportion of undernourished people in the developing regions has fallen by almost half since 1990, from 23.3 per cent in 1990–1992 to 12.9 in 2014-2016 (United Nations 2015) and some developing countries reached the 1st MDG.

However, malnutrition is still challenging national and international development actors. As an example, in Sub-Saharan Africa (SSA), even though the poverty rate and prevalence of undernourishment declined between 1990–1992 and 2014–2016, the total number of undernourished people continues to increase with an estimated 217.8 million in 2014–2016 compared to 175.7 million in 1990–1992 (Madzivhandila et al. 2016). Furthermore, in in the Democratic Republic of Congo (DRC), one third of the total population is undernourished between 2014 and 2016 (FAO, IFAD, and WFP 2015) as well as in other countries of the same region such as Burundi, Chad, and Eritrea (Conway 2012).

Globally, child malnutrition (undernourishment and overnutrion) increased. At one of the hand, the Standing Committee on Nutrition of United Nations System (UNS/SCN) estimates that one in four children who survive malnutrition in early childhood are stunted, and 51 million children suffer from wasting. This situation implies 161 million futures people permanently compromised; thus. their lives and abilities are inextricably connected to progress towards goals to end extreme poverty and preventable child deaths (due to malnutrition). At the other hand, obesity has nearly doubled since 1980.; two billion people are overweight and obese, and at least 2.8 million people die every year from these conditions. The number of overweight children under 5 has doubled since 1990, reaching 43 million in 2013 while rates of childhood overweight are expected to nearly double again by 2025 (UNS/SCN 2015). Moreover, particularly for the SSA, some authors have very recently estimated that even within the new global development agenda, the Sustainable Development Goal (SDG) will leave, by 2030, around one-in-five sub-Saharan African children living in poverty (then hungry and/or malnourished); these children will account for 43% of global poverty (Watkins and Quattri 2016).

As it is illustrated above, it is evident that there are several international and national organizations that are still facing this challenge and invest in different ways to eradicate hunger and malnutrition worldwide especially in developing countries and many authors agree on the fact that food and water warrant a goal separate from poverty; in fact, ending hunger and malnutrition is a critical prerequisite for sustainable development and inclusive economic growth (Nicole et al. 2012).

To reach the last-mentioned goal, innovative ways (tools and system management) in food production and use are fundamental. Already in 1985, in fact, the adoption of technological innovations in agriculture has attracted considerable attention among development economists because the majority of the population of less developed countries derives its livelihood from agricultural production and because new technology seems to offer an opportunity to increase production and income substantially (Feder et al.1985). However, introduction of many new technologies has met with only partial success due to many constraints to the rapid adoption of innovative factors. Some of these constraints are the lack of credit, limited access to information, aversion to risk, inadequate farm size, inadequate incentives associated with farm tenure arrangements, insufficient human capital, absence of equipment to relieve labour shortages (thus preventing timeliness of operations), chaotic supply of complementary inputs (such as seed, chemicals, and water), inappropriate transportation infrastructure, etc.(Feder et al. 1985) This various constraints to agricultural and rural development in developing countries have also confirmed by other authors operating in rural India and in D R Congo suggesting the importance of adoption of new ways to overcome the food production stagnation (Bertoni et al. 2015). Other authors have supported that innovations are most successful when they are accomplished within 'innovation systems. In fact, it not enough to bring advanced production means where they do not exist but more promising may be an inclusive approach of the targeted stakeholders at the beginning, in the implantation and in the evaluation. This may allow the stakeholders to assess the accuracy and the adequacy within the theoretical tools of the change. This implies the use of tools and methods to carrying out adequate monitoring and evaluation to obtain data from research and development institutions understandable by intermediaries between those institutions and farmers to permit the expected difference in the change (Millstone, Van Zwanenberg, and Marshall 2010).

In the paragraph below, it has been illustrated such an approach of innovative ways of the agriculture as an "innovation of a hole system" rural people livelihood in developing countries.

1.4 Importance of appropriate food production in developing countries

The present doctoral work and this related thesis are one of the research activities within the framework of the project "*Appropriate food production: Sufficient, Safe and Sustainable*". The Italian original name of the project is: "***Produzione di cibo appropriato: sufficiente, sicuro e sostenibile***" which has been subsequently given the acronym of **C3S**. This project began in 2012 designed as an innovative model with a multi-sectorial approach of innovation and know-how transfer to overcome subsistence agriculture (hunger and malnutrition) and rural poverty in developing countries and of a sustainable agro-system management in developed ones. The overall aims of C3S project have been clarified as following:

- Establishment, within the Faculty of agricultural, environmental and foods sciences of the Università Cattolica del Sacro Cuore of Piacenza, of a group with specific skills needed for facing the problems of global malnutrition, especially in developing world;
- Delineation of a new model for rural development in developing countries based exclusively on local human resources (although with expert support from the Faculty of Piacenza);
- Involvement of the Faculty in the agriculture of developed countries in order to reconcile the needs for high productivity and genuine sustainability, mistakenly held to be in conflict (Bertoni et al. 2015)

More explicitly the C3S project aims:

- To get an estimate of the nutritional needs of a human being based on general criteria of Mediterranean diet: grains, vegetables, fruits, legumes, but also animal products (from livestock) and fish, avoiding excesses;
- To get valid data to verify the feasibility to produce, season by season and in different climatic and socio-economic conditions food needed to compose a Mediterranean type diet outside of borders of Mediterranean environment;
- To provide appropriate techniques to preserve food products to minimize losses and to ensure the health and hygiene safety for consumers (especially in India and DRC);
- To carry out an assessment of the actual environmental sustainability through Life Cycle Assessment(LCA) of agriculture and energy production obtained with techniques aimed to favour the conservation agriculture (in Italy) and the innovation aspects in India and DR of Congo;
- To develop appropriate procedures for the technical training of farmers, to enable them to maximize production efficiency while minimizing the food hazards and the impact on the environment; all in accordance with economic sustainability.

To explicit what an appropriate food is, this thesis adopted the meanings given in the framework of C3S project. The authors support that the most important meaning of the term *appropriate* relates to nutrition, considering not only any single foodstuff but the whole diet (Bertoni et al. 2015). Here in paragraph, the meaning of food sufficiency, safety and sustainability are also defined:

- a food can be considered sufficient only to the extent that it meets all the nutritional needs of the populations, not just their calorie requirements;
- Safe foodstuff is food that does not constitute a risk to the consumer's health. This implies the correct application of the techniques for production, processing, and storage. Just as important, however, is the final stage of management, by the consumer: correct home storage, and suitable procedures for detoxification and sanitization, such as cooking;
- Sustainable food is related to the situation in which the achievement of food production, that is sufficient to feed the global population, is of appropriate nutritional quality, and relates to agronomic, environmental, economic, ethical sustainability in any type of society. Moreover, it must be considered that agricultural workers and their families have to live, not just eat, and for this they need a fair income giving the opportunity of development to all elements of the population, including future generations (Bertoni et al. 2015).

The importance of shifting from the "only agriculture" concept to nutrition has also been reported by Madzivhandila et al. 2016 on the African project named "Agriculture to Nutrition (ATONU)" that focuses on understanding how agriculture can deliver positive nutrition outcomes to smallholder farm families through the generation of research-based evidence. ATONU is implementing nutrition-sensitive interventions that are: (i) distribution of high-producing chicks to households through the distribution of chicken with superior genetics; (ii) Social Behavior Communication Change Communication (SBCC) intervention on poultry-specific aspects of nutrition, water, sanitation and hygiene), women's empowerment, and use of income; and (iii) vegetable production and home gardens intervention. The results of these trials are expected in early 2018 (Madzivhandila et al. 2016).

About pathways to be followed to innovate agriculture and nutrition in the post-2015 development agenda, many aspects should be considered especially related to the smallholder farmers.

Although there is no single definition of what smallholders are, FAO has defined them as the small-scale farmers, pastoralists, forest keepers, fishers who manage areas varying from less than one hectare to 10 hectares and some of whom are characterized by family-focused motives such as favouring the stability of the farm household system, using mainly family labour for production and using part of the produce for family consumption (FAO 2012a). In addition to this definition, other characteristics should be considered such as low-technologies production system, limited capacity of marketing, limited capacity of farm administration, limited capacity of and limited capacity of processing and storage (Calcaterra 2013). In sub-Saharan Africa and Asia, Eighty percent of the farmland is managed by smallholders and they supply up to 80% of the foods in these areas (FAO 2012a).

However, despite the substantial progress it has made since 2000, appropriate food production in developing countries continues to be a challenge especially in rural contexts where producers are smallholders. Toward the end of agenda 2000 regarding MDGs, some authors stated that it is possible to eradicate extreme poverty, hunger and malnutrition by increasing yields decoupled from the unsustainable exploitation of water, energy, fertilizer, chemicals, and the land (Dobermann and Nelson 2013). Furthermore, the motto of EXPO Milan 2015, *Feeding the planet. Energy for Life*, included anything related to the power supply, the problem of lack of food in some areas of the world, nutrition education, and even up to the issues related to GMOs advocating the need to adapt technological innovation and environmental protection in food production systems (case by case), the guarantee for human needs (Bertoni et al. 2016). At the international level, the agenda of the post-2015 development aims to be transformational allowing future generations to have a proper nutrition in a way that nobody goes to bed hungry, and that all children are able to survive and prosper and live to their full potential (UNS/SCN 2015). Some authors considered that development goals should be focused on outcomes, but in some cases, input, output or process goals and targets could be appropriate (Nicole et al. 2012). The same authors continued suggesting that the poste 2015 development agenda-among the others targets-should: a) express the many dimensions of human well-being, yet include a limited number of targets; b) address the complexity of development, yet exploit the charm of simplicity; c) embody agreed principles, yet allow for quantitative monitoring, d) reflect global priorities and universal standards, yet be tailored to the domestic situation and local challenges; e) specify the destination, yet spell out the journey for getting there; and f) combine comprehensiveness with conciseness; complexity with simplicity; principles with measurability; universality with country specificity; and ends with means (Nicole et al. 2012).

In this situation, smallholder producers are likely key point to face the complexity of the intervention aiming to reduce malnutrition, hunger and poverty in poor countries. About the importance of the role of smallholder's farmers and farms, it has also been reaffirmed by FAO stating that smallholders form a vital part of the global agricultural community and they manage over than 80 per cent of the world' farms. Moreover, recent researches have illustrated that in poor countries food production is based on subsistence agriculture, which occupy 70-80% of population. In this conditions, families have too small amounts of foodstuff to avoid malnutrition and to rise their income (Bertoni et al. 2016). In addition, continues, the author "except for emergency, the problem cannot be solved with bringing food from abroad, but it requires a local agricultural development including: increasing of crop productivity, improvement of food processing and conservation techniques (providing more and wider variety of food), besides better consciousness of hygienic and nutritional rules. The current level of underdevelopment requires that any intervention will include the population and addresses simultaneously to all the above aspects"(Bertoni et al. 2016).

Recently, it has been supported that smallholder agriculture continues to play a key role in African agriculture. In the investigations carried out in East Africa through case studies of Kenya, Ethiopia, Uganda and Tanzania, it was found that in these agriculture-based economies, smallholder farming accounts for about 75 percent of agricultural production and over 75 percent of employment. Nevertheless, contributions of smallholder farming, and agriculture in general, to the region's growth have remained limited during the last few years. It is also clear that this growth was driven by services, in particular trade. Moreover, it was found that-at national levels-there is weakness of institutions and even the small observed growth is limited to access to markets and credit. These reasons, including a lack of infrastructure, have constrained productivity growth of smallholder farming. In these conditions, measures needed to improve productivity of smallholder farmers include ease of access to land, training to enhance skills and encourage technology adoption and innovation, and removal of obstacles to trade addressing also regional trade barriers (Salami et al. 2010).

Other authors considered that smallholders farmers concern may include also a global view affirming that there is awareness of the links between climate change, the pressure on natural resources, demographic growth of the population of the rural areas that's why there is a need of a thorough renovation of the agriculture production systems both in developing and developed countries (Bertoni 2015). This global point of view on the need of agriculture systems innovation, has been also implicitly announced, especially about food security. It has been estimated that if we could add up all of the world's production of food and then divide it equally among the world population, each man, woman, and child would receive a daily average of over 2,800 calories, enough for a healthy lifestyle (if we consider the hunger). But food is not divided in this way (nor is the income) and it is unrealistic to expect it will happen in the near, or even distant, future (Conway 2012).

The aforesaid motivations explain why production of appropriate food, referring to each specific geographical zone, wherever in the world, and especially for developing countries, shall follow the needed steps to be implemented from subsistence towards a sustainable intensification of the agriculture. Thus, in our opinion adequate ways of technologies transfer and innovation in developing countries could help to shift from subsistence agriculture to the sustainably intensified one.

1.5 Importance of a sustainable intensified agriculture

Agriculture has a strategic role to ensure food for 7-9 billion people worldwide, nevertheless, the burden of agriculture effect on the planetary crisis of environment and available water, should be also judged wisely. For instance, greenhouse gas emissions (GHE) from agriculture represent approximately 15% of the total world emission; in addition, only 1/10 of total dry land (1/3 of the world land considered as being for agricultural purpose) is cultivated and represents the part that most greatly puts the environmental sustainability at risk. With regard to water, approximately 70% of the total used by humans around the world, is for irrigation; nevertheless, 60-80% of the agricultural production depends exclusively on rainwater (which is without any other alternative use), and consequently does not use irrigation (Connor et al., 2009). Indeed, this rainwater anyway would remain in the "green" water cycle, that evaporates and returns to "wet the land" (Bertoni et al. 2016)

As regard the answer to the "biggest question" of whether the world can be fed and the planet be saved, the answer is yes but the following ways should be followed (Foley et al. 2011) :

- Stop expanding agriculture's footprint (particularly by stopping the expansion of agriculture – both cultivated or grazing lands);
- Close the world's yield gaps (particularly by boosting production in developing countries that still use obsolete cultivation techniques);
- Use resources much more efficiently (for example, by adopting precision agriculture and drip irrigation);
- Shift diets away from meat using more of crops to feed people directly and less to fatten livestock; (the use of "cereals" to produce bio-fuels should also be contended);
- Reduce food waste (eliminating production losses as much as possible and adopting a more simple lifestyle).

As it is elucidated above**,** to feed the world it is essential to maintain the current production levels of developed countries, in order to have production that is compatible with the environment's needs while in developing countries, research and innovation must face the sustainable intensification of subsistence agriculture. Thus, many of the actual means and technics used in food production in developing countries have to be suitably innovated (Bertoni et al. 2016):

- Elevated manual labor yet low productivity for all operations – hoeing, seeding, weeding, harvesting, transporting everything on their heads or shoulders, and preserving food products and rendering them suitable for consumption (cooking). Furthermore, finding water for domestic use (for drinking, washing, cooking, etc.), and wood (less and less available) for cooking are also on the list of operations done manually causing the shortage of time for crop cultivation (and animal husbandry). Especially for land cultivation, climatic changes disrupts the family farming agenda leading to and additional time shortage for food production.

- Scarce use of working animals, particularly in central-eastern Africa, and their use is often limited to ploughing or just to a few other operations such as "threshing" the rice, wheat, teff and legumes. Even when available, animals are rarely used for transporting, even for water or harvested goods.
- Lack of technical means to boost agricultural yields. For example, availability of organic fertilizers is very limited because there are few animals and the technique to recover the organic waste is not well known. On the other hand synthetic fertilizers are also rarely used because of their cost and the difficulty to purchase them.
- Lack of means and facilities for animal and plant protection against parasites, and against water excesses (violent or prolonged rains) and drought.
- Lack of availability of improved plant varieties – improved in comparison to the local and self-reproduced varieties – and selected for their adaptability to the pedo-climatic conditions and the local biotic adversities.
- Insufficient and inappropriate structures to manage the harvests in an efficient way and protect the yields against mold, insects, rodents and thieves such as community warehouses and workplaces that enable the drying, the detoxification of the cassava, or the extraction of oil etc.
- Inadequate sales, transport, handling and packing systems, especially for the fresh produce that requires an unbroken cold chain.
- Poor hygienic conditions, mostly because of the absence of potable and clean water and ignorance of the correct behavioral norms.
- Lack of highly nutritious foods, especially animal source foods (ASF), fruits and vegetables, whose absence renders impossible to have a correct diet, particularly for children;
- Lack of community hygiene and sanitation for rural families in addition to the lack of individual one.

Given the abovementioned characteristics of subsistence agriculture, the authors continued suggesting that it is unrealistic and wrong to think about proposing the intensive agricultural systems of developed countries such as occurs in "land grabbing" (or in Missionaries 'farms). More likely, the main problem is not what to do (that however must be accessible), but how to transfer the innovation in a concrete manner in countries where the population has a low level of education, where public systems for the technical knowledge transfer do not exist (assistance or extension services), and where the availability or cost of production means are a big problem. These are the reasons why only rarely the success occurred after development projects of governments, both in bilateral and multilateral projects, and of non-governmental organizations (NGOs) in developing countries and aimed to give structural assistance through realization of roads, schools, health services, trade, large intensive farming, food processing and food preservation plants etc.

In the paragraph below, some concrete examples from our recent experience in DR Congo and India illustrate some of the actions we have taken (and that we suggest for development project) as pathways of field implementation of the project "Appropriate Food Production: Sufficient, safe, sustainable" designed as a model of innovation to overcome subsistence agriculture (hunger and malnutrition) and poverty in rural areas of developing countries.

1.6 Innovation transfer to overcome subsistence agriculture in developing countries: case of rural India and DR Congo

In the framework of the above-mentioned C3S project, for the implementation of the new rural development model, 3 pilot centres (PCs) have been created in 3 countries with high, middle and low development level; respectively Piacenza (Italy), Darenchigre area (Meghalaya State of India) and Kabinda in the province of East Kasai (DR Congo). The two PCs in developing countries (India and DR Congo), have been organized to be understood as "*local pole of development*" with a permanent connection with the Faculty of Agriculture, Food and Environmental Sciences in Piacenza.

The importance of such a multi-sectorial approach has also very recently supported even by African authors suggesting that malnutrition is better addressed through a multi-sectorial approach involving agriculture, nutrition, gender, health, water and sanitation, and education (Madzivhandila et al. 2016).

Furthermore, other authors insist suggesting women's inclusivity and empowerment. As an example, when income generating activities (IGA) are successful within a developmental project, the translation of the increased income into better family nutrition depends on a series of intra-household factors and processes in which the women knowledge and capacities are important (Hawkes et al. 2015). For this reason, women's interventions empowerment should include: nutrition knowledge, social inclusion and behaviour change on advancement of women's status, health-related practices, decision-making power, income generation, access and use of health care and sanitation services, etc. Moreover, nutrition education encourages people to adopt healthy diets, and is also a way to increase demand for local agricultural produce and encourage local suppliers, such as producers, processors and retailers, to supply foods with better nutritional values (Madzivhandila et al. 2016).

Here below it is suggested how technological transfer can be done in an "innovative" way as inspired by an experience very recently gained over a three-year period in Meghalaya (India) and East Kasai (Democratic Republic of the Congo, DRC). In this paragraph, what is needed to pass from pure subsistence agriculture to intensive and sustainable rural development will be illustrated through 10 main points (corresponding to the same 10 points of subsistence agriculture defects) as it has been stated by the authors as following (Bertoni et al. 2016):

1) Increasing the potential of the work must be a general objective, for example with animals (and small mechanization), not only for tilling, but also for threshing and for small transportations (water, farm harvests, products to sell, manure, wood, etc.). In fact, on one hand it can contribute to the reduction of fatigue, but more important is the time savings - especially for women - who everyday are burdened by routine tasks (baby care, meals preparation starting from raw food collection: harvesting, cleaning, first treatment in a mortar, etc. with the final cooking of almost everything to be eaten, household water supply, washing clothes, etc.). Moreover, there are also less-routine, but strategical, activities that are *time-consuming*: the soil preparation and everything that is necessary from the sowing until the harvest of staple foods (cereals, tubers

roots), but also vegetables, flavourings, fruits etc., but also animal husbandry. In addition, there are treatments to make edible some seasonal products, but needed all the year (rice, maize, teff, cassava), by drying, sporadic grounding or husking, etc., often without mechanical means but with only manual rudimentary tools (mortar, stones, wood sticks, etc.). These are the reasons why there is no time left for other activities such as training, handcrafting, etc.

2) Introduce technological innovation in the fields and in the successive phases of agricultural production in order to increase the yield per hectare and per unit of labor.

Innovations to be underlined are:

- Use of improved plant varieties, more resistant to adversities and results as a substantial increase of yields with respect to those of subsistence agriculture. As an example, in the experience of the authors in India, the use of improved rice seeds variety within the "*System of Rice Intensification*" (Glover 2011)permitted the increase of the paddy yield form 2.0-2.5 to 3.5-4.5 Mg per hectare. Such varieties (not necessarily GMO) are often already available at governmental research centers, but are distributed with great difficulty for use because they have to be purchased because are not produced directly by the farmers(FAO 2015).
- Improved sowing techniques, passing for example from broad casting to precision sowing, thereby saving seeds, reducing lodging and improving the fight against weeds.
- Optimization of rotations and intercropping to best exploit the effects of succession planting and to maximize the growing season.
- Favor agronomic fertility of the soils using available organic material such as dry grass and crop residue as mulch (rather than burning it), cultivation of cover crops to be later interred into the soil, and make compost with the organic wastes and the animal manures.
- Improve the harvest, drying, processing and preservation techniques of the agricultural products, also by building community warehouses for storage.

3) Increase animal production aimed to improve the diet (with animal source foods), to empower working force and access to organic fertilizers. Such an increase can be achieved in the following ways:

    - Introduce genetically improved breeds-lines, vaccinated against the most serious illnesses. A prime example is replacing local chickens with "Kuroiler" chickens, as it has been done in a rural development project in Meghalaya (India). The "Kuroiler" chickens were given the same food rations as the local, but being rustic and capable of finding food on the ground around the huts, they reached a weight of over 3 kg in 6 months – twice compared to the local breeds – and they laid eggs almost daily, compared to the total of 20-30 eggs laid by the local hens in the 2-3 breeding seasons each year (Bertoni, 2015).
    - Identify any possible sources of suitable feed, especially for monogastrics-chicken leftovers and any other waste from garden vegetables, cereal byproducts and/or wastes, oil cake, fermentation residues or "forage crops" such as water narcissus. For ruminants, it is important to correctly manage the spontaneous grasses colonized by *Imperata cylindrica* so as to obtain young shoots of a quality good enough for grazing or even for making hay. Straw (from cereals) and legume crop residues should also be made a better use, though their use implies storing them, as well as having rudimental forms of shelter where the animals can receive products and where it is possible to collect the solid animal manure for further use (mainly fertilizer).

4) Improve the availability of food throughout the year. For cereals, legumes and cassava this means correctly drying the harvested grain/bean/root etc. and protecting them by storing. Fruit and vegetables, that are typically seasonal products, can be dried and/or made into jams/compotes, or they can be fermented, pickled or fried in stable oils (such as palm oil). In addition, fruit and vegetable cultivation should be extended into the less favourable seasons using simple irrigation systems and covering the plants with branches or grass to shade them in order to reduce evapotranspiration phenomena. Products of animal origin (milk, eggs, fish, meat) generally have the advantage of being available all year round (being animals alive) without a particular need to preserve or conserve them.

5) Increase the trade opportunities, not only with the "*banquet*" in front of the house and beside roads or in their immediate vicinity (village market and / or small cities), but also involving bigger cities. This implies the procurement of suitable transport means (motorized) and not rapidly perishable products. In fact, fresh fruits and vegetables require cold chain, often impossible to obtain or too expensive. As it is valid for other improvement tools, also in this case, the trade opportunity would benefit the big advantage offered by the cooperative (association) groups well managed as it is discussed further. Moreover, it may be useful, in these cases, the ability to organize the groups so that the unsold fresh material is recycled as feed for chickens, pigs, ducks, etc.
6) Create a training/education system that supplies knowledge that is elementary but essential for implementing the innovations related to crop cultivation, animal rearing, storage/conservation, correct use of foods for humans etc. Agricultural teachings throughout the primary school, together with some practical experience, will help to instil a sense of need with regard to the family farm, to reduce the risk of considering the agriculture as an inferior activity avoided by the young as soon as they have reached a minimum level of education (Tabaglio et al. 2013). In the same time it is necessary to provide permanent training for adults, for farming families; this will be an essential role of associations and cooperatives.
7) Establish groups, associations and cooperatives of women (and men) interlinked among themselves at a second level of aggregation to facilitate a sufficient acquisition of all the innovations suggested until now (to which forms of micro credit, technical assistance and insurance schemes could be added). Without a doubt, almost none of the innovations referred are available for the very small farmer with 1-2 ha individually. Thus, considering that there can be no development and no fight against poverty without innovation, the joining of rural forces becomes an essential need.

The three final points refers to health and hygiene prerequisites. Good health induces a greater predisposition to work; in addition, nutritional demands should drive the food production processes. Nutrition and Agriculture should be recognized as a binomial that provides health (and permits full human development), and as such it should drive food production towards being that which properly nourishes rather than which just only satiates.

8) Bring about improvements in the kitchen and the technology of food preparation.
    - Use slightly more advanced tools to assist food preparation as mortars, stone grinder, mechanical mills for grinding hard materials such as seeds, and knives to remove inedible parts of vegetables, meat etc. The intent is to increase food's appeal and digestibility, and its hygienic state. The habit of cooking almost all the foods probably derives from the need to kill pathogens, but this simultaneously causes the loss of many vitamins (that together with proteins and micro-minerals are the most common deficiencies). From this derives the necessity to develop and apply methods for preparing fresh foods that can be safely consumed without intensive cooking. Some trials with grilling for example have shown that it is possible to kill the microorganisms on the surface, without excessively damaging the texture and the nutrients of some vegetables.
    - Improve the efficiency of the cooking fire thereby limiting the wood or charcoal consumption, which has two advantages consisting first in the reduction of deforestation and second the reduction of spread of smoke in the houses (a cause of lung damage in women and children in particular).
    - Associate foods and divide them through the day in a correct nutritional manner, taking restrictions and local habits into consideration. In DRC, in order to better balance starch and proteins, replace of the traditional foufou (a type of polenta almost completely made up of carbohydrates from cassava and maize) has been tried with a mixture of cassava and peanuts, thereby obtaining a food with a better protein and lipids balance via a very simple preparation method. No less important is the association of complementary foods to achieve a better nutritional balance; cereals/cassava can be associated with protein foods: leaves, legumes, fish, eggs, meat (each one would be available to be eaten at least once a week).
9) Teach the main concepts of a correct diet/nutrition in relation to critical phases in life: pregnancy and breastfeeding, but particularly weaning and early years

growth (up to 8-10 years). It is fundamental to manage both the phase of breastfeeding and weaning when mothers cannot feed the baby for all day long and the process of weaning. The supplementary foods cannot be constituted solely by adult foods, as is currently the norm, but should be based on suitable sources of protein, vitamins and trace minerals (ongoing trials appear to be promising). Similar care should be taken in early childhood given that a child's digestive utilization is not comparable to that of an adult and their nutritional demands are greater. Attention should also be given to the diet of the pregnant and breastfeeding women, and to that of the elderly, also to avoid problems of overweight (at least in the future).

10) Increase through all possible ways, the availability of drinking water or sanitized water (filtered, solar-treated) for personal hygiene purposes and for use in the house, including in the kitchen and the toilet. Gastrointestinal illnesses (often the most numerous after malaria) are in fact due to the absence of safe water; it should be noted that these illnesses also contribute to the problems of malnutrition due to poor nutrient absorption and increased nutritional needs during the illness. This poor state of health can even trigger further illnesses, as confirmed by our studies in India (Ndereyimana et al. 2015).

In here above it has been largely cleared that food systems, if appropriately strengthened, have the potential to deliver adequate availability, access, utilization, and supply stability of both macro-and micronutrients that contribute to a real nutrition security. Moreover, it has been shown how these food systems include all processes and infrastructure involved in feeding a population: seeding, growing, harvesting, processing, packaging, transporting, marketing, consumption and disposal of food and food-related items.

In this chapter, it has been reviewed the general nutritional status worldwide focusing mainly to the related problems in developing countries. In the following parts of the present doctoral labour, there will be illustrated the three specifics research objectives; for each one it has been dedicated a chapter before stating the general conclusions.

1.7 References

African Union. 2013. “Declaration of the High Level Meeting of African International Leaders "Towards African Renaissance": Renewed Partnership for a Unified Approach to End Hunger in Africa by 2025 under the Framework of the Comprehensive Africa Agriculture Development Program,” 1–5. http://www.fao.org/africa/perspectives/end-hunger/en/.

Bertoni, G., Tabaglio, V., Ganimede, C., Trevisan, M., Pellizzoni, M., Anaclerio, M., Cappa, F., Grossi, P., Fiorani, M., Ndereyimana, A., Minardi, A., Sckokai, P., Guastella, G., Chiesa, M., Rossi, L. Food Production and Use. Security, Safety and Sustainability, Egea, Milano (2015): 150 [http://hdl.handle.net/10807/72037]

Bertoni, G. & Minardi, A. 2015. Agriculture and animals: different roles and ethical considerations. in World Food Production. Facing growing needs and limited resourses.

Bertoni, G., Grossi, Fiorani, M., Ndereyimana, a., Minardi, A., Ganimede, C., Tabaglio, V. 2016. Innovation to overcome subsistence agriculture on family farms in developing countries :the role of catholic church. Rivista Internazionale di Scienze Sociali, 12 pp, pp.199–211.

Bhutta, Z.A., Das, J., Rizvi, A., Michelle F.G., Walker, N., Horton, S., Webb, P., Lartey, A., Black, R.E., 2013. Evidence-based interventions for improvement of maternal and child nutrition: What can be done and at what cost? The Lancet, 382 (9890), pp.452–477.

Black R, Victora C, Walker S, Bhutta Z, Christian P, de Onis M, Ezzati M, Gran- tham-McGregor S, Katz J, Martorell R, Uauy R, and the Maternal and Child Nu- trition Study Group. 2013 Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet, 382 (9890), pp. 427-5

Commission of European Communities. 1974. “Communication from the Commission to the Council World Food Conference,” no. December: 1–9. http://aei.pitt.edu/15701/1/SEC_(74)_4955_final.pdf.

Deaton, Angus, and Jean Drèze. 2009. “Food and Nutrition in India: Facts and Interpretations.” *Economic and Political Weekly* 47 (7): 42–65. doi:10.2307/40278509.

Dobermann, A, and R Nelson. 2013. “Opportunities and Solutions for Sustainable Food Production. High-Level Panel of Eminent Persons on the Post-2015 Development Agenda.” *IRRI*, no. January 2013: 25.

Duwarah, Sourabh, Samiran Bisai, and Himesh Barman. 2015. “Prevalence of Undernutrition among Preschool Children under Five Attending Pediatric OPD in a Tertiary Care Hospital of Northeastern India” 3 (16): 527–33.

FAO 2004. Incorporating Nutrition Considerations into Development Policies and Programmes. Brief For Policy-Makers and Programme Planners in Developing Countries, p.76.

FAO. 2016. Nutrition in protracted crises.

FAO 2012. Smallholders and family farmers, FAO 2015. Voluntary Guide for National Seed Policy Formulation,

FAO, IFAD & WFP (2015). The State of Food Insecurity in the World: Meeting the 2015 international hunger targets: taking stock of uneven progress, Available at: http://www.fao.org/3/a4ef2d16-70a7-460a-a9ac-2a65a533269a/i4646e.pdf.

FAOSTAT 2011. Food and Agriculture Organization of the United Nations. Available at: http://faostat.fao.org/.

FAO, IFAD, and WFP. 2015. *The State of Food Insecurity in the World: Meeting the 2015 International Hunger Targets: Taking Stock of Uneven Progress. FAO, IFAD and WFP.* doi:I4646E/1/05.15.

FAOSTAT. 2011. "Food and Agriculture Organization of the United Nations." http://faostat.fao.org/.

Foley, Jonathan A, Navin Ramankutty, Kate A. Brauman, Emily S. Cassidy, James S. Gerber, Matt Johnston, Nathaniel D. Mueller. 2011. "Solutions for a Cultivated Planet." *Nature* 478 (7369): 337–42. doi:10.1038/nature10452.

Glover, Dominic. 2011. "Science, Practice and the System of Rice Intensification in Indian Agriculture." *Food Policy* 36 (6). Elsevier Ltd: 749–55. doi:10.1016/j.foodpol.2011.07.008.

Grantham-McGregor, Sally, Yin Bun Cheung, Santiago Cueto, Paul Glewwe, Linda Richter, and Barbara Strupp. 2007. "Developmental Potential in the First 5 Years for Children in Developing Countries." *Lancet* 369 (9555): 60–70.

Hawkes, Corinna, Trenton G. Smith, Jo Jewell, Jane Wardle, Ross A. Hammond, Sharon Friel, Anne Marie Thow, and Juliana Kain. 2015. "Smart Food Policies for Obesity Prevention." *The Lancet* 385 (9985). Elsevier Ltd: 2410–21. doi:10.1016/S0140-6736(14)61745-1.

Haytowitz, D.B., P.R. Pehrsson, and J.M. Holden. 2002. "The Identification of Key Foods for Food Composition Research." *Journal of Food Composition and Analysis* 15 (2): 183–94.

Millstone, Erik, Patrick Van Zwanenberg, and Fiona Marshall. 2010. "Monitoring and Evaluating Agricultural Science and Technology Projects: Theories, Practices and Problems." *IDS Bulletin* 41 (6): 75–87.

Ndereyimana, A., Edakkarottu S., J., Ndayisenga, V., Fiorani, M., Carlomagno, A., Bertoni, G. 2015. "Impatto dell'uso di filtri domestici per l'acqua da bere sulla salute in una popolazione rurale Indiana."

Nicole Bates-Eamer, Barry Carin, Min Ha Lee and Wonhyuk Lim, and Mukesh Kapila 2012. "Post-2015 development agenda: goals, targets and indicators. Special report."

Richard Connor, William Cosgrove, George de Gooijer, Denis Hughes and Domitille Vallée. 2009. "Water in a Changing World." *World Water*. Vol. 11.

Salami, A., Kamara, A.B., Brixiova, Z. 2010. "Smallholder Agriculture in East Africa: Trends, Constraints and Opportunities." African Development Bank. Workin paper N°105

Tabaglio, Vincenzo, Giuseppe Bertoni, Paolo Barone, Cristina Ganimede, and Paolo Grossi. 2013. "Revisione Dei Sistemi Colturali Nei Paesi Sviluppati E in Quelli in Via Di Sviluppo In Intensificazione Sostenibile E La Sicurezza Alimentare." pp 54–56.

The World Bank. 2013. "Improving Nutrition through Multisectoral Approaches."

Tshilidzi Madzivhandila, Simbarashe Sibanda, and Farai Alice Gwelo. 2016. "Achieving Food Security and Nutrition." In *AFRICA AGRICULTURE STATUS REPORT 2016*, edited by AGRA, 19.

Unicef/, and WHO/Wolrd Bank. 2016. "Levels and Trends in Child Malnutrition: Key Findings of the 2016 Edition." *Midwifery*.

United Nations. 2015. "The Millennium Development Goals Report." *United Nations*, 72.

UNS/SCN. 2015. "Nutrition and the Post 2015 Development Agenda: Seizing the Opportunity." *SCN News* 41 (1564–3743): 116.

Victora, Cesar G., Linda Adair, Caroline Fall, Pedro C. Hallal, Reynaldo Martorell, Linda Richter, and Harshpal Singh Sachdev. 2008. "Maternal and Child Undernutrition: Consequences for Adult Health and Human Capital." *The Lancet* 371 (9609): 340–57.

Vinicius J. B. Martins, Telma M. M. Toledo Florêncio, Luciane P. Grillo, Maria do Carmo P. Franco, Paula A. Martins, Ana Paula G. Clemente, Carla D. L. Santos 1, Maria de Fatima A. Vieira and Ana Lydia Sawaya 2011. "Long-Lasting Effects of Undernutrition." *International Journal of Environmental Research and Public Health* 8 (6): 1817–46.

WFP. 2014. "Logistics | WFP | United Nations World Food Programme - Fighting Hunger Worldwide." http://www.wfp.org/logistics.

World Food Program. 2016. "Democratic Republic of the Congo: Current Issues and What the World Food Programme Is Doing." *Www.Wfp.Org*. https://www.wfp.org/countries/congo-democratic-republic.

2 OBJECTIVES OF THE RESEARCH

This doctoral thesis is part of the project "Production of appropriate food, sufficient, safe sustainable_C3S" The overall objectives of C3S project were briefly summarized in paragraph 1.4. The specifics research purposes of this thesis, have been the following:

- To estimate the consumption of some groups of foods of animal and plant origin in an Italian population through some biomarkers and some nutritional status indexes;
- To get a nutritional status assessment of some population from rural D.R.Congo and Meghalaya State of India;
- To set some sustainable proposals applicable in field to reduce food and waterborne diseases in developing countries towards better hygiene and diets.

For each of these objectives, it has been dedicated a specific chapter in the rest of the present work before reaching the final general conclusions.

3 AN ESTIMATE OF THE CONSUMPTION OF SOME GROUPS OF ANIMAL AND PLANT SOURCE FOODS IN AN ITALIAN POPULATION THROUGH SOME BIOMARKERS.

3.1 Introduction

3.1.1 Overview

When in the mid-900 Keys (1953) reported the relationship between saturated fatty acids and atherosclerosis mediated by elevated cholesterol levels, the scientific basis was an epidemiological research (the so-called study of the 7 countries). From these studies, it emerged that the Mediterranean diet was characterized by cereals, vegetables, legumes, fish and olive oil, while meat, eggs, milk, cheese, and wine were consumed in low or moderate quantities (Fidanza 1991). Over the '80s, the aversion, in particular to cholesterol (also consequence of Keys'studies), led to consider animal products (the only ones that supply it pre-formed), the main cause of cardiovascular disease (CVD) and cause of the progressive "*vegetarianization*" of the Mediterranean diet.

In the same period, the '80s, the relationship between the consumption of animal source products and cancer was stated (Armstrong and Doll 1975), but in some measure also with obesity (and the related metabolic syndrome) that, being related to an excess of energy-rich foods, was easy to attribute to animal fats and meats with the result of greatly favoring carbohydrates (USDA 2010) (Hite et al; 2010). Whatever it is evidenced by the fact that in the 2010 report of the Guidelines for Americans Committee is still alleging that a healthy diet is mainly based on carbohydrates and in any case on vegetal source foods (VSF) (USDA 2010)(Hite et al, 2010). Very likely it was the result of the resolution of a US Senate committee in 1977 that suggested the"dietary goals" for the USA: eat less high fat, read meat, eggs and dairy and replace them with more calories from fruits, vegetables, and especially carbohydrates; that disregarded the consequences registered in the US in the three decades between the end of 1900 and beginning of

2000: the dramatic increase in overweight and obesity (Flegal et al., 2002; USDA 2010 Hite et al 2010). Eventually, in the last decade, especially the last five years, there are increasing researches in order:

- To show that the relationship between animal sources foods and degenerative diseases (atherosclerosis, cancer, diabetes, obesity) are nonexistent or much less certain than above illustrated;
- To make an evidence that epidemiological research - that are commonly used to demonstrate these relations - are most useful to find an association between the consumption of certain foods and the frequency of degenerative diseases (vascular diseases, cardiac and neural, certain forms of cancer, type 2 diabetes and obesity), but not the cause / effect ratio that would imply research of the long periods, with subjects regularly fed with food which effects have to be determined (Klumfela 2015).

With regards to the relationship between diseases and animal source foods, it seems important to draw attention to some meta-analysis recently published and related to milk and dairy products. Pufulete (2008) has been already shown the inconsistency of a relationship between these foods with cancer of the colon and rectum while Lovegrove and Hobbs (2016) more recently pointed out the inconsistent relationship between dairy food and the effects on CVD. (In opposite, they claim that milk and dairy products would have favorable effects on blood pressure and hardening of the artery walls (serious risk factors for vascular diseases), as already observed before by Aslibekyan et al. (2012). Moreover, it was already evidenced that meat consumption was not responsible for the breast cancer (Alexander et al. 2010), and more recently also for colon-rectal cancer (Alexander et al. 2015). In this regard, it is interesting the comparison – on meta-analytical basis - between omnivores and vegetarians operated recently in the UK where the overall mortality is similar (in the two groups) but with small differences in favor of one or the other group depending on the considered degenerative disease (Appleby et al. 2016)

On the other side, the positive effect of the consumption of fruits and vegetables on disease prevention (mainly cancer) is not always demonstrated (Molfetta et al. 2010); furthermore, according to Key et al. (2009), colon-rectal cancer seems to be a little more frequent in populations without meats.

So, it is interesting to note that blood cholesterol, the most emphasized risk factor at least for CVD, is now rehabilitated; some authors (Ravnskov et al. 2016) have in fact concluded that the hypothesis of the associations between low-density lipoprotein cholesterol (LDL-C) with increase of mortality for CVD and for whichever cause was not true.

Even in this case, it is shown that an association - in this case between high blood levels of cholesterol (especially in its form of low-density lipoprotein) and diseases - does not mean necessarily a cause/effect relationship. Going back to the second aspect highlighted before: the considerable limitations of epidemiological research, we consider of great significance the quiet recent review "The great fat debate: taking the focus off saturated fat". The author states that the hostility to the fat (as a cause of degenerative diseases) was based on epidemiological data, on blood markers (e.g. cholesterol) and on tests with animals (Mozaffarian 2011). Nevertheless, when more accurate checks were carried out on the relationship with the onset of degenerative diseases (including CVD, cancer, etc.), it was excluded the close relationship with the total intake including saturated fats. Moreover, it was stressed that the individual nutritional aspects cannot be seen in itself, but in the context of an overall diet. For example, if fats even saturated are replaced with carbohydrates, especially if refined, the risk gets worse; on the contrary, the risk decrease if fats are substituted by fiber-rich foods (fruits, vegetables, whole cereals, etc.). Also interesting is the fact that the improvements are not seen in terms of the blood level of cholesterol, but of objective risk factors: blood pressure, systemic inflammation, insulin resistance etc. These Mozzafarina's considerations recall what already highlighted by other researchers some years before, namely that, for a healthy diet, it is more important to increase the consumption of recommended foods: vegetables, fruits, whole grains, fish, milk and low-fat derivatives, rather than lowering the unfavorable ones (Trichopoulou et al. 2011). In fact, the same authors refer the attempts to "label" the Mediterranean diet with specific foods, but none showed a predominant effect, so they prefer to define a Mediterranean diet as characterized by the variety and freshness of the foods, as well as a correct lifestyle.

These suggestions pose again some doubts on the epidemiological method, as a possible mean to identify the dietary causes of degenerative diseases as CVD, cancer, diabetes, metabolic syndrome and obesity. Furthermore, the second objection to this kind of epidemiological research concerns if is it possible to correctly estimate the intake of individual foods (at least as part of relatively homogeneous groups) within large populations and during relatively long periods.

3.1.2 Assessing dietary intake

According to Macdiarmid and Blundell (1998), few people will dispute the fact that one of the most fundamental problems facing nutritional research is the inability to assess accurately the dietary intake in humans. Despite efforts to develop more accurate methods and the improvement of the existing ones to assess dietary intake, we fail to escape the rudimentary problem of under-reporting. Indeed, during the 1950 and 1960s nutritionists started to develop questionnaires for the assessment of the habitual food intake based on a checklist of food consumed over a set time period because of problems encountered with 24 h recall techniques and weighed inventories. After further refinements, revision and appraisal during the 1980s, food-frequency questionnaires (FFQ) have become one of the key research tools in nutrition epidemiology. However, despite considerable advantages in terms of ease of administration of the questions and data analysis, FFQ may be limited in their usefulness and, through poor design and inappropriate use, may not yield the required information. These difficulties have lead Prentice et al. (2002) to suggest that the epidemiological studies of the last 3 decades were not able to establish appropriate relationships between diets and chronic diseases also because others factors can be important as physical activities, smoking, etc. Other researchers argued in fact that to measure the amount of ingested food is among the most complex problems, but not less complex is the evaluation of the diet to the whole (Thorogood et al. 2007). More recently, Scagliusi et al. (2008) argued that in the surveys on diet and health, nutritional exposure is assessed as intake of a single or a few nutrients or foods. However, diet is something far more complex and ends up being artificially reduced and simplified when a single nutrient or food is analyzed separately. Thus, this approach may have several limitations. First, individuals do not eat isolated foods and nutrients, there is an interaction and synergy between these foods and nutrients, which are disregarded in the single food or nutrient approach. Second, the effect of one nutrient may be confounded by the effect of the eating pattern. Third, there is a high correlation between nutrients, since one single food can provide many nutrients. Finally, the effect of a single food may be too small and undetectable, but the cumulative effect of various foods may be sufficiently large. Therefore, most authors are using one of the following procedures to assess validity of the dietary pattern analysis: (a) comparing the patterns based on one dietary assessment method with the nutrient intake obtained through another dietary assessment method; (b) comparing dietary patterns obtained by different dietary assessment methods; (c) correlating dietary patterns to concentration of biomarkers; (d) determining the risk of developing a disease according to dietary patterns.

Among the above-suggested procedures, interesting can be the use of appropriate biomarkers; already Bingham (2002) has suggested: "There will always be an error in dietary assessments. The challenge is to understand, estimate, and make use of the error structure during analysis.' The ability to do this, however, has only become possible with the advent of biological markers in biological specimens such as blood, urine or hair, which reflect intake sufficiently closely to act as objective indices of true intake. These biomarkers could also replace estimates of intake based on traditional methods. Thus Blanck et al. (2003) observed that nutritional biomarkers can serve as measures of nutritional exposure, or to use the nomenclature of environmental biomarkers, internal dietary dose. In this latter context, the nutritional biomarkers go beyond being indicators of dietary intake and are an aid, in our understanding, of causal mechanisms between diet and diseases. More recently, Jenab et al. (2009) aware of the difficulties to properly evaluate the real effects of foods, have suggested the use of some biomarkers, not only as measures of dietary intake and nutrient status but also as predictors of disease risk. Unfortunately there are several nutrients and dietary aspects, therefore also biomarkers are numerous; the actual equipment has suggested the metabolome: a complex of thousands of components that rise from intra and extracellular metabolic pathways and external sources such as food (Gibney et al. 2005; Beckonert et al. 2007; Lodge 2009; Favé et al. 2009).

According to Nettleton et al. (2009), the opportunities to validate the use of biomarkers via dietary are limited for most nutrients and/or food items and, in some cases, require special sample preparation. Despite the biomarkers are objective (as opposed to self-reported food intakes), these markers are imperfect traces of nutrients. Thus, non-dietary factors that affect circulating concentrations of biomarkers, often need to be considered in the interpretation. As an example, some large epidemiological studies have taken advantage of the well-known associations of dietary carbohydrate and fat with plasma lipids (namely, TAG and HDL-cholesterol). Moreover, taking into account the effects that many nutrients exercise on the metabolism of nucleic acids, Kussmann et al. (2008) have also suggested the mRNA variations in certain tissues (although the interpretation does not appear to be easy).

A part these promising opportunities of biomarkers, our major interest is for a simpler relationship, namely for the identification of biomarkers useful to track intake of specific foods. For example:

- The dairy products: The content of 15:0 and 17:0 fatty acids (which are only synthesized by rumen bacteria) in the subcutaneous adipose tissue might be a valid biological marker of long-term milk fat intake in free-living individuals in populations with high consumption of dairy products (Wolk et al. 1998) as confirmed by Aslibekyan et al. (2012). According to Smedman et al. (1999), also plasma level of 15: 0 fatty acid can be used as a marker for intake of milk fat;
- Meat: the omnivores can be distinguished from vegetarians through the evaluation of the amount of 1-methylhistidine excreted in the urine. Moreover, the concentration of this metabolite can be used to estimate the greater or lesser ingestion of meat. Less useful is the 3-methylhistidine, since it is mainly influenced by muscle catabolism (Myint et al. 2000). Vegetal foods: their intake can be estimated through the evaluation of plasma pigments, which are related to the consumption of fruits and vegetables, such as carotenoids (Bingham et al. 1997). In particular, the alfa-carotene appears to be better correlated with the food intake than the beta-carotene. Nevertheless, the latter is much better correlated ($r = 0.48$) to the food intake assessed by weighing with respect to the Food Frequency Questionnaire ($r = 0.15$) and the 24 hours recall ($r = 0.08$). What is said for the beta-carotene is true, albeit to varying degrees, for other plasmatic carotenoids: lutein, beta-kryptoxanthin, lycopene, and cis-carotene. These data confirm that the estimation of ingested diet is better reflected in biomarkers.

The markers can be also utilized to check the effects of food preparation in the presence of some nutrients in foods (Sanders 2015). However, the use of biomarkers is not free from limitations, especially when they are utilized such as indices of nutritional status of individuals, and especially when it is not possible to apply sophisticated and expensive techniques. This clearly appears from the supplement 3 of British Journal of Nutrition, 99 (2008) which contains the Proceedings of the EURRECA Workshop and Working Party of New Approaches for Measuring Micronutrient status. However, the topic is widely known (Ommen et al. 2008).

Thus, also for this last reason, the aim of the study ignores the meaning of the nutritional status indicators and merely uses biomarkers as a counterpart of the declaration of food consumption, collected through questionnaires (EPIC or 3 days).

3.2 Materials and methods

Definition of the population group - In the framework of Moli-sani project, n=200 subjects aged 35-65 years were randomly recruited. The selected participants were recalled in 2012 and 77% of them (n=153) responded to an anamnestic questionnaire and to two different dietary questionnaires: the EPIC- FFQ and the three days' questionnaire (n=104).

Definition of chronic disease and common risk factors - The subjects recruited were free from cancer and cardiovascular disease (CVD) including myocardial infarction, unstable angina, cardiac revascularization, ischemic and hemorrhagic stroke. Moreover, subjects were also free from hypertension and diabetes. Hypertension was defined as systolic blood pressure (BP) ≥ 140 mm Hg and/or diastolic blood pressure ≥90 mm/ Hg, or when using pharmacological treatment for hypertension. Values of systolic BP ≥130 and <140 or diastolic BP ≥85 and <90 were set to define prehypertension. Diabetes was defined by current antidiabetic treatment. Subjects were also classified as never-smokers, current smokers or ex-smokers (quitting from at least 1 year). Education was used as a proxy for socioeconomic status and was based on the highest qualification attained and was categorized as low (up to lower secondary school, approximately ≤ 8 years of study) or high (upper secondary education or higher; approximately > 8 years of study). Physical activity was assessed by a structured questionnaire (24 questions on working time, leisure time and sports participation) and expressed as daily energy expenditure in metabolic equivalent task-hours (MET-h). Body mass index (BMI) was calculated as kg/m^2 and then grouped into three categories as normal (≤25), overweight (25-30) or obese (≥30).

Dietary assessment-The recall phase has been carried out from May to Septembre in 2012**.** The European prospective investigation into cancer and nutrition-food frequency questionnaire (EPIC- FFQ) adapted for Italian population were used to determine usual nutritional intakes in the past year. The NAF software (Nutritional Analysis of Food Frequency Questionnaires, National Cancer Institute, Milan, Italy) was used to transform information about food composition into daily intake of food items, energy, macro- and micro nutrients and total antioxidant capacity (TAC) (Pala et al. 2003); (Agnoli et al. 2011). Nutrient data for specific foods were obtained from the food composition database for epidemiological studies in Italy integrated with the TAC values of a number of foods representative of the average Italian diet, such as fruits, vegetables, oils, beverages, spices, dried fruits, sweets, cereals, pulses and nuts (Ed & Europeo 1998; Pellegrini 2006; di Giuseppe et al. 2012).

Three days questionnaire-Each participant received a short photographs atlas and guidance notes to estimate portion sizes developed on the basis of EPIC-SOFT picture book ("Van Kappel AL, Amoyel J, Slimani N, Vozar B, Riboli E. EPIC-SOFT Picture Book for Estimating Portion Sizes. IARC Press, Lyon, France (1994) and a hard-copy diary structured by meal, where to self-record all the information on food consumption three days before the interview (one day was a weekend day). All foods and drinks consumed (including tap and bottled water), both at and outside the home, were recorded. For every eating occasion, subjects were asked to carefully record and recall: time, place of consumption, detailed description of foods (or beverages), quantity consumed and brand (for manufactured foods). Portion sizes were reported by subjects with the help of a picture booklet. Moreover, it was asked if they were following a particular diet and if the consumption they had reported differed from their usual consumption.

The data management system INRAN-DIARIO 3.1 developed by INRAN (Leclercq et al. 2009; Sette et al. 2011; Agnoli et al. 2011) in previous surveys was used for data coding, data entry and data processing. This software includes several checkpoints to ensure the accuracy and completeness of the data recorded and allows each interviewer to create new temporary food codes for all the food items and recipes that are not present in the databanks. Four databases were used to transform the data reported by subjects into the weight of single foods, raw ingredients and into the amounts of nutrients consumed. The portions estimated by subjects with the help of the picture booklet are linked to the specific weight of each food item. This database contains a total of 9,450 entries (weight of standard portions of specific dishes or units of measurement) for 2,460 foods, i.e. on average approximately four entries per food. Any missing food consumed during the survey was added to the food composition database.

Blood sampling and laboratory analysis-During the recall phase, blood samples were collected with disposable vacuum sterile tubes within lithium heparin coating balanced with lyophilic calcium and safety closing

The sample collection was done between 07:00 and 09:00 from participants who had fasted overnight and had refrained from smoking for at least 6 h. At the end of recruitment, blood samples were immediately subjected to centrifugation and extraction of the plasma, which will have been stored at -80 ° C in several fractions in the Moli-sani biobank. Successively, a fraction of each plasma sample was transferred in dry ice to the laboratory of the *Istituto di Zootecnica, Università Cattolica del Sacro Cuore* in Piacenza.

Total cholesterol, HDL, triglycerides, glucose, C-reactive protein, and creatinine were analyzed using commercial colorimetric enzyme kits (Instrumentation Laboratory, Milan, Italy) and an automatic analyser (IL 350, Instrumentation Laboratory) at the laboratory of *Centro Giovanni Paolo II* of Campobasso. Quality control was assured using commercial (high and low) laboratory standards and an in-house plasma pool. Coefficients of variation (CV) for the high-level external standards were 5.0% for triglycerides, 6.1% for HDL cholesterol, and 5.0% for glucose. CVs for the low level of external standards were 7.9% for triglycerides, 7.0% for HDL cholesterol, and 7.6% for glucose. CVs for the in-house plasma pool were 3.5% for triglycerides, 5.3% for HDL cholesterol and 3.8% for glucose.

The other blood samples were analysed at the laboratory of *Istituto di Zootecnica, Università Cattolica del Sacro Cuore* in Piacenza with an automated clinical analyser (ILAB 600, Instrumentation Laboratory, Lexington, MA) using the analytical methods developed in the same institute. In particular, have been determined the following parameters:

- energy metabolism: fructosamine;
- protein metabolism: urea and uric acid;
- inflammatory responses: positive acute phase proteins: haptoglobin, ceruloplasmin, and creatinine; negative acute phases proteins: albumin, paraoxonase (PON)
- oxidative stress: reactive oxygen metabolites (ROMs)
- vitamins and pigments: retinol for vitamin A, tocopherol for vitamin E, D, beta-carotene (BC), lycopene (LC), lutein-zeaxanthin (LZ) and cryptoxanthin (CX)
- minerals: calcium, zinc, cobalt, iodine, and selenium.

The main points of the different parameters analysed with ILBA 600 are summarized in (Calamari et al. 2016). For the determination of Se, I and Co, minerals were extracted adding 0.3 mL of trichloroacetic acid (10% v/v) to 0.3 mL of plasma, samples were mixed and centrifuged at 3500 g per 10 min. At 0.3 ml of supernatant was added 2.7 mL of Millipore® water and mixed. The content of each mineral was determined always on the same solution by inductively coupled plasma mass spectrometry (ICP MS Agilent 7900, Agilent Technologies, USA) equipped with collision cell, operating in High Energy Collision Mode with helium. Several isotopes of the elements were monitored, with ^{72}Ge and ^{89}Y like internal standards. The trichloroacetic acid solution at the same concentration of the samples was used as blank and to prepare the calibration curve diluting external standard (Merck, Darmstadt, Germany) to 100, 250, 500, 1000 ppM. The accuracy of results was verified using a mineralized solution of SRM 1577b (National Institute of Standards and Technology or NIST).

Blood content of carotenoids (BC, LC, LZ and CX) and vitamins (D, retinol for A and tocopherol for E) were determined by their extraction from 0.5 ml of plasma added of 0.5 ml of absolute ethanol, and 5 ml of n-hexane. After the centrifugation (at 5 ° C, 3520 g x 5 minutes), 4 ml of supernatant are taken and dried in a nitrogen flow. Then, the residue is suspended with 0.4 ml of methanol: tetrahydrofuran (THF) solution (80:20) and analysed by HPLC (LC-4000, Jasco Europe Srl, Cremella, Italy) using a C18 column (ZORBAX Eclipse Plus, 4.6 x 150mm, 3.5μ; Agilent Technologies, Santa Clara, CA, US) with a guard pre-column (ZORBAX 4.6 x 12.5 mm and 5 μm of diameter;

Agilent Technologies, Santa Clara, CA, US). The mobile phase employed has been Methanol-THF solution (80:20) at a constant flow of 1 ml/minute.

Data analysis - To carry out a comparative study of the two systems used to the evaluate the nutritional status (EPIC-SSQ system and 3D food diary consecutive) and their correspondence with the nutritional state measured with blood biomarkers, the foodstuffs were gathered into 9 categories: starchy foods, fruits and vegetables, legumes, meat, fish, milk eggs and dairy products, cheeses fats and dessert. Foodstuffs group for EPIC and 3D questionnaires are summarized in table 1 while the corresponding biomarkers are summarized in table 2.

Data description related to the food consumption (g/d), anthropometric parameters and blood biomarkers have been performed calculating the minimum (Min), the maximum (Max), the average (x') and the standard deviation (SD) reporting the data within subjects divided into 3 tertiles (low, middle and high) respect to the consumption of each food category corresponding to EPIC and 3D surveys.

It was subsequently fairy an evaluation of blood markers testing whether high levels of these markers correspond or not to the quantities of the corresponding food groups that contain them most. As regards carotenoids, especially fruit and vegetables, to standardize their effect, a standardized index (SI) was calculated for each pigment (β-carotene, lycopene, β-kryptoxanthin, lutein and zeaxanthin) taking into account its variability within the population. The blood content of the four carotenoids was summed for each subject and successively the SI was set up for each with the following formula:

$$SI = \Sigma\ [(Xi-Mi)\ /SD]$$

Where:

Xi = the individual analytical value

Mi = the arithmetic mean of the studied population

SD = the standard deviation of the studied population calculated fitting the values for each subject of the population.

The correlations between food intakes, anthropometric indicators of nutritional status and potential plasma biomarkers of the food intake, as well as the corresponding p-values, were calculated using the software SAS version 9.3. They have been considered meaningful results with p-values of 0.05 and slightly significant those with 0.1. Furthermore, multivariate linear regression analysis has been performed to evaluate the relationship between carotenoids (through the aforesaid index) and the consumption (g/d) of the different foodstuffs categories.

3.3 Results and discussion

3.3.1 Diets consumed, anthropometric data and blood parameters

To define the magnitude of nutrients ingested by a person, it is necessary first to specify the nature and consumption of individual foods, because their chemical-nutritional characteristics are very different. Although studies of recent decades tend to show that this is not enough, since the overall effect of each food is fundamental (Mozaffarian 2011 and Givem 2017), all these data are indispensable for each controlled person, especially when studying the relationship between diet and other parameters (e.g. blood markers, health status, etc.). In our case, for the above estimate, two types of questionnaire were used: EPIC and 3 days. Unfortunately, the first difficulty encountered was the lack of homogeneity in food identification between the two questionnaires. As can be seen from tables 3.1 and 3.2, the specific distribution of individual foods in the 9 categories is quite different: in fact, for EPIC is much better (e.g. the meat is divided into several types, so the milk and its derivatives) while the 3days tends to identify food categories, not always perfectly homogeneous. To improve the comparability, it has been possible to group the related foods and present within the 2 questionnaires to reach 9 categories: starch, fruit and vegetables, legumes, meat, fish, egg-milk and related fresh derivates, fat and dessert.

Given this premise, it was doubtful whether the individual ingested nutrients could be estimated, separately for the two questionnaires and to compare them, too randomly being the possibility of identifying an average value of the nutritional characteristics for each food category. In contrast, attempts have been made to establish a relationship between food consumption (although by categories and within each questionnaire), anthropometric data and blood parameters (these latter in the hypothesis that, to some extent, such parameters are correlated to the quantity of one or more food categories, as shown in table 3.3).

Table 3.1 Main categories of foods and the included single foods according to EPIC questionnaire

Starchy foods	Fruits and vegetables	Legumes	Meat	Fish	Milk and eggs	Cheeses	Fats	Desserts
Potatoes	Raw vegetables	Legumes	Beef	Fish	Milk	Fresh cheese	Vegetable oils	Soft drinks
Rice	Cooked vegetables		Veal	Dried fish	Yogurt	Seasoned cheese	Olive oil	Sugar and sweets
Bread	Cooked tomatoes		Pork	Canned fish	Eggs		Butter	
Breakfast cereals	Fruits		Mutton/Lamb	Crustaceans, molluscs			Margarines	
Pizza			Chicken (Turkey)				Animal fats	
Pasta			Processed meat					
			Rabbit					
			Other meat					
			Offals					

Table 3.2 Main categories of foods and the included single foods according to 3 days questionnaire

Starchy foods	Fruits and vegetables	Legumes	Meat	Fish	Milk and eggs	Cheeses	Fats	Desserts
Cereals	Fruits	Legumes	Meat	Fish	Milk	Fresh cheese	Oil and butter	Sweets
Potatoes	Vegetables				Yogurt	Seasoned cheese		Candies
					Eggs			

Table 3.3 Blood biomarkers which could be affected by the different intake of each category of foods

Starchy foods	**Fruits and vegetables**	**Legumes**	**Meat**	**Fish**	**Milk and eggs**	**Cheeses**	**Fats**	**Dessert**
Glucose	Tocopherol	Urea	Urea	Urea	Urea	Urea	Total cholesterol	Glucose
Fructosamine	Retinol	Glucose	Uric acid	Total cholesterol	Total cholesterol	Total cholesterol	HDL	Fructosamine
	Beta carotene		Total cholesterol	Vitamin D	Vitamin D	Vitamin D	LDL	Total cholesterol
	Lutein +Zeaxanthin		Vitamin D	Retinol	Selenium	Selenium	Triglycerides	
	Lycopene		Retinol	Selenium	Iodine	Cobalt (vitamin B12)		
	Beta cryptoxanthin		Selenium	Iodine	Cobalt (Vitamin B12)	Retinol		
			Cobalt (Vitamin B12)	Cobalt (Vitamin B12)	Retinol			

3.3.2 Estimating food consumption by comparing different questionnaires

The first necessary comparison was between the estimated EPIC consumption and 3 days; for this reason, the subjects were subdivided according to the consumption (g/d) of the individual categories of food, separately for both questionnaires. The relevant data are collected in table 3.4 and refers only to the 99 subjects for whom the two questionnaires were compiled at the same time. The mean (with standard deviation) - after subdividing into tertiles - the consumption (g/d) of the main categories of foods separately for the two questionnaires are reported. First of all, it is noted that the magnitude of the values measured with the two questionnaires is similar, at least for the starchy and for fruit and vegetables, but very different are the average values found for the consumption of meat, fish, milk, cheese, fat and dessert in the various tertiles. However, even for the first two categories, the comparison for individual subjects - except that the same subject is not always in the same tertile of the two questionnaires - revealed that 40% of them have values of the food consumption, recorded with the two questionnaires, which differ by more than 40%. On the other hand, there is no correlation between the consumption observed with the two types of a questionnaire for any category of food.

About the consumption of foods that differ within the surveys of each questionnaire, with EPIC or 3days, the correlations appear generally modest but above all, they are not univocal between the two questionnaires (present in one and not the other). Only between starch and meat consumption, there is a positive correlation ($r = 0.39$; $p < 0.001$ in the EPIC questionnaire and $r = 0.26$; $p < 0.02$ in the 3 days) questionnaire) in both (probably indicating that in major meat consumers there is the tendency to privilege bread and pasta). However, the most important aspect remains the degree of reliability of the consumption values provided by the 2 questionnaires which, given the above results, would seem rather low and with little certainty because of the inability to determine if and which of the two questionnaires provided more reliable results. Therefore, we limit ourselves just to observe that is confirmed the not negligible randomness while estimating food consumptions, for individuals, through the compilation of food questionnaires (Macdiarmid and Blundell 1998).

Table 3.4 Averages values (Mean±SD) of consumption (g/d) of the different categories of foods within EPIC and 3 days questionnaire in the same subjects divided into tertiles (low, meddle, upper)

Categories of foods	**EPIC questionnaire**					**3 days questionnaire**				
	N° of Subjects	Low tertile	Middle tertile	Upper tertile	All data	N° of Subjects	Low tertile	Middle tertile	Upper tertile	All data
Starchy foods	97	153±42	240±30	377±56	254±56	97	146±36	241±22	360±68	248±100
Fruits and vegetables	91	291±69	453±43	722±168	491±168	90	250±80	475±73	770±159	501±242
Legumes	99	10±4	23±4	47±17	27±17	99	0±0	0±0	27±16	9±16
Meat	92	60±20	105±12	157±40	107±40	92	28±16	80±14	151±58	86±61
Fish	93	22±7	39±7	65±15	42±15	93	0±0	15±11	92±49	36±50
Milk and eggs	97	39±20	140±35	294±149	159±149	99	23±18	99±35	211±48	111±85
Cheeses	94	15±7	37±7	80±34	44±34	93	36±19	117±35	303±111	153±132
Fats	97	16±3	22±1	30±5	22±5	97	22±6	34±3	50±12	35±14
Dessert	86	21±10	59±16	183±69	89±69	87	86±25	183±26	288±50	153±102

3.3.3 Relationships between anthropometric data and food consumption.

In table 2.5, are reported averages values (with standard deviation) of some anthropometric (and clinical, means blood pressure) parameters of the controlled population with both questionnaires (99 subjects). For each parameter, subjects were subdivided into tertiles reporting the mean. It is noted that age is confirmed by the initial choice: controlling a middle-aged population with comparable numbers between the two sexes (47 males vs. 52 females). A partial result of this choice is the values of anthropometric and clinical data collected in the same table:

- the tendency to have relatively high weights, given the height (the maximum of the 2nd tertile is 80 kg per 167 cm);
- this is confirmed by BMI, as the 3rd tertile (the highest) contains obese people, while the 2nd contains overweight people (with a minimum of 24.9 and a maximum of 29.7), while no underweight people are noticed (the minimum is 20.3 in all population);
- Waist, Hip, and WH ratio values, confirm substantially, what BMI indicates;
- in some ways, unexpected is the behavior of the blood pressure values, since only in the upper tertile, there are people with values above 140 mmHg for maximum and 90 for the minimum (maximum limits for acceptability, especially for people of relative ages advanced). In the third tertile, the minimum is 132.0 and a maximum of 181.5; in part explaining the fact that some of the controlled people were under antihypertensive therapy (particularly, among the 21 hypertensive patients declared, 18 were under pharmacological treatment).

Referring to the verification of the correlations between the parameters in table 3.5, although likely obvious, it is important to emphasize - also because significant - the positive correlation of weight with BMI ($r = 0.76$; $p < 0.0001$) and with the hip ($r = 0.73$; $p < 0.0001$) but also with blood pressure, even if lower ($r = 0.24$; $p < 0.01$). The possible relationship between anthropometric parameters and consumption of the main categories of foods has been studied by comparing the correlations within the 146 subjects undergoing EPIC and within the 99 submitted also to the 3days questionnaire. The correlations appear at least diversified between the two sets of data and not always in the same direction (the r reported refer only to EPIC). Nevertheless, only for starches there is a significant and positive correlation with the weight ($r = 0.29$, $p < 0.001$ and $r = 0.25$, $p < 0.02$ in the EPIC 3 days questionnaires respectively), confirming that the excess of carbohydrates is among the major causes of overweight (Flegal et al. 2012 Sen et al. 2017).

Table 3.5 Anthropometric and clinic data divided into tertiles for each parameter for subjects which food consumption (g/d) survey has been done with both EPIC and 3D questionnaires

Parameters	**Units**	**Reference ranges**	**N° of Subjects**	**Low tertile**	**Middle tertile**	**Upper tertile**	**Mean±SD**
Age males	Years	40-70*	47	46±3	53±2	59±16	54±7
Age females	Years	40-70*	52	47±3	54±2	63±3	55±7
Weight	kg	-	99	57±5	72±5	90±8	73±15
Height	cm	-	99	151±5	163±3	174±6	163±10
BMI	kg/m^2	18.5-25	99	23±1	27±1	33±5	28±5
Waist	cm	<94 (M); <80 (F)	99	82±5	101±1	109±9	95±12
Hip	cm	<89 (M); <68 (F)	99	94±3	94±3	111±10	102±10
WH Ratio	-	<0.95(M); <0.85 (F)	99	0.85±0.04	0.94±0.02	1.01±0.03	0.93±0.07
Blood pressure							
Systolic	Hg mm	90-120	99	111±7	126±3	144±12	127±16
Diastolic	Hg mm	60-80	99	69±5	78±2	88±7	78±9

* Reference age range criteria of the population for data collection in this study

3.3.4 Blood parameters and food consumption

As above mentioned, the only objective parameters in our possession are the anthropometric (and clinical) and the blood ones gathered in table 3.6. There are reported the means (with standard deviation) of blood indices, divided by metabolic-nutritional domains, depending on the possible relationship between the consumption of the main categories of foods. Again, the values are related to the population surveyed with both questionnaires and were subdivided into the tertiles of each parameter.

At the same time, the data of the 146 subjects were analysed to establish possible correlations between blood parameters, but also between these and the anthropometric-clinical parameters as well as the consumption of foods. For those with consumption, correlations with all parameters were determined using the consumption observed with both questionnaires (EPIC and 3days, 146 and 99 subjects respectively). Again, even in this case, the only correlations of some interest are within the blood parameters between them and with the anthropometric ones (but will be discussed later), while for the food consumption, only for the starchy foods, there is a positive correlation in both questionnaires - already mentioned - with the weight ($r = 0.29$).

Among the blood parameters and the type of diet based on consumption, there is also little consistency over the 2 types of questionnaire, there is always a (positive) correlation between starchy foods and triglyceride but also between starchy foods and HDL but, in this case negative with $r = -0.22$; $p < 0.05$ and only for consumption detected with 3days. Not that this is a major surprise, as it is already shown in table 3.3, there are few blood parameters which variations are attributable to a single category of food. It is also right to observe that most of the blood indices are subjected to various mechanisms and these can act on various metabolic processes by modifying several parameters (e.g. glucose, urea, cholesterol).

In the case of inflammatory parameters, it should be noted that the causes of these processes are numerous and that some foods can accentuate them, but others prevent or otherwise attenuate them Trichopoulou et al. 2011 and Appleby et al. (2016). Even with these difficulties, it is of great interest - for the purposes of our study - for any relationship between blood parameters (as well as anthropometric data) and diet. Therefore, some clarification on the significance of these parameters, not so much of a clinical nature, but referring to nutrition and hence to the categories of foods for which ingestion is estimated.

Particularly table 3.6 shows that:

- among the indicators of the energy-lipid metabolism, glucose and fructosamine (glycated protein in the blood) are similar for meaning even if the second is in relation to the long-term glucose levels. Hence the hypothesis of some possible relation to the intake of foods rich in carbohydrates and particularly in glucose. For both, we note that hypoglycemic values are almost absent in our population, while those above the standard are relatively few and only in the upper tertile (confirming relatively few cases of diabetes). Even for the 4 "lipid" indicators, we can make similar considerations: values above normality are in the high tertile (except for HD cholesterol which, being favorable if high, shows too low values in the 1st tertile). Particularly, the 3 cholesterol indices are representative of circulating lipoproteins: totals, the LDLs with the function of triglycerides transportation (and other lipid-soluble substances), especially the HDLs used in the recovery of various fractions rich in cholesterol, otherwise included in the atherosclerosis causes. Their presence in the blood, rather than dietary cholesterol, is influenced by fats absorbed in the intestine (re-esterified in triglycerides) and by triglycerides synthesized ex novo in the liver in the presence of excess diet's carbohydrates. In both cases, triglycerides are circulated around the lipoprotein (these are structurally rich in cholesterol, so it is synthesized if not enough that from the diet). With these premises, it is also understood the importance of measuring triglycerides that at high values (like LDLs) denote dyslipidemia with an increased risk of atherosclerosis. Finally, it should be noted that "all" lipophilic substances (including vitamins, pigments, hormones, etc.) are to some extent transported to the blood by lipoproteins. Therefore, the positive correlations of total cholesterol with different forms of lipoproteins (HDL with $r = 0.27$, $p < 0.001$ and LDL with $r = 0.95$, $p < 0.0001$), but also with lipid-soluble vitamins and generally with pigments carotenoids: beta-carotene ($r = 0.23$, $p < 0.005$), lutein zeaxanthin ($r = 0.38$; $p < 0.0001$), beta - cryptoxanthin ($r = 0.18$, $p < 0.05$) and lycopene ($r = 0.36$; $p < 0.0001$);

- About the indicators of protein metabolism, there are two (urea and uric acid) as catabolites of nitrogen metabolism: urea is a true protein (ureagenesis is done to eliminate, in non-toxic form, the NH_3 from amino acid catabolism or absorbed by the digestive tract), while uric acid is derived from the bases from the catabolism of nucleic acids which are abundant in foods rich in cells and therefore also proteins. This last statement, however, is not always true because there are foods (especially those of animal origins, such as milk and eggs) rich in protein, but not of nucleic acids. Hence the opportunity to keep separate the two indicators, although it may be useful to consider them at the same time, remembering their different origins. Within our population, we notice that there are no too low values that, for urea, would be indicative of severe protein deficiency. On the other hand, they are very high, though only in the upper tertile, indicating a possible excess of protein and/or nucleic acids. In general, it can be considered that diets consumed by the population studied are considered adequate in terms of protein intake. However, we should not neglect the fact that both indexes may be elevated due to renal failure (there was no specific requirement in the questionnaires, but the choice of subjects to be controlled was based on a normal health state).
- The third indicator included in this group, the albumin, is a protein fraction of plasma made up of many different proteins, predominantly synthesized in the liver and able to perform innumerable functions, especially to avoid the excessive loss of water from the bloodstream with fluid build-up in the subcutaneous (edema). For this parameter, both low values and too high values are found. In the first case, the cause could be a protein deficiency (which, however, rarely urea confirms) or, more likely, a modest renal dysfunction which is, among other things, the cause of loss of albumin in urine, already hypothesized for very high values of urea and uric acid. About the high values of albumin, the most probable cause is usually hemoconcentration (stress, dehydration, etc.), which however is difficult to confirm.

- The 4th indicator, creatinine, is reported separately between men and women for the significant difference existing. The significance of this parameter is at least twofold: a renal function indicator, since the excreted daily dose should be constant (except for a renal impairment); However, it is also an indicator of the amount of creatine released by the muscle tissue which, in normal condition, is higher when greater is the mass that then modifies the blood content. Thanks to these indications, the fact that females are separated from males confirms that in the first muscle mass is proportionally lower. The data in table 3.6 show that there are no particularly high values so that forms of nephropathy could be excluded (as previously hypothesized). Conversely, the values are often low, the first tertile has values always lower than the minimum limit for women, while in men only a part of that tertile is less than that limit. These low values suggest that - at least in advanced age - the risk of sarcopenia increases, especially in women. In fact, no correlation between age and creatinine was demonstrated, so low values seem to be justified by adipose tissue accumulation and sex: BMI is in fact only negatively related to creatinine in females ($r = -0.21$) although not statistically significant ($p < 0.1$). To confirm the different behavior between the sexes about the muscular and fat masses, there seems to be a correlation between weight and creatinine: negative, though not significant in females indicating the prevalence of fat mass while positive in males (more muscle mass).
- the vitamins that we controlled are only lipid-soluble (vitamin B12, will be discussed later), so their presence in may be affected by lipoprotein levels (as already anticipated). In addition, especially for retinol (Trevisi et al. 2013) and to a lesser extent for tocopherol (Trevisi et al. 2013) but also vitamin D, the blood level may be influenced by the presence of proteins vectors (Retinol Binding Protein for Retinol) that are often synthesized in the liver when well-functioning. In our population, we do not observe values below the minimum, at least for retinol (so it would be possible to exclude them), while they are frequent for vitamin E (tocopherol) and D. For these two last vitamins, there could be some inadequate subjects (especially for E, the overall average of 6.27 μg/ml, is not far from the minimum of 5 μg/ml, and the whole lower tertile, basically below this limit, plausible some spread of

this deficiency). About values above the maximum limit, they are observed only for retinol and vitamin D and in the upper tertile. Their meaning is difficult to say. The foods that mostly bring these vitamins are fruits and vegetables for retinol (such as beta-carotene and other carotenoids) and tocopherol, while vitamin D is only made from food of animal origin (however, it is well known that in plants there is 7- dehydrocholesterol, vitamin D precursor if activated by sunlight). Animal products are also good suppliers of retinol and tocopherol;

- for minerals, it is appropriate to distinguish between calcium and cobalt compared to others. For calcium, it is known that blood levels have no relation to the diet because they are subject to rigid hormonal regulation. For cobalt, it is known that it has a biological function only and as it is present in vitamin B12. Its values, considered normal in the blood (150-300 ng/l) would, however, correspond to Co levels close to the lower tertile (0.1-0.2 nmol/l). Therefore, the values we have found, being on average 6.27 nmol/l, are likely to be attributed to the presence of cobalt as a chemical element and thus of no utility for nutritional purposes. The lack of vitamin B12 determination in the plasma - initially present in the protocol - is only due to our inability to conduct the analysis within the brief time of its stability in the plasma itself. It should not be overlooked that there are many confirmations of a good relationship between plasma levels and milk consumption (Mendonça et al. 2016). Among the other blood minerals, they are poorly related to nutrition (eg Na and Cl), others well present in human foods and therefore rarely at serious risk of serious deficiency (e.g. K and partly P and Mg); for others, many deficiencies and excesses are possible, depending on the circumstances. Among the latter, there is the iodine of which is essential for the synthesis of thyroid hormones (T3 and T4), but there are also additional amounts of protein-bound iodine (PBI) in the blood, probably in reserve. While the first fraction (hormonal) is under endocrine control, the second is mainly due to nutritional availability (as well as urinary excretion), the values observed in table 3.6 tend to be low (only the upper tertile has values within the reference range: 0.6 - 1, 4 μmol/l). Being a mountain area (Campobasso), this may correspond to the reality as confirmed by Aiello et al.(998) that approximately 30% of school age children of this area are affected by goiter (1A and 1B) of probable chronic origin. For selenium in the blood there is, as for iodine, a functionally-linked part of enzymes (e.g. glutathione-peroxidase) and a minority part only transported by proteins. For low values, there is certainly the possibility of suspected food shortage, but in our case the minimum and maximum values are within the reference range. For zinc, there are many similarities with selenium, but it must be added that rapid drops of the element in the plasma also occur in the case of inflammatory processes (acute phase response), so low values should also be interpreted considering these processes. Table 3.6 shows both low zinc (few) values, but also high values, at least in the upper tertile. These latter values do not have a certain motivation, but they do not seem to be dangerous, because they are frequently caused by the pollution of the blood sample taken without specific cautions;

- the pigments present in the plasma are of predominantly vegetable origin (only beta carotene can be relatively abundant in beef, horses and chickens as well as in dairy products (of bovine origin only) and particularly from leaves and fruits, hence the hypothesized report (non-starchy or leguminous) nutrients in the present study are all hydrocarbon-like carotenoids, so lipophilic substances are used for their absorption and transferring in the blood with lipids (with lipoproteins, therefore cholesterol, as anticipated earlier). Apart β-carotene, which is also pro-vitamin A, they perform some functions especially as antioxidants. It should also be noted that it is possible a substance that could go ignored at least at the blood level; this is confirmed by the fact several species are those having white plasma (pigs, rabbits, sheep, goats, etc.). But it confirmed by the amplitude of the reference range shown in table 3.6. This need clarification since, unlike what is seen for most parameters, values do not indicate a field within which the organism does not have any negative consequences, but simply an observed oscillation range (coming out of which, probably, nothing happens). This does not alter our interest because it is linked to the ability to act as a biomarker for the consumption of foods that contain them, although to a very different extent depending on the specific carotenoid: mainly leaves (or green parts) for lutein and zeaxanthin, equally green parts of plants, but also red-yellow fruit for β-carotene; especially some colored fruits for lycopene and β-cryptoxanthin. Therefore, due to the different presence of individual pigments in different foods and their seasonal availability, blood intake may change considerably. Very noticeable, as a factor of variation, is the sex effect; Although it is not equally evident for all pigments, it was considered useful to report separately the values of males and females. In any case, the data shown in table 3.6, show the following:

- at least for the mean values of the dataset, for all pigments, values that fall within the reference range are observed. In fact, for β-carotene, lutein + zeaxanthin and lycopene also the means of the tertiles fall perfectly in that range (only some subjects leave but for β carotene and lycopene only relate to extremely high values: up to 183.4 µg/100ml of the first and 1273.2 µg/l of the second). Vice versa for β-cryptoxanthin, the 1st and 2nd tertiles 'averages are lower than the minimum value of the reference range;
- the variability of the values found in our population is generally very large and there is certainly sex among the causes. Especially, the values in females are higher for β-carotene, β-cryptoxanthin and lutein + zeaxanthin, while they are lower for lycopene. However, only for the first 3, the differences are significant (with $p < 0.001$, $p < 0.1$ and $p < 0.05$ respectively). Some differentiation between males and females for these pigments had already been observed by Olmedilla et al. (2001), although in evident manner only for β-carotene and β-cryptoxanthin. However, the great variability observed is, as a probable main cause, the different intake of foods more or less rich in such pigments and the trend at higher values in women would be justified in the fact that they tend to consume more vegetables or fruit (albeit in relative terms since the absolute values are higher in males). The reversal situation of lycopene appears to be the

result of increased consumption of tomato-based foods in males (all year with pasta, pizza, etc.);

    - based on these last observations, regarding the causes of the enormous variability of pigments, it is reasonable to think that they can constitute a biomarker of the subjects' eating habits, especially for the consumption of vegetables and fruits.

- For inflammatory process indicators, it has already been reported that a relationship with the diet can only be indirect, even with more than one mechanism: variation in immune system response capacity (therefore different risk of disease), induction of inflammatory processes mainly through microbial phenomena in the digestive tract (e.g. for excess carbohydrates as recently demonstrated by Sen et al. (2017), but also possible attenuation of any ongoing inflammatory process (e.g. antioxidants, fatty acids ω 3, etc.). However, some consideration of our data still appropriate; first, it is necessary to distinguish between acute response indices (C-reactive protein, haptoglobin, ceruloplasmin, and ROMs) and chronic response indices (paraoxonase and albumin). The first group covers 3 specific proteins of the acute inflammatory phase; among them, C-reactive protein is more considered in the human species, whereas haptoglobin appears to be of little relevance and ceruloplasmin has an intermediate relevance.
- For the latter data, to be feared because of their high values in table 3.6 appear to be exclusively those related to ceruloplasmin (the upper tertile has an average high and in any case all subjects are above the maximum limit); since this does not occur simultaneously for C-reactive protein (as well as for haptoglobin), it is difficult to judge whether the high values of ceruloplasmin actually denote inflammatory processes in progress. In fact, the results in some ways unexplainable could contribute to the lack of synchrony between the variations of the proteins. In fact, the response times are very different, extremely fast for C-reactive protein C and haptoglobin, very slow for ceruloplasmin (especially for returning to the standard that this implies for many weeks). The 4th acute response indicator, ROMs, is far less specific for inflammatory processes, so high values - found increasingly in intermediate and superior patients - can simply be linked to oxidative stress, regardless of

the inflammatory processes, of which we do not know the reasons. The two subsequent parameters, paraoxonase, and albumin, are respectively an enzyme and a set of proteins (which have already been mentioned for food protein supply) whose blood level is also influenced by liver synthesis; Therefore, their reduction may be caused by lower liver synthesis and this may also occur in the case of inflammatory processes (especially if prolonged).

The values reported in table 3.6 would, however, exclude prolonged inflammatory phenomena since few subjects with low albumin, while paraoxonase is never lower than the minimum considered to be normal to confirm that chronic inflammatory phenomena are poorly relevant if present. As already noted, inflammatory and/or oxidative processes can be influenced by the food that can accentuate them (serious deficiencies due to lesions or metabolic disorders, food rancidity, digestive abnormalities, etc.), but also attenuate them (fatty acids ω3, substances antioxidants, etc. notoriously anti-inflammatory); In our case, obviously, the last ones would have been prevalent. This circumstance is, however, very important as it confirms the "good" state of health - as preconceived - of the subjects on which the survey was conducted. The correlations between certain indices of inflammatory processes are also highlighted. Especially the positive ones among CRP (the most important acute positive protein in humans), ceruloplasmin ($r = 0.44$, $p < 0.0001$) and ROMs ($r = 0.46$, $p < 0.0001$). On the other hand, there is no positive correlation with the other positive acute protein, that is, haptoglobin who is in fact considered to be of little importance in humans. Vice versa, the correlation between CRP and some negative acute phase proteins: PON ($r = -0.30$, $p < 0.0002$) and retinol ($r = -0.14$, $p < 0.1$); in the latter case, probably due to lower hepatic synthesis of Retinol Binding Protein due to inflammation (especially if important).

Table 3.6 Plasma Biomarkers data divided into tertiles of each parameter for subjects which food consumption (g/d) survey has been done with both EPIC and 3 days questionnaires (parameters were clustered according to metabolic aspects and supposed food relationship)

Parameters	Units	Reference ranges	Subjects	Low tertile		Middle tertile		Upper tertile		Mean±SD
				Min	Max	Min	Max	Min	Max	
Biomarkers of energy and lipid metabolism (starchy foods-animal products-desserts)										
Glucose	mg/dL	70-110	99	69	88	89	96	97	149	93±14
Fructosamine	µmol/L	50-150	90	5	203	204	254	256	345	213±74
Triglycerides	mg/dL	115-200	94	43	87	88	123	126	234	109±43
Total cholesterol	mg/dL	85-125	99	131	184	188	216	217	276	201±32
LDL	mg/dL	200-285	97	60	112	113	136	137	187	125±28
HDL	mg/dL	40-80	99	26	47	47	56	57	83	52±13
Biomarkers of protein metabolism (legumes-animal products)										
Urea	mmol/L	2.1-7.1	99	2.95	5.83	5.91	7.13	7.18	12	6.76±1.70
Uric acid	mg/dL	3.5-7.1	99	3.22	5.34	5.37	6.29	6.32	8.72	5.91±1.19
Albumine	g/L	2.5-6.2	99	26.2	44.6	44.6	46.3	46.3	56.4	45.4±3.7
Creatinine males	mg/dL	0.7-1.3	47	0.54	0.78	0.80	0.92	0.92	1.11	0.84±0.12
Creatinine females	mg/dL	0.6-1.1	52	0.42	0.59	0.59	0.67	0.70	0.81	0.64±0.11
Vitamins (fruits and vegetables-animal products)										
Retinol	µg/100ml	30-120	99	40.0	82.0	82.1	99.3	99.7	143.7	90.1±19.6
Tocopherol	µg/mL	5-18	99	2.06	5.66	5.77	7.64	7.68	14.60	6.69±2.31
Vitamin D	ng/mL	5-75	99	3.9	25.0	25.1	31.7	32.4	54.5	29.2±10.1
Minerals (animal products)										
Calcium	mmol/L	2.15-2.50	99	2.04	2.32	2.33	2.41	2.42	2.66	2.37±0.11
Cobalt	nmol/L	0-20	95	0.08	3.19	3.27	6.63	6.87	34.17	6.27±6.06
Iodine	µmol/L	0.6-1.4	98	0.20	0.33	0.33	0.40	0.41	1.04	0.37±0.11
Selenium	µmol/L	0.80-1.90	98	0.92	1.43	1.44	1.57	1.59	1.98	1.51±0.20
Zinc	µmol/L	10.7-18.4	98	11.2	16.0	16.0	17.5	17.5	24.9	17.2±4.6

Table 3.6 (continue) Plasma Biomarkers data divided into tertiles of each parameter for subjects which food consumption (g/d) survey has been done with both EPIC and 3 days questionnaires (parameters were clustered according to metabolic aspects and supposed food relationship)

Parameters	Units	Reference ranges	Subjects	Low tertile		Middle tertile		Upper tertile		Mean±SD
				Min	Max	Min	Max	Min	Max	
Pigments (fruits and vegetables)										
Beta carotene	µg/100 mL	50-300	99	10.3	29.2	29.8	52.0	53.3	183.4	48.8±30.6
Beta cryptoxanthin	µg/L	20-400	99	3.3	13.6	14.3	28.3	31.3	181.1	32.6±31.6
Lutein +Zeaxanthin	µg/L	50-550	99	56.9	119.9	120.2	161.4	164.9	291.5	148.5±52.1
Lycopene	µg/L	30-550	99	53.2	302.6	306.4	481.3	486.7	1273.2	443.0±245.5
Inflammation biomarkers (different nutritional aspects)										
C-Reactice Proteina	µg/mL	0-10	98	0.02	0.62	0.63	1.99	2.00	7.72	1.65±1.51
Haptoglobine	g/L	0.3-2	99	0.59	0.82	0.82	0.99	1.01	2.28	0.96±0.29
Ceruloplasmin	µmol/L	1.4-3.81	99	0.53	3.32	3.32	3.93	3.96	6.65	3.66±0.87
Paraoxonase	U/mL	53.3-355.0	99	137.6	221.3	221.8	244.2	245.4	316.4	234.2±28.6
Albumine	g/L	39-51	99	26.2	44.6	44.6	46.3	46.3	56.4	45.4±3.7
ROMs	mg H2O2 /dL	20-24	99	27.5	34.6	34.7	38.8	38.9	58.1	37.2±5.7

Table 3.7 Reference range values of biomarkers from different literatures and laboratories

Parameters	Units	Reference range values	Specimen	References
Biomarkers of energy and lipid metabolism (starchy foods-animal products-dessert)				
Glucose	mg/dL	74-106	Plasma or serum (fasting)	(Duh and Cook 2005)
		56-115	Plasma or serum (fasting)	(den Elenzen et al. 2017)
Fructosamine	µmol/L	200-285	Serum	(Kotus 2014)
Triglycerides	mg/dL	50-150	Serum	(Farinde 2014)
Total cholesterol	mg/dL	115-200	Serum	(Farinde 2014)
		150-284.6	Serum	(den Elenzen et al. 2017)
LDL	mg/dL	85-125	Serum	(Farinde 2014)
HDL	mg/dL	40-80	Serum	(Farinde 2014)
Biomarkers of protein metabolism (legumes-animal products)				
Urea	mmol/L	2.1-7.1	Serum	(Duh and Cook 2005)
		2.5-7.5	Serum	(den Elenzen et al. 2017)
Uric acid (males)	mg/dL	4.5-8.0	Serum	(Duh and Cook 2005)
		3.3-8,0	Serum	(den Elenzen et al. 2017)
Uric acid (females)	mg/dL	2.5-6.2	Serum	(Duh and Cook 2005)
		3-5.8	Serum	(den Elenzen et al. 2017)
Albumine	g/L	39-51	Serum	(Duh and Cook 2005)
		34-48	Serum	(den Elenzen et al. 2017)
Creatinine (males)	mg/dL	0.7-1.3	Serum	(Duh and Cook 2005)
		0.7-1.2	Serum	(den Elenzen et al. 2017)
Creatinine (females)	mg/dL	0.6-1.1	Serum	(Duh and Cook 2005)
		0.5-1.02	Serum	(den Elenzen et al. 2017)
Vitamins (fruits and vegetables-animal products)				
Vitamin A (Retinol)	µg/100mL	30-120	Plasma	(ARUP LABORATORIES 2017)
Vitamin E (tocopherol)	µg/mL	mag-18	Serum	(Duh and Cook 2005)
Vitamin D	ng/mL	mag-75	Serum	(Farinde 2014)

Table 3.7 (continue) Reference range values of biomarkers from different literatures and laboratories

Parameters	Units	Reference range values	Specimen	References
Minerals (animal products)				
Calcium	mmol/L	2.15-2.50	Serum	(Duh and Cook 2005)
		2.15-2.56	Serum	(den Elenzen et al. 2017)
Cobalt	nmol/L	0-20	Serum	(Mao et al. 2011)
Iodine	µmol/L	0.6-1.4	Plasma	(Allain et al. 1993)
Selenium	µmol/L	0.80-1.90	Serum or plasma	(Combs 2001)
Zinc	µmol/L	10.7-18.4	Serum	(Duh and Cook 2005)
Pigments				
		3.22-96.10	Plasma	(Olmedilla et al. 2001)
Beta carotene (males)	µg/100mL	4.83-118.11	Plasma	(Olmedilla et al. 2001)
Beta carotene (females)	µg/100mL	22.11-33.17	Plasma	(Olmedilla et al. 1994)
Beta cryptoxanthin (males)	µg/L	71.87-591.55	Plasma	(Olmedilla et al. 1994)
Beta cryptoxanthin (females)	µg/L	22.75-409.54	Plasma	(Olmedilla et al. 2001)
Lutein +Zeaxanthin (males)	µg/L	25.60-381.10	Plasma	(Olmedilla et al. 2001)
Lutein +Zeaxanthin (females)	µg/L	42.95-1138.16	Plasma	(Olmedilla et al. 2001)
Lycopene (males)	µg/L	42.95-1138.16	Plasma	(Olmedilla et al. 2001)
Lycopene (females)	µg/L	21.47-1105.95	Plasma	(Olmedilla et al. 2001)
Inflammation biomarkers				
C-Reactice Proteina	µg/mL	0-10	Serum	(Gabay and Kushner 1999)
Haptoglobine	g/L	0.3-2	Serum	(Wilczynski 2014)
Ceruloplasmin	µmol/L	1.4-3.81	Serum or plasma	(Safavi et al. 2012)
Paraoxonase	U/mL	53.3-355.0	Serum	(Maturu et al. 2013)
Albumine	g/L	34-48	Serum	(den Elenzen et al. 2017)
ROMs	mg H2O2 /dL	20-24	Serum	(Mancinelli et al. 2013)

3.3.5 Possible estimation of food consumption in individual subjects

As we have seen till now, it tends to confirm that among the parameters in our possession, objective only, are anthropometric-clinical and blood parameters ones because they are directly detected on the subjects considered. Of course, the fact that the behavior of the correlations observed, among the parameters in the 99 subjects submitted to both EPIC and the 3days questionnaire, is somewhat of the same, that they are completely overlapping (albeit not identical) with respect to the 146 subjects coincide the previous 99 and the other 47 subjects controlled only with EPIC. When reference is also made to the consumption of food declared with the questionnaires: EPIC and 3days, the absolute values are very different between the two and there is also no correlation between the declared consumption by the same subjects with the two questionnaires. These observations are sufficient to justify the fact that only rarely have been correlated between blood parameters and dietary consumption; However, other reasons may be:

- many of these parameters have control systems; they may be endocrine and non-endocrine; but also of other nature the vector proteins of specific nutrients (lipids, minerals, vitamins), as well as mechanisms for regulating excretion absorption according to needs, etc.);
- very different foods may help to change the same parameter as they are rich in the same nutrients.

However, the low level of reliability of the consumptions with the EPIC and 3days questionnaires remains, among other things, in poor relation with the anthropometric parameters, which should also have a more direct response to the magnitude of the individual consumption of certain foods. Of this relationship we find a trace, but only for starches showing a positive correlation with the weight ($r = 0.29$; $p <0.001$ and $r = 0.25$; $p <0.02$ for EPIC and 3D questionnaires respectively). However modest, it can be assumed that there is some link between them: between adipose tissue accumulation - also because the weight is often correlated with BMI - and carbohydrate-rich foods that are considered principal factors for such accumulation (Sen et al. 2017).

As discussed above, it, therefore, confirms that it is difficult to establish a link between unmanageable food consumption and anthropometric-clinical parameters (in themselves objective, but also influenced by our uncontrolled lifestyle), but also with parameters at least those associated with the main metabolic aspects (carbohydrates, lipids, proteins, and minerals). Conversely, our results seem to show that there is some relationship between diet type (though with coarse consumption) and blood parameters that are little or nothing controlled within the body and are mainly related to a specific category of foods: fruits and/or vegetables i.e. pigments. The behavior of these pigments is well known in table 3.6 (of the 99 subjects submitted to both questionnaires) with the net variations between the means of the tertiles.

However, further separation of the subjects (in this case 146) was made based on the pigment index and, presumably, of the consumption of fruit and vegetables. Accordingly, we calculated the mean of consumption, of all blood parameters and of anthropometric-clinical parameters of all subjects together (table 3.8), or separately for sex (tables 3.9 and 2.10). The separation between the sexes was made because already Olmedilla et al. (1994 and 2001) had found significant differences between males and females in pigment values. In general, differentiation among tertiles is remarkable for the 4 pigments and is always highly significant within the entire population or separately between the sexes. About the 3 pigments, β-carotene, β-cryptoxanthin and lutein + zeaxanthin pigments, the values in females are higher respectively at 58.9 vs 37.5 μg/100 ml ($p < 0.05$), 38.7 vs 25.9 μg/l ($p < 0.01$) and 156.6 vs 139.4 μg/l ($p <0.05$). The situation is reversed for lycopene, higher in males (460.2 vs 427.4 μg/l) but without meaningful differences. Analogous behavioral differences between males and females are also noticed when the comparison is done within the entire population.

Table 3.8 Differences between the average values of the different parameters (blood parameters, anthropometric, clinical) and food consumption (g/d) of the different food categories divided according to the lower (1), middle (2) and higher (3) level of the pigments index of the all population (males and females together)

Parameters	Units	(1)	(2)	(3)	(1) vs (2)	(1) vs (3)	(2) vs (3)	p<	Symbol	p<
Biomarkers of energy and lipid metabolism (starchy foods-animal products-dessert)										
Glucose	mg/dL	96.27	87.43	94.38	**		**		+	<0.1
Fructosamine	µmol/L	219.77	215.32	209.01					*	<0.05
Triglycerides	mg/dL	139.67	104.63	130.40	+			0.0652	**	<0.01
Total cholesterol	mg/dL	191.61	202.14	227.15	+	**	**	0.0932		
LDL	mg/dL	115.83	127.75	147.60	*	**	**			
HDL	mg/dL	50.45	53.47	54.17						
Biomarkers protein metabolism (legumes-animal products)										
Urea	mmol/L	7.01	6.50	6.80						
Uric acid	mg/dL	6.08	5.78	5.92						
Albumine	g/L	45.69	45.14	45.00						
Creatinine	mg/dL	0.76	0.73	0.70		*				
Vitamins (fruits and vegetables-animal products)										
Vitamin A (Retinol)	µg/100ml	88.73	91.89	94.58						
Vitamin E (tocopherol)	µg/mL	6.31	6.20	7.96		**	**			
Vitamin D	ng/mL	26.48	31.13	28.76	*					
Minerals (animal products)										
Calcium	mmol/L	2.37	2.37	2.39						
Cobalt	nmol/L	5.46	6.47	5.42	*					
Idine	µmol/L	0.36	0.39	0.37						
Selenium	µmol/L	1.51	1.56	1.52						
Zinc	µmol/L	17.87	16.94	16.45			+	0.0865		
Pigments										
Beta-carotene	µg/100mL	28.29	43.85	68.89	**	**	**			
Lutein + Zeaxanthin	µg/L	121.47	174.17	193.97	**	**	+	0.0592		
B-cryptoxanthin	µg/L	16.20	24.07	52.55	*	**	**			
Lycopene	µg/L	336.37	400.18	659.87		**	**			

Table 3.8 (continue). Differences between the average values of the different parameters (blood parameters, anthropometric, clinical) and food consumption (g/d) of the different food categories divided according to the lower (1), middle (2) and higher (3) level of the pigments index of the all population (males and females together)

Parameters	Units	(1)	(2)	(3)	(1) vs (2)	(1) vs (3)	(2) vs (3)	p<	Symbol	p<
Inflammation biomarkers										
C-Reactive Protein	μg/mL	2.21	1.87	1.94					+	<0.1
Haptoglobine	g/L	0.99	0.91	0.96					*	<0.05
Ceruloplasmin	μmol/L	3.66	3.71	3.85					**	<0.01
Paraoxonase	U/mL	235.24	235.57	230.80						
Albumine	g/L	45.69	45.14	45.00						
ROMs	mg H2O2 /dL	37.41	37.14	38.34						
Anthropometric and clinic parameters										
BMI	Kg/m2	29.13	26.76	27.65	*					
Weight	Kg	78.43	70.00	69.99	**	**				
Height	Cm	164.49	161.55	159.10		**				
Waist	Cm	99.41	92.31	95.77	**					
Hip	Cm	103.90	100.41	100.98	+			0.052		
WH ratio	NA	0.96	0.92	0.95	*		+	0.051		
Blood pressure										
Systolic pression	Hg mm	132.00	125.33	126.96	*					
Diastolic pression	Hg mm	79.59	77.89	78.00						
Heart frequency	NA	63.54	64.10	62.30						
Food categories										
Starchy foods	g/d	264.75	246.37	225.02		*				
Fruits and vegetables	g/d	410.81	450.41	546.36		**	*			
Meat	g/d	117.03	96.75	95.13	*	*				
Fish	g/d	43.26	41.94	37.19						
Cheeses	g/d	48.19	44.53	45.68						
Milk and eggs	g/d	178.90	179.90	155.56						
Legumes	g/d	26.95	29.63	33.45		+		0.057		
Fats	g/d	22.71	23.34	21.52						
Dessert	g/d	85.33	108.63	116.74						

Table 3.9 Comparison of the means of the parameters (blood parameters, anthropometric, clinical) and food consumpion (g/d) of tertiles classified by pigment index in females (n = 76)

Parameters	Units	Average values in low (1), middle (2) and upper (3) tertile			significance levels			
		(1)	(2)	(3)	(1) vs (2)	(1) vs (3)	(2) vs (3)	p-value
Biomarkers of energy and lipid metabolism (starchy foods-animal products-dessert)								
Glucose	mg/dL	93.60	84.38	91.60	*		+	0.0663
Fructosamine	µmol/L	236.96	218.73	192.93		*		
Triglycerides	mg/dL	92.24	97.46	126.32		*	*	
Total cholesterol	mg/dL	199.24	202.42	238.40				
LDL	mg/dL	122.75	125.08	154.10		**	**	
HDL	mg/dL	58.04	57.85	59.04				
Biomarkers protein metabolism (legumes-animal products)								
Urea	mmol/L	6.81	6.89	6.60				
Uric acid	mg/dL	5.35	5.11	5.48				
Albumine	g/L	44.82	45.13	44.59				
Creatinine	mg/dL	0.63	0.65	0.63				
Vitamins (fruits and vegetables-animal products)								
Vitamin A (Retinol)	µg/100ml	116.54	116.09	127.34	**	**		
Vitamin E (tocopherol)	µg/mL	6.30	6.02	7.89		**	**	
Vitamin D	ng/mL	23.93	32.84	27.88	*			
Minerals (animal products)								
Calcium	mmol/L	2.36	2.35	2.41			*	
Cobalt	nmol/L	6.86	7.41	5.18				
Idine	µmol/L	0.39	0.42	0.39				
Selenium	µmol/L	1.44	1.58	1.47	*			
Zinc	µmol/L	16.47	16.63	16.93				
Pigments								
Beta-carotene	µg/100mL	34.39	50.75	78.26	**	**	**	
Lutein + Zeaxanthin	µg/L	129.31	186.95	188.05	**	**		
B-cryptoxanthin	µg/L	13.56	24.56	69.93	**	**	**	
Lycopene	µg/L	328.87	371.22	671.33		**	**	

Table 3.9(continue) Comparison of the means of the parameters (blood parameters, anthropometric, clinical) and food consumpion (g/d) of tertiles classified by pigment index in females (n = 76)

Parameters	Units	Average values in low (1), middle (2) and upper (3) tertile			significance levels			
		(1)	(2)	(3)	(1) vs (2)	(1) vs (3)	(2) vs (3)	p-value
Inflammation biomarkers								
C-Reactive Protein	µg/mL	3.13	1.43	2,1				
Haptoglobine	g/L	0.88	0.90	0,96				
Ceruloplasmin	µmol/L	4.41	3.98	4,12	+			0.056
Paraoxonase	U/mL	225.71	237.33	235				
Albumine	g/L	44.82	45.13	44,59				
ROMs	mg H2O2 /dL	42.34	38.70	40,85	*			
Anthropometric and clinic parameters								
BMI	Kg/m2	30.28	25.84	27.75	*			
Weight	Kg	72.88	62.23	65.04	**			
Height	Cm	155.48	155.81	153.32				
Waist	Cm	107.80	99.42	101.56	**			
Hip	Cm	101.12	89.04	94.60	**			
WH ratio	NA	0.94	0.89	0.93	*			
Blood pressure								
Systolic pression	Hg mm	134.74	122.88	127.98	*			
Diastolic pression	Hg mm	79.66	75.19	78.96				
Heart frequency	NA	69.20	64.19	63.60		+		0.0721
Food categories								
Starchy foods	g/d	235.10	225.04	190.16		*		
Fruits and vegetables	g/d	385.54	412.13	480.63		*	+	0.08
Meat	g/d	106.37	98.06	90.20				
Fich	g/d	43.28	38.23	37.50				
Cheeses	g/d	52.05	36.67	50.34				
Millk and eggs	g/d	189.43	177.84	182.68				
Legumes	g/d	37.10	29.57	30.32				
Fats	g/d	24.24	21.97	21.58				
Dessert	g/d	69.94	139.61	107.48	*			

Table 3.10 Comparison of the means of the parameters (blood parameters, anthropometric, clinical) and food consumpion (g/d) of tertiles classified by pigment index in males (n = 70)

Parameters	Units	Average values of in low (1), middle (2) and upper (3) tertile			significance levels			
		(1)	(2)	(3)	(1) vs (2)	(1) vs (3)	(2) vs (3)	p-value
Biomarkers of energy and lipid metabolism (starchy foods-animal products-dessert)								
Glucose	mg/dL	94.78	95.92	96.74				
Fructosamine	µmol/L	201.20	203.15	235.42				
Triglycerides	mg/dL	141.74	162.96	133.09				
Total cholesterol	mg/dL	188.52	198.00	213.26		**		
LDL	mg/dL	116.18	124.80	140.46		**	+	0.0514
HDL	mg/dL	45.96	46.13	47.70				
Biomarkers protein metabolism (legumes-animal products)								
Urea	mmol/L	6.85	6.57	6.91				
Uric acid	mg/dL	6.41	6.75	6.63				
Albumine	g/L	46.20	45.43	45.64				
Creatinine	mg/dL	0.86	0.82	0.79		+		0.0527
Vitamins (fruits and vegetables-animal products)								
Vitamin A (Retinol)	µg/100ml	143.75	141.53	154.75				
Vitamin E (tocopherol)	µg/mL	5.83	6.95	7.96		**		
Vitamin D	ng/mL	30.17	28.02	29.91				
Minerals (animal products)								
Calcium	mmol/L	2.37	2.37	2.39				
Cobalt	nmol/L	4.65	5.20	5.18				
Idine	µmol/L	0.34	0.33	0.36				
Selenium	µmol/L	1.59	1.54	1.57				
Zinc	µmol/L	17.65	18.68	16.27			+	0.0925
Pigments								
Beta-carotene	µg/100mL	25.32	34.60	56.21	*	**	**	
Lutein + Zeaxanthin	µg/L	116.06	152.83	202.80	*	**	**	
B-cryptoxanthin	µg/L	16.95	25.85	33.02	+	**		0.0684
Lycopene	µg/L	323.93	447.63	648.46	*	**	**	

Table 3.10 (continue) Comparison of the means of the parameters (blood parameters, anthropometric, clinical) and food consumpion (g/d) of tertiles classified by pigment index in males (n = 70)

Parameters	Units	Average values of in low (1), middle (2) and upper (3) tertile			significance levels			
		(1)	(2)	(3)	(1) vs (2)	(1) vs (3)	(2) vs (3)	p-value
Pigments (continue)								
Ceruloplasmin	mcmol/L	3.34	3.12	3.37				
Paraoxonase	U/mL	240.67	240.08	224.45		+	+	0.053 & 0.059
Albumine	g/L	46.20	45.43	45.64				
ROMs	mg H2O2 /dL	35.38	33.56	34.27				
Anthropometric and clinic parameters								
BMI	Kg/m2	27.62	28.59	27.05				
Weight	Kg	80.39	81.67	76.41				
Height	Cm	170.61	168.79	168.13				
Waist	Cm	100.52	101.92	99.17				
Hip	Cm	96.26	98.88	95.48				
WH ratio	NA	0.96	0.97	0.96				
Blood pressure								
Systolic pression	Hg mm	128.13	130.29	124.61				
Diastolic pression	Hg mm	80.33	79.79	77.28				
Heart frequency	NA	61.41	60.08	60.93				
Food categories								
Starchy foods	g/d	267.37	276.07	285.64				
Fruits and vegetables	g/d	415.17	533.24	612.66	+	**		0.08
Meat	g/d	111.03	112.75	100.25				
Fish	g/d	44.11	44.83	37.47				
Cheeses	g/d	39.77	53.42	45.68				
Milk and eggs	g/d	191.90	151.31	132.24				
Legumes	g/d	19.35	26.75	36.36		*		
Fats	g/d	22.90	22.77	21.69				
Dessert	g/d	86.23	107.28	108.94				

The distribution of males and females has also been made necessary to prevent the effect of the different presence of males and females in the sexes obtained based on the pigment index due to the sex influence on the plasma concentration of pigments (in fact, in table 3.8, the first tertile contains 19 females and 30 males, while in the third there are 29 females and 19 males). This could have affected some of the parameters of anthropometric species, which differ not least among males and females. In the three tertiles it is noted that the difference between the first (less fruit and vegetables) and the other two are visible and significant only in females, as shown in table 3.9 (greater weight, BMI, waist, and hips highest) tendency in them to accumulate adipose tissue more. This is typical of the female sex, but could have contributed to the fact that low consumption of fruit and vegetables results in significantly higher starch consumption (235 g/d vs 190 g/d, $p < 0.05$). Have a merit to be mentioned, although the behaviour is similar between the sexes (table 3.9 and 3.10), the differences in the two forms of cholesterol associated with the transport of blood lipids (both LDL and total) are both higher (often significantly) in 3rd tertile (more pigments). As discussed above, this is justified by the lipid transport mechanism to which pigments also belong; it should be considered because:

- higher cholesterol values are not considered justified by higher consumption of fruit and vegetables (though estimated by pigments) but may in part contribute to the higher pigment values;
- therefore, cholesterol values, especially if very high or very low, are used to "correct" the estimation of fruit and vegetable consumption obtained based on plasma pigment values.

In any case, from those tables emerges, in addition to what has already been illustrated:

- a clear separation for fruit and vegetable consumption. It should be noted, however, that in females - albeit with higher pigments - the declared absolute values are tendentially lower (400-500 g/d than males 400-600 g/d), perhaps for the most accurate aptitude in compiling the questionnaires or perhaps because they absorb them most. Meat and starchy foods are also differentiated (especially in females); but in the reverse direction of fruit and vegetables; for example, the meat passes from 106 to 98 and 90 g/d respectively in the three-thirds of the females, even without significant significance, while for starches it passes from 235 to 190 g/d with $p < 0.05$ between the first and the third tertile;

- even for anthropometric data there is an interesting separation (but only in females), since subjects of the first tertiary (low blood pigments and presumably low fruit and vegetable consumption) are significantly heavier (73, 62 and 65 kg, respectively) and, however, with higher BMI and hip circumference values (108, 99, 101 cm), as previously commented;
- always female subjects with low pigments values exhibit higher blood pressure (only significant for systolic and in the tertiles 1 and 2);
- at the blood level, among the tertiles, some indicators of lipid metabolism are distinguished: total cholesterol and LDL cholesterol, which - in both sexes - have the same pattern of pigments and the differences between tertiles are often significant. This result has already been widely commented, but remember that it is purely "mechanical". Indeed, significant ($p < 0.01$) is the difference for tocopherol in the two sexes and probably linked to the higher ingestion of vitamin E, with the increase in fruit and vegetables of the 3rd tertile.

Given the importance of sex in modifying pigments, it seemed useful to recall the behavior of correlations between the pigment index (separately for the two sexes) and the other set of parameters. This behaviour strongly confirms the above: the correlation is positive with vegetables and fruits ($r = 0.36$ in females = 0.43 in males; $p < 0.01$) but negative (only in females) with starches and meat (respectively $r = -0.3$, $p < 0.01$ and $r = -0.18$, but not significant). Equally negative is the correlation with some anthropometric parameters in females (more fruit-vegetables consumed and less fat-accumulation) with significant values only for weight and hips ($r = -0.28$ and $r = -0.26$, $p < 0.05$). In both sexes, the positive correlation exists with total cholesterol ($r = 0.34$-0.40; $p < 0.01$) and LDL, $r = 0.36$-0.47; $p < 0.01$). It is interesting the positive correlation between the index and individual pigments is very high - especially in females - for beta-carotene, beta-cryptoxanthin and lycopene ($r = 0.7$-0.8, $p < 0.01$), but a little lower for lutein - zeaxanthin ($r = 0.34$; $p < 0.01$). Conversely, in males, the correlations are equally good, but with fewer r values. Interestingly, the absence of correlation between the index of pigments and the anthropometric parameters of males is in some respects compensated by the negative and significant correlation of beta-carotene with the main anthropometric indices, meaning that the higher consumption of fruit and vegetables, from which beta-carotene, is still associated with lesser fattening (even in males).

Finally, the purpose of our research (to distinguish our population according to diet) would thus be possible with the separation of the 146 subjects in thirds compared to the values of the pigment index which in turn would find people with different consumption of fruit-vegetables. Although this may be considered a limitation, since it is a single category, in fact, fruit and vegetable consumption is probably the least significant factor in discrimination at least in the first instance - the most important discrimination factor between a healthy diet (e.g. Mediterranean) and a risky diet for health Woodside et al. (2017) reached to similar considerations, although they express doubts about the accuracy of the method for estimating the consumption of fruit and vegetables which, however, should be interpreted in the light of the differences between sexes and those linked to the transport of fats both during absorption. But especially in the bloodstream as lipoproteins (which is the index of cholesterol, LDL species). On this basis, and considering only the two extreme tertiles, we could consider the subjects as belonging to two populations characterized by quite different diets, a) with little and b) with lots of fruit-vegetables:

- are subjects of the 1st tertile, with males (30) predominating on females (19) and are characterized by the index of pigments to have a relatively poor diet of fruit-vegetables (411 g/d). However, it is not a defective diet since our pigment values are within the ranges found by Ormedilla et al. (1994) and Olmedilla et al. (2001), but also because of the corresponding EPIC consumption - albeit casual - is close to 400 g/d suggested by (WHO & FAO 2003). It should be noted that in this population, only females have an inverse behavior of starch consumption with a maximum of 235 g/d compared to 190 g/d found in the third tertile ($p < 0.05$). Meat consumption is always higher in females, and is 106 g/d to 90 g/d of the 3rd (diet b) but not significantly;
- are subjects of the 3rd tertile, in which females (29) prevail on the males (19) and this contributes in part to the highest index of pigments in absolute values and therefore more fruits and vegetables. From this point of view,

high pigments may, however, not contribute to higher consumption values, since the females have lower values. Apart from the detail, there was a greater consumption of fruit and vegetables (between 500 and 600 g / d) in this population, with very high values (if real), especially in males (confirming what said above), about the corresponding differences in the consumption of starches and meat, lower than the diet a), they concern females and only for starches and are significant ($p < 0.05$).

Therefore, as was expected, given the index used, the two diets differ mainly for the consumption of fruit and vegetables, for the other categories of food, the differences are modest or not-existent, but it is well known (Hassen et al 2010) that the higher consumption of these foods tends to reduce cardiovascular disease, while the same is not required to reduce the meat (except the preserved ones) (Mozaffarian 2011) This promised, the main results that emerge from the comparison of these "two populations" were the following:

- female subjects with diet a) (low in fruits and vegetables and higher in starches) tend to accumulate more fat, since the weight is significantly higher than the same height, 73 to 65 kg ($p < 0.05$); this is confirmed by the tendency of higher BMI values. There is no such thing in the males, maybe because starchy and meats were eaten change little. This result could mean that even in terms of the effects of diet the role of sex is important;
- constantly with diet a) total cholesterol levels and LDL (low-density lipoproteins) are lower than the diet richer in fruits and vegetables, both in males and in females. However, this should not be interpreted as the effect of higher consumption of fruit and vegetables on cholesterol, but rather as a kind of drift that the high values of lipoproteins have on plasma pigments. It follows that this is not worrying, but rather that lipoprotein levels should be taken to "correct" lipid-soluble pigment levels when they are used to estimate fruit and vegetable consumption (for the purpose, further research will be required).

3.3.6 Conclusive considerations

The results of the above-described tests seem to demonstrate the following:

- the two questionnaires we used (EPIC and 3days) provided very different consumption values and referred to categories of foods that are difficult to be defined. Hence, it is not easy to calculate reliable nutritional intakes and in any case to compare the two questionnaires or to use only them, in estimating diets and their effects on human health, because not enough accurate;
- the data we collect from questionnaires cannot be used separately, but for better interpretation other most objective data are needed i.e : anthropometric-clinical and some blood parameters. In fact, the most interesting data showed by questionaires are those concerning the positive and significant correlation between the consumption of starchy foods and meat. Of major interest seems otherwise the positive and significant correlation between starchy food consumption and weight observed in females who tend to deposit the excesses mainly as fat in adipose tissue (justifying also a good relationship with BMI). This example shows the helpful utilization of anthropometric parameters as well as for blood ones. In fact, the may be associated, althought unspecifically (because there are many interfering factors), to some macronutrients as well as micronutrients. Nevertheless, the most commonly controlled blood parameters do not seem to have been of great utility as they are not generally correlated to the food intake. This, however, would be caused by the low riability of food consumption; it can be therefore presumed that the relationship could be better in case of more precise data of food intake, especially for macro-nutrients: carbohydrates, proteins, and lipids.
- Interesting, however, appeared to be:
    - creatinine, which, without renal problems, almost impossibile in clinically healthy subjects, able to indicate the state of muscle mass and is therefore significantly lower in females as well as in too fatty subjects;

    - some vitamins - in our case the E - but probably also the B12 and the D - can be a good index of body availability and hence the consumption of foods known as good suppliers of them (fruits and vegetables for E, meats and dairy products for B12);
    - among the minerals, especially iodine - as we have seen in our experience - but probably also plasma levels of selenium, magnesium, and phosphorus could be useful indicators of their nutritional status;
    - pigments of hydrocarbon nature present in the plasma have fruits and vegetables as the main diet source, moreover they have no endogenous control systems; so their levels can be valid biomarkers of the consumption of these foods. Nevertheless, these pigments are also influenced by sex and by the level of lipoproteins; they are in fact lipid-soluble and conveyed in blood by low-density lipoproteins (LDL);
    - among the blood indicators of inflammatory processes (both positive and negative acute phase proteins) and the food intake, there may be some interesting relationships but the substantial indeterminacy of certain data (intake) makes unrealistic every attempt of deepness;
- Many of the tools often used to estimate food consumption or the nature of diets are insufficient for many distinct reasons. However, our results seem to show that pigments offer some additional possibilities. In fact, subjects with higher values of an index that "links" the individual content of plasma pigments, are characterized by higher consumption of fruit and vegetables, particulary with reference to the values measured with EPIC. At the same time, even if only in females, the higher pigment index is accompanied by lower consumption of starchy foods and meat (that the lower weight and BMI values tend to confirm).

We can, therefore, conclude that whatever the purpose of dietary controls, but particularly if they want to ascertain the long-term effects on health, food consumption measurement cannot be exclusive, but blood type indicators (e.g. pigments, certain vitamins, creatinine, etc.) and anthropometric ones may also be useful. Specifically, on the blood indicators, our research has allowed to find a new index based on the 5 major plasma pigments: β-carotene, β-cryptoxanthin, lutein + zeaxanthin and lycopene - whose values seem to be well correlated with the ingestion of fruits and vegetables (at least those typical of central Italy). However, more research seems to be appropriate for:

- clarify the relationships and possible interferences of this index with sex and with lipoprotein levels;
- to ascertain whether, in other climate-environmental conditions and food traditions, the index requires some adjustment;
- better define the relationship between pigment index and diet composition in order to use it in epidemiological studies on diet/health relationships;
- expanding the availability of indices (e.g. vitamin B12, vitamin D, etc.) that can improve the estimate of the really ingested diets.

3.3.7 Reference

Agnoli, Claudia, Vittorio Krogh, Sara Grioni, Sabina Sieri, Domenico Palli, Giovanna Masala, Carlotta Sacerdote, et al. 2011. "A Priori–Defined Dietary Patterns Are Associated with Reduced Risk of Stroke in a Large Italian Cohort." *The Journal of Nutrition* 141 (8): 1552–58.

Alexander, Dominik D, Libby M Morimoto, Pamela J Mink, and Colleen a Cushing. 2010. "A Review and Meta-Analysis of Red and Processed Meat Consumption and Breast Cancer." *Nutrition Research Reviews* 23 (2): 349–65.

Alexander, Dominik D., Douglas L. Weed, Paula E. Miller, and Muhima a. Mohamed. 2015. "Red Meat and Colorectal Cancer: A Quantitative Update on the State of the Epidemiologic Science." *Journal of the American College of Nutrition* 34 (May 2015): 1–23.

Antimo AIELLO, Gabriella RUZZI, Antonio CAROVILLANO, Maria Rosaria CRISTOFARO, Tiziana De VITO, Raffaele PARISI, Gaetano LOMBARDI. 1998. "Progetto Di Prevenzione Della Patologia Tiroidea E Di Educazione Alla Iodoprofilassi Nella Provincia Di Campobasso." *An.Ist.Super.Sanità* 34: 423–24.

Antonia Trichopoulou, M.D., Tina Costacou, Ph.D., Christina Bamia, Ph.D., and Dimitrios Trichopoulos, M.D. 2011. "New England Journal of Medicine," 1991–2002..

Appleby, Paul N., Francesca L. Crowe, Kathryn E. Bradbury, Ruth C. Travis, and Timothy J. Key. 2016. "Mortality in Vegetarians and Comparable Nonvegetarians in the United Kingdom." *American Journal of Clinical Nutrition* 103 (1): 218–30.

ARUP LABORATORIES. 2017. Retinol Reference Interval.pdf

Aslibekyan, S., H. Campos, and A. Baylin. 2012. "Biomarkers of Dairy Intake and the Risk of Heart Disease." *Nutrition, Metabolism and Cardiovascular Diseases* 22 (12). Elsevier Ltd: 1039–45.

Beckonert, Olaf, Hector C Keun, Timothy M D Ebbels, Jacob G Bundy, Elaine Holmes, John C Lindon, and Jeremy K Nicholson. 2007. "Metabolic Profiling, Metabolomic and Metabonomic Procedures for NMR Spectroscopy of Urine, Plasma, Serum and Tissue Extracts." *Nature Protocols* 2: 2692–2703.

Bingham, S. , C Gill, Welch, Cassidy, S Runswick, S Oakes, R Lubin 1997. "Validation of Dietary Assessment Methods in the UK Arm of EPIC Using Weighed Records, and 24-Hour Urinary Nitrogen and Potassium and Serum Vitamin C and Carotenoids as Biomarkers." *International Journal of Epidemiology* 26 Suppl 1 (1): S137–51.

Bingham, Sheila A. 2002. "Biomarkers in Nutritional Epidemiology." *Public Health Nutrition* 5 (6A). pp821–27.

Blanck, Heidi Michels, Barbara A Bowman, Gerald R Cooper, L Myers, and Dayton T Miller. 2003. "Biomarkers of Nutritional Exposure and Nutritional Status Laboratory Issues : Use of Nutritional Biomarkers 1." *Environmental Health*, no. 7: 888–94.

Bruce Armstrong and Richard Doll. 1975. "Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices." *Int. J. Cancernt. J. Cancer* 631: 617–31.

Calamari, Luigi, Annarita Ferrari, Andrea Minuti, and Erminio Trevisi. 2016. "Assessment of the Main Plasma Parameters Included in a Metabolic Profile of Dairy Cow Based on Fourier Transform Mid-Infrared Spectroscopy: Preliminary Results." *BMC Veterinary Research* 12 (1). BMC Veterinary Research4

Cem Gabay and Irving Kushner 1999. Acute -phase proteins and other systemic responses to inflammation. The New England Journal of Medicin

Combs, G.F. 2001. Selenium in global food systems. *British Journal of Nutrition*, 85(5), pp.517–547.

di Giuseppe, R, Arcari, M Serafini, Di Castelnuovo, F Zito, De Curtis, S Sieri 2012. "Total Dietary Antioxidant Capacity and Lung Function in an Italian Population: A Favorable Role in Premenopausal/never Smoker Women." *European Journal of Clinical Nutrition* 66 (1): 61–68

Duh and Cook 2005. Laboratory References ranges values. , 1(1975), pp.2–7

den Elzen, Wendy P.J. Joop van Gerven, Paul W. Schenk, Anna Uit Den Boogaard, Ria Kroon, Erica Klaassen, Bart E.P.B. Ballieux and Christa M. Cobbaert. 2017. How to define reference intervals to rule in healthy individuals for clinical trials? *Clinical chemistry and laboratory medicine*, 55(3), pp.9–11.

Farinde, A.A. 2014. Laboratory Reference Ranges in Health Adults. , (Mcv), pp.1–2.

Favé, G., Beckmann, M.E., Draper, A.J., Mathers, J.C., 2009. Measurement of dietary exposure: A challenging problem which may be overcome thanks to metabolomics? *Genes and Nutrition*, 4(2), pp.135–14

Fidanza, Flaminio. 1991. "The Mediterranean Italian Diet: Keys to Contemporary Thinking." *Proceedings of the Nutrition Society* 50 (1991): 519–26.

Flegal, Katherine M, Margaret D Carroll, Brian K Kit, and Cynthia L Ogden. 2012. " Prevalence and Trends in Obesity Among US Adults, 1999-2000." *American Medical Association* 307 (5): 491–97.

Gibney MJ, Walsh M, Brennan L. 2005 Metabolomics in human nutrition: opportunities and challenges. Am J Clin Nutr 82, 497–503.

Key, T. J., P. N. Appleby, E.Spencer, R.C.Travis, W Roddam, and N E Allen. 2009. "Cancer Incidence in Vegetarians: Results from the European Prospective Investigation into Cancer and Nutrition (EPIC-Oxford)." *Am.J Clin Nutr* 89 (5): 1620S–1626S.

Keys, A. 1953. "Atherosclerosis: A Problem in Newer Public Health." *Journal of the Mount Sinai Hospital, NY* 20: 118–39.

Kotus, J., 2014. Fructosamine: Reference range, Interpretation, Collection and Panels.pp1-2

Kussmann, Martin, Serge Rezzi, and Hannelore Daniel. 2008. "Profiling Techniques in Nutrition and Health Research." *Current Opinion in Biotechnology* 19 (2): 83–99.

Leclercq, Catherine, Davide Arcella, Raffaela Piccinelli, Stefania Sette, Cinzia Le Donne, and Aida Turrini. 2009. "The Italian National Food Consumption Survey INRAN-SCAI 2005-06: Main Results in Terms of Food Consumption." *Public Health Nutrition* 12 (12): 2504–32.

Lodge, John K. 2009. "Symposium 2: Modern Approaches to Nutritional Research Challenges: Targeted and Non-Targeted Approaches for Metabolite Profiling in Nutritional Research." *The Proceedings of the Nutrition Society* 69 (1):pp95–102.

Lovegrove, Julie A., and Ditte A. Hobbs. 2016. "Plenary Lecture 2: Milk and Dairy Produce and CVD: New Perspectives on Dairy and Cardiovascular Health." *Proceedings of the Nutrition Society* 44 (July 2015): 1–12.

Macdiarmid, J, and J Blundell. 1998. "Assessing Dietary Intake: Who, What and Why of under-Reporting." *Nutrition Research Reviews* 11 (2): 231–53.

Mancinelli, R., Barlocci, E., Ciprotti, M., Senofonte, O., Fidente, R.M., Draisci, R., Attilia, M.L., Vitali, Fiore, M., Mauro Ceccanti, M. 2013. Blood thiamine, zinc, selenium, lead and oxidative stress in a population of male and female alcoholics: clinical evidence and gender differences. Ann Ist Super Sanità 2013 | Vol. 49, No. 1: 65-72

Mao, X., Wong, A. Crawford, R.W.2011. Cobalt toxicity — an emerging clinical problem in patients with metal-on-metal hip prostheses? MJA • Volume 194 Number 12.

Maturu V. Nagarjuna, Nidhi Gupta, Gagandip Singh, Kirandip Gill, Yash Paul Sharma, Surjit Singh. 2012. Serum Paraoxonase (PON1) Activity in North-West Indian Punjabi's with Acute Myocardial Infarction. Ind J Clin Biochem (July-Sept 2013) 28(3):248–254

Mendonça, Nuno, John C. Mathers, Ashley J. Adamson, Carmen Martin Ruiz, Chris J. Seal, Carol Jagger, and Tom R. Hill. 2016. "Intakes of Folate and Vitamin b12 and Biomarkers of Status in the Very Old: The Newcastle 85+ Study." *Nutrients* 8 (10): 1–15.

Mozaffarian, Dariush. 2011. "The Great Fat Debate: Taking the Focus Off of Saturated Fat." *Journal of the American Dietetic Association* 111 (5). Elsevier Inc.: 665–66.

Myint, Tun, Gary E. Fraser, Kristian D. Lindsted, Synnøve F. Knutsen, Richard W. Hubbard, and Hannelore W. Bennett. 2000. "Urinary 1-Methylhistidine Is a Marker of Meat Consumption in

Black and in White California Seventh-Day Adventists." *American Journal of Epidemiology* 152 (8): 752–55.

Nettleton, Jennifer, Cheryl L Rock, Youfa Wang, Nancy S Jenny, and David R Jacobs. 2009. "Associations between Dietary Macronutrient Intake and Plasma Lipids Demonstrate Criterion Performance of the Multi-Ethnic Study of Atherosclerosis (MESA) Food-Frequency Questionnaire." *The British Journal of Nutrition* 102 (8).

Olmedilla, B.A, & Lorencio, F.G., 1994. "Seasonal and Sex-Related Variations in Six Serum Carotenoids , Retinol , and -Tocopherol Variations," American Journal of Clinical Nutrition · July 1994.

Olmedilla, Ä, Fernando Granado, Susan Southon, Anthony J A Wright, Inmaculada Blanco, Enrique Gil-martinez, Henk Van Den Berg, et al. 2001. "Serum Concentrations of Carotenoids and Vitamins A , E , and C in Control Subjects from Five European Countries," no. 2001.

Ommen, Ben van, Susan Fairweather-Tait, Andreas Freidig, Alwine Kardinaal, Augustin Scalbert, and Suzan Wopereis. 2008. "A Network Biology Model of Micronutrient Related Health." *The British Journal of Nutrition* 99 Suppl 3 (2008): S72–80.

P Allain, S Berre, N Krari, P Laine-Cessac, A Le Bouil, N Barbot, V Rohmer, J C Bigorgne 1993. Use of plasma iodine assay for diagnosing thyroid disorders. J.Clin Pathol 1993;46:453-455.

Pala, Valeria, Sabina Sieri, Domenico Palli, Simonetta Salvini, Franco Berrino, Manuela Bellegotti, Graziella Frasca, et al. 2003. "Diet in the Italian EPIC Cohorts: Presentation of Data and Methodological Issues." *Tumori* 89 (6): 594–607.

Pellegrini N, Serafini M, Salvatore S, Del Rio D, Bianchi M, Brighenti F. 2006. "Total Antioxidant Capacity of Spices, Dried Fruits, Nuts, Pulses, Cereals and Sweets Consumed in Italy Assessed by Three Different in Vitro Assays." *Mol Nutr Food Res*, 2006.

Prentice, Ross L, Elizabeth Sugar, C Y Wang, Marian Neuhouser, and Ruth Patterson. 2002. "Research Strategies and the Use of Nutrient Biomarkers in Studies of Diet and Chronic Disease." *Public Health Nutrition* 5 (6A). Universita Cattolica Sacro Cuore: 977–84.

Pufulete, M. 2008. "Intake of Dairy Products and Risk of Colorectal Neoplasia." *Nutrition Research Reviews* 21 (September): 56–67.

Ravnskov, Uffe, David M Diamond, Rokura Hama, Tomohito Hamazaki, Björn Hammarskjöld, Niamh Hynes, Malcolm Kendrick, et al. 2016. "Lack of an Association or an Inverse Association between Low-Density-Lipoprotein Cholesterol and Mortality in the Elderly: A Systematic Review." *BMJ Open* 6

Safavi, S. M., Ziaei, R. RezaMaracy, M. 2012 Association of SerumCeruloplasmin LevelwithObesity: Some Components ofMetabolic Syndrome andHigh-Sensitive C-Reactive Protein in Iran. Journal of Obesity Volume 2012, Article ID 951093.

Salvini S, Parpinel M, Gnagnarella P, Maisonneuve P, Turrini A. 1998 Banca Dati di Composizione degli Alimenti per Studi Epidemiologici in Italia. Ed. Istituto Europeo di Oncologia, Milan, Italy

Sanders, Thomas A. B. 2015. "How Important Is the Relative Balance of Fat and Carbohydrate as

Sources of Energy in Relation to Health?" *Proceedings of the Nutrition Society* 44 (June): 1–7.

Scagliusi, Fernanda B, Eduardo Ferriolli, Karina Pfrimer, Cibele Laureano, Caroline S F Cunha, Bruno Gualano, Bárbara Lourenço, and Antonio H Lancha Jr. 2008. "Under-Reporting of Energy Intake Is More Prevalent in a Healthy Dietary Pattern Cluster." *British Journal of Nutrition* 100 (5): 1060–68.

Scalzo, Roberto L O, Marta Fibiani, Giuseppe Mennella, Giuseppe L. Rotino, Monica Dal Sasso, Maria Culici, Alessandra Spallino, and Pier Carlo Braga. 2010. "Thermal Treatment of Eggplant (Solanum Melongena L.) Increases the Antioxidant Content and the Inhibitory Effect on Human Neutrophil Burst." *Journal of Agricultural and Food Chemistry* 58 (6): 3371–79

Sen, Tanusree, Carolina R. Cawthon, Benjamin Thomas Ihde, Andras Hajnal, Patricia M. DiLorenzo, Claire B. de La Serre, and Krzysztof Czaja. 2017. "Diet-Driven Microbiota Dysbiosis Is Associated with Vagal Remodeling and Obesity." *Physiology and Behavior* 173. Elsevier Inc.: 305–17.

Sette, S., C. Le Donne, R. Piccinelli, D. Arcella, A. Turrini, and C. Leclercq. 2011. "The Third Italian National Food Consumption Survey, INRAN-SCAI 2005-06 - Part 1: Nutrient Intakes in Italy." *Nutrition, Metabolism and Cardiovascular Diseases* 21 (12): 922–32.

Smedman, A E, I B Gustafsson, L G Berglund, and B O Vessby. 1999. "Pentadecanoic Acid in Serum as a Marker for Intake of Milk Fat: Relations between Intake of Milk Fat and Metabolic Risk Factors." *The American Journal of Clinical Nutritionournal of Clinical Nutrition* 69 (1): 22–29.

Thorogood, Margaret, Iveta Simera, Elizabeth Dowler, Carolyn Summerbell, and Eric Brunner. 2007. "A Systematic Review of Population and Community Dietary Interventions to Prevent Cancer." *Nutrition Research Reviews* 20 (1): 74–88.

Trevisi, E., G. Bertoni, R. Lombardelli, and A. Minuti. 2013. "Relation of Inflammation and Liver Function with the Plasma Cortisol Response to Adrenocorticotropin in Early Lactating Dairy Cows." *Journal of Dairy Science* 96 (9). Elsevier: 5712–22.

USDA. 2010. "Report of the Dietary Guidelines Advisory Committee on the Dietary Guidelines for Americans."

Van Kappel AL, Amoyel J, Slimani N, Vozar B, Riboli E. 1994. EPIC-SOFT picture book for estimating portion sizes. IARC Press, Lyon, France (1994)

Wilczynski, C., 2014. Haptoglobin. Reference Range., pp3-5. Medscape.

WHO & FAO, 2003. Diet, nutrition and prevention of chronic diseases: report of a joint WHO/FAO expert consultation - Ginevra 28 January - 1 February 2002

Wolk, A., Vessby, B., Ljung, H., Barrefors, P. Peter 1998. Evaluation of a biological marker of dairy fat intake. Am J Clin Nutr 1998;68:291–5

Woodside, J.,V., Draper, J., Lloyd, A., McKinley, M.C 2017. Use of biomarkers to assess fruit and vegetable intake. Nutrition Society Summer Meeting 2016 held at University College, Dublin on 11–14 July 2016

4 ASSESSMENT OF NUTRITIONAL STATUS THROUGH ANTHROPOMETRIC INDICATORS AND DIET CHARACTERISTICS IN RURAL INDIA AND D.R. CONGO

4.1 Introduction

Nutritional assessment utilise the interpretation of anthropometric, clinical, biochemical (laboratory) and dietary data to establish whether a person or groups of people are well nourished or malnourished as well as over-nourished or under-nourished (Hartog et al. 2006). Nutritional assessment is useful in field program interventions to meet nutrition needs. Nutrition security differs from food security in that it also considers the aspects of adequate caring practices, health and hygiene in addition to dietary adequacy (FAO, IFAD, and WFP 2015). A typical nutritional screening includes anthropometric measurements, clinical data (e.g. ongoing and past diseases) and dietary story (including feeding education), and possibly laboratory data (from biochemical analysis). Moreover, a full nutritional assessment may include more details such as measure of dietary intake, estimation of nutritional requirements, body composition examination, sexual and skeletal maturation, etc.(Maqbool, Olsen, and Stallings 2008).These methods are completed with socioeconomic and ecological information (Vlismas, Stavrinos, and Panagiotakos 2009). The aforesaid methods can be used singularly or in combination depending on the scope of a specific study and/or due to the availability of tools or feasibility for a given situation. As regard to the availability and feasibility, choice of some tools instead of others can be justified by the inaccessibility of some laboratory methods in many developing countries compared to the anthropometric ones (FAO 2002), especially in refugees' camp situation.

Anthropometry science has followed a variety of paths and this fact is both a richness and its bane. Description of different rules and tools used in specific anthropometric studies can be found elsewhere (WHO 1986; ISAK 2001; Nucara et al. 2012, etc.) In a given community anthropometry can be used to assess the entity of change in the "body composition" of the people. Anthropometric indicators of nutritional status are useful to determine the impact and the performances of development projects especially regarding maternal health and infant survival.

Already in 1986, World Health Organization (WHO), has considered the anthropometry to be the most useful tool for assessing the nutritional status in developing countries. In the analysis of data, the basic measurements such as age, weight, height, etc. are used to establish indices. The indices are used to classify an individual situation as well as to establish an entire population distribution. In both cases, standard deviation (SD) scores or z-scores are preferred. Cut-offs of -2SD to +2SD are used as range of normal nutritional status referred to the standard population in different indices (WHO 1986). The advantages of anthropometry as tool for nutritional status assessment in developing countries have been also reconfirmed by FAO (2002) supporting that:

- the methods are precise and accurate, provided that standardized techniques are used;
- procedures use simple, safe and non-invasive techniques;
- equipment required is inexpensive, portable and durable, and can be made or purchased locally;
- relatively unskilled personnel can perform measurement procedures;
- information is generated on past nutritional history;
- methods can be used to quantify the degree of undernutrition (or overnutrition) and provide a continuum of assessment from under to overnutrition;
- methods are suitable for large sample sizes such as representative population samples;
- methods can be used to monitor and evaluate changes in nutritional status over time, seasons, generations, etc.;
- methods can be adopted to develop screening tests in situations such as nutrition emergencies to identify those at elevated risk.

The anthropometric indicators are used to monitor the reduction of the percentages of children with growth retardation in in the age range of 24 to 60 months with the determination of percentage of children having a less than -2 of the standard deviation (SD) related high-for-age z-scores and weight insufficiency related to the weight-for-age z-scores (Cogill 2003). Furthermore, other studies have suggested to consider mother's nutritional status and anthropometrics indicators to understand the trends of children growth even from the foetal age. In fact, already in 1997, some authors explained that maternal nutrition is associated with foetal growth restraint; poor maternal nutrition as indicated by low weight, height, and body mass Index (BMI) are associated with smaller and shorter babies with smaller heads; i.e. short and thinner women had babies who had low birth weight (Thame et al. 1997). In the anthropometric methods of nutritional status assessment, z-scores have a number of advantages: first, they are calculated based on the distribution of a reference population (mean and standard deviation), and thus reflect the "normal" distribution; second, as standardized quantities, they are comparable across ages, sexes, and anthropometric measures; third, the z-scores can be analysed as a continuous variable in studies (Nucara et al. 2012). While undernutrition in low and middle income countries, expressed with wasting (weight for height z-score <-2) and stunting (height for age z-score <-2) during childhood continue to burden the poorest regions in the developing world (Richard SA, Black RE, and Checkley W. 2012), anthropometry is still the most used method for nutritional status due to its simplicity, and availability even in remote rural areas as are the areas of interest of the present studies since we operated in rural DR Congo (Kandala et al. 2011) and the Meghalaya State in northeast India (Duwarah, Bisai, and Barman 2015)

Despite the abovementioned advantages, the use of anthropometry may be difficult especially in adolescents because anthropometric indices change rapidly with age and sexual development. Moreover, survey and reference populations may differ in the age at which certain pubertal landmarks are attained, requiring adjustment for differences between survey and reference populations (reference standards suggested by WHO). Adolescent populations may also differ by ethnicity in various body proportions that affect anthropometric indices. Woodruff and Duffield (2002) supported that weight-for-height could be used for prepubertal adolescents and BMI-for-age could be used for post-pubertal adolescents. Moreover, since cut-off points are age-specific, age should be collected as accurately as possible for all adolescents measured during screening or survey activities. WHO has recognized that reference population of US adolescents is inappropriate in most populations of adolescents and this implies also the fact that, while using anthropometry in adolescent nutritional assessment, the surveys should never be carried out in isolation, but other population subgroups should be included to get valid data for practical decisions. To obviate these well-known limitations, anthropometry is combinate with the other aforesaid methods. The use of different methods in nutritional status assessment is useful to mitigate the different anthropometry limitations such as those illustrated by FAO in 2002 regarding for example:

- the influence of other environmental factors such as infections (surveys on actual diseases), despite several diseases can be consequence of malnutrition;
- the relative insensitivity to detect changes in nutritional status following inadequacy of food over short periods of time. This weakness is obviated through a careful food surveys using for instance food frequency questionnaires (FFQ);
- the inability to distinguish the effect of specific nutrient deficiencies (e.g. zinc, iron, Iodine and vitamin A deficiencies) that affect growth in children from that due to inadequacy of food in general. To overcome this limit, laboratory analysis to determine nutrient content of food and, if possible, blood markers of food and some nutrient intake are useful. These last techniques are useful to evaluate whether recommended daily (RDA) of nutrients and energy are less or more met (Hartog et al. 2006);
- the inability to pinpoint the principal causality of undernutrition, as the poor nutritional status may be the result of factors such as repeated insults owing to infections and poor care in children; etc., reason why nutritional status assessment requires the recourse to survey on present and/or past diseases among the population under study. Especially malaria and diarrheal diseases are among the most harmful threats to desired nutritional status in developing countries (Lanata and Black 2006).

About the use of laboratory methods of nutritional status assessment, although are less available in developing countries compared to the others above illustrated, they are more objective and precise approach than the anthropometric, dietary methodology, or clinical assessment methods (Christakis 1973). Furthermore, laboratory tests potentially offers a reproducible quantitative means of measuring specific nutrients that can be of great use to clinicians, nutritionists, and researches (Falcão 2000). These methods utilize biochemical tests, to measure levels of nutrients in biological fluids such as plasma, serum, urine, stool and solid body sample such as hair, nails, etc. (Falcão 2000; Maqbool et al. 2008) to evaluate certain biochemical functions which are dependent on an adequate supply of nutrients. However, not all nutrients can or should be assessed by laboratory methods. Generally, laboratory methods are used to determine deficiencies: serum protein (particularly albumin), blood-forming nutrients (iron, folacin, vitamin B6, and vitamin B12, etc.), water-soluble vitamins (thiamine, riboflavin, niacin, and vitamin C); fat-soluble vitamin (A, D, E, and K); minerals (iron, iodine and other trace elements such as selenium, cobalt, etc.), levels of blood lipids (cholesterol and triglycerides), glucose and various enzymes which are implicated in heart disease, diabetes, and other chronic diseases (Christakis 1973). Another limit of the laboratory approach is the fact that the interpretation of laboratory data is often difficult and does not necessarily always correlate with either clinical or dietary findings (Christakis 1973). Moreover, most laboratory tests of nutritional status are too specific, and the measurements may not correlate with values at other times, in other body pools, or with deficiencies of other nutrients. Furthermore, many drugs, diseases, and environmental conditions not related to nutrition can affect measured levels of nutrients. As other methods, none single test gives a completely satisfactory picture of the global nutritional state. Thus, laboratory assessment of nutritional status requires an intelligent selection of tests to fit the clinical situation and should augment the dietary, clinical, and anthropometric data that should also be gathered and evaluated and should not be excluded from nutritional assessment (Falcão 2000).

Clinical methods of nutritional status assessment consist mainly of collection of historic information and physical examination (White et al.2012). History taking can be done by controlling the weight loss, oedema, anorexia, vomiting, diarrhoea, chronic diseases, decreased or unusual food intake, etc. Ph ysical examination recall the presence or absence of clinical signs such as jaundice, cheilosis, glossitis, loss of subcutaneous fat, muscle wasting, oedema, etc. (Baker et al. 1982). These same authors considered also that clinical methods of nutritional status assessment are the oldest, simplest and probably the most widely used methods of nutritional status assessment (Baker et al. 1982). Recently, other researchers supported that clinical outcomes may be important feature of all nutritional surveys as the goal is to assess the health status of individuals or groups within a population in accordance with the food consumed (Shrivastava et al. 2014). According to the same authors, the presence of two or more clinical signs of a specific nutritional deficiency increases the diagnostic significance. Glorio et al (2000) have illustrated different clinical signs related to nutritional disorders (anorexia and bulimia nervosa) but these are for pathological cases with high psychological morbidity (Fairburn and Harrison 2003); therefore cannot be used for our scope (surveys within groups in a "normal" population). Furthermore, clinical appraisals cannot quantify the exact level of nutrient deficiency because most of clinical signs of nutrient deficiency are nonspecific and require (for a more accurate survey), biochemical analysis to identify the specific nutritional status (Shrivastava et al. 2014).

Dietary methods of nutritional status assessment are used to get food habit and consumption in term of nutritional characteristics and intake (Hartog et al. 2006).The appropriate tool of dietary methods of nutritional assessment will depend on the purpose for which it is needed such as nutrients measurement, foods intake or food habit and consumption (Wrieden et al. 2013) but also available tools to be used in specific context especially while operating in less developed countries. Several of dietary tools are not able to assess correctly nutrients intake. However, they are useful to assess and estimate the intake of each specific food. Some of determinations performed within dietary surveys of nutritional status are: (i) the identification of the foods, (ii) determination of the frequency with which each food is eaten; (iii) quantification of the portion size of each food item (through weighed Food records); and (iv)calculation of the nutrient intake (Wrieden et al. 2013). Particularly, the calculation of the nutrient intake can be done using the following formula: portion size (g) x frequency x the specific nutrient content per g. The nutrient content can be found by chemical analysis or from food composition tables (Hartog et al. 2006). As regard the identification of foods, some choice may be clarified: firstly, few key foods data are practically manageable, secondary nutrients intake targeted have to be taken into account; thus, the only key foods, that are the major contributors of different targeted nutrients needed to shift a given unbalanced diet to an appropriate one will be considered (Haytowitz et al. 1996). Therefore, not every available food samples will be analysed as well as for nutrients calculations from food tables (Haytowitz, Pehrsson, and Holden 2002)

The determination of the frequency with which each food is eaten is usually performed with food frequency questionnaires (FFQ). According to Cade et al. (2002), a FFQ is a questionnaire in which the respondent is presented with a list of foods and is required to say how often each is eaten in broad terms such as x times per day/per week/per month, etc. Foods are usually chosen for the specific purposes of a study and may not assess total diet. Some of the limitations of FFQs have been reported some decades ago and, regards especially the accuracy and appropriateness when more precise estimates of actual food intake are desired (Sempos 1992). Nevertheless, FFQs have several advantages that make them to be the tools of choice (in our concern especially in developing countries): (i) they minimize the very high intra-individual, day-to-day variability in nutrient intake without relying on multiple-day assessment of actual foods consumed, (ii) they are cost-effective in monitoring individual dietary intake in large intervention studies, (iii) they are sensitive to the behaviour changes targeted by interventions, (iv) they are easily modifiable to include new food items, etc. (Kristal, Shattuck, and Williams 1992). In our concern, the diet survey carried out within this study is based on general criteria of Mediterranean diet (MD) and its related lifestyle. The importance of MD (even in developing countries) has been supported also by Alexandratos (2006) suggesting that promotion of the MD holds a huge potential in mitigating the undesirable effect of both diet poverty (burdening in many African countries) and diet transition (accompanied with obesity and other diet related non-communicable diseases in Asian populations).

The other important aspect to be investigated in nutritional status assessment, especially in developing countries, are the socio-economic conditions (SEC) of the targeted population. Vlismas et al. (2009) have reported that SEC influence dietary habits as well as health of populations. In fact, education, occupation and income have been demonstrated to be relevant in influencing, individually, the relationship between SEC and diet (Galobardes, Morabia, and Bernstein 2001). Education is related to diet and health outcomes through better lifestyle behaviours, problem-solving capacity, etc. (Vlismas, Stavrinos, and Panagiotakos 2009). Better occupational levels enable one to have the funds for better housing and better nutrition (Azpiazu et al. 2003). Income is likely to mirror the availability of economic and material resources, and therefore influences dietary quality by making healthy food more or less affordable and accessible for a single people, families or in a given community (Turrell and Kavanagh 2006).

4.2 Objectives

In the framework of the project "C3S" of *Università Cattolica del Sacro Cuore*, the objectives of this third chapter of the present doctoral thesis have been:

- to get an updated estimate of the overall nutritional status of the rural people of Darenchigre (in India) and Kabinda (in DR Congo) as baseline to identify priorities for appropriate proposals of improvement;
- to get diet characteristics to estimate (approximately) nutritional needs of rural people of Darenchigre (in India) and Kabinda (in DR Congo) based on general criteria of Mediterranean diet: grains, fruits, vegetables, legumes, fish, but also animal products, etc., from which to perform evidence based solutions to improve the diet and nutritional status of the two pilot centres within the C3S project, bearing in the mind the different degree of development of the two countries.

4.3 Material and methods

General target population definition-Two randomly selected rural populations from villages of Darenchigre in West Garo Hills in Meghalaya State (India) and from Kabinda in Eastern Kasai province Democratic Republic of Congo (DRC) were surveyed**.** To carry out the general survey in India and DRC, an appropriate questionnaire was drawn up to administer to a certain number of families, randomly chosen in the village of the two pilot centres (PCs). The questionnaire has two main parts (general family data and specific topic related data) in which are inserted questions regarding the 5 approaches (anthropometric socioeconomic, dietary and laboratory methods and, clinical histories) that are used here to assess the nutritional status of the target population. All ages and both females and males for general data were considered within each family. The overall age structure by sex of the two population groups has been illustrated through a population pyramid graph. Four years clusters were used to set the age groups in both males and females for the pyramid population construction. The total population of India and DRC (730F and 687M in India; 256F and 214M in DRC) have been divided in five main age clusters for general data description: from 0 to 2 years (40F and 49M in India; 16F and 13M in DRC), from 0 to 5 years (111F and 113M in India; 59F and 60M in DCR), from 3 to 10 years (174F and 158M in India; 106F and 94 M in DRC), from 5 to 19 years (259F and 229M in India; 115F and 110M in DRC) and over 19 years. Some of the age clusters are overlapping in some extent, but this is because in literature there are different clusters 'separation (WHO 2006). The age range from 3 to 10 years has been introduced by us to evaluate the nutritional status post-breastfeeding, till the overcoming of the post weaning crisis of children. For body mass index (BMI) determined for all the population, in addition to the previous three age clusters of infant stages, we have introduced four age clusters for both areas resulting in India: 11-18 years (138 F and 124M); 19-30 years (192 F and 156M); 31-60 years (174F and 181M) and over than 60 years (12F and 18M); in DRC, for the same age clusters, the number of females and males were (50F and 60M), (25F and 22M), (55F and 24M) and (4F and 1M).

- **Socioeconomics indicators -** Within the second part of the general questionnaire, socio-economic data have been collected regarding educational levels, type of occupation/employment, and health conditions in both India and RDC population groups. In addition to these general information, a simple questionnaire on child growth within 16 families has been administrated in Kabinda PC (in DRC) to assess whether child weight gain is related to the family occupation type. Thus, the 16 families were ranked in 4 groups having 4 families each and, in which the family head is farmer, teacher, dealer or a public officer. Body weight of each baby was noted on the questionnaire every 15 days for 6 months. Some additional information about mother diet was also collected. A similar study was carried out also in India to establish whether there is relationship between parent's education levels and their children's nutritional status have been evaluated.
- **Anthropometric indicators measurements -** Using the abovementioned questionnaire**,** a trained staff carefully collected age (years), weight (kg), height (cm), mid-upper-arm circumference (cm), and waist (cm) in both males and females in each country. Subsequently, to assess the nutritional status of populations with anthropometric methods, World Health Organization new standard have been used (WHO 2006). Average values (±SD) of abovementioned single measurement by age (according to WHO standards age ranges), and sex in both India and DRC have been calculated.

The determination of the different z-scores of each measurement have been performed using a series of mathematical calculations that take into account the not normally distributed values as described by (WHO 2008) ion in the reference population.

The general following formula used is:

$$z - score = \frac{(observed\ value\ \div M)^{L} - 1}{L\ \times S}$$

In this formula, M, L and S are values for the reference population. M is the reference median value which estimates the population mean. L is the power needed to transform the data to remove skewness (i.e. to normalize the data). S is the coefficient of variation (or equivalent). This formula (sometimes called the LMS formula) were used to calculate z-scores for BMI-for-age (BAZ), height-for-age (HAZ), weight- for-age (WAZ), weight-for-height (WHZ), weight-for-length (WLZ), and Mid-upper-arm circumference (MAZ).

Definitions of the anthropometric indices used to establish the indicators of nutritional status related to the difference gender, and age ranges in the both Indian and DRC populations are summarized below:

- Body Mass Index (BMI). BMI is an index of weight-for-height that is commonly used to classify underweight, overweight and obesity in adults. It is defined as the weight in kilograms divided by the square of the height in meters (kg/m2). BMI average values (±SD) have been calculated for all the above 7 age clusters considered.
- Body Mass Index (BMI)- for-age z scores (BAZ). BAZ indicator is a screening tool to assess risk of underweight and overweight in children and adolescents. According to WHO (2006) it must be used from 5 to 19 years.
- Height –for-age z-scores (HAZ). HAZ are indices using height (cm) related to the age (weeks, months or years) to assess whether an individual or a group of a population is stunted (chronic undernutrition), tall or very tall and classify him or her relatively to the international or national reference population. In this study, HAZ was utilized to assess stunting (chronic malnutrition) from 0 to 19 years in both India and DRC populations groups.

Four age clusters have been used: from 0 to 2 years, from 0 to 5 years, from 3 to 10 years and, from 5 to 19 years.

- Weight-for-length z-scores (WLZ) or weight-for-height z-scores (WLZ). WLZ or WHZ are indices that expresses whether a child, is wasted (suffering from acute malnutrition) or not. These indicators are used especially for under five years' children and are particularly important when age children are unknown like in the case of refugees or when such an information is difficultly to be obtained as in some vulnerable rural population in developing countries. Furthermore, weight-for-length z-scores (WLZ) in the reference standards are used for children from 45 cm to 110 cm of stature. Children under 2 years are measured lying down (length) while those from 2 to 5 years are measured standing up (height). Weight-for-height z-cores (WHZ) are used in children from 65 cm to 120cm. WLZ or WHZ was used in the present study for the age clusters from 0 to 2 years and from 0 to 5 years to evaluate wasting.
- Weight –for-age z-scores (WAZ). These indicators are used to assess whether a child is wasted, or obese (over-nourished) or not. Weigh (kg) related to the age (weeks, months, or years) are used as basics measurements. We used this index for under five children and from 3 to 10 years to establish their global nutritional status (GS).
- Mid-upper-arm circumference for-age z scores (MAZ). MAZ was determined for infant (from 0 to 5 years in this study) to assess nutritional status relating to arm muscles and subcutaneous fats. To describe the nutritional status in each age range population group, appropriate classes of all above mentioned indices have been established.

The infant nutritional status classification used in this chapter, using different z-scores indices are shown in the table 1 and that using BMI and BMI for age z scores indices is given in the table **2** (WHO 2008).

Table 4.1 Description of the ranges of the nutritional status classifications in children

Legend	Abbreviation	Range	Index
Severe Acute Malnutrition	SAM	z score< -3	WLZ or WHZ
Severe Acute Malnutrition	SAM	z score< -3	WLZ or WHZ
Moderate Acute Malnutrition	MAM	-3 <z score< -2	WLZ or WHZ
Severe Chronic Malnutrition	SCM	z score< -3	HAZ
Moderate Chronic Malnutrition	MCM	-3 <z score< -2	HAZ
Global Severe Malnutrition	GSM	z score< -3	WAZ
Global Moderate Malnutrition	GMM	-3 <z score< -2	WAZ
Global Normal Nutritional Status	GNS	-2 <z score< 2	WAZ
Global Overnutrition (ON)	GON	z score > 2	WAZ
Normal Nutritional Status	NNS	-2 <z score< 2	(HAZ, WAZ, MAZ, and WL or LH)

Table 4.2 Ranges of the nutritional status classification using BMI and BMI for age z scores indices (WHO 2008)

Meaning	Abbreviation	Range
BMI classification		
Severe thinness	ST	BMI<16
Moderate and Mild thinness	MMT	16<BMI<18.5
Normale Weight	NW	18.5<BMI<25
Overweight	OV	BMI>25
BMI for age z scores (BAZ) classification		
Severe Thinness	ST	z score< -3
Thinness	TH	-3<z score< -2
Normal Weight	NW	-2<z score< +1
Overweight	OV	z score > +1

Diet characterization- Screaming of the diet of the two population have been carried out through an appropriate FFQ, weighed food portions, chemical analysis of nutrient contents and calculation of nutrient intake. The FFQ has been used to estimate the quantity and frequency of consumption of a list of foodstuffs determined with the reference to literature (FAO 2012b) and relevant to the two PC areas. This part of the questionnaire was drawn up to cover the whole nuclear family, often by the mother as the person responsible for meal preparation (Bertoni et al. 2015). The survey was carried out using interviews conducted by local team, who had had suitable training on right filling of the forms. Moreover, during the 12 visits to pilot centres (6 visits by PC, twice every year for 3 years), several samples of single foodstuffs and ready meals relevant for the diet were collected and subsequently analysed in the food quality control laboratories of the *Università Cattolica del Sacro Cuore* of Piacenza. Chemical analyses have been done to determine nutrients' content (macro and micronutrients) of the different food samples collected. Macronutrient mainly starch, crude proteins, lipids and fiber and minerals (macro and microelements) were determined. The different analytical methods used are below summarized.

- humidity was determined by difference of weigh (in %) after maintenance of each sample in a ventilated oven at 65 ° C until reaching a constant weight. In the same way, dry mater (DM) was calculated refereed expressed as a percentage of the whole sample;
- crude proteins (CP) were determined using the VARIO MAX CN elemental analyzer. This requires weighing 0.5 g of sample in a quartz crucible, that the instrument inserted in a furnace at 800 ° C by analyzing the gases produced. Yield is the amount of elemental nitrogen present, which is converted into percentage content of proteins by multiplying the fixed coefficient 6.25;
- fiber was determined with the instrument Ankom and expressed as % of neutral detergent fiber (NDF). To prepare the neutral detergent solution, sodium lauryl sulfate-30 g, EDTA-18,61 g, sodium borate decahydrate-6, 81 g, anhydrous disodium phosphate-4.56 g, ethylene glycol monoethyl ether-10 ml and 1000 ml of distilled water, have been used and all reagents were heated for 1 hour at 100 ° C according to NDF termination routine;
- starch was determined by solubilizing of the polymer in a hot and acid environment. This was followed by enzymatic hydrolysis with amyloglucosidase and subsequent enzymatic-colorimetric determination (Trinder) of the liberated glucose;
- lipids were determined through Soxhlet method which uses acid hydrolysis.
- Minerals content determination (macro and microelements), 0.5 g of sample was weighted in 50 mL DigiTubes (SCP Science, Baie d'Urfé, Canada) adding 1 mL of hydrogen peroxide (30% v/v, Merck Darmstadt, Germany) and 2 mL of nitric acid (65% v/v, Carlo Erba, Arese, I). Nitric acid solution at the same concentration of the samples was used as blank and to prepare the calibration curves diluting external standards (Merck, Darmstadt, Germany). Minerals and standards content in final solutions were determined by inductively coupled plasma optical emission spectrometry (ICP-OES 5100, Agilent Technologies, USA). Selenium content was

determined always on same solution by inductively coupled plasma mass spectrometry (ICP MS Agilent 7900, Agilent Technologies, USA);

- Ashes were determined placing the sample in a muffle furnace at a temperature of 550°C, until reaching a constant weight, and then weigh the residue expressed in percentage;
- average estimate of protein and energy intake for an adult in India and D R Congo has been carried out using the results from food frequency questionnaires (FFQ). Referring to the assumed quantities of the foods utilised, the values of protein (as % of total energy) and energy (Kcal) intake have been calculated with the Italian program "*dieta ragionata*" refering to an average theoretical subject of a family composed by father, mother and 3-4 children. Food habit and consumption between the two populations, have been evaluated also throught the food consumption scores (FCS) according to World Food Programme (2008) guidelines. More precisely, we calculate the FCS using the aforesaid 7-days data and the food groups and related weight showns in table 4.3.

Table 4.3 Food groups and related weight used to calculate the Food Consumption Scores (WFP 2008)

	FOOD ITEMS (*examples*)	**Food groups (definitive)**	**Weight (definitive)**
1	Maize, maize porridge, rice, sorghum, millet pasta, bread and other cereals Cassava, potatoes and sweet potatoes, other tubers, plantains	Main staples	2
2	Beans. Peas, groundnuts and cashew nuts	Pulses	3
3	Vegetables, leaves	Vegetables	1
4	Fruits	Fruit	1
5	Beef, goat, poultry, pork, eggs and fish	Meat and fish	4
6	Milk yogurt and other diary	Milk	4
7	Sugar and sugar products, honey	Sugar	0.5
8	Oils, fats and butter	Oil	0.5
9	spices, tea, coffee, salt, fish power, small amounts of milk for tea.	Condiments	0

- Clinical information collection-Glucose blood content was analyzed in a representative sample of adults from Kabinda PC population to check the presence of diabetes given huge consumption of carbohydrates-based diet with the almost only foufou, a kind of polenta made with maize and cassava flour singularly or mixed (Bertoni et al. 2015). 50 subjects including 33 females and 17 males over the 35 years; without evident diseases, have been checked. Using the analytical instrument Glucocard G+, the blood sampling was done in the morning before any food. Every tip of the finger to be picked was appropriately disinfected; a new lancet was insert on the appropriate pen and then puncturing the finger and taking a drop of blood of each subject. The determination of blood glucose (mg/dl) occurred following the instructions provided with Glucocard G+.
- Statistical data analysis-Generalized Linear Model (GLM) within the software SAS version 9.3 has been used for statistical analysis. Statistical significance has been established for $p<0.05$ and slight significance has been set for $p<0.1$ (sometimes).

4.4 Results and discussion

4.4.1 Demographic and socio-economic characteristics of the Indian and DRC populations

The age structure of the two surveyed populations is shown by population pyramids in the figure 4.1. On the vertical axis of each pyramid are reported age ranges with an interval of 5 years while at the basis are reported percentage of population repartition of females (on the right) and males (on the left). From these pyramids, it is evident that in the Indian population there are more old people than in DRC; therefore, about 30 % of the Indian population are under 30 years while in DRC they are above 50%. These results suggest that rural Indian population have more life expectancy than Congolese one. Similar results have been found in India by Kowal et al (2012) who showed an average of life expectancy at birth and at 60 years, of 60 and 17 years respectively. Moreover, difference between the 2 countries is confirmed by recent report of the Population Reference Bureau (PRB) in 2016 where life expectancy in India is reported to be 70 and 67 years in women and men respectively; while in DRC it is only 52 and 49 in women and men respectively. Nevertheless, comparing these data within gender our results are in contrast with the above report. In fact, in the figure1, males tend to have more life expectancy than females at least in India. This may be partly justified by the fact that while in cities (included in the PRB report) men and women tend to have the same access to SEC and opportunities, the situation may be inverse in rural deprived areas. According to Vimard & Fassassi (2011) in D R Congo, the very rapid demographic growth, is associated with general poverty and high mortality compared to countries where the opposite situation is observed. Comparing livelihood of the two populations our first speculation based on these data is to assume that, because longevity in RD Congo is lower than in India, thus, socio-economic and life style (including food habits and consumption) conditions also tend to be worse than in India.

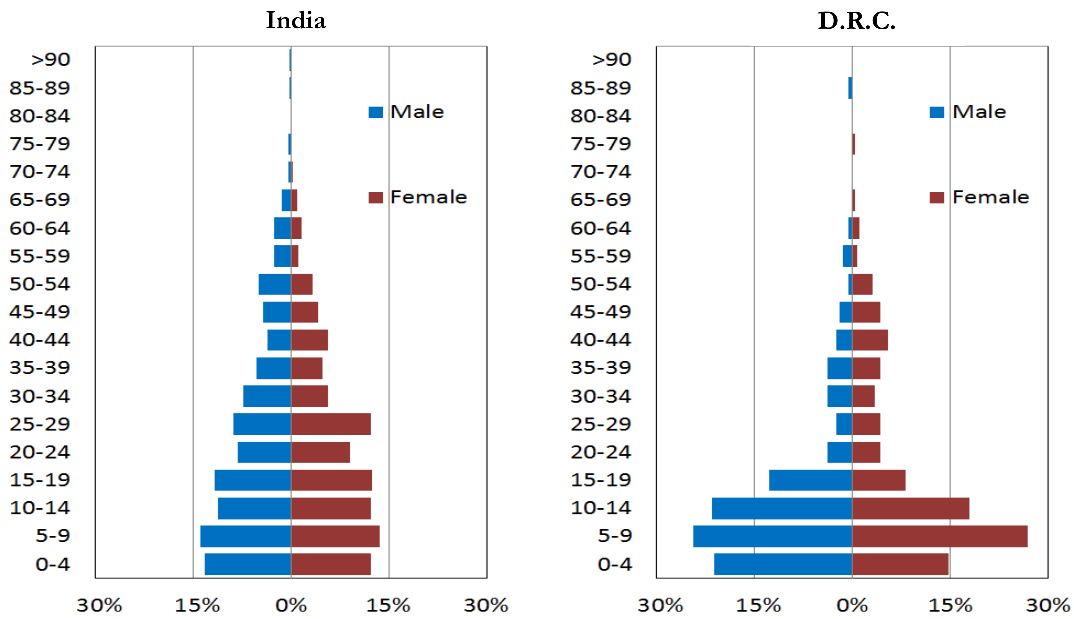


Figure 4.1 Descriptive population pyramids of the assessed rural areas of India and DR Congo. On the left axis of each pyramid are reported age ranges with an interval of 5 years while at the basis are reported percentage share of females (on the right) and males (on the left).

These findings are in accordance with those of Smith & Baghurst (1992) where it was demonstrated that disadvantaged groups have, among other factors, worse dietary profiles which increase mortality.

As aforesaid in this chapter, socio-economic conditions (SEC) influence positively or negatively household life style including food consumption. The effect of SEC could be more sensitive in tender age of children. After 6.5 months from the birth, children having farmers and teachers as parents registered lower weight gain (4.71kg and 5.08 kg respectively) compared to those whose parents are dealers and public officers. The children of these last social categories in fact resulted to have respectively 5.35 kg and 6.25 kg of weight gain in the same period of survey and growing time. The analysis of variance with Tukey test did not give significant differences between the average weight gains in the different parent's categories.

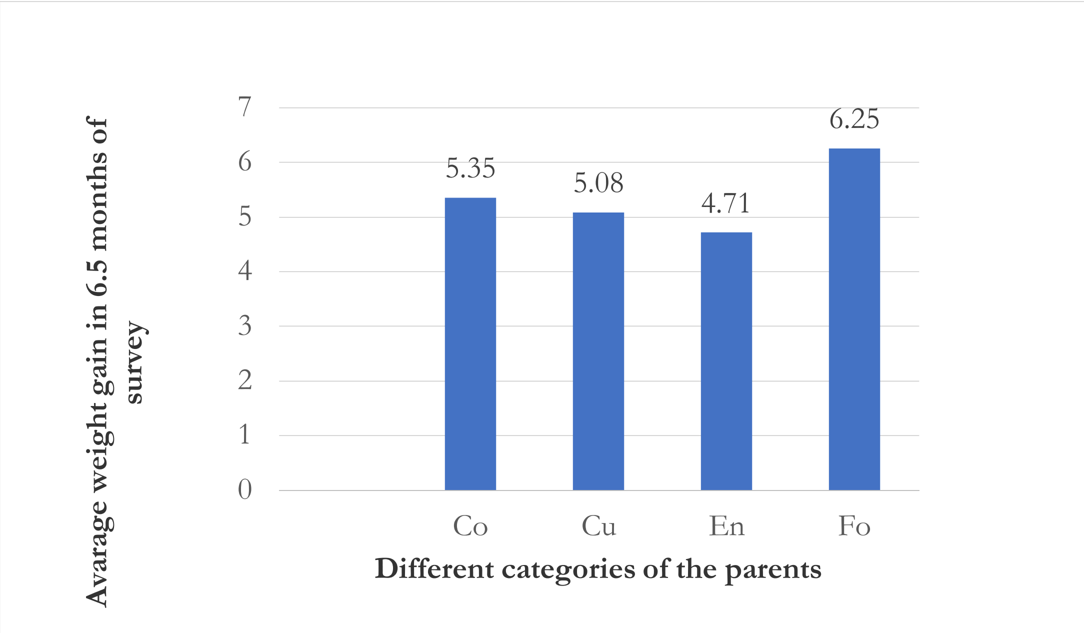


Figure 4.2 Average weight gain of children (from 1 to 6.25 months old) from the different social categories of parents (Co: dealers, Cu: farmers, En: teachers and Fo: public officers) of Kabinda PC.

Nevertheless, the data of figure 4.2 may suggest that, in general, children whose parents have better household socio-economic conditions tend to grow better than those whose parents (especially mothers) are poor and/or have a low education levels. This fact has been shown also by Kandala et al. (2011) where is reported that malnutrition in children is linked to poor socio-economic conditions including the insufficiency of hygiene while feeding babies (a condition more frequent in less educated parents).

A study that is like to be similar to the abovementioned outcomes has been carried out also in India. Nevertheless, the results of the evaluation of relationships between anthropometric measurements of Indian children (0-5 years) and the education levels of the parents (however peasants) are shown in the table 4.3. These data show that most of the anthropometric measurements in Indian children tend to be higher in those whose parents have secondary school. level or above than those with only primary school. These results are like other previous researches affirming that the education parents (especially mothers) and training is a key factor in child deployment child mortality prevention/reduction in developing countries (Cleland and van Ginneken 1988) and, especially in India it has been demonstrated that maternal education increases significantly child survival (Basu and Stephenson 2005).

Table 4.4 Relationships between anthropometric measurements of Indian children from (0 to 5 years old) and the education levels of the parents

	Education Level of mothers			Education Level of fathers		
Parameters	Primary	Secondary	p<	Primary	Secondary	p<
Weight (kg)	14.73	15.28	ns	14.23	14.71	ns
Height (cm)	88.04	91.72	ns	87.27	87.36	ns
BMI (kg/m^2)	19.98	18.48	ns	19.59	20.37	ns
Waist (cm)	48.79	52.14	*	48.98	49.47	ns
MUAC (cm)	14.91	15.36	ns	14.46	15.16	ns
N° of subjects	97	29	-	84	55	-

4.4.2 Anthropometric indicators of nutritional status of Indian and DRC populations

Although socio-economic indicators are useful to understand the nutritional status within a given population, more measurements are necessary to give an overall judgement on the nutritional status of a population. In the following paragraphs, are described our findings within the two population of India and D.R Congo through different anthropometric indicators of nutritional status across different age ranges in both sexes. The average values (±SD) of the anthropometric measurements of weight (kg), height (cm), waist circumference (cm), and arm circumference (cm) according to WHO 2006 standards age ranges in both males and females of India and DRC, are shown table 4.5. The age cluster from 3 to 10 years is not given by the WHO 2006 standards, it has been added in our study to evaluate the nutritional status of preschool and preadolescence children supposed to be greatly susceptible to malnutrition after breastfeeding and before adulthood while eating - despite early age - the same food of adults in the family. Results of anthropometric measurements, the related indices and prevalence of nutritional status assessments of the Indian and Congolese populations are shown in the tables 4.5 to 4.9. These results show that in general, the anthropometric measurements of Indian population across the different ages ranges in both sexes are higher than in DR Congo (except weight and height of females over 19 years). In the different age ranges some other interesting aspects were:

- In the children of the age range 0-2 years, there is evidence that arm circumference (MUAC) of males and females from DRC was higher than that of Indians (p<0.1) while there are also differences but not significant in other indices (table 4.5). It is interesting to observe that body weight is not different in the two countries nor does the height; suggesting that measurements per se could not be suitable to compare the nutritional status in very young children. In this same age range in fact the prevalence (%) of the different form and degree of nutritional status (table 4.7) does not confirm the situation illustrated above shown (table 4.5). In table 4.8 it can be observed that the severe acute malnutrition (SAM) or wasting - which considers weight and high - is higher in India than in DR Congo in both males (15% vs 7.7%) and females (5.3 % vs 0%). A smaller difference occurs for severe chronic malnutrition (SCM) or stunting - which considers high and age - lower in India than in DR Congo both in males (44.9% vs 46.2%) and females (40% vs 43.8%) while the difference is again important for severe global malnutrition (GSM) - which summarizes the previous wasting and stunting because it considers weight and age - resulted higher in Indian than in Congolese children in both males (73.5% vs 23%) and females (50 % vs 6.3 %). The better situation in Congo is confirmed by the global normal nutritional status (GNS) - absence of wasting and/or stunting - is much lower in Indian children than in Congolese ones in both males (14.3% vs 53.8%) and females (20 % vs 75 %). Furthermore, in Indian Children there are cases of children obesity which much higher in females (22,5 %) than in males (2%), contrary, in DRC none baby was found to be obese. These results seem to demonstrate that there are big differences at the extreme conditions: thin or fats, particularly in Indian children. In fact, as regard to moderate malnutrition, acute (MAM) and chronic (CMM) individually or together (GMM), Congolese children registered higher prevalence than Indian ones in males, respectively 15.4% and 23.1% or 23.1% (Congo) vs 2.2% and 6.1% or 10.2% (India) while this trend is not followed in the females where the preference values are respectively 6.25% and 12.5 % or 18.8 % (Congo) vs 13.2% and 7.5% or 7.5% (India). These results show that in many nutritional status indicators, Congolese children seem to have better conditions than Indians ones, in the specific age range (within 3 years).

- Although international standards support that ethnicity plays a small role in nutritional status revealed with anthropometric measurements (WHO 1986), this assessment have shown that since SAM is referred to weight-for-age z scores, these differences may be justified by ethnicity (Garo children are “smaller” than Congolese ones) than differences in livelihoods of the families (particularly in this age range where breastfeeding is prevalent); suggesting that for this nutritionals status indicator, national or regional even tribal standards may be better as references than international ones. BMI is criticized when it comes to children, but the results we have observed (Table 4.8) point to less important differences between the two countries than previously seen. In the table 4.8 in fact, emerges that in India there is more severe thinness than in D R Congo (ST) in both males 43% vs 38 % (India) and females 38 % vs 25 % (D R Congo). The presence of overweight in children in India with 22 % and 23 % in males and females respectively and not in Congo tends to confirm that. Furthermore, with regards to obesity and child overweight and the related consequences on the health, remain controversial because children are growing, the link between adiposity, or "true fatness," and the ratio of their weight to their height may be looser than that of adults (Anderson and Butcher 2006).
- Considering the age range of children from 0-5 years, the data of table 4.5 show a surprising change of situation: in India, the children are totally different respect to 0-2 years, in Congo the changes are very small and data of the 2 countries are similar or significantly higher in India for weight than in RDC ($p<0.05$). There were no significant differences between males and females of the same country in both India and RDC. No significant differences were found in other measurements. In the table 4.7 is observed that males resulted more malnourished than females in India with 7.3 % of SAM and 40.4 % of SCM in males versus 1.9% and 30.6 % in females. The same trend is observed in R D Congo with respectively 6.7 % and 65% in males versus 0 % and 61% in females. The same data show more children with normal nutritional status in India (77.22%) than in DRC (50%) contrary to what is observed in the age range from 0 to 2 years. This last situation is confirmed by average values of z-scores reported in table 4.6 and the

nutritional status assessed by BMI (table 4.8). Considering the different development levels of the two countries, these data suggest that the assessment of the nutritional status of zero to five years' children suggested by WHO can be considered as a mistake because it mixes two completely different periods: breastfeeding one and weaning-post weaning.

- The third age rang of our interest has been the age range of preschool and schooling pre-adolescent children (3-10 years). From the table 4.5, results basic anthropometric measurements show that both males and females body weight, height, and AC of Indian children was significantly higher than in DRC children ($p<0.05$) but waist was only slightly higher in Indians than in RDC children ($p<0.1$). Females resulted to have slightly more waist circumference than males in India while in DRC the situation is inverse. The differences z scores (table 4.6) also show a better condition of nutritional status in India than in Congo, and this situation is confirmed by BMI (table 4.5 and table 4.8) and nutritional status prevalence (table 4.7). These better condition of nutritional status in Indian Children than Congolese ones is easily understandable likely due to the better socio economic conditions of Indian population than DRC (where worse are also SEC and other livelihood conditions) and this is well-known in other Asian countries (Jesmin et al. 2011). Nerveless, table 4.7 permits to remark that severe chronic malnutrition even in India is still a nutritional issue (27, 2 % and 24.5 % in males and females respectively) but the same situation is much more burdening in DRC (60.6 % and 60.4 in females and females respectively). As a general comment, it can be observed that 3-10 years period is much more useful than 0-5, particularly to judge the more critical stage: end of breastfeeding to self-capacity to digest usual foods. The disappearance of SAM observed in both countries in this age range, suggests an early prevention of SAM would focus on 2 to five years old children (which perhaps could be the best period to verify malnutrition, may be better than 3-10 years).
- Schooling (from 5 to 10/11 years) and adolescent (10-19 years) children together represent about 30% (India) and more (D R Congo) of the population of this study (figure 4.1). As aforesaid, WHO recommends the

use of BMI-for- age z scores to assess the nutritional status of schooling and adolescents (5-19 years). From the results shown in the table 4.5, we can appreciate that, in the basic anthropometric measurements, both males and females body weight, height, waist and MUAC of Indian children was significantly higher than in DRC children ($p<0.05$) while there was none significant difference between males and females in the same country. This situation confirms the better nutritional status encountered in Indian than in R D Congo in other age ranges. Furthermore, also using BMI-for- age z scores (table 4.6) that are specific in assessing the nutritional status of schooling and adolescents (5-19 years), confirm the same situation. Within this last indicator, females' nutrition status resulted better than males in both population groups with respectively -0.35 ± 1.61 (India) and -0.57 ± 1.31 (D R Congo) versus -0.44 ± 2.05 (India) and -0.95 ± 1.42 (D R Congo). These outcomes are confirmed by the data from table 4.8 reporting prevalence of the different nutritional status in this age ranges from both Indian and Congolese population groups.

- In adults, (>19 years), significant differences and variations between countries and gender are observed. In males, only MUAC resulted higher in India than in RDC ($p<0.05$). There are not significant differences in other parameters (table 4.5). In female's weight and height were greater in DRC than in India ($p<0.05$), but there is no difference in waist. While contrary, AC is greater in Indian than in Congolese women. The most interesting observations from table 4.5 are however the tendency of Congo population to recover the slower growth from 3 to 19 years. Particularly in females the weight and height are significantly higher in Congo. Again, this confirms some ethnical difference in favour of Congo. Comparing genders in the same countries, significant differences were found in India, all the measurements are significantly greater in men than in women ($p<0.05$). Contrary, in DRC, the situation is more variable. In fact, there is no significant difference in height between men and women while body weight in men is slightly higher than in women ($p<0.1$) whereas waist was higher in women than in men ($p<0.05$).

- Differences between India and Congo are mainly explained by those in the general nutritional status conditions (better SEC and lifestyle in India than in R D Congo). Nevertheless, we do not agree with Lean et al. (2001) suggesting that ethnicity contributes only slightly in anthropometrics measurements outcomes in different populations. In addition to the results reported above, data from table 4.8 confirm the situation with lower prevalence of severe thinness in adult Indian population. Another contrary in Indian adults is some prevalence of overweight (and obesity). This can be part of the transition in nutrition phenomena: the better availability of usual foods - rich in carbohydrates - increases their intake and obesity risks. In fact, when the nutrition is closer to the requirements: 0-2 years for breastfeeding and above 19 years perhaps for the possibility to better manage the food intake, the Congo population appears to have "bigger size". Thus, a genetical component of these patterns can be recognized.

Table 4.5 Average values (±SD) of the anthropometric measurements: weight, height, waist, and arm circumference (MUAC) by age (according to WHO 2006, standards age ranges), and sex in India and DRC

India

Age range (Y)	Males				Females			
	Weight Kg	Height cm	Waist cm	MUAC cm	Weight kg	Height cm	Waist cm	MUAC cm
0-2 (n=40F, 49M)	9.87±2.82	72.90±16.84	44.91±6.90	13.04±2.34+	10.26±4.12	74.50±18.36	44.89±4.89	13.19±2.38*
0-5 (n=111F, 114M)	14.07±5.35*	84.24±24.10	48.45±7.36	14.59±2.66	14.09±5.02	87.33±20.23	48.96±6.09	14.86±2.84
3-10[#] (n=174F, 158M)	21.28±6.17*	107.59±20.75*	54.77±7.09+a	17.34±3.11*	21.63±7.01*	109.18±20.64*	56.12±7.73*a	17.45±3.22
5-19 (n=259F, 229M)	35.70±12.82*	133.20±20.02*	64.99±8.74*	21.99±4.36*	35.59±11.11	134.05±19.13	65.52±8.86*	21.62±4.01*
>19 (n=360F, 344M)	54.27±6.86b	158.58±7.06b	76.93±5.52 b	27.44±2.59*b	46.86±6.16*b	149.69±6.45*b	74.04±6.45b	25.52±2.59*b

DRC

Age range (Y)	Males				Females			
	Weight Kg	Height cm	Waist cm	MUAC cm	Weight kg	Height cm	Waist cm	MUAC cm
0-2 (n= 16F, 13M)	9.85±2.54	77.00±6.14	45.31±9.07	14.08±1.44+	10.25±1.48	77.44±5.53	45.63±6.32	14.08±1.44*
0-5 (n=59F, 60M)	12.27±2.65*	85.02±7.88	48.93±6.61a	14.92±1.39	11.95±2.44	84.88±9.63	47.69±4.40a	14.69±1.31
3-10[#] (n=106F, 94M)	15.22±3.78*	96.73±13.29*	51.51±5.20+a	15.37±1.41*	15.65±4.19*	99.35±13.68*	49.82±4.23*a	15.32±1.47
5-19 (n=115F, 110M)	24.55±9.68*	120.64±17.50*	56.17±5.97	17.24±2.55*	25.43±11.79	120.38±19.40	55.69±9.11*	17.56±3.03*
>19 (n=82F, 44M)	53.48±11.20a	157.98±9.62	71.40±8.10b	24.11±2.81*	51.17±8.21*a	156.33±4.75*	74.40±6.42b	23.62±1.80*

*: significant differences between Indian and DRC within the same sex (p<0.05)
+: slightly significant differences between Indian and DRC within the same sex (p<0.1)
b: significant differences between females and males within the same country (p<0.05)
a: slightly significant differences between females and males within the same country (p<0.1)
#: The age range of 3-10 years has been added to evaluate the nutritional status of preschool and preadolescence children supposed to be greatly susceptible to malnutrition after breastfeeding and before adulthood while eating the same food of adults in the family

Table 4.6 Average values (±SD) of nutritional status indices BMI, HAZ, WAZ, MAZ, WL/HZ, and BAZ by age (according to WHO standards age ranges), and sex in India and D R Congo

India

Age range (Y)	**Males**						**Females**					
	BMI	HAZ	WAZ	MAZ	WL or HZ	BAZ	BMI	HAZ	WAZ	MAZ	WL or HZ	BAZ
0-2 (n=40F, 49M)	20.03±7.82	-3.59±5.70	-1.32±2.43	-1.94±2.33	1.31±4.08	-	19.30±6.32	-1.77±87	-0.60±2.78	-1.38±2.24	1.14±3.29	-
0-5 (n=111F, 113M)	20.85±7.83	-2.96±4.89	-0.46±2.18	-1.09±2.15	2.14±3.79	-	19.20±6.54	-1.77±4.54	-0.27±2.17	-0.79±1.95	1.53±2.94	-
3-10# (n=174F, 158M)	19.28±6.52	-1.64±3.24	-0.20±1.59	-	-	-	18.72±5.92	-1.54±3.77	-0.27±1.94	-	-	-
5-19 (n=280F, 252M)	19.41±4.69	-1.89±2.23	-	-	-	-0.44±2.05	19.61±4.41	-1.89±2.30	-	-	-	-0.35±1.61
>19 (n=360F, 344M)	21.62±2.75	-	-	-	-	-	20.92±2.87	-	-	-	-	-

DRC

Age range (Y)	**Males**						**Females**					
	BMI	HAZ	WAZ	MAZ	WL or HZ	BAZ	BMI	HAZ	WAZ	MAZ	WL or HZ	BAZ
0-2 (n= 16F, 13M)	16.44±2.93	-2.867±2.01	-1.75±1.89	-0.95±1.28	-0.42±2.28	-	17.11±2.03	-0.91±2.27	-0.91±1.29	-0.57±1.23	0.59±1.29	-
0-5 (n=59F, 60M)	16.88±2.59	-3.55±1.46	-1.88±1.56	-0.83±1.07	0.40±1.99	-	16.83±4.21	-2.46±2.94	-1.89±1.57	-0.98±1.10	0.47±1.90	-
3-10# (n=106F, 94M)	16.24±2.35	-3.51±1.62	-2.49±1.51	-	-	-	15.89±3.44	-3.47±1.67	-2.45±1.49	-	-	-
5-19 (n=115F, 110M)	16.19±2.46	-3.80±1.55	-	-	-	-0.95±1.42	16.65±3.11	-3.20±1.74	-	-	-	-0.57±1.31
>19 (n=82F, 44M)	21.29±3.54	-	-	-	-	-	20.92±3.06	-	-	-	-	-

BMI: Body Mass Index (used to classify underweight, overweight and obesity in in adults)
HAZ: Height-for-age z-scores (stunting or chronic malnutrition).
WL or HZ: Weight-for-length or height z score (wasting or acute malnutrition).
WAZ: Weight -for-age z-scores (both stunting and wasting in children).
BAZ: Body Mass Index (BMI)- for-age z scores (nutritional status for children and adolescents)
MAZ: Middle Upper Circumference for-age z scores (arm muscles and subcutaneous fates to assess nutritional status especially in children).

Table 4.7 Prevalence (%) of the nutritional status by age and sex in children from 0 to 10 years in India and D.R. Congo

India

Age range (Y)	**Male**								**Females**							
	SAM	MAM	SCM	MCM	GSM	GMM	GNS	GON	SAM	MAM	SCM	MCM	GSM	GMM	GNS	GON
0-2 (n=40F, 49M)	15.2	2.2	44.9	6.1	73.5	10.2	14.3	2.0	5.3	13.2	40.0	7.5	50.0	7.5	20.0	22.5
0-5 (n=111F, 114M)	7.3	2.8	40.4	6.1	7.0	8.8	77.2	7.0	1.9	7.6	30.6	7.2	9.0	7.2	72.1	11.7
3-10 (n=174F, 158M)	0.0	0.0	27.2	8.9	2.5	10.1	81.7	5.7	0.0	0.0	24.7	11.5	2.3	5.7	87.4	4.6

DRC

Age range (Y)	**Male**								**Females**							
	SAM	MAM	SCM	MCM	GSM	GMM	GNS	GON	SAM	MAM	SCM	MCM	GSM	GMM	GNS	GON
0-2 (n= 16F, 13M)	7.7	15.4	46.2	23.1	23.1	23.1	53.8	0.0	0.0	6.25	43.8	12.5	6.3	18.8	75.0	0.0
0-5 (n=59F, 60M)	6.7	6.7	65.0	21.7	21.7	28.3	50.0	0.0	0.0	8.47	61.0	16.9	22.0	20.3	57.6	0.0
3-10 (n=106F, 94M)	0.0	0.0	60.6	22.3	36.2	29.8	34.0	0.0	0.0	0.0	60.4	24.5	32.1	27.4	40.6	0.0

Legend:
SAM: Severe Acute Malnutrition GSM: Global Severe Malnutrition MAM: Moderate Acute Malnutrition GMM: Global Moderate Malnutrition SCM: Severe Chronic Malnutrition GNS: Global Normal Nutritional Status MCM: Moderate Chronic Malnutrition GON: Global Overnutrition

Table 4.8 Prevalence (%) of nutritional status assessed with BMI in the Indian and DRC population with different age ranges.

India								
	Males				Females			
Age range (Y)	ST	MMT	NW	OV	ST	MMT	NW	OV
0-2 (n=40F,49M)	43	10	24	22	38	13	28	23
0-5 (n=111F, 114M)	36	15	22	27	37	20	26	17
3-10 (n=174F, 158M)	39	21	23	18	36	26	28	10
11-18 (n=138F,124M)	11	27	53	9	8	17	67	7
19-30 (n=192F, 156M)	1	4	84	11	2	8	78	12
31-60 (n=174F, 181M)	0	14	76	10	2	22	68	8
>60 (n=12F,18M)	0	22	78	0	25	8	58	8
DRC								
	Males				Females			
Age range (Y)	ST	MMT	NW	OV	ST	MMT	NW	OV
0-2 (n=16F,13M)	38	38	23	0	25	44	31	0
0-5 (n=59F, 60M)	25	52	23	0	42	42	12	3
3-10 (n=106F, 60M)	39	47	14	0	62	31	5	2
11-18 (n=50F,60M)	45	37	17	2	26	40	30	4
19-30 (n=25F, 22M)	8	32	64	5	0	20	68	4
31-60 (n=55F, 24M)	0	17	58	25	4	18	69	9
>60 (n=4F,1M)	0	0	1	0	0	25	25	50

Table 4.9 Prevalence (%) of nutritional status established with BMI-for-age z scores (BAZ) of schooling and adolescent children (from 5 to 19 years) in India and DRC

Nutritional status (NS)	India (n=229M; 259F)		RDC (n=110M; 115F)	
	Males	Females	Males	Females
Normal weight (NW)	65	66	65	77
Overweight (OV)	28	28	10	8
Thinness (TH)	5	3	20	9
Severe thinness (ST)	3	3	5	6

4.5 Some diet characteristics of India and D.R Congo

4.5.1 Frequency of weekly consumption of foodstuffs of Indian and Congolese population

Frequency of weekly consumption of foodstuffs of India and Congolese diets are respectively shown in figures 4.3 and 4.4, if consumed at last once a week. Figure 4.3 tells us that in the Indian surveyed population, rice, and fresh chili peppers are the only items eaten daily, while fish, often dried, is the only animal source food consumed about 3 times per week. By contrast, various sorts of plant foodstuffs are consumed at least 3 times per week, for example potatoes, bitter melon, lettuce, jack fruit, and mango; tomatoes, peas, hibiscus leaves and flowers, and cabbage leaves at least twice. Other vegetable items are present once or twice per week; more complex the dependent use of sugar, especially utilized in tea, as well as the use of condiments such as turmeric and ginger, but also chili and pepper. These results are similar to those found by Murugkar & Pal (2004) supporting that the diet in the three main tribes (Garos, Khaisis, and Jaintias) is mainly based on many types of plant source foods, meat is eaten rarely. Thus, there are correlate nutrients deficiencies (Ion, calcium and animal source vitamins) especially risky for some specific categories such as pregnant women (Marak 2010). Furthermore, other studies have reported that, except cereals, other foods are insufficient to cover the Recommended Daily Allowance (National Institute of Nutrition 2011). The apparent variety of foodstuffs in these population (especially in rural area) does not mean necessary the adequate intake of required nutrients. Interventions to shift the traditional diet to a better one are therefore needed, especially including mothers "nutritional education".

In figure 4.4 is shown the frequency with which foodstuffs are consumed across a week in D.R. Congo. Here, three items are consumed daily: cassava and maize flours and palm oil. The first two are the essential ingredients of foufou, the typical Congolese dish, which is like polenta. The only animal-origin product in the diet is dried fish, consumed about 1.5 times per week. The limited variability of the DRC diet constitutes a negative aspect of the diet. In addition, some items (fruit and vegetables, except leaves) are only seasonally present and not always included in the daily intake. The leaves (amaranth, cassava, hibiscus, and others), however, are important source of proteins; leaves are always present in the diet and most probably alternate according to the season and their availability. Our results are similar to those found by Lyana & Manimbulu (2014) demonstrating cassava is the main staple food, both for adults and young children, while the variety of foodstuffs still limited in several tribes of Congo although there is huge biodiversity in many natural surrounding regions (Termote et al. 2012). This suggests that more interventions - also at education level - to improve diet adequacy (especially in rural areas) are needed (Bahwere & Philippe, 2012).

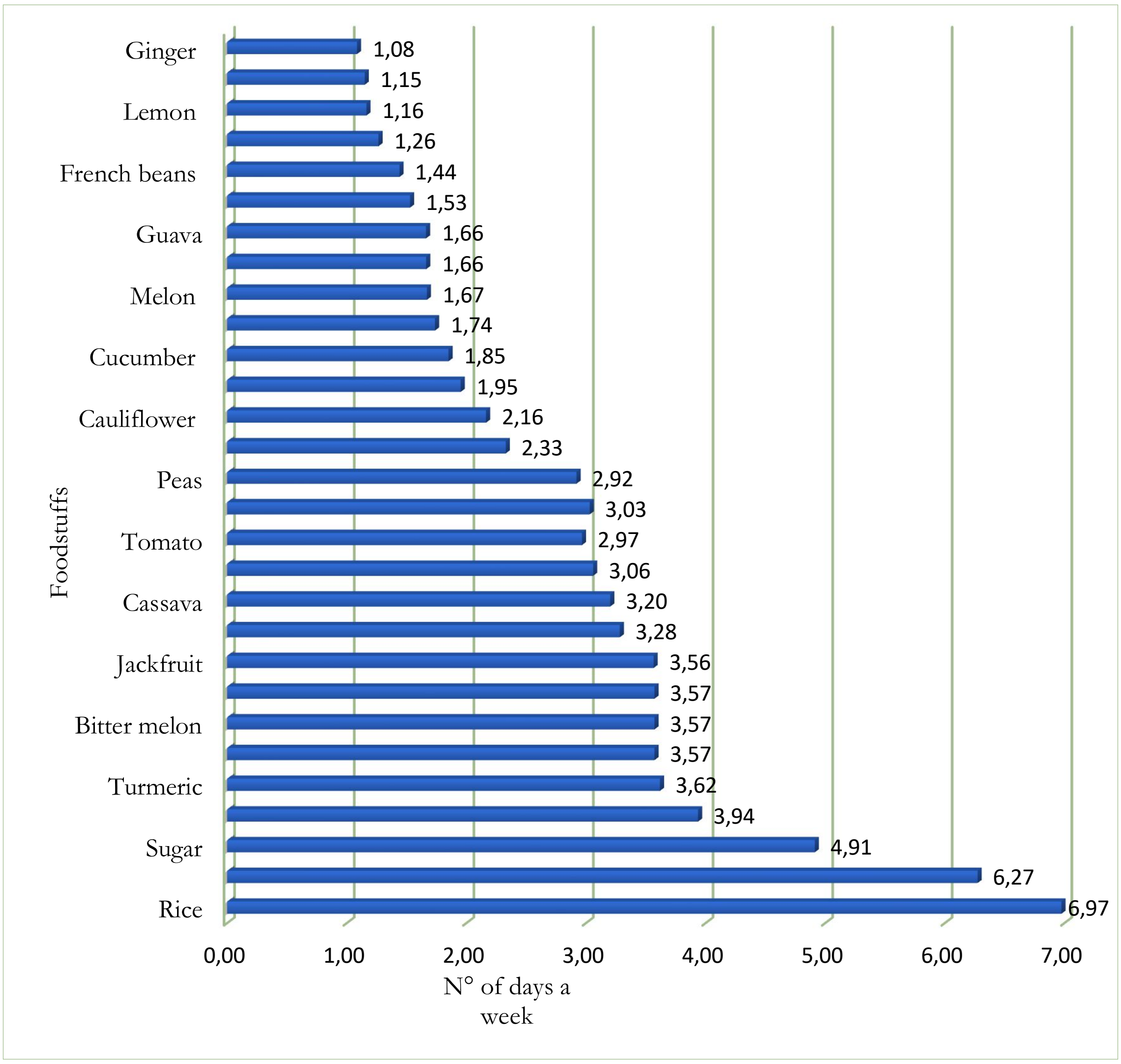


Figure 4.3 Frequency of weekly consumption of foodstuffs among the surveyed population of Darenchigre (state of Meghalaya-India) in spring season.

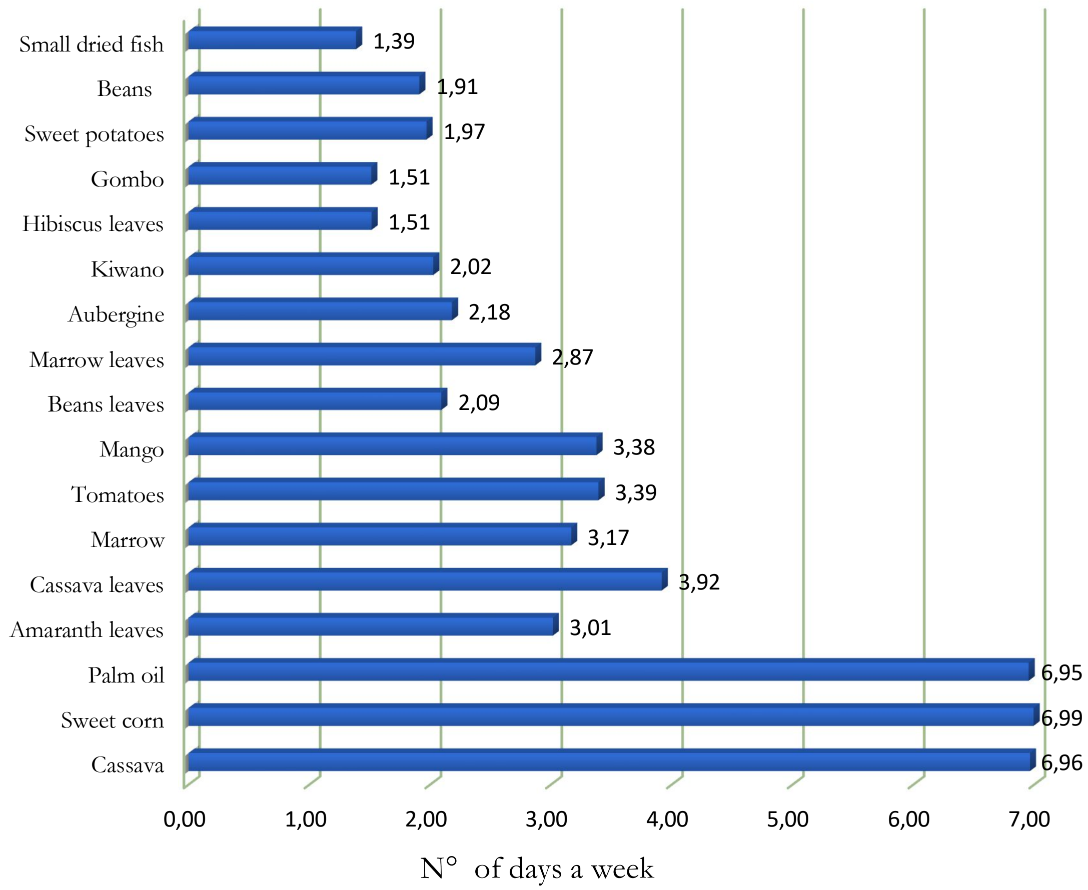


Figure 4.4 Frequency of weekly consumption of foodstuffs among the surveyed population, Kabinda, DRC.

4.5.2 Chemical-nutritional characteristics of foods in India and DR Congo

Chemical-nutritional values of some Indian foodstuffs and dishes

The chemical-nutritional characteristics of food sample collected In Indian and in DRC diets are summarized in paragraphs below. The values are expressed in % of each component on dry mater (DM).

- Cereals: the only cereal encountered in the Indian meals into families is rice. Two main kind of rice are consumed in Garo dishes, the plane rice and the Hill rice. In addition to these, there is also the so-called steaky rice (Oryza sativa var. glutinosa with low amylose). The rice is the main source of carbohydrates. The plan rice is richer in starch content (82.1 %) and lower in protein (10.0 %), whereas the hill rice contains less starch (72.4 %) and more protein (11.7 %) and a higher presence of lipid (1.7 % vs 0.30 %). Compared to plan and hill rice, the steaky rice has more starch (86,9%), less proteins (7.9%) and a moderate amount of lipids (1.4%). Another type of cereal that was found in the Garo meals is fresh maize; its chemical-nutritional values can be found elsewhere (Gopalan et al. 2012).

- Legumes: several raw legumes consumed in Garo dishes were analysed to verify their nutritional values. In general, their chemical-nutritional characteristics show that they are poor in lipid (1-3%); Chana groundnut, peas and Daal; rich in protein (21-28 %) and starch (30-42%). Fiber, determined as NDF (Neutral Detergent Fiber) varies from a type to another, lower in Daal (26%) and higher in different black grams (44%) and peas (46%) and in the Chana groundnut (29%). Our data are in accordance with the values reported in literature (Gopalan, Rama Sastri, and Balasubramanian 2012). However, the nutritional values contribution of legumes in rural Garo dishes are very small mainly because they are consumed only few times a week and used in very small amount. More probably, these are the reasons why, excepted peas, for many legumes are not reported the frequency of weekly consumption of foodstuffs in Darenchigre (figure3). Merely, this misreporting of the several types of daal consumed may have been occurred due to the very small amount used as soups (as proportion) in the rice-based recipes, about 50 g vs more than 2000 g of rice are used.
- The seeds of the oil crops (peanuts, and sesame) are also rich in protein (21-23 %) and very rich in lipid (40-53 %), but poor in starch. During this study, we found that, these foodstuffs are often consumed in very small amount (less than the abovementioned legumes) often as snacks or condiments is different soups; their nutritional contribution is modest while they could be used to increase the protein intake of good biological values (particularly peanuts) of rice-based meals.
- Fruits: in figure 3 shows that several fruits are available and their chemical-nutritional values are accessible in literature (Gopalan, Rama Sastri, and Balasubramanian 2012). Unfortunately, the declared fruits are consumed only a few days a week: lemon, sweet orange, guava, pineapple, melon,

mango, and Jackfruit are eaten from 1 to 4 times a week only and often in small amount. Furthermore, we found that some fruits (papaya, jackfruit, etc.) are often eaten cooked; thus, with the reduced nutritive values (especially for hydrosoluble vitamins and antioxidants) for which they are the main source for a balanced and healthy diet, although some of the tropical fruits encountered in Garo population are the best source of some nutrients such vitamin C in Guava (184 mg/100g) and carotenoids in mango (1800 µg/100 g)(Rodriguez 2001) and eaten raw.

- Vegetables: among the vegetables, there are two kind of leaves (Hibiscus and pumpkin) very rich in proteins (27-38%); the bamboo shout is also rich in protein (28.6%), but also the leaves of colocasia (33.5%) and cassava (33%). Unfortunately, the problem of cooking "everything" mainly for hygiene purpose, as abovementioned about some fruits occurs here to. Therefore, such types of vegetables cannot be considered in this food habits as reliable sources of vitamins (especially vitamin C and B complex ones). There is lack of raw or less cooked vegetables consumption in the surveyed population even for the few that could be consumed raw such as onions, carrots are only used as condiments in soups. Their nutritional contribution within their peculiarities remain mainly minerals, some carotenoids (Wojciech J. et al 2009) and proteins. However even for these, the above-illustrated content may be ten times reduced if reported on raw material before cooking resulting in very low intake, particularly of vitamins. The nutritional benefits of antioxidant (especially carotenoids) may be low if these vegetables are consumed with "less-fatty" dishes such as tapioca and rice based ones, because of the reduction of their absorption (Rodriguez 2001).
- Roots and tubers: colocasia, cassava, and potatoes have been encountered in are Garo food habit but they are consumed by Garo few times a week (colocasia and cassava) or in small amount (potatoes). Cassava and potatoes are also used to make fried chips in rural areas. Roots and tubers are mainly starch source foods (68%) with low amount of proteins (2%) and lipids (0.3%). The chemical-nutritional values above given refer to the giant taro roots from our analysis; however more nutritive values are given elsewhere (Gopalan, Rama Sastri, and Balasubramanian 2012).

- Ready meals: several Garo dishes have been analysed. From the FFQ survey, the only animal source food declared is dry fish (normally Nakam). However, during our complementary survey on food habit and consumption at Darenchigre, different chickens (the so-called Do. O Kappa and Do. O Pura) were encountered; declared to be traditional or typical dishes, but also pork in the so-called Wark Gomindà meal. Although these last dishes may be related to tradition and culture (Marak 2010), their name referring to meat do not mean that meat is often eaten nor is the main ingredient of the typical dish. Indeed, in general, complex ready dishes have a significant amount of lipids even if huge variables (5-34%) but less proteins (2-10 %) excepted the abovementioned meat-based foods reaching more than 26 % and 53% in pork and chickens-based foods respectively. Also, legume-based soups have a good amount of proteins: from 15% (in different daal-based soups) to 20% in beans-based ones. More available samples of chips - often eaten by pupils at school - have a good amount of lipids (27-32%) and less proteins (1.8-4%); on the contrary, less available snacks reaches 15 % of proteins and 30% of lipids. Vegetable based potages such one with Hibiscus leaves and fish resulted rich in protein (40.4 %), as leaves and fish are rich in protein, but low lipid content confirming our diet survey of Garo population which is low of oil: about 20 g per day (often mustard oil) in family with seven members.

All the above illustrated data suggest that some nutrient deficiencies and related health problems in rural Garo population may occur not just due to the absence of "high nutrient-dense foods" (animals source foods, legumes, fresh fruits and vegetables). Nevertheless, the presence of foods does not mean enough of each one needed to make a balanced diet. Indeed, although some so-called traditional dishes are contain pork meat (Wark Gomndà) or chichen (Do.O kappa and Do o Pura) there are eaten occasionally and in few quantities as confirmed by the higher prevalence of iron deficiencies leading to anaemia as stated by Marak (2010). This is in fact the real problem: a plenty of starchy foods (rice, roots, cassava, etc), but a small amount of protein-lipids rich foods (meats, milk, eggs, fish, legumes etc.). The main reasons of this situation are:

  - in low technology conditions, the higher crop/labour ratio is offered by starchy foods;
  - the low education condition does not allow the people to understand the importance to produce (and properly utilise for their family) the more nutritious foods (despite costly).
- Mineral contents: macro and microelements of several food samples have been determined. Unfortunately, the main foods of Indian diet are seed or reserve storage organs (seeds, roots, tubers, etc.) and therefore with a small content of cell-tissues (where minerals are more often present). Rice, cassava, colocasia etc. have in fact showed a very poor content of micro elements (except phosphorus in the whole seeds, but not in polished rice). Good suppliers of minerals are otherwise the foods of animal origin, but of them only fish (fresh or dry) is available in good amount. Also, good minerals 'suppliers are vegetables derived from leaves (Hibiscus, pumpkin, colocasia, cassava, etc.) as well as bamboo shoots. Also interesting is the very high level of sodium in prepared dishes (often above 5% on D.M); the reason is not only salt use, but also sodium bicarbonate added for a better cooking and flavouring. Nevertheless, the garo tradition was to use a "soda" syrup from banana-palm leaves ashes; this however, is rich in potassium and not in sodium (the latter risky for high artery pressure).

Chemical-nutritional values of some Congolese foodstuffs and dishes

The chemical-nutritional characteristics of foods sample collected from DRC diets are summarized in paragraphs below. All values are expressed in % of each component on dry mater (DM).

- Cereals: maize is almost the only available cereal largely used in the surveyed Congolese population of Kabinda to make foufou (with or without cassava flour) Obviously, maze has a high content of starch (60-71%) and is better than rice in protein (9-13%) and lipids (3.7-4.8%). During this study, polished rice resulted to be consumed only sometimes.
- Legumes- there are different varieties of beans (especially of niébé) contributing (even with a small amount and few days a week) in the dishes of Kabinda rural villages. They have a significant amount of starch, although

more variable (22-35%) and protein (28-30%) but the content of lipids (0.8-1.34%) is very low.

- Oilseeds are mainly peanuts and soybeans (very rarely). They are rich in proteins and oil but peanut is richer in lipids (39-50%) than in proteins (29%). Roasted peanuts are usually eaten as a snack outside of the house. Contrary, soybean is richer in proteins (38%) than in lipids (16-20%).
- Fruits. In addition to mango and banana, other fruits local analyzed are palm oil fruits, afromum (Afromumu alboviolaceum) and safu (Dacryodes edulis). Some tropical fruits i.e. afromum and safu are harvested in the bushes and not yet "domesticated". Compared to many other fruits, they have more protein content (7-8%) and an appreciable amount of lipids reaching 11 % and 28% in Safu and Afromum respectively (edible part).
- Vegetables-The only vegetables encountered in rural villages of Kabinda that are especially cultivated are amaranth (different comestibles species of the gender Amaranthus) and Gombo (Abelmoschus esculentus), also named Okra. Other vegetables are mainly cassava and moringa leaves. Dilombolombo (Piper umbellatum) is a wild vegetable. Amaranths are the richest in protein (38%) followed by the cassava leaves (20-34%) moringa leaves (27%) and dilombolombo (26%) sprouts. Contrary, dilombolombo is the richest in lipids (8.3%), followed by cassava leaves (3-6%) while amaranths are the poorest (1,5%). Nutritive values of Gombo reported elsewhere show that it also has a good protein content (17%) but fewer lipids (1,7%) and its nutritional quality tends to be more appreciate for antioxidants properties and fatty acids profile of its seeds wich are part of the Okra pods (Gemede et al. 2015). The protein content of leaves is high but part of them is nitrates; nevertheless, the content in cassava leaves is very low (0.05-0.1% on dry matter as $NO3$) while in amaranth it would be toxic (1.8% on a dry matter basis). Moreover, even in the amaranth, the risk of the toxicity - particularly in monogastric - is very small, while the overestimation of proteins corresponds to only 2.5 points. Moreover, some studies have reported that proteins from cassava leaves have a well-balanced amino acid profile - with a good content of several essential amino acids - excepted for

methionine and may be for isoleucine(Montagnac, Davis, and Tanumihardjo 2009).

- Roots and tubers: Cassava (Manihot esculenta: bitter and sweet varieties) and taro (Colocasia esculenta) are the two roots mainly used. Sweet varieties of cassava are eaten boiled while bitter ones are mainly used in foufou preparation (the main energy supplier dish) but previously detoxified. Bitter varieties furnish also raw material of different chips made frying dry cassava root pieces - previously rehydrated - in palm oil (often as snacks among the other street foods rather than household dishes). Cassava (the main foodstuff) is also the principal source of carbohydrates almost only starch (85-90%), very low lipids (0.5-1.25) and protein (less than 1%). According to some researchers, proteins from roots may be increased by solid-state fermentation via Aspergillus niger while also reducing cyanogen content by up to 95%.; this may help to optimize the nutritional values of cassava especially because nutrient deficiencies are more prevalent in the diets where cassava is used as staple food (Montagnac, Davis, and Tanumihardjo 2009).
- Ready meals: Some dishes have been analyzed. Foufou (made with cassava and/or maize) resulted with more proteins (9-12%) than cassava because of the use of maize flour in the mixture; lipids resulted relatively low (2-4%) but only when maize is prevalent. Different vegetables used as accompaniment of foufou controlled such as hibiscus (leaves and flowers), amaranths and aubergines, have more added lipids (19%) and some time in very high content (50%) because are fried with palm oil. Other ready meals are represented by insects (often eaten in small amount with foufou). Insects - and sometimes also small dry fish (fretin) - are the principal animal source food eaten at Kabinda. They consist in caterpillars, ants, termites, etc.: they are all very rich in proteins (approximately 50-70% on a dry mater basis, of which a part may be made up of chitin, polysaccharide of N-acetyl glucosamine), but in some forms winged. They have also abundant lipids (often close to 20 % but sometimes close to 50%, probably because males with much sperm).
- Minerals: What previously written for mineral in Indian foods, can be utilized in Congo: plenty of starchy foods such as maize, cassava, colocasia,

etc. which are poor in minerals (except phosphorus). Good suppliers of minerals are the leaves (amaranths, cassava, moringa, etc.) as well as fish, while animal foods are only rarely available. Mineral composition of insects can be also useful, but our available data are too few.

Finally, Congolese family diet still deficient in proteins because of low consumption of proper sources and can be deficient of some minerals. Nutritional issues could include: calcium, sodium and selenium, in addition to the iodine known to be among the burdening nutritional deficiencies in DRC especially in children and adolescents (Barclay et al. 2003) but also iron (especially for women) since animal foods are eaten in very low amount. Caution may be taken also about magnesium, copper and, phosphorus because of the high prevalence of starchy foods in Congolese population in general and, at Kabinda particularly. About selenium, Ndereyimana et al. (2016) have recently reported that *Moringa oleifera* may play a role of supplement to fulfil the RDA (both in India and in D R Congo). Furthermore researches would consider that micronutrients deficiencies relay on several types including vitamins, here not determined, as reported also by other studies(Barclay et al. 2003).

4.5.3 Average estimate of family protein and energy intake in India and D R Congo.

Our previous results of the estimates of protein (as % of total energy) and energy (Kcal) intake in India and DR Congo, referred to a theoretical subject (average of a family composition: father, mother and 3-4 children) for each area, are shown in table 4.9. In India, there is an acceptable energy intake (2000 Kcal/d), in which the proteins accounted for about 12-14% of energy. Therefore, there would be some protein deficiency which explains the malnutrition, particularly in children having higher needs and worsened by the fact that after weaning children eat the same foods of adults. Contrary in DR Congo, there is a low energy intake (about 1550 Kcal) and a low protein intake, corresponding to about 12% of calories, which is of course poor in terms of absolute intake (referring to few total calories).

Table 4.10 Average estimate of protein (as % of energy) and energy (Kcal/d) intake for a theoretical average subject of a family composed by a father, a mother and 3-4 children in India and D R Congo

Country	% protein/die	Kcal/d
India	12-14	2000
RD Congo	12	1550

These differences in food habit and consumption between the two populations, are confirmed by the food consumption scores (FCS) that we have calculated according to World Food Programme (2008) guidelines. We used the 7-days food frequency questionnaires data illustrated in paragraph 4.5.1 to calculate the FCS herein reported. FCS of Indian and Congolese diets resulted 56 and 41 respectively. Although both FCS classify the two diets in the same threshold (*acceptable*), the Indian diet is better than the Congolese one mainly due to the higher frequency of highly "weighted" foods (table 4.3) with 15 points more of FCS suggesting that food habit and consumption in D R Congo are less adequate. Nevertheless, these FSC that classify the two diets as acceptable do not mean that the diets are fully appropriate, and World Food Programme guidelines suggest consider other factors specific for each area. As an example, the daily diet of Garo population is taken in 3 meals while in Congo the largest part of foods is eaten in the evening meal. However, the difference in FCS between Indian and Congolese populations is useful here to confirm the gap between the two realities but less explain the diet adequacy in those populations.

An issue related to diet and nutritional status, particularly in Congolese population, is linked to the imbalance of the diet with some extent to excess of carbohydrates. This latter aspect is confirmed by the data of table 4.11 on blood glucose content where 20% of a representative sample of the same studied population-selected over than 40 years old have values higher than normal limit (> 100 mg/dl). This last result suggests that diabetic cases could not be neglected and more applied researches to improve preventive food habit and consumption are required.

Table 4.11 Blood concentration of glucose (mg/dl) in the Kabinda population

	(≤ 40 years)	(≥ 40 years)	Total
N° of subjects by group	7	43	50
N° of subjects with > 100 mg/dl	1	9	10
% on total of subjects (> 100 mg/dl)	2	18	20

4.6 Clinical indicators of nutritional status in India and D.R Congo populations

The most common health problems in the Darenchigre and Kabinda populations are presented in Figure 4.5 and 4.6. In the Indian situation (figure 4.5.), 33 % of the total surveyed population (1438) reported intestinal problems, but the incidence of malaria was much greater, affecting 55% of them. Malaria has been reported to be also the most common illness in the RDC 60% (figure 4.6) as 280 out of 470 people were affected. Intestinal problems were also important because 136 people have experienced them (33%). Of some interest in DRC the 18% as prevalence of people who were affected by measles, a viral disease. Prevalence of gastrointestinal diseases alone by age groups in DRC population is given in figure 4.6. Despite what is reported in some studies supporting that high incidence of diarrhoea is only in the 2 years of life (Lanata and Black 2006), our results, in DRC show a high prevalence even in schooling and preadolescent children (3-10 years) with a peak in zero-five years old children. Our data are in general in accordance with other researchers supporting that diarrheal diseases are the leading cause of childhood morbidity and mortality (Lanata and Black 2006). The amebiasis and colitis (intestinal) are the other burdening gastro-intestinal problems in DRC. Our results show a slight reduction of diarrhoea in adult but accompanied by a higher presence of colitis and amebiasis [figure 6 c (n=199), d (n=110) and e (n=123)]; the last two gastrointestinal problems may be reciprocally related because amebiasis may cause intestinal colitis when amoebic trophozoites (the active form of *Entamoeba histolitica*) penetrate in the intestinal mucous layer and the same explanation may be valid also for tender age (0-5 years) including the presence of other intestinal parasites as suggested by some studies (Bellomo, De Angelis‡, and Preziosi§ 2003). The intestinal candida (caused by the fungi *Candida albicans*) resulted also important healthy problem especially for very young children and it is knowns to be related to other gastrointestinal track diseases such as ulcerative colitis, gastric ulcer Crohn's disease, etc.(Kumamoto 2012). To sum-up, all these healthy problems encountered in both Indian and Congolese populations are known to contribute to malnutrition. In fact. Gastrointestinal diseases impair nutrient absorption while any disease increases the nutrient requirements. On the other hand, malnutrition impairs the immune capacity with an increase of morbidity and mortality in developing countries.

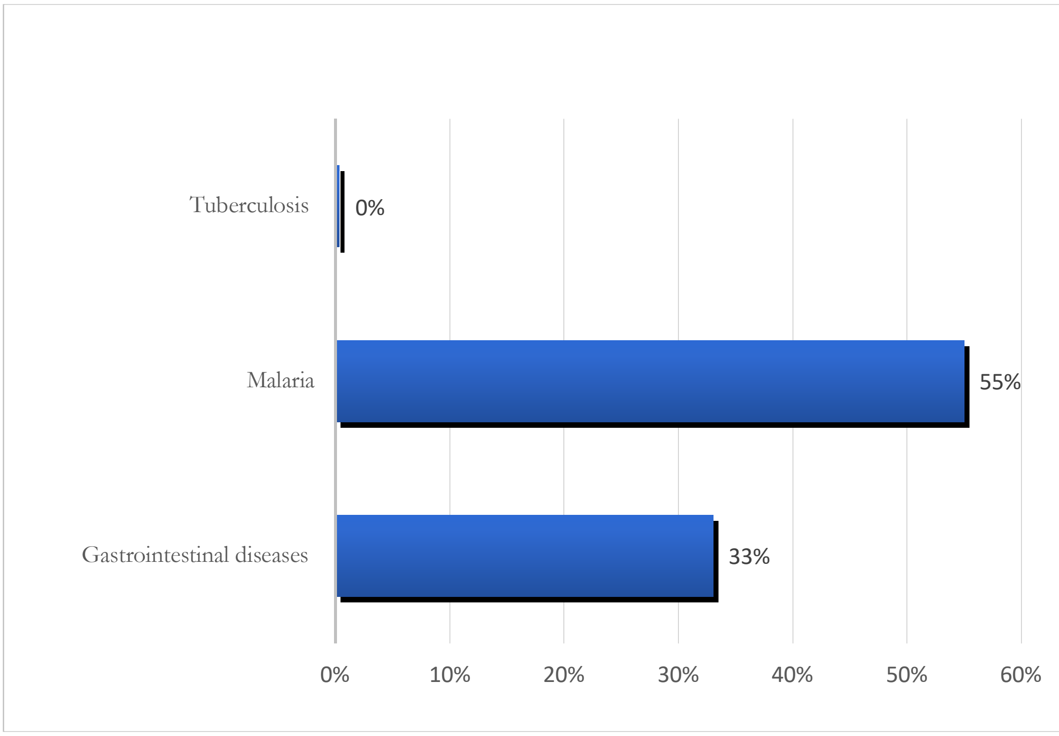


Figure 4.5 Prevalence of health problems (%) in total population in India

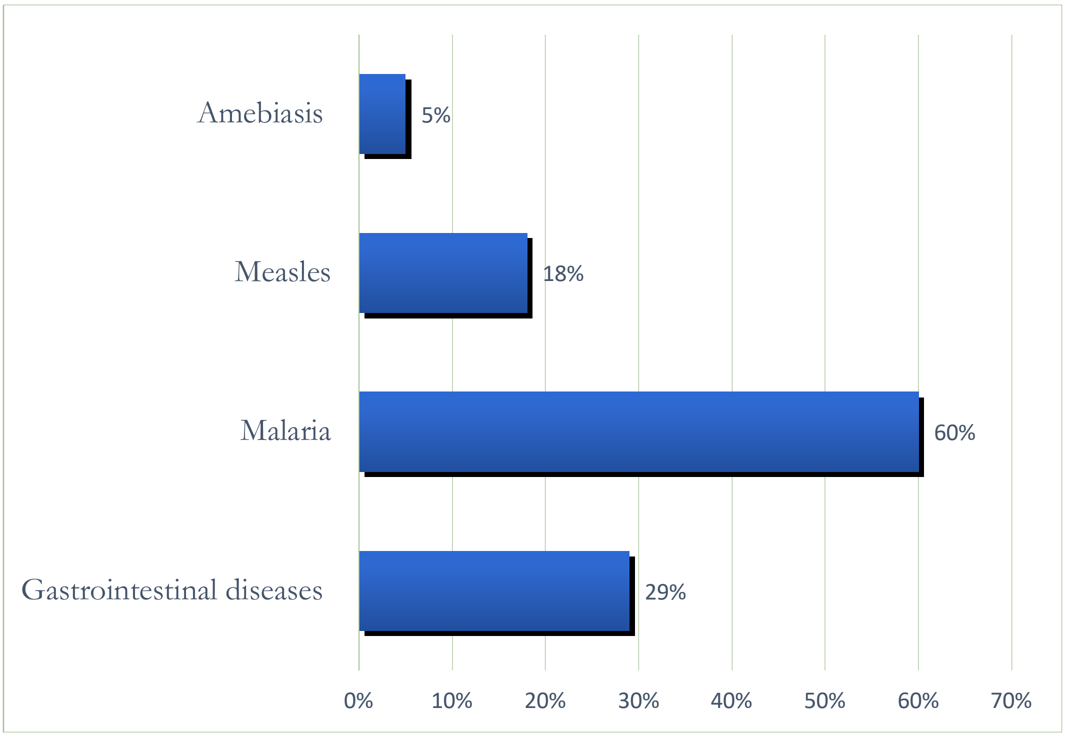


Figure 4.6 Prevalence of health problems (%) in total population in D R Congo

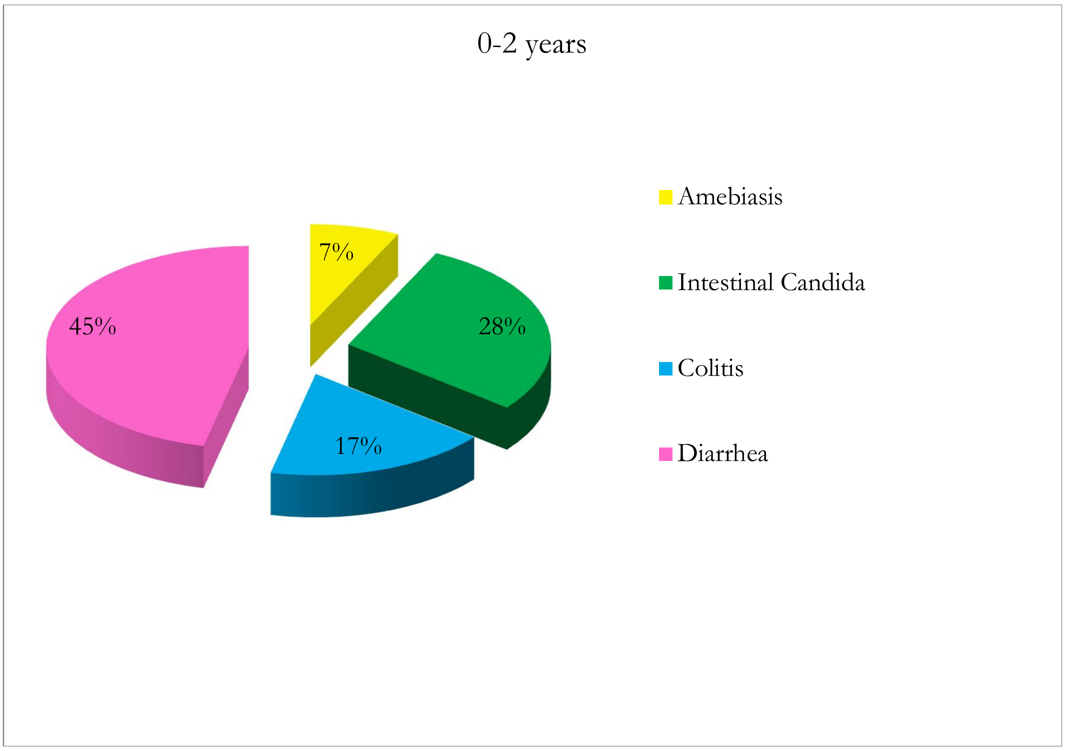


Figure 4.7 Prevalence of specific gastrointestinal diseases from 0 to 2 years in DRC children

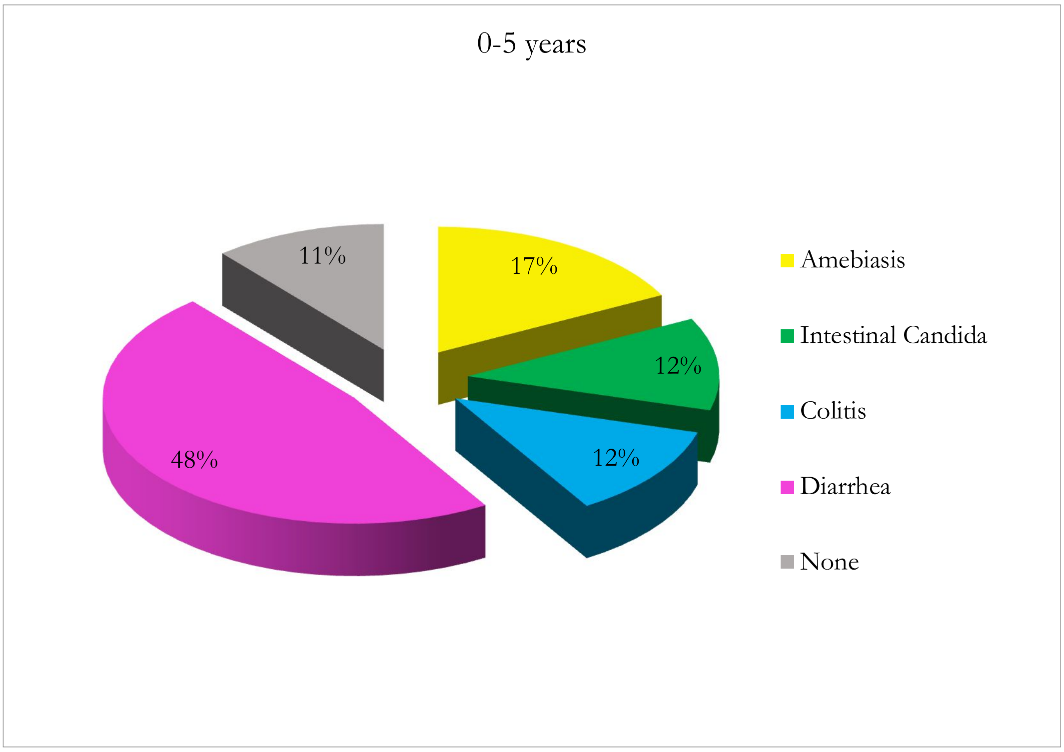


Figure 4.8 Prevalence of specific gastrointestinal diseases from 0 to 5 years in DRC children

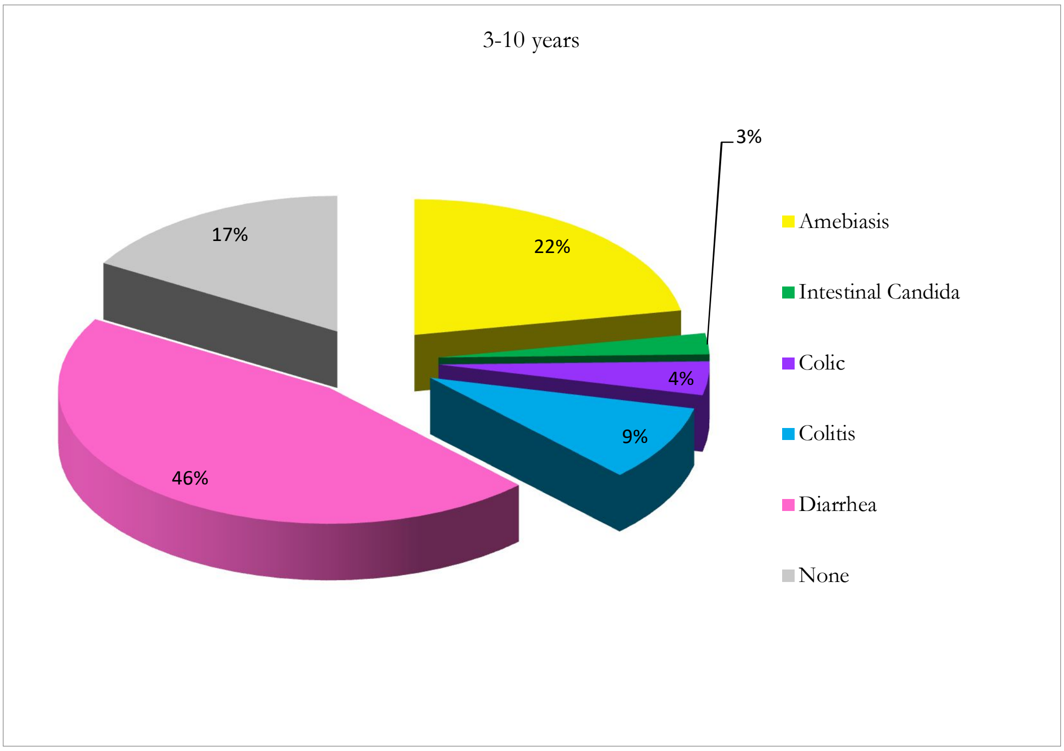


Figure 4.9 Prevalence of specific gastrointestinal diseases from 3 to 10 years in DRC children

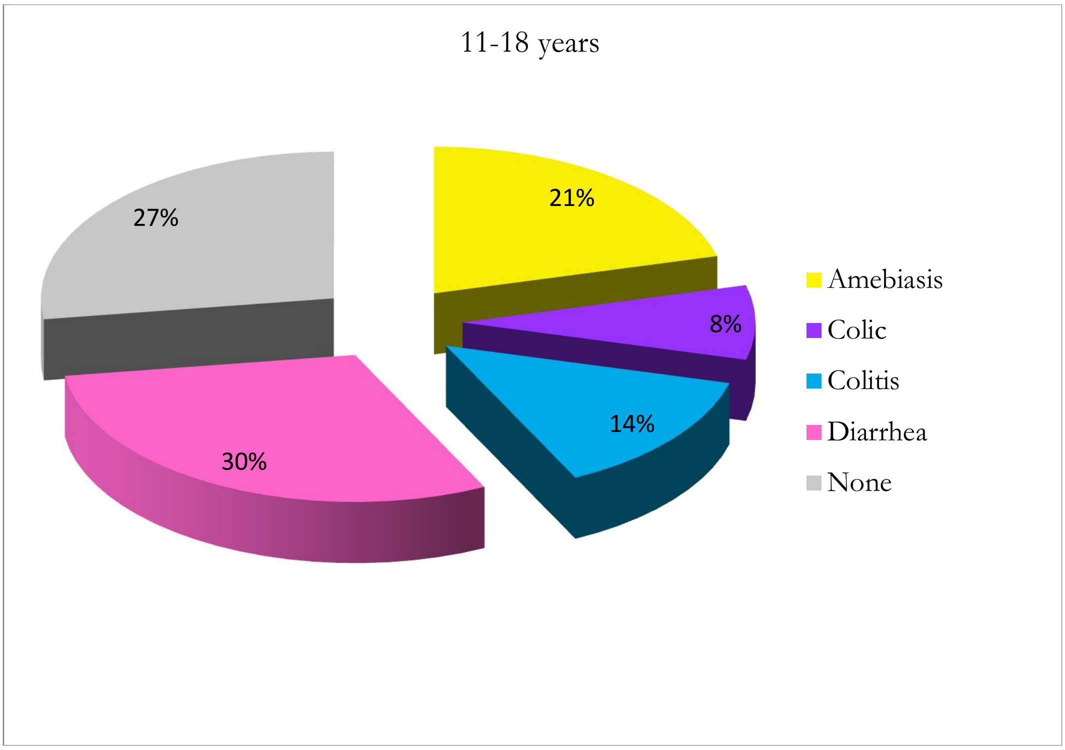


Figure 4.10 Prevalence of specific gastrointestinal diseases from 11 to 18 years in DRC pre-adolescent and adolescent population

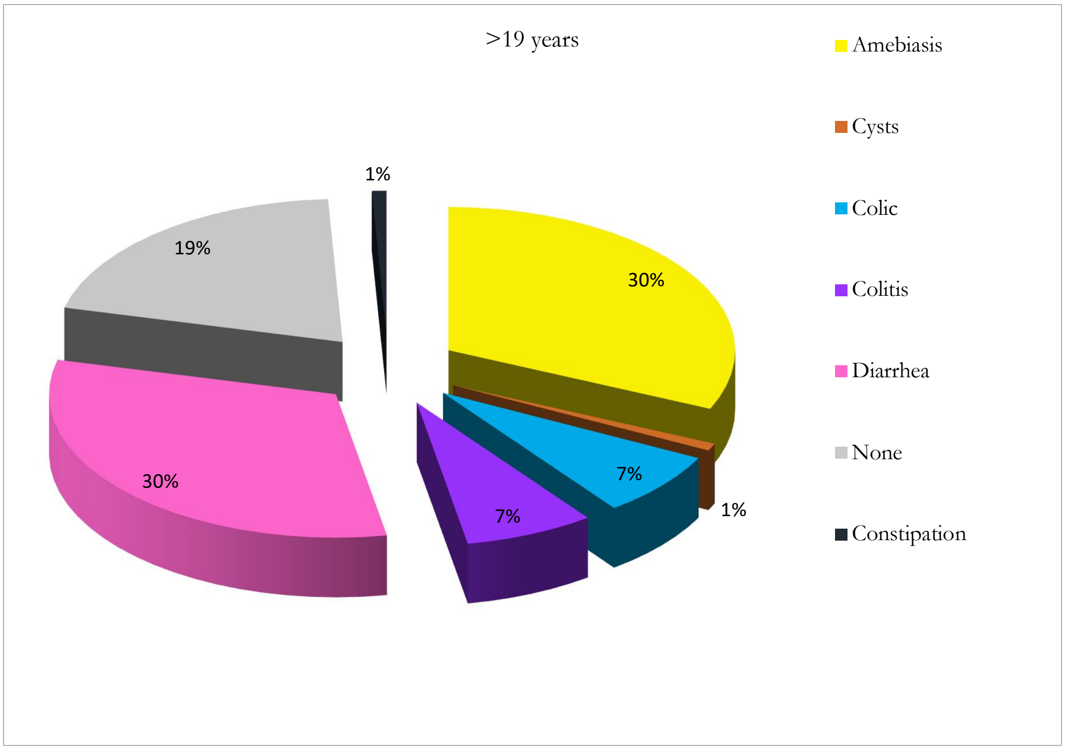


Figure 4.11 Prevalence of specific gastrointestinal diseases in DRC in adult population (> 19years)

4.7 Conclusions

The main objectives of this research have been to estimate, with different criteria, the nutritional status of the main age categories and the chemical-nutritional characteristics of foods to better define the existing diets in order to envisage some improvement hypothesis, according to the Mediterranean diet basis, but also taking into account the health conditions of the examined populations. The most important aspects that emerged have been the following:

- single anthropometric measurements are useful, but in children they must be converted into the more precised indices (z scores);
- the outcomes of our study demonstrated that India has better nutritional indicators than DR Congo, but even in India severe chronic malnutrition, except for 0-2 years and above 19 years is still an issue (with an average of 26 %). The difference between the two counrties is more evident from 3rd to 5th years where the situation is worse in DRC, with almost three-time higher (more than 60 % of severe chronic malnutrition). Therefore, our findings suggest that mitigation interventions must focus particularly above two and under-five years old children. That means after weaning stage. Nevertheless, particularly in Congo, malnutrition remains an issue at least till the adolescence.

Our findings on food habit and consumption in Garo population of Darenchigre, suggest that some nutrient deficiencies and related health problems may occur not just due to the absence of "high nutrient-dense foods" (animals source foods, legumes, fresh fruits and vegetables) but also for the lack of nutritional education about a well-balanced food combination and appropriate household food preparation.

Furthermore, food consumption of Kabinda population, is very imbalanced with an excess of starchy foods in the diet and very low protein and lipids intake. These macronutrient deficiencies in addition to micronutrients ones (especially vitamins and mineral microelements) lead to the aforesaid manifestations of malnutrition. Of great interest is the fact that in the two periods with good nutrition (breastfeeding, 0-2 years), or acceptable at least for quantity (adults above 19 years), the anthropometric data are better in Congo or like Indian ones (suggesting a genetic “superiority” of this population).

- many of the health problems encountered in both Indian and Congolese populations are known to be correlated with malnutrition (especially malaria and gastrointestinal diseases) in causing morbidity and mortality in developing countries and suggest that more efforts are still needed to contrast their harmful effect on physical development, especially with a preventive lifestyle concerning hygiene conditions and water potability.
- the chemical-nutritional characteristics of the foods surveyed in India and Congo did not add much to what was already known; therefore even in terms of mineral contents, their composition was not so relevant. Remains the certainty that in both countries, but especially in the Congo, starches are prevalent with more or less serious scarcity of protein, fat and micronutrients (for the latter also due to the prevalent use of "well-cooked" foods).

Since this study is the first one using several nutritional status assessment methods in these rural areas, it is one of the more complete studies that allow to appreciate the magnitude of the problem of malnutrition in the target population compared to single topic assessments reported in literature and, this may be considered a strength. As limitation in our study is that ages were reported only in years (as it was difficult to get exact date of birth); this may augment measurement error in the estimates (where age is relevant as reference parameter, especially in under-five children);

Finally, we recommend a multidisciplinary empowerment (e.g. hygiene and nutrition education; basic baby care education, water sanitation practices, etc.) particularly for mothers as a key factor of family integrated development in developing countries in general and especially in Garo (India) and at Kabinda (D R Congo) populations. A further objective of this empowerment and strategic for proper physical and cognitive capacities is the hygiene.

4.8 References

Agrahar-Murugkar, D. & Pal, P.P.2004. Intake of nutrients and food sources of nutrients among the Khasi tribal women of India. *Nutrition*, 20(3), pp.268–273.

Alexandratos, N.2006. The Mediterranean diet in a world context. *Public Health Nutr*, 9(1A), pp.111–117.

Anderson, P.M. & Butcher, K.E.2006. Childhood obesity: trends and potential causes. *Future.Child.*, 16(1), pp.19–45.

Baker, J.P., Detsky, A.S., Wolman, S.L., Steward, S., Whitell, J., Larger, B., Khursheed, N.J. 1982. Nutritional Assessment. A comparison of Clinical Judgement and Objective Measurements. *The New England Journal of Medecine*, pp.969–972.

Barclay, D.V., Maurona , J., Blondela , A., Cavadinia, C., Verwilghenb, A.M, Van Geertb, C., Dirrena, H. 2003. Micronutrient intake and status in rural Democratic Republic of Congo. *Nutrition Research*, 23(5), pp.659–671.

Basu, A.M. & Stephenson, R.2005. Low levels of maternal education and the proximate determinants of childhood mortality: A little learning is not a dangerous thing. *Social Science and Medicine*, 60(9), pp.2011–2023.

Bellomo, N., De Angelis‡, E. & Preziosi, L.2003. Amebiasis. *Journal of Theoretical Medicine*, 5(2), pp.111–136.

Bertoni, G., Tabaglio, V., Ganimede, C., Trevisan, M., Pellizzoni, M., Anaclerio, M., Cappa, F., Grossi, P., Fiorani, M., Ndereyimana, A., Minardi, A., Sckokai, P., Guastella, G., Chiesa, M., Rossi, L. 2015 Food Production and Use. Security, Safety and Sustainability, Egea, Milano (2015): 150 [http://hdl.handle.net/10807/72037]

Cade, J., Thomson, R., Burley, V., Warm, D. 2002. Development, validation and utilisation of food-frequency questionnaires - a review. *Public Health Nutr.*, 5(4), pp.567–587.

Carol A. Kumamoto (2012). Inflammation and gastrointestinal Candida colonization. , 14(4), pp.386–391.

Christak.G 1973. Nutritional Assessment in Health Programs - Foreword. *American Journal of Public Health*, 63, pp.R3–R5.

Claudio F. and Lanata Robert E.Black 2006. Diarrheal Diseases. In *Nutrition and Health in Developing Countries.*

Cleland, J.G. & van Ginneken, J.K.1988. Maternal education and child survival in developing countries: The search for pathways of influence. *Social Science and Medicine*, 27(12), pp.1357–1368.

Cogill, B.2003. Guide de Mesure des Indicateurs Anthropométriques Guide de Mesure des Anthropométriques.

David B. Haytowitz, Pamela R. Pehrsson, Julie Smith, Susan, E. Gebhardt, Ruth H. Matthews, A.B.A.A., 1996. Key foods : setting priorities for nutrient analyses. *Journal of food composition and analysis*, 364(9), pp.331–364.

Den Hartog, A.P., Van Starveren, W.A., Brouwer, I.D. 2006. Food Habits And Consumption In Developing Countries: Manual for Field Studies.

Duwarah, S., Bisai, S. & Barman, H.2015. Prevalence of Undernutrition among Preschool Children under Five Attending Pediatric OPD in a Tertiary Care Hospital of Northeastern India. , 3(16), pp.527–533.

Fairburn, C.G. & Harrison, P.J.2003. Eating disorders. *The Lancet*, 361(9355), pp.407–416.

FAO. 2002. Measurement and assessment of food deprivation and undernutrition.

FAO.1997. Tabella dei fabbisogni delle popolazione dei Paesi in via di sviluppo.

FAO.2012. West African food composition table [Table de composition des aliments d´Afrique de l´Ouest].

FAO, IFAD & WFP.2015. The State of Food Insecurity in the World: Meeting the 2015 international hunger targets: taking stock of uneven progress.

Galobardes, B., Morabia, A. & Bernstein, M.S. 2001. Diet and socioeconomic position: does the use of different indicators matter? *International Journal of Epidemiology* , 30(2), pp.334–340.

Gavin Turrell and Anne M Kavanagh 2006. Socio-economic pathways to diet : modelling the association between socio-economic position and food purchasing behaviour. , 9(March), pp.375–383.

Gemede, H.F., Ratta, N., Haki, G.,D., Woldegiorgis, A.Z., Fekadu, B., 2015. Nutritional Quality and Health Benefits of Okra (Abelmoschus Esculentus): A Review. *Global Journal Of Medical Reseacrh: K Interdisciplinary*, 14(5), pp.29–37.

George Christakis 1973. Nutritional Assessment in Health Programs. *American Public Health Association*, 63.

Glorio, R., Allevato, M., De Pablo, A., Abbruzzese, M., Carmona, L., Savarin,M., Ibarra, M., Corina Busso, C., Mordoh,A., Llopis, C., Haas, R., Bello, M., Æoscoff, A. 2000. Prevalence of cutaneous manifestations in 200 patients with eating disorders. *International journal of dermatology*, 39, pp.348–353.

Gopalan, C., Rama Sastri, B.. & Balasubramanian, S.2012. Nutritive value of Indian foods. *Nutritive value of Indian foods*, p.52.

Haytowitz, D.B., Pehrsson, P.R. & Holden, J.M.(2002). The Identification of Key Foods for Food Composition Research. *Journal of Food Composition and Analysis*, 15(2), pp.183–194.

ISAK (2001). International Standards for Anthropometric Assessment.

Jesmin, A., Yamamoto, S.S., Malik, A.A., Aminul Haque, Md.A.2011. Prevalence and determinants of chronic malnutrition among preschool children: A cross-sectional study in Dhaka City, Bangladesh. *Journal of Health, Population and Nutrition*, 29(5), pp.494–499.

Kandala, N-B., Madungu, T.P., Emina, J.B., Nzita, K.P., Cappuccio, F.P 2011. Malnutrition among children under the age of five in the Democratic Republic of Congo (DRC): does geographic location matter? *BMC public health*, 11(1), p.261.

Kowal, Paul, Williams, Sharon, Jiang Yong, Fan, wu, Arokiasamy P, Chatterji, S., (2012). Aging, Health, and Chronic Conditions in China and India: Results from the Multinational Study on Global AGEing and Adult Healt (SAGE). , (January), pp.415–437.

Kristal, A.R., Shattuck, A.L. & Williams, A.E.(1992). Food frequency questionnaires for diet intervention research. *Proceedings of the 17th*, pp.110–125.

Lean, M., Han, T.S., Bush, H., Anderson, A.S., Bradby, H., Williams R. 2001. Ethnic differences in anthropometric and lifestyle measures related to coronary heart disease risk between South Asian, Italian and general-population British women living in the west of Scotland.

López-Azpiazu, I., Villegas, A.S., Johansson, L., Petkeviciene, Prättälä, Gonza´lez, M.AM., (2003). Disparities in food habits in Europe: Systematic review of educational and occupational differences in the intake of fat. *Journal of Human Nutrition and Dietetics*, 16(5), pp.349–364.

Lyana, A. & Manimbulu, N.(2014). Culture and Food Habits in Tanzania and Democratic Republic of Congo. *J of Human Ecology. J Hum Ecol*, 48(1), pp.9–21..

Maqbool, A., Olsen, I.E. & Stallings, V.A. (2008). Clinical Assessment of Nutritional Status.

Mário Cícero Falcão (2000). CLINICAL AND LABORATORY ASSESSMENT OF. *Rev. Hosp. Clín. Fac. Med. S. Paulo*, 55(1), pp.1–2.

Montagnac, J.A., Davis, C.R. & Tanumihardjo, S.A.(2009). Nutritional value of cassava for use as a staple food and recent advances for improvement. *Comprehensive Reviews in Food Science and Food Safety*, 8(3), pp.181–194.

Natasha Marak (2010). Dietary practices of the Garo tribe. Influence of Socio-Economic Factors and Cultural Beliefs on Nutritional Status. Saarbrücken, Lambert Academic Publishing.

National Institute of Nutrition (2011). Dietary Guidelines for Indians.p.139.

Ndereyimana A., Ndayisenga V., A.Minardi, G.Bertoni, F.Piccioli-Cappelli (2016). Selenium content in food of rurale areas of Kabinda in the Democratic Republic of Congo and Of Meghalaya State of India.

Nucara, A., Pietrafesa, M., Rizzo, G., Scaccianoce, G., 2012. Handbook of Anthropometry. pp.91–114.

Paluku Bahwere and Philippe Donnen (2012). Nutritional status of the populations of the Congo Basin: Today and tomorrow.

Population Reference Bureau (2016.) 2016 World Population Data Sheet, p.23.

Richard SA, Black RE & Checkley W. (2012). Revisiting the relationship of weight. *Advances in Nutrition*, 3(2), pp.250–254.

Rodriguez, D.(2001). A Guide to Carotenoid Analysis in Foods.

Sambra and Bloem (2006). Nutrition and Health in Developing Countries Second.

Sempos CT (1992). Some limitations of semiquantitative food frequency questionnaires. *AM J Epidemiol*, 135, pp.1127–1132.

Shrivastava et al. (2014). Assessment of Nutritional Status in the Comunity and Clinical Settings. *Journal of Human Nutrition and Dietetics*, 27(2), pp.105–106.

Smith, a M. & Baghurst, K.I. (1992). Public health implications of dietary differences between social status and occupational category groups. *Journal of epidemiology and community health*, 46(4), pp.409–416.

Termote, C., Meyi, M.B., Djailo, B.D., Huybregts, L., Lachat, C., Kolsteren, P.,Van Damme, P.2012. A biodiverse rich environment does not contribute to a better diet: A case study from DR Congo. *PLoS ONE*, 7(1).

Thame, M., Wilks, R., Mcfarlane-Anderson, N., Bennett, F., Forrester, T. 1997. Relationship between maternal nutritional status and infant's weight and body proportions at birth. *European Journal of Clinical Nutrition*, 51, pp.134–138.

United States Department of Agriculture (2016). Agricultural Research Service USDA Food Composition Databases.

Vimard, P. & Fassassi, R.(2011). Démographie et développement en Afrique : éléments rétrospectifs et prospectifs. *Cahiers québécois de démographie*, 40(2), p.331.

Vlismas, K., Stavrinos, V. & Panagiotakos, D.B., (2009). Socio-economic status, dietary habits and health-related outcomes in various parts of the world: A review. *Central European Journal of Public Health*, 17(2), pp.55–63.

World Food Programme (2008). "Food Consumption Analysis - Calculation and Use of the Food Consumption Score in Food Security Analysis." *Technical Guidance Sheet*, 1–24.

WHO 1986. Use and interpretation of anthropometric indicators of nutritional status. , 64(6), pp.929–941

WHO. 2006. The new WHO child growth standards. *Paediatria Croatica Supplement*, 52(SUPP.1), pp.13–17.

Wojciech J. Florkowski, Robert L. Shewfelt, Bernhard Brueckner and Stanley E. Prussia 2009. Nutritional Quality of Fruits and Vegetables. In *Postharvest Handling: A Systems Approach*. pp. 56–106.

Woodruff, B. A. & Duffield, A. (2002). Anthropometric assessment of nutritional status in adolescent populations in humanitarian emergencies. *European journal of clinical nutrition*, 56, pp.1108–1118.

World Health Organization (2008). Training Course on Child Growth Assessment.

Wrieden, W, H Peace, J Armstrong, and K Barton. 2013. "A Short Review of Dietary Assessment Methods Used in National and Scottish Research Studies." *Group on Monitoring Scottish Dietary …*, no. September: 1–17.

5 IMPROVEMENT OF NUTRITIONAL STATUS IN DEVELOPING COUNTRIES THROUGH REDUCTION OF FOODBORNE DISEASES AND BETTER DIETS.

5.1 Introduction

5.1.1 Overview

The state of nutritional status in developing countries and the importance of an appropriate diet to have a healthy and productive life for individuals and community development have been widely discussed in the previous chapters of this doctoral thesis. Especially, the introductive chapter and the nutritional status assessment results - where about 30% of children in India and 60% in Congo resulted malnourished - showed that hunger and malnutrition in rural Garo (in North East India) and Kabinda (East Kasai in DR Congo) population still burdening issue as one of the most challenging problem to meet the sustainable development goals within families. Diets of the two populations resulted unbalanced both at macronutrients and micronutrients levels with the worst situation in Congo compared to India. To worsen the situation is the insufficient access to potable drinking water and for food preparation - especially those eaten fresh - and other potable uses (washing hands for example), in addition to the ignorance of domestic and community good practices of hygiene. As result, gastrointestinal, especially diarrheal diseases take the lead in the threatening the health of these populations creating the vicious circle: illnesses-malnutrition both in children and adults. These gastrointestinal diseases tend to impair nutrient absorption which worsen the malnutrition situation causing more illnesses in its turn.

Since our previous awareness on this health and diet related problems is confirmed by assessment results of the specific rural areas of Kabinda and Darenchigree, we decided to carry out a variety of trials including strategies for diet diversification and food (and water) safety. This chapter will illustrate results of our experiments to suggests practices to improve and diversify the diet both in children and adults where domestic meals (eaten at home) and outside food consumption aspects have been considered. Our proposals include food preparation practices that may contribute to the nutrient balance (considering both macro and micronutrient deficiencies), their preservation and use by human body while preserving or enhancing the safety of the ingested food.

5.1.2 The problems of household food and waterborne diseases in developing countries

Foodborne diseases (FBD) are defined as illnesses that result from the ingestion of pathogenic bacteria, viruses, parasites, chemical or natural toxins (e.g. aflatoxins) that contaminate food (Grace 2015 a and b). FBD are household and public health concern both in developed and in developing countries but some evidence based outcome of researches suggest that they appear higher and more burdening in developing counties (WHO 2016).

Indicators of the magnitude of food contamination in developing countries is the information available about infant diarrhoea, child (particularly after 6 months) and adult malnutrition, conditions closely related to contaminated food and drinking water (KÄFERSTEIN 2003). Especially for water, many experts suggest that most cases of diarrhoea and cholera are solely waterborne-related more than person-to-person contact (KÄFERSTEIN 2003). As reported by different researchers, there are mainly three classic strategies to prevent foodborne diseases especially under five years children, known to be at high risk to contract these types of diseases compared to adults : water sanitation practices (Fewtrell et al. 2005), vaccination (KÄFERSTEIN 2003) and the promotion of breastfeeding (Arifeen et al. 2001). The problem of foodborne parasites, such as worm (and amebiasis), can only be controlled if food safety measures are applied (Grace 2015a). Furthermore it is well known that food contamination often increases malnutrition worldwide and particularly in low-income countries because nutrients and calories are lost through diseases (Motarjemi et al. 1993 and Ngure et al. 2014). In many poor rural families, there is no access to many hygiene facilities and commodities (Scott 2003) such as fuel for cooking, refrigeration systems causing very difficult or impossible to get a safe food supply (KÄFERSTEIN 2003). The huge magnitude of an unhygienic food preparation in poor families, essentially due to unclear kitchen amenities, poor personal hygiene, insufficient cooking, the conservation of ready-to eat food (fresh foods, cooked remaining after meals, etc.) in inappropriate conditions and insufficient re-heating increase foodborne diseases risk in developing countries (Muinde and Kuria 2005). The United Nation World Water Development Report (2016) estimated that 663 million people lack ready access to improved sources of drinking water, while some 2.4 billion people do not use improved sanitation facilities. Furthermore, the access to improved sources is significantly lower among poorer communities in low-income countries (UNWWAP 2017).

It is thus clearer that household's strategies to improve potable water access (especially drinking water) as well as hygiene in food preparation and conservation remain a big challenge in reducing foodborne diseases and related malnutrition problems. In Indian and Congolese rural areas, to which we put major interest during the present research, many other previous studies have demonstrated how much is important implementing researches and practices to improve the above illustrated conditions. Diarrheal diseases has been reported to be among the major causes of morbidity and mortality, especially within children in India (Mckenzie and Ray 2005) as well as in other developing countries (Clasen et al. 2006). The faecal contamination of water for human consumption, is one of the biggest risks for public health in India (Mckenzie and Ray 2005). Although largely preventable and / or treatable, diarrhoea is estimated to be the cause of over 840,000 deaths each year (Who and Unicef 2014). In India, it has been reported that 47 per cent of households do not use any method of purification for water coming from sources often contaminated (Jalan et al. 2003). The use of unimproved water resources for drinking water is a general problem also in many Sub-Saharan countries. Onabolu et al. (2011) estimated that in Nigeria, it was estimated to reach an average of 37% and 58% in rural areas. These conditions are worse in D R Congo where only 26 per cent of the population (17,6 millions) has access to potable water while the average in all sub-Saharan countries where estimated to 60 percent in the same period (PNUE 2011). Although the situation was estimated to be improved until to a national access of 49% in 2015, the trend remained less promising in rural areas where the access would reach only 29% in the same year (Lwanga and Offosse 2012).

Interventions most used for the improvement of drinking water quality at the household level in low-income countries are, among others, chlorination, filtration, Solar Water Disinfection (SODIS), combined flocculation disinfection and appropriate storage (Clasen et al. 2006). Moreover, the same researchers reported that interventions to improve the microbiological quality of drinking water are effective in the prevention of diarrhea, for both adults and children. Other researchers have later demonstrate that the use of household candle ceramic water filters in rural areas is an appropriate solution to reduce microbial contamination (Simonis and Basson 2011 and Mwabi et al. 2011). Furthermore, a meta-analysis of 976 studies, has shown that among all interventions of households improvements of drinking water quality, filtration provides more consistent, more effective and more sustainable results in the prevention of waterborbne diseases in developing countries (Clasen et al. 2006). The aforesaid SODIS is another low-cost solution to improve household drinking water quality in rural area of poor countries. SODIS is a technique that consists of placing water into transparent plastic or glass containers - usually 2 L PET beverage bottles - which are then exposed to the sun. Exposure times vary from 6 to 48 h depending on the intensity of sunlight and sensitivity of the pathogens (McGuigan et al. 2012). SODIS effectiveness on E.coli strains (known to be the more resistant to the lethal effect of sunlight among pathogenic enterobacteria) has been proven some decades ago (Wegelin et al. 1994). Other studies confirmed the inactivation of different pathogenic bacteria supporting that SODIS method is revealed to be a suitable home-based low-budget water purification method, especially in developing countries (Dejung et al. 2007). SODIS method remained attractive research topic especially about variety of material and acceleration of the inactivation of viruses and bacteria Nalwanga et al. 2014).

In addition to unsafe water as vehicle of hazards leading to FBD, there are unsafe ready-to-eat foods, especially fruit and vegetables, that transmit infectious microorganisms and related foodborne gastrointestinal illnesses (Sabbithi et al. 2014 and Grace 2015). Other studies, carried out in some developing countries, suggested that even where disinfection is applied to fruits and vegetables, it is not sufficient and other water sanitation methods are required for other household foods handling activities (Jeddi et al. 2014). This explains the habit to cook daily any kind of vegetable, no matter if needed or not, to make them more comestible (removing antinutritional factors, increase digestibility, to improve taste, etc.). This practice cause loss of some essential nutrients such as vitamin C, B complex vitamins (especially thiamine and riboflavin), and other thermolabile nutrients.

Grilling is one of the technics that may contribute to increase the safety of fruits and vegetables without compromising their nutritional value and - in some cases - even by increasing the nutritional quality. Some available researches on the effect of this technic have focused on the nutritional value. It has been reported that mild heat treatment can be used to increase the bioavailability of carotenoids and vitamins in green leafy vegetables by releasing bound carotenoids from the food matrix and binding proteins (NSW Food Authority, 2009) and enhances the absorption of non-haem-Fe and Zn in tubers but not in cereals and legumes (Marfo et al. 1990). Unfortunately, in poor countries, cooking is done with elevated temperatures and mild heat treatments technologies are not available. Grilling has been demonstrated to induce a significant increment of free radical scavenging capacity in different genotypes of eggplant more than does boiling (Scalzo et al. 2016). Okra is still known as a tropical vegetable having good antioxidant capacity (Gemede et al. 2015). The increase of antioxidant capacity by heat treatment is studied also in other vegetables such tomato (Kamiloglu et al. 2014) but less available are information about the effect on microbiological quality.

In our concern, the importance of increasing the bioavailability of nutrients from vegetables in poor communities is indisputable. However, since vegetables - one of the highly nutritious food category- are in the same time among the challenging vehicles of biological hazards (pathogenic bacteria, viruses, intestinal worms, etc) and related foodborne diseases, their safety at the microbiological point of view may be a precondition in food preparation methods that aim to increase their consumption especially in rural areas of developing countries.

5.1.3 Field practices to prevent macro and micronutrients deficiencies in developing countries.

There are three common strategies to improve diet preventing macro and micronutrient deficiencies:), food fortification, food supplementation and food based strategies (FBS). According to (WHO and FAO 2006) food fortification is the practice of deliberately increasing the content of essential micronutrients (vitamins and minerals, trace elements included), in a food so as to improve the nutritional quality of the food supply and to provide a public health benefit with minimal risk. As regard supplementation, it is the provision of relatively large doses of micronutrients, usually in the form of pills, capsules or syrups with optimal amount of specific one or more nutrients, in a highly absorbable form. While food fortification and supplementation aim to eliminate micronutrient deficiencies (WHO and FAO 2006), food based strategies (FBS) seek to reduce the overall malnutrition. Thus, FBS include diet diversification by of new foods (Sibhatu et al. 2015) , food preparation practices, food intakes, etc. In developing countries, diet diversification is considered to be the most important factor in providing a wide range of micronutrients better than fortification and/or supplementations that target specific micronutrient only (Tontisirin et al. 2002, WHO and FAO 2006). Thus, although supplementation and fortification, are commonly used as cost-effective and relatively easy to extent, and to deliver (Allen 2003), increasing dietary diversity means increasing both the quantity and the range of micronutrient-rich foods consumed. The relationship of diet diversity and nutritional status has been also recently confirmed by Shashikanta et al. (2016) in rural female population of India where they demonstrated that diet diversity is related to a better nutritional status. Diet diversification regards meals that are consumed at home as well as different type of foods that are eaten outside. In this last case, street foods (SF) sector seems to be among the more spread forms in developing countries. The nutrients contribution derived from SF vary according to the categories of each food item used and from country to country and from community to community according to the diverse levels of socioeconomic development. Foods items are more diversified and nutritious in countries or communities with better socioeconomic conditions. In urban areas SF include more processed foods, animal fats and proteins than rural areas (Levin et al. 1999). About differences between countries, for example it has been reported that in Haiti 146 different street foods types (Steyn et al. 2013) while in low-socioeconomic areas of Nairobi (in Kenya), 53% of street foods vendors sold one items of food only (Mwangi et al. 2002). Furthermore, while in Abekuta (Nigeria) 50 % of meat and fish and 60% of legumes have been estimated to be supplied as street foods (Steyn et al. 2013) , in Kumba (Cameroon) about 36% SF sold are only carbohydrate products (Acho-Chi 2002). Other studies have shown that snacks are among the main foods groups found in streets foods sector and other out-of-home food consumptions contributing significantly in nutrient and energy intake (Mwangi et al. 2002 and Nago et al. 2010). Other recent studies conducted in Accra (Ghana) demonstrated that when SF are mainly home-made snacks (sold by 25% of the vendors in Accra), they are dominated by starchy foods (roasted yam, fried plantain, bread, etc.) reaching more than 63% of all home-made snacks sold (FAO 2016, Ficano 2017). Furthermore, the same study reported that only about 15% of the SF consist of fruits (banana, mango, pineapple, papaya, etc.) while vegetables salads are sold only by 6% of all SF vendors. However, while coming to cooked dishes, they are dominated by animal source foods mainly fish and chicken that are sold by 60% and 35, 7% of the total SF vendors respectively (but only in towns).

In our experience also, out of-home food habits and consumptions, remain important in Garo and Kabinda villages. During our field visit and food sample collection, some of the ready to eat food samples were collected bought as street foods. In India, these foods include *Samosa* (made with wheat flour, peas, onions, potatoes, mustard oil), several variety of chips made with Jackfruits, potatoes, cassava, etc. In rural DRC, it has been easier to get sample of roasted peanuts, cassava chips and "*fritters*" called beignets. Beignets are also made almost exclusively with cassava fours (often adding sugar) or plains and fried in palm oil. All aforesaid foods are mainly carbohydrates suppliers while macronutrient's unbalance of Indian and Congolese diets is mainly caused by low intakes of proteins and fats (Robillard and Christon 1993). In our concern, interventions to empower the production and use of such type of foods may also focus the extend to rural communities. Indeed, processing and preserving technics suggested may be locally available, low-cost and safe. About the last aspect, there is a lack of studies on appropriate food processing and preservation technics reliable for poor communities of developing countries. Furthermore, research could focus on the nutritional quality of food items utilized is such a sector. Since diverse studies agree that out-of-home food may more contribute in protein and lipid, sometimes even in micronutrients intakes, all the ingredients may be locally available and the nutrient balance must be considered.

Also in this approach, more attention may be put on infant especially about complementary foods of breastfeeding. In fact, health care services in developing countries tend to stand upon the WHO's recommendation regarding the introduction of complementary breastfeeding foods only after at least 6 months of age (Kathryn and Kenneth 2003). However, many researches have shown that the introduction of different foods in the very young children is done very early, before 3 months as it has been demonstrated in Ghana where only 35% of children between 0 and 3 months are exclusively breastfed (Aidam et al. 2005) and with unsuitable foods, especially poor in proteins, lipids and micronutrients (vitamins and minerals), situation that worsen the problems of malnutrition (Ekerette and Olukemi 2016). According to some researchers, among the other causes of early introduction of the different foods in the very young children before the recommended age are the fact that mothers employment, poverty and consequent low-income, lack of education of mothers, etc. (Alzaheb 2016). As regard the RD Congo one of the areas of interest for us, recent studies have demonstrated that locally made complementary foods (if carefully formulated) may contribute to alleviate the aforesaid problems substituting common commercial ones while obtaining the same results (Bisimwa et al. 2012). Also in our rural working context in developing countries the aforesaid hypothesis- using locally available nutrients dense foods - especially in the way to improve energy and nutrients intakes of children coupled with hygiene and nutrition education of mothers - may be suitable verified with the aim of contributing to better child growth and general conditions of their nutritional status and health.

5.2 Objectives

The objectives of this chapter have been:

- evaluate the use of domestic water filters and solar water disinfection (SODIS) to reduce waterborne diseases in rural India area (filters) and DR Congo (SODIS);
- development and use of a macronutrient balanced flour for children to improve complementary feeding of breastfed babies of Kabinda (D.R. Congo);
- Evaluate the possibility to produce a snack also suitable to improve the diet of schooling children of rural areas in developing countries;
- to verify the effectiveness of grilling in the reduction of pathogenic bacteria while preserving the content of vitamins and sensory quality in tomato, zucchini, aubergines and okra to improve the diet of the rural populations of developing countries.

5.3 Material and methods

Use of domestic water filters in rural India-Ceramic candle filters (made in India) were distributed to 136 families in the rural area of Darenchigre. After 6 months, two types of questionnaires were administered, one in 100 families with filters (FF) and another to as many families without filters (FWF). The compilation of the questionnaires was done by experts going from house to house. The information required, in addition to those related to family members, concerned the origin of water, the use of filtered water or not and the type of health problems observed whithin the families. The questionnaires were given twice: the first time for all two family groups (still without filters) to identify the health problems in the six months before the use of filters; the second time after six months only to the FF families group who had used the filters. Summary statistics such as the average, maximum, minimum, and standard deviation of household members and the frequency (%) of the different health problems in controlled families have been calculated.

Solar water disinfection-A plastic container of 20 liters having an ermetic closure has been used to transport and storage the water, visibly clear but supposed to be microbiologically contaminated, from an open canal in the San Bonico area of Piacenza (Italy). The choose of this place was motivate by the need to simulate a contaminated water as may be in any congolese river. SODIS was applied the following day using transparent PET bottles that were sealed and sterile until the day of treatment. A becker was used to fill PET bottles. 5 bottles were filled and after about ¾ of the filling were vigorousely shaked (to better oxygenate them) and then were completely filled and tightly closed. After the bottles were hermetically sealed, they were put on a stainless steel reflective surface exposed to the sun in the South direction. One liter (1000 ml) of water was used as control sample (without SODIS treatment) for microbiological analysis that has been performed according to the official method for microbiological quality of drinking water in Europe (CE 1998). In one of the 5 bottles exposed to the sun, a temperature sensing probe was inserted. Exposure to the sun was conducted for a total of 7 hours and 30 minutes; then 5 bottles of water treated with SODIS technic were microbiologically analyzed.

Development and use of a macronutrient balanced flour for children complementary feeding-The flour has been developed using 3 ingredients that are corn, peanuts and soy that are easily accessible on the market in the pilot centre area. The raw material was appropriately selected verifying, with visual appreciation, the absence of signs of development of fungi or other strange material, adequate drying, etc. The ingredients were grinded separately. Soy has been always toasted before the grinding while corn and peanuts have not undergone any pre-treatment before grinding. The 3 types of flour obtained were stored in a dry and cool place. The mixture was formulated by putting together 5g of soybean and 5g of peanuts. We called the mixture of these two ingredient "***protein core***" (PC). To these 10 grams of PC, 20 g of corn flour was added, thus obtaining a unique mixture of 30 g made of PC and corn. Subsequently, 40 children aged between 2 and 3 months to whom mothers were giving foufou as a substitute for breastmilk during the absence of mothers (working for long hours far from home) have been randomly selected. In all the 40 children, weight and health information was reported every two weeks during a period of 3 months. Only 20 - as "treated" sample - received the mixture (30 g per day by child). The cooking of the mixture was done after an addition of sufficient amount of clean water visually estimated. The cooking time varied from 10 to 15 minutes depending on the quality of the fire, mixing everything and without adding anything else (without salt nor sugar) and then given to the baby with a spoon.

Development and preservation of a snack made with local ingredients-Peanuts roasted with their shells, maize and plantain have been used as raw material. Peanuts have been shelled before making the different treatments for preservation study. Maize and palm oil (most accessible in the rural area of interest) have been used to make popcorn using a closed pot. The plantain raw bananas have been peeled, washed and cut in to have 2 mm of thickness for each piece. Then, they have been rinsed and fried in palm oil for about 3-5 minutes. All of three foods have been preserved in two different modalities of packaging: a) keeping in small packages after using household vacuum food packaging machine (Va) and b) kept in the same type of small packages sealed without vacuum packaging (NVa). In addition, but only for plantain, in small earthenware with cover and kept at room temperature(RT). Two temperature conditions have been considered: a room temperature that varied between 20 and 22 °C, and controlled temperature of 28-30 ° C. Five conservation times have been considered in the evaluation of shelf-life of each food: 15, 30, 45, 75 and 105 days. To assess the hygienic conditions of the entire process, the general microbiological quality evaluation has been carried out through laboratory analysis on water activity (a_w), total microbial charge to evaluate the possibility for bacteria to grow and the presence or absence of yeasts and moulds in colony forming unit per gram (ufc/g) of each product. Lipids stability has been evaluated. After an adequate extraction, we proceeded with *Folch method* and subsequently with the determination of the peroxides number (indicator of lipids level of oxidation). In addition to the general microbiological quality control of these 3 foods intentionally considered as ingredients to make nutrient balanced snack, water content (%) and macronutrients (carbohydrates, protein, lipids) have been determined and expressed as % on dry mater.

Grilling of tropical vegetables - Four categories of vegetables (tomatoes, zucchini, aubergines and okra), normally used well cooked in the diets of the rural peoples of Darenchigre (India) and Kabinda (DRC) were used to carry out a process of moderate grilling. The vegetables have been chosen ensuring that they have the same degree of maturation to make as uniform as possible the concentration of antioxidants and vitamins among the different samples of the same matrix. For the tomato, it was attempted to select those with an appropriate ripeness to be cut, and suitably inserted in a commercial grid modified for this research. *Charcoal Companion model grid* was used, but modified fixing it on an iron steak auxiliary that makes it vertical so that the slices of vegetables inserted therein may remain fixed during grilling on side of a "three stones cooking fire" usually used to cook rice or foufou (figure 5.1)

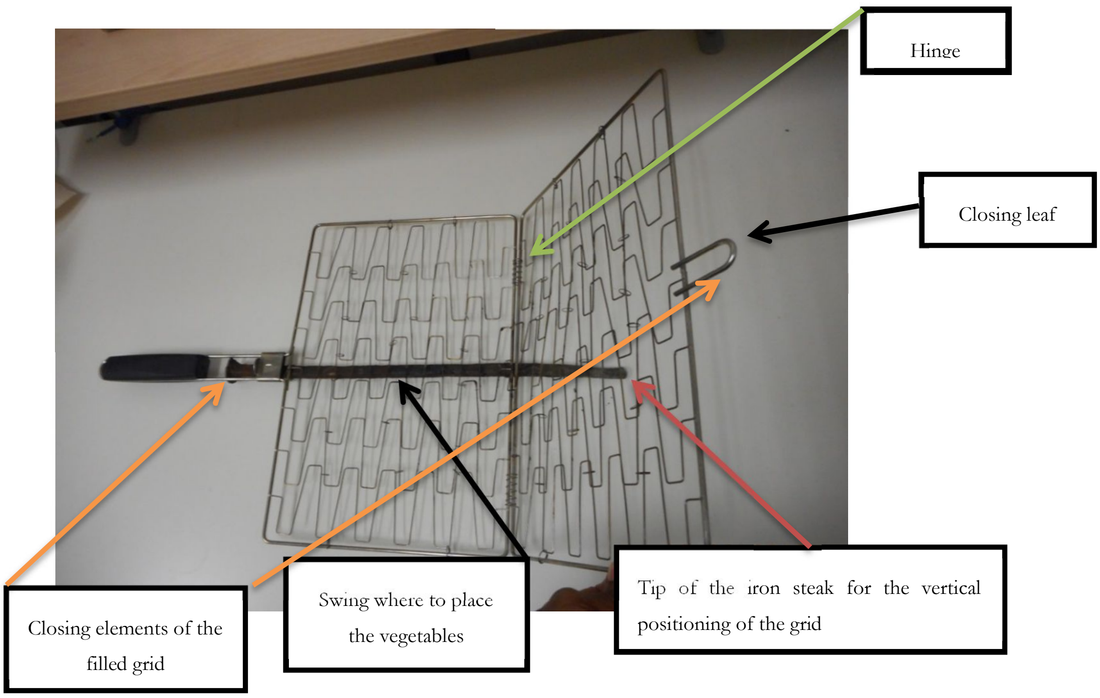


Figure 5.1 Modified grid with an additional iron steak to fix it in front of the three-stone fire

The three-stones fire was used to simulate the conditions of the most popular cooking fire places used in India and especially in DRC. Firewood were used as fuel. Using a stainless-steel kitchen knife and a plastic cutting board, the different vegetables were sliced to have as homogeneous thickness as possible for each category of vegetables.

To ensure the presence of pathogenic bacteria, an artificial contamination have been performed. Firstly, a simulation of local (contaminated) water (previously analyzed) has been performed washing the vegetables with water from an open canal to make sure that the vegetables were microbiologically contaminated. The magnitude microbiological contamination of the water used in this preliminary test was later determined. Systematic microbiological efficiency of the grilling was subsequently carried out. Artificial standardized contamination of the vegetables has been performed before the grilling. Thus, standard concentration of *E. coli and Enterococcus faecium* strains where used to obtain the challenging bacteria contamination. The preparation of the culture inoculum was performed as previously described by Vescovo et al. (2006). Active cultures of single *E. coli* strains, grown in 12 hours at 30°C and *Enterococcus faecium* grown in 24 hours at 37° and centrifuged at 3000 rpm for 10 min to obtain cultures' pellets. The pellets were subsequently washed in sterile saline solution (0.8%), centrifuged and resuspended in the same solution to reach a cell density of 6 log colony forming unit per ml (cfu /ml) for both bacteria groups. The suspensions were immediately used to inoculate the experimental vegetables. All the vegetables types were sprayed with the pathogenic bacterial suspensions before cutting them. Furthermore, worst-case scenario was performed by spraying the pathogens on internal slices of eggplant that has a spongy structure which facilitates microbial penetration and consequently able to hold more bacteria than the other vegetables used in this study. The contaminated vegetables were subsequently sliced as described above and loaded into the grill one category at a time to make grilling homogeneous. The completion of the grilling was appreciated observationally as it is done in normal food preparation, but a stopwatch has been used to check the time the exposure of both sides of the grid. The samples were collected for microbiological analysis and antioxidant capacity control. Microbiological analysis was performed in the microbiological laboratory of the Faculty of agriculture food and environmental sciences of "*Università Cattolica del Sacro Cuore*" of Piacenza in accordance with microbiological criteria as established by the regulation on foodstuffs (EC 2005). Microbiological quality of the vegetables has been controlled before (control samples) and after (treated samples) each heat treatment (grilling). Total charge of Enterobacteriaceae (TE) and total charge of microorganisms (TM) were determined.

Total polyphenols content and 2,2- diphenyl-1-picrylhydrazyl (DPPH) determination

a) Total polyphenols

After the extraction of polyphenols, the solution was analyzed using the PerkinElmer (Ontario, Canada) Lambda 12 spectrometer at a wavelength of 765 nm. Considering the previously constructed calibration curve, the polyphenols content was obtained through the ratio of 0.001443, as follows:

$$mg_{\ (gallic\ acid)}/100\ \mathrm{g} = A\ (765\ nm) * 5/0.001443$$

b) Antioxidant activity (DPPH)

After the solubilization of the samples in the ethanol, 2 mL were added of 2 mL of DPPH solution were added at 0.1 mol / L in 96% ethanol; after inserting it into instant readout cuvets of the UV (λ = 517 nm) by subsequent absorbance readings for at least two hours with cadence to scale up to a constant absorbance value. From the absorbance A value of DPPH (2,2-diphenyl-1-picrylhydrazyl) in equilibrium control, the AAC index is calculated with respect to the sample absorbance value at equilibrium (eq) and at the time zero (t_0).

$$\text{Cellurar Antioxydant Activity (CAA)} =$$

$$100 * (\text{A sample}^{eq} - \text{A control}^{eq}) \div (\text{A controlo}^{t0} - \text{A control}^{eq})$$

If the sample contains an antioxidant, it degrades the colored radical, generating the colorless non-radical species. Then the absorbance drop is proportional to the radical scavenging power of the sample.

Sensory analysis-Sensory analysis has been performed with a trained group of 8 panellists (6 males and 2 females). Obviously, here in used vegetables where not intentionally contaminated. The evaluation has been carried out in DR Congo and all the recruited panellists where Congolese. After the preparation of the all the vegetables, it was asked to each group member to mark his/her appreciation or not on an evaluation schedule. Sensory analysis of zucchini in RD Congo has not been done due to its absence in the horticultural products of the pilot centre. The non-application of this technique in India is attributable only to our impossibility to get the grid reasonable times to run the experiment within deadlines scheduled within this study. In addition, this sensory analysis has been simultaneously done on grilled foufou. Four main sensory attributes have been evaluated. These attributes were the appearance, olfactory intensity, taste and general appreciation. The appreciation used three levels of appreciation classified as unpleasant, pleasant or very pleasant.

5.4 Results and discussions

5.4.1 Results of the use of domestic water filters in a rural population of India

The results comparing the health outcomes responses of two population groups before and after six months one using water filters (FF) another - the control group - (FWF) are showns in the figures 5.2 and 5.3. Analysis of the questionnaires, revealed that the FF (91 responses) and FWF (94 responses) were grouped in the same water supply conditions. The average number of family members was 6 ± 2 for both FF and FWF with a minimum of 2 and a maximum of respectively 10 and 12. The source of the drinking water was the open well (for 99% of the FF and the 100% of the FWF), known to be easily subject to faecal contamination (Mckenzie and Ray 2005). In FF, the filtered water was used by all members and almost exclusively for drinking, one family of all FF said also use it for cooking. Major health problems reported before the use of the filters were found to be similar in both groups of families and dominated by diarrhea (51% of FF and 28% of the FWF) and in any case by abdominal pain (47% of FF and 45% of the FWF). With the use of the filters, the families who have declared to have no health problems has increased significantly. There was also a nearly-disappearance of diarrhea and abdominal pain dropped respectively from 51% and 47% to only 1%. Problems related to the general malaise, jaundice, fever and headaches disappeared completely even if they were declared to be infrequent (1%).

It is interesting to observe that FF have declared a higher presence of diarrhea and abdominal pain with reference to the period before the use of the filters.Without excluding that in the two periods the water contamination was different, it is possible that the remembrance of the problems were amplified in who subsequently were free of these problems (FF group). These results are consistent with those obtained by other authors, where it is stated that interventions to improve the microbiological quality of drinking water are effective in the prevention of diarrhea, for both adults and children (Clasen et al. 2006). In addition, a meta-analysis of 976 papers, has shown that among all entries by home improvement of drinking water quality, filtration provides more consistent results, more effective and more sustainable in the prevention of the above mentioned health problems (Clasen et al. 2006). Still to understand why 15-27% of households without filters did not report health problems (do they have better located wells?).

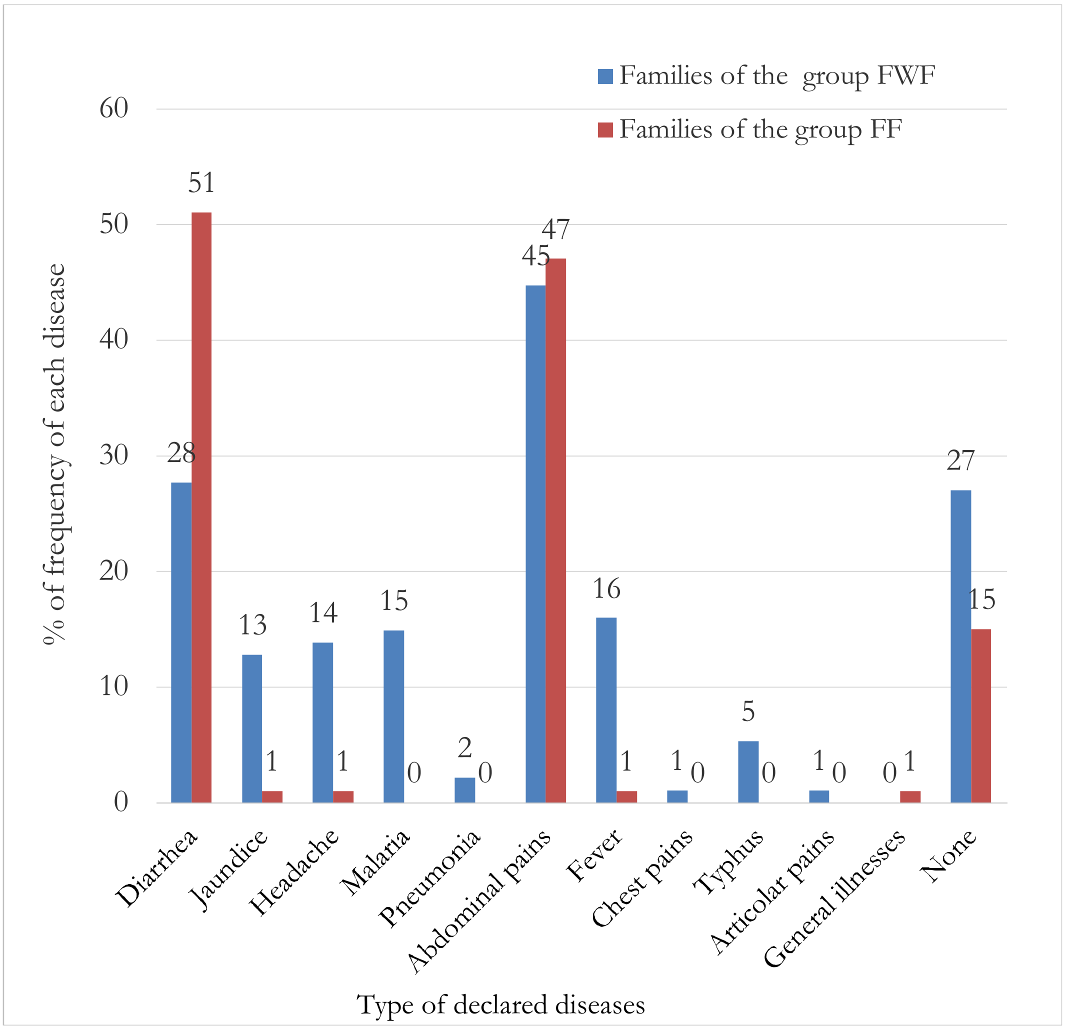


Figure 5.2 Frequency of each disease in both FWF and FF groups of families in the absence of filters (6 months, period before filter adoption in FF group)

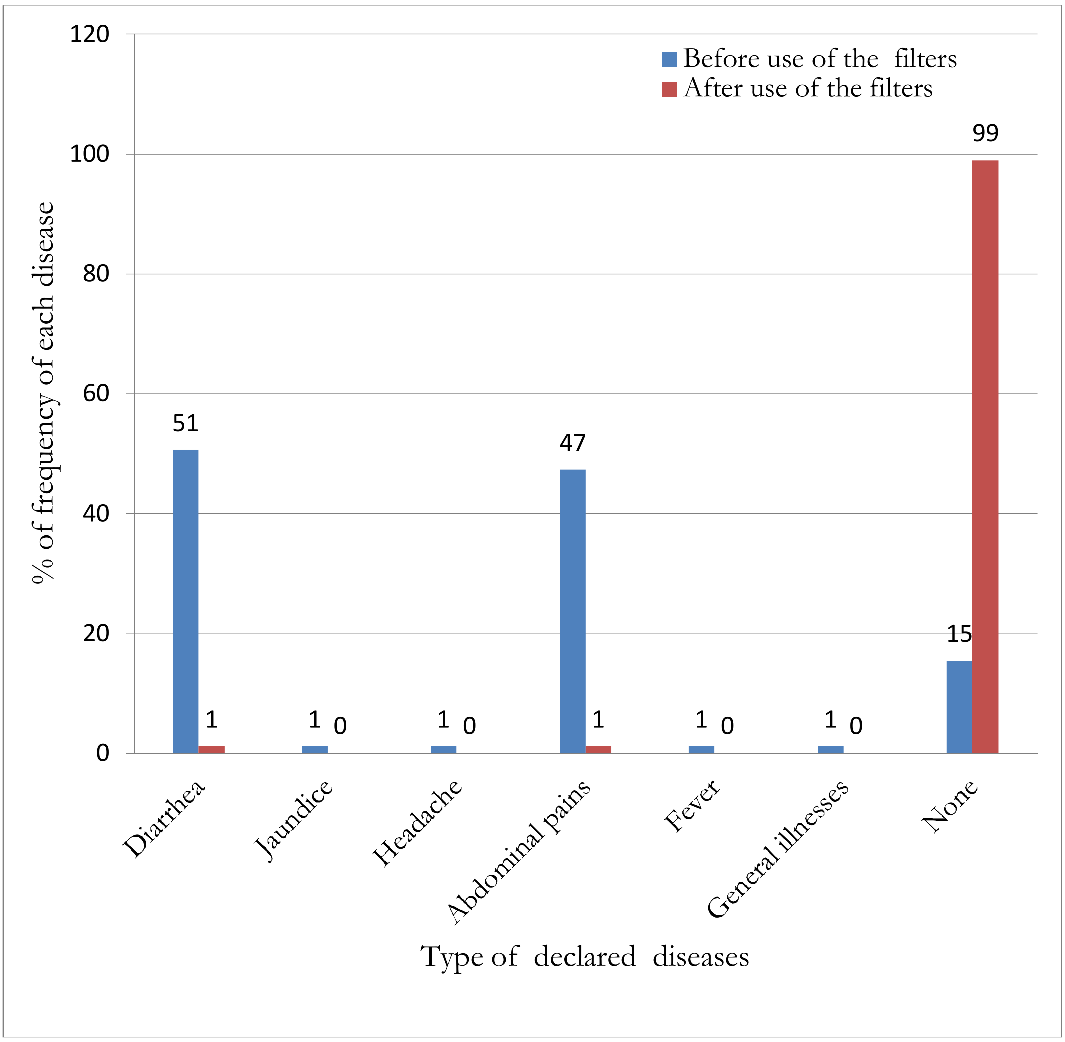


Figure 5.3 Comparison of the frequency (%) of diseases in the group of families that used the filters before and afters their utilization.

5.4.2 Solar water disinfection as household water sanitation in rural R D Congo

The results of bacterial concentration before (control) and after solar water disinfection within 5 PET-bottles using water from an open canal are shown in the table 5.1. From that table it can be observed that coliforms and enterococci bacteria, the direct fecal contamination markers of water have undergone a significant reduction as known in different literature (Wegelin et al. 1994 and McGuigan et al. 2012). The application of SODIS have been more effective for the enterococci where the limits allowed by law for drinking have been reached (roughly) while in the other bacterial groups the concentration remainded higher than limits (CE 1998). This may be caused by the very high initial challenging contamination suggesting that SODIS may be applied accuratly according the real water source used for drink within a given community.

Table 5.1 Bacterial concentration before (control) and after solar water disinfection within 5 PET-bottles using wateter from an open canal

Bacterial groups	Control	After SODIS application in 5 replicas				
		1	2	3	4	5
Total coliforms bacteria	630/ml	1/ml	1/ml	33/100ml	30/100ml	40/100ml
Enterococcus bacteria	14/ml	1/100ml	1/100ml	0/100ml	0/100ml	0/100ml
Total bacteria charge 22°C	50 000/ml	7000/ml	10000/ml	500/1ml	900/1ml	1800/1ml
Total bacteria charge 37°C	53 000/ml	20000/ml	200000/ml	3300/ml	3000/1ml	2800/1ml

Table 5.2 Allowed bacterial limits for drinking water according to European legislation (CE 1998)

Bacteria groups	Allowed limits (cfu/100ml)
Total coliforms bacteria	0/100ml
Enterococcus bacteria	0/100ml
Total bacteria charge 22°C	0/100ml
Total bacteria charge 37°C	0/100ml

5.4.3 Use of a macronutrient balanced flour for children complementary feeding in R D Congo.

Data comparing the weight gain of 20 children that have received the complementary food (CF) for infant made with maize, soybean and peanut flours against other 20 who have not received this meal for 3 months of the "treatment"(from 4^{th} to 6^{th} months of the breastfeeding) are shown in figure 5.4. Children who consumed the CF (tratt) had an increase of 1.6±0.7 kg compared to 1.4±0.6 kg of those who of the "control" group (CTR).

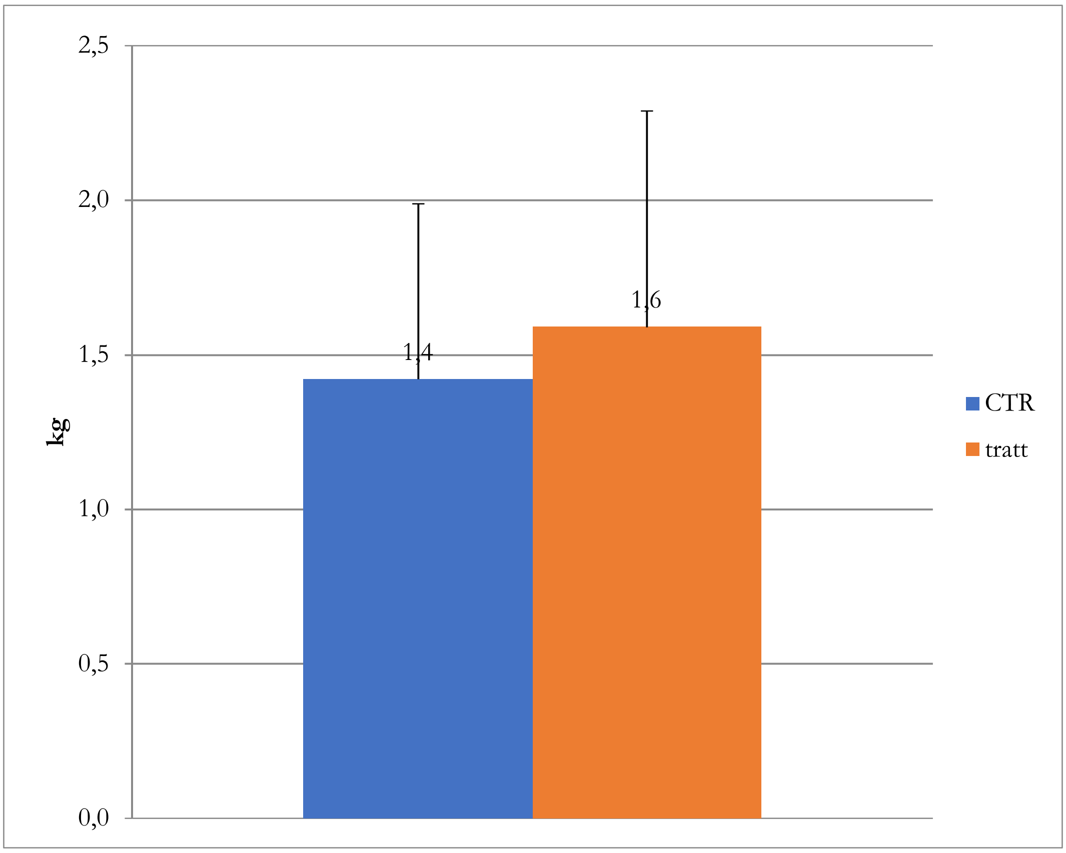


Figure 5.4 Comparison of total weight gain (kg) between children fed with the Complementary Food (tratt) vs the control group (CTR)

The difference of weight gain between the children who received the CF and the children of the control group resulted statistically relevant ($p < 0.01$). The use of locally available complementary food to improve nutritional status of children in rural DR Congo have been demonstrated to be useful even by previous researches (Bisimwa et al. 2012). Although World Health Organization (WHO) suggests the exclusive breastfeeding at least until 6th month (WHO 2001), our results showed that appropriate complement of breastfeeding in the age between 3rd and 6th months is necessary for better growth of children in those conditions where mothers cannot adequately breastfeed for several reasons, especially work shortly after delivery and work for long hours (Kimani-Murage et al. 2015). However, any other research is needed to evaluate the integration to macronutrients balanced complement with micronutrients, especially mineral microelements and vitamins (possibly with local foods) combined with mothers' education on other topic such food and drinking water hygiene.

5.4.4 Development and preservation of local ingredients that may be used to produce a homemade snack in developing countries

Water activity and the presence microbes on the food items-Water activity (aw) and the presence of the different microorganisms (total bacteria, moulds and yeasts) in plantain chips resulted very variable according the different conservation times (CT) and the type of packaging as shown in tables from 5.3 to 5.5. From those data, it evident that the more challenging food item to preserve is plantain chips since it has an initial high activity water (0.83) as shown in table 5.3. In addition, the aw remained high and sometimes increased up above 0.90 known to be generally enough for growth of bacterial and fungal microorganisms (Ntuli et al 2017). Thus, without any other antimicrobial treatment, that aw is suitable for the development of various microorganisms including those for our interest. In fact, the lowest aw at which growth of bacteria has been reported is 0.75 while fungal microorganisms (yeasts and moulds in our case) are more tolerant and can growth even at levels as low as 0.61(Ntuli et al. 2017). The high aw occurred both in vacuum and without vacuum plastic packaging and, sometimes, is confirmed by the microbial growth especially moulds and bacteria (table 5.3). The activity water in peanuts and popcorn (shown in table 5.4) is always too low for microbial growth. This result is confirmed by the absence of growth of bacteria, moulds and yeasts. In fact, only few increase from the initial 10 cfu/g occurred but only in popcorn (table 5.5). As regard the samples preserved in earthenware recipients, they yield the best results about the presence of microorganism in different conservation period. However, higher bacteria presence has been observed at 45th day till 2x105 cfu/g. Since in all other 4 earthenware recipients (including that open at 105th day) there are very low number of cfu/g, the deterioration of that sample could be attributed to different causes including eventual defect of the recipient but not to the process.

Table 5.3 Variation of activity water (aw) and the presence total bacteria, moulds and yeasts in plantain chips within the different preservation technics: vacuum packaging, without vacuum packaging and in earthenware and stored at controlled 30°C and at room temperature (RT) for prolonged period of conservation time (CT).

Activity water (aw)

CT	No vacuum packaging		Vacuum packaging		earthenware	
	RT	30°C	RT	30°C	RT	30°C
0	0.83	0.83	0.83	0.83	0.83	0.83
15	0.91	0.65	0.93	0.60	0.51	0.48
30	0.97	0.97	0.94	0.94	0.53	0.7
45	0.88	0.83	0.96	0.93	0.43	0.54
75	0.92	0.97	0.88	0.62	0.34	0.53
105	0.47	0.59	0.48	0.66	0.34	0.61

Moulds (cfu/g)

CT	No vacuum packaging		Vacuum packaging		earthenware	
	RT	30°C	RT	30°C	RT	30°C
0	10	10	10	10	10	10
15	$4.8x10^5$	10	$3.7x10^5$	30	10	10
30	10	10^5	$1.7x10^5$	$3.8x10^4$	20	10
45	$6x10^4$	$1.8x10^4$	40	$9.8x10^5$	10	10
75	$6x10^4$	200	$4x10^5$	$3x10^4$	10	10
105	30	20	30	$1.8x10^2$	100	30

Total Bacteria (cfu/g)

CT	No vacuum packaging		Vacuum packaging		earthenware	
	RT	30°C	RT	30°C	RT	30°C
0	30	30	30	30	30	30
15	$4x10^5$	20	3.10^5	50	10	10
30	$6.6x10^4$	$4x10^5$	$1.6x10^4$	$4x10^4$	50	220
45	$6x10^4$	$2x10^4$	$3.1x10^4$	10^6	$2x10^5$	$1.8x10^2$
75	$1.8x10^5$	$4.7x10^5$	$4.5x10^5$	$3.2x10^4$	$3x10^3$	$2.4x10^2$
105	90	30	30	$2x10^3$	300	250

Yeasts (cfu/g)

CT	No vacuum packaging		Vacuum packaging		earthenware	
	RT	30°C	RT	30°C	RT	30°C
0	10	10	10	10	10	10
15	10	10	10	10	10	10
30	$4.5x10^4$	$1.5x10^5$	$5.5x10^4$	10	40	150
45	10	10	$8.1x10^2$	10	$8x10^4$	50
75	$5x10^4$	$8x10^4$	$2.8x10^4$	$2x10^3$	$2.5x10^2$	$1.8x10^2$
105	10	10	10	10	80	190

Table 5.4 Variation of activity water (aw) in peanuts and popcorn during the different conservation times (CT) with or without vacuum packaging at room temperature (RT) and at controlled temperature of 30°C

CT	Peanuts				Popcorn			
	No vacuum packaging		Vacuum packaging		No vacuum packaging		Vacuum packaging	
	RT	30°C	RT	30°C	RT	30°C	RT	30°C
0	0.49	0.49	0.49	0.49	0.08	0.08	0.08	0.08
15	0.48	0.47	0.48	0.45	0.3	0.20	0.12	0.21
30	0.47	0.54	0.47	0.51	0.23	0.30	0.30	0.37
45	0.47	0.56	0.47	0.52	0.30	0.38	0.29	0.40
75	0.38	0.48	0.41	0.48	0.29	0.39	0.31	0.41
105	0.35	0.51	0.34	0.50	0.30	0.45	0.32	0.45

Table 5.5 Presence total bacteria, moulds and yeasts in popcorn during the different conservation times (CT) at room or at controlled (30°C) temperature with or without vacuum packaging.

CT	No vacuum packaging		Vacuum packaging	
	RT	30°C	RT	30°C
0	10	10	10	10
15	20	20	20	20
30	10	10	40	10
45	20	20	10	10
75	10	10	10	10
105	10	10	10	10

In general, the above illustrated data permit a comparison of the different preservation technics, the earthenware has the best results because the lowest aw is accompanied with the lowest microorganism's growth during all the 5 conservation times of preservation (figure 5.4). This result - likely due to the water passage (loss) throughout the "terra-cotta" wall - is encouraging since terra-cotta is an easily available utensil in developing countries. Our findings suggest that earthenware could be used as household preservation of homemade plantain chips at least for 3 months without evident foodborne diseases risks. However, further research is necessary to establish the presence or not of specific pathogenic bacteria that could growth on such foods.

Oxidation of lipids-The different food items had 73.2, 2.64 and 0 of initial peroxide numbers (PN) expressed in milliequivalents of active oxygen by kilogram of lipids (meq

/kg) from peanuts, plantains chips and popcorn. The various peroxide values in the different storage times, ways of packaging and temperature conditions of preservation are shown in the table 5.6. Lipids contained in the plantain and popcorn did not show any risk for all 5 storage times in diverse ways of packaging and temperature conditions. Plantain and popcorn in fact, have had always peroxide numbers lower than the critical limit of 20 (meq) of active oxygen per kg of lipids. While for the popcorn, the low lipid content contributes to the result, for plantain, the type of palm fats, saturated exclusively can be the main reason. On the contrary, all the peanut samples had values above the permissible limit values (80-100 meq / kg). This last result is probably due to a long-time storage of the peanuts (although roasted within their shells) in the food shop before our trials. Given that this is due to the high content of lipids and in turn rich in polyunsaturated fatty acids. Nevertheless, for peanuts, further trials are necessary with better source as the original material of the present one was already altered with values of 73 meq / kg of lipids.

Table 5.6 Peroxide number (PN) in meq of active O_2 /kg of plantain chips, peanuts and popcorn in the different conservation times (CT) in days, without vacuum (NVa) and with vacuum (Va) in room temperature (RT) and in controlled temperature of 30 ° C.

	Plantain chips						Peanuts				Popcorn			
CT	NVa		Va		Earthenware		NVa		Va		Nva		Va	
	RT	30°C	RT	30°C	RT	30°C	RT	30°C	RT	30°C	RT	30°C	RT	30°C
15	4.2	0.84	2.36	3.4	1.2	1.18	76.28	71.03	99.05	93.19	0.16	0	18.92	19.09
30	2.6	0.93	3.84	0.96	1.29	0.48	62.87	64.37	44.45	64.25	0	0	0	0
45	2.06	1.56	2.04	0.88	0.66	1.07	55.47	56.04	62.79	51.11	0	0	0	0
75	1.23	1.45	3.35	4.86	3.03	4.28	88.72	119.3	71.84	105.9	0	2.93	0	2.47
105	0.8	0	0	1.4	3.6	3.2	85.9	151.1	69.6	73.2	0	0	0	6.6

Chemical-nutritional values of peanuts, plantains' chips and popcorn used-As previously mentioned, the objective of these preservation study was to verify if the 3 food items may be used as ingredients to produce homemade snack that could contribute to improve diet in rural areas of developing countries especially for schooling children. Chemical-nutritional values of the different ingredients are reported in table 5.7 expressed in percentage of macronutrient on dry mater (DM). As expected, the peanuts used in this study have good amount of proteins (19%) and much lipids (39%) that make it a good ingredient to formulate a macronutrient well-balanced snack. In fact, the other two ingredients are mainly starchy foods although making popcorn and chips increase their content in lipids. The combination of these 3 ingredients may allow to produce a good snack also thanks to the well-known good sensorial quality of peanuts. Other trials are necessary to develop a snack with appropriate combination of these 3 ingredients in one "commercial" package considering their different costs.

Table 5.7 Chemical-nutritional values of the studied peanuts, plantains' chips and popcorn with proteins, lipids and starch expressed in percentage on dry mater.

Ingredient	Water	Proteins	Lipids	Starch
Peanuts	2	19	39	5
Plantain chips	7	3	26	62
Popcorn	10	12	5	60

5.4.5 Effect of grilling on pathogenic bacteria and on the content of vitamins and sensory quality in tomato, zucchini, aubergines and okra to improve the diet of the rural populations of developing countries

Visible appreciable aspect of grilling was obtained after an average of time exposure of 10 minutes for aubergines and okra, 4 for zucchini and 5 minutes for tomato (half by side of the grid with frequent changing the sides during the grilling).

Microbiological results-Microbiological water quality used in the preliminary trials of simulation are shown in the table 5.8. These results show that the surface water used in the simulation of the local condition had cfu concentration higher than limit admitted (0 cfu /100 ml) for clean water for all controlled groups of bacteria. This result ensured us that water used and therefore the vegetables washed with it were contaminated. The total charge of enterobacterial groups (TE) and the total charge of microorganisms (TM) before and after the grilling are shown in table 5.9. The bacterial charges of both TE and TM in the control group were highly out of allowed limits precooked vegetables. Contrary both TM and TE bacterial charges results admissible for these types of foodstuffs according to European microbiological criteria (EC 2005). These preliminary trials yield the best results on TE represented by *E. coli* with 5 cfu/g of grilled vegetables while the European limits are 100 and 1000 cfu/g as minimum and maximum limits respectively. Also, the presence of spores has been drastically reduced. Results of the two abovementioned pathogenic bacteria groups and spores on aubergine, okra, tomato and zucchini are reported in table 5.10. In general, the obtained results are comparable to those illustrated above from the sample made with the mix of the 4 vegetables. However, some differences occurred. The lower microbial reduction is observed in okra with TE ($2x10^3$ cfu/g) slightly above the acceptable values (10^3cfu /g). This outcome may be justified by the presence of external hairs in okra that hold bacteria more than in the other 3 types of vegetables causing an inhomogeneous thermal death. Another presence of relatively high number of cfu is observed in zucchini but it less worrying because TM bacteria include also non-pathogenic strains.

Table 5.8 Microbiological quality of the water used to simulate the local conditions

Bacterial groups	Unit	Values
Enterobacteriaceae	(cfu/100ml)	$5.6x10^4$
Steptococcus	(cfu/100ml)	50
Total microrganisms (36°C)	(cfu/ml)	$1.4x10^4$
Total microbial charge at 22 °C	(cfu/ml)	$1.5x10^4$

Table 5.9 Average values (cfu/g) of preliminary tests on the 4 vegetables (tomato, okra, zucchini and aubergine) where microbiological analysis has been done on mixed samples

Replicas	Control			Grilled		
2	TE	TM	Spores	TE	TM	Spores
2	$3.5x10^5$	$1.5x10^7$	$1.5x10^2$	5	$2.8x10^2$	6

The preliminary test leaded as to deepen our study on the microbiological quality of the grilled vegetables by standardizing the contamination through known concentration of pathogenic bacteria strains to obtain the challenging contamination. The average levels of colony forming units (cfu) of the standardized artificial contamination in the challenge test are reported in table 5.10. The pathogenic bacteria strains used have been *E. coli* and *Enterococcus faecium*. We used these strains because they are known to be among the main indicators of microbiological contamination that occur during food processes and manipulation (McGowan et al. 2006; Mritunjay and Kumar 2017) and cause of foodborne diseases especially in developing countries (Grace 2015a). We performed the challenging test on aubergines and okra because they resulted most challenging in the previous test using contaminated water (table 4.9). We simulated the worst-case scenario by spraying the pathogens on internal slices of eggplant that has a spongy structure which facilitates microbial penetration and consequently able to hold more bacteria than the other vegetables used in this study. The results in table 5.10 show that there is a good homogeneity of contamination for each bacterial strain to allow comparison after the grilling. The results on concentration of the two pathogens before and after the grilling of okra and aubergine are shown in table 5.11. It is evident from that table that both *E. coli and Enterococcus faecium* presence have been drastically reduced and fulfil the above reported European limits for precooked fruits and vegetables excepted for the worst-case scenario represented by the internal aubergine slices.

Table 5.10 Average values of colony forming units (cfu) of the Enterobacteriaceae, total microorganisms and sporogenic bacteria groups of the test on 4 vegetables (tomato, Okra, zucchini and aubergine) where control samples have been obtained spraying on the vegetables contaminated water from an open canal.

Vegetable	Replicas	Control			Grilled		
		TE	T M	Spores	TE	T M	Spores
Aubergine	2	$6x10^3$	$1.5x10^4$	10	10	$2x10^2$	$4x10^2$
Okra	2	$9x10^3$	$1x10^6$	$1x10^2$	$4x10^2$	$2x10^3$	70
Tomato	2	$2x10^3$	$2.5x10^4$	10	5	$2x10^2$	5
Zucchini	2	$1.6x10^3$	$3.6x10^4$	25	10	$2x10^3$	$3x10^2$

Table 5.11 Average levels of colony forming units (cfu) of the standardized contamination in the challenge test using E. coli and Enterococcus strains

Vegetables	Replicas	E. coli	Enterococcus
Aubergine external slices	4	$4.8x10^4$	$1.5x10^6$
Aubergine internal slices	4	$1.2x10^5$	$1.9x10^6$
Okra whole fruit	4	$3.8x10^5$	$4.8x10^6$

Effect of the grilling on total polyphenols (polyp) and on the antioxidant capacity (DDPH)- Polyphenols are one of the major groups of food compounds acting as antioxidants. The results of total polyphenols (polyp) and the antioxidant capacity (DPPH) respectively expressed in milligram of Galic Acid equivalent by 100 g of the analyte (mg

GAE/100 g) and in micromoles of Trolox equivalent (µM trolox eq.) are reported in table 5.12. The data of table 5.12 are expressed calculated considering the different vegetables as fed (not on dry matter). We can observe that in general, the grilling has significantly increased both total polyphenols content and antioxidant capacity ($p < 0.01$). Similar results have found also by Lo Scalzo et al. (2010) and Kamiloglu et al. (2014). However, in the aubergine the increase of total polyphenols has been accompanied by the reduction of antioxidant capacity ($p < 0.01$), contrary for other herein studied vegetables and what is reported in some literature (Lo Scalzo et al. 2010). The low increase of polyphenols in tomato may be explained by the fact that the Naringenin chalcone known to be the main content of tomatoes' polyphenols as supported by Martí and Cebolla-Cornejo (2016), has been demonstrated by Slimestad and Verheul (2011) to be largely degraded during any heat treatment. Comparing data expressed on samples as fed and data on dry matter (DM), some parallelism occurred. In fact, the absolute values of total polyphenols concentration and antioxidant activity are always lower in control (not grilled) samples regardless they are expressed on DM or not. (figures 5.5 to 5.8). Several reasons could explain this increase. Firstly, in the case of data expressed on samples as fed, grilling leads to higher dry matter (DM) by water evaporation and consequently higher polyphenols concentration (figure 5.5). Obviously, absolute values are higher when expressed on DM of each sample (graph 7); at least about five times higher because of the higher water content in fresh vegetables. Secondary, heat treatment augments the efficiency of the extraction of different phenolic compounds (e.g. chlorogenic acid in aubergine) as illustrated by Lo Scalzo et al. (2016), partially justifying the higher values of antioxidant capacity (figures 5.6 and 5.8). Both latter reasons are in the favor of the significant increase of polyphenols and antioxidant capacity observed in grilled vegetables of shown in table 5.11 as illustrated above. Our results are comparable to those found in literature (Chumyam et al. 2013 and Scalzo et al. 2016) demonstrating a high correlation between phenolic compounds concentration and antioxidant capacity determined with DPPH. Nevertheless, polyphenols content differs according to different thermal stability of some specific compounds (Lo Scalzo et al. 2016) and differently in different fruits (or vegetables) (Lingua et al. 2016). Some causes of the increase the antioxidant capacity reported in literature state that as antioxidant compounds in plants are mainly present as covalently bound forms with insoluble polymers, is therefore possible that heat disrupts the cell wall and releases antioxidant compounds, leading to an increase in antioxidant capacity (Choi et al. 2006). In addition, polyphenols have been reported to be the main contributors of antioxidant capacity in food plants (Velioglu et al. 1998). This confirms the aforesaid high correlation between polyphenols content and antioxidant capacity, again in accordance with our results. We can conclude that such grilling technic preserve some nutritional values of vegetables and - as it has been illustrated in paragraphs above - contributing in prevention of foodborne diseases as well.

Table 5.12 Total polyphenols (polyp) and the antioxidant capacity (DPPH) respectively expressed in milligram of Galic Acid equivalent by 100 g of the analyte (mg GAE/100 g) and in micromoles of Trolox equivalent (μM trolox eq.) raw (CTR) and grilled (TRATT) aubergines, okra, tomatoes and zucchini.

Vegetable	Polyp and DPPH	CTR	TRATT	p<
Aubergine	Polyp	17.48	22.76	**
	DPPH	4.92	3.91	**
Okra	Polyp	21.26	58.45	**
	DPPH	7.25	16.95	**
Tomato	Polyp	10.15	10.30	ns
	DPPH	3.14	3.67	**
Zucchini	Polyp	12.85	29.90	**
	DPPH	2.09	3.01	**

** refer to significance levels with $p < 0.01$

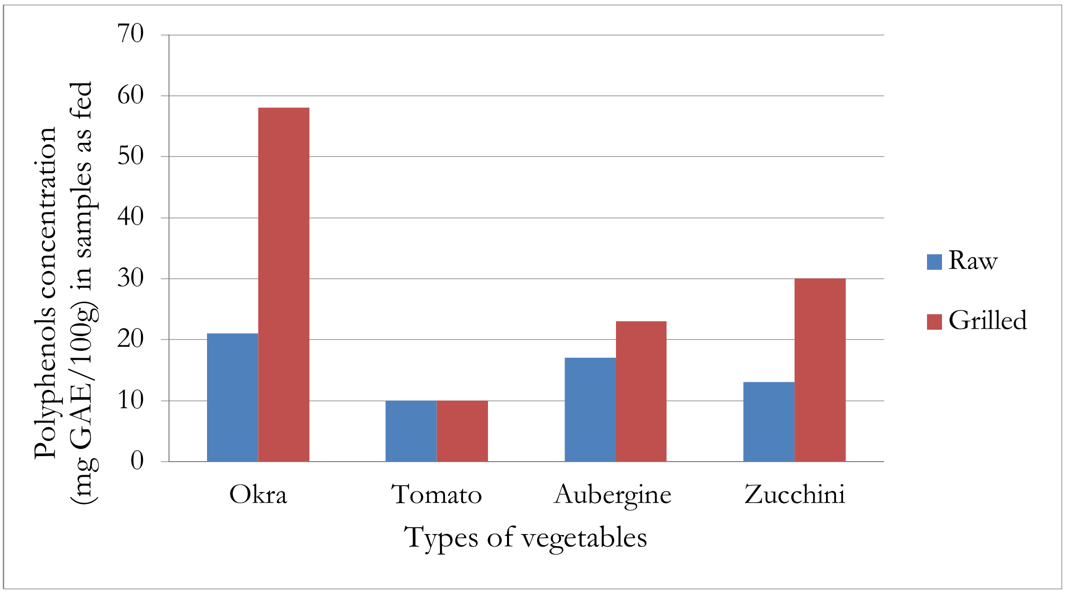
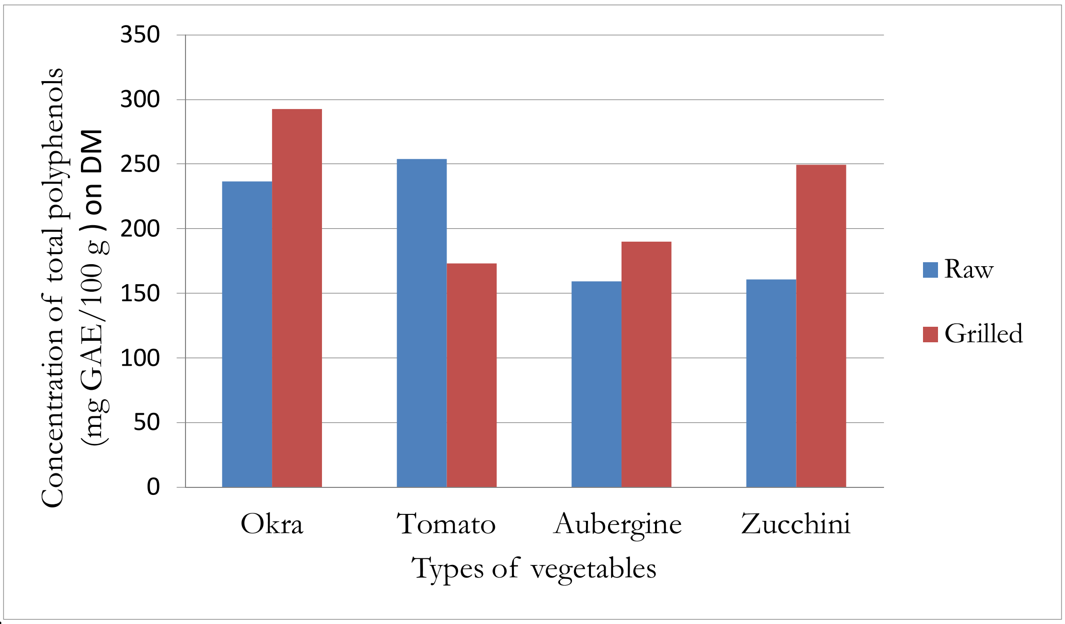


Figure 5.5 Total polyphenols (mg GAE/100g) in samples as fed of Okra, Tomato, Aubergine and Zucchini before (Raw) and after (Grilled) grilling.



.

Figure 5.6 Total polyphenols (mg GAE/100g) expressed on dry matter (DM) in Okra, Tomato, Aubergine and Zucchini before (Raw) and after (Grilled) grilling.

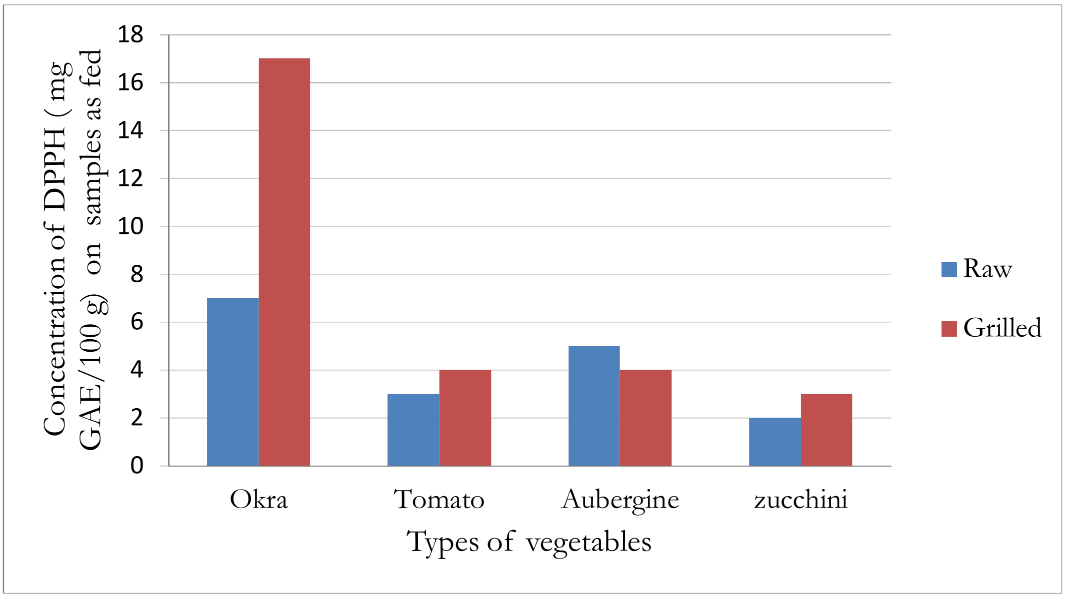


Figure 5.7 Antioxidant capacity expressed in mg GAE/100g of DPPH in samples as fed of Okra, Tomato, Aubergine and Zucchini before (Raw) and after (Grilled) grilling.

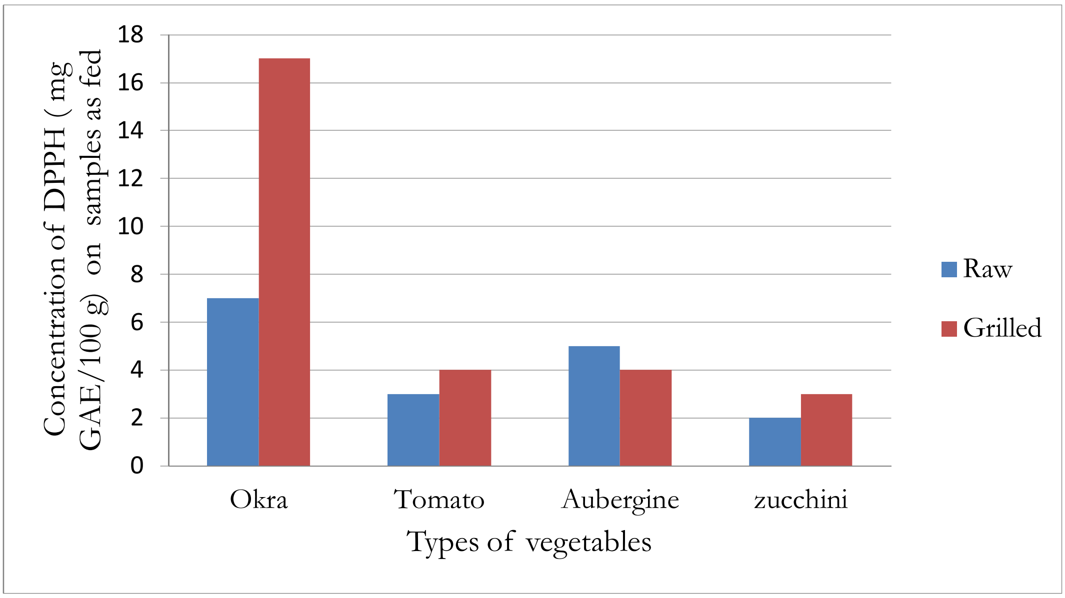


Figure 5.8. Antioxidant capacity expressed in mg GAE/100g of DPPH on dry matter (DM) of Okra, Tomato, Aubergine and Zucchini before (Raw) and after (Grilled) grilling.

Sensory analysis outcomes of the grilled vegetables and "roasted" foufou-The results of the sensory analysis that has been performed with a trained group of 8 panelists (6 males and 2 females) are given in the figure 5.9 Outcomes of sensory analysis of aubergine, Okra, tomato and foufou on appearance (a), olfactory intensity (b), taste(c) and general appreciation (d). Results expressed in percentage of the number of panelists who reported the judgement for each sensory attribute and for each grilled vegetable and foufou. The appearance has been judged generally good excepted for aubergine where 60% of panelists qualified it as unpleasant (figure 4. 5 a). As regard aromatic attribute, the okra yielded the best appreciation among the grilled vegetables with 80 % of panelists finding it as having a pleasant olfactory intensity. Considering the all 4 foods together, it is the foufou that got highest percentage of good aroma (90%). Okra and tomato have been judged to have a good taste as well as foufou (60%), contrary for aubergine (30%). As regard general appreciation combining appearance, appearance, aroma and taste, okra and tomatoes have been appreciated to good but less than foufou. To summer up grilled okra and tomato have been judged to have good sensorial quality while the contrary occurred for aubergines. The roasted foufou has yielded the best results among the 4 foods.

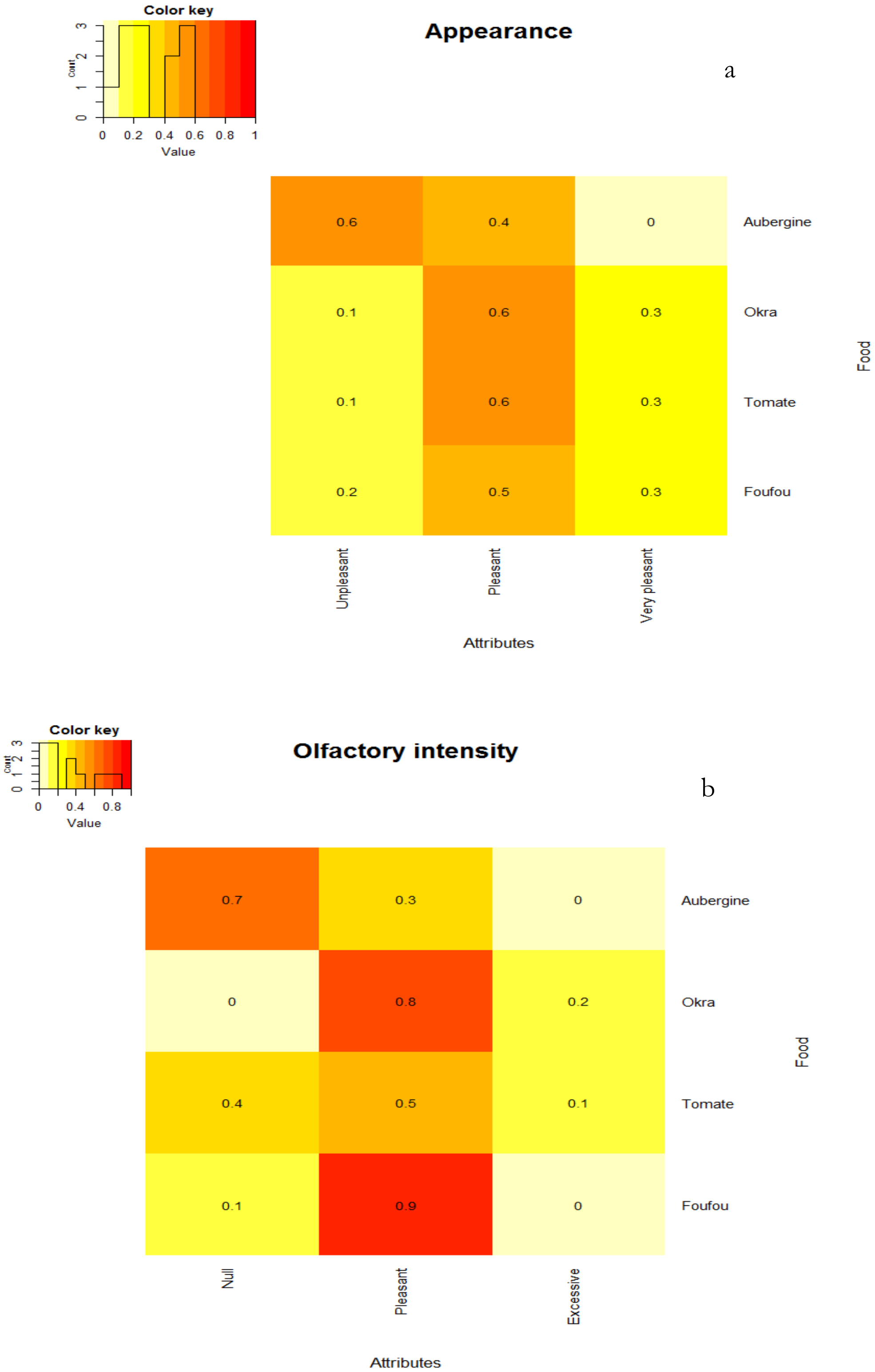
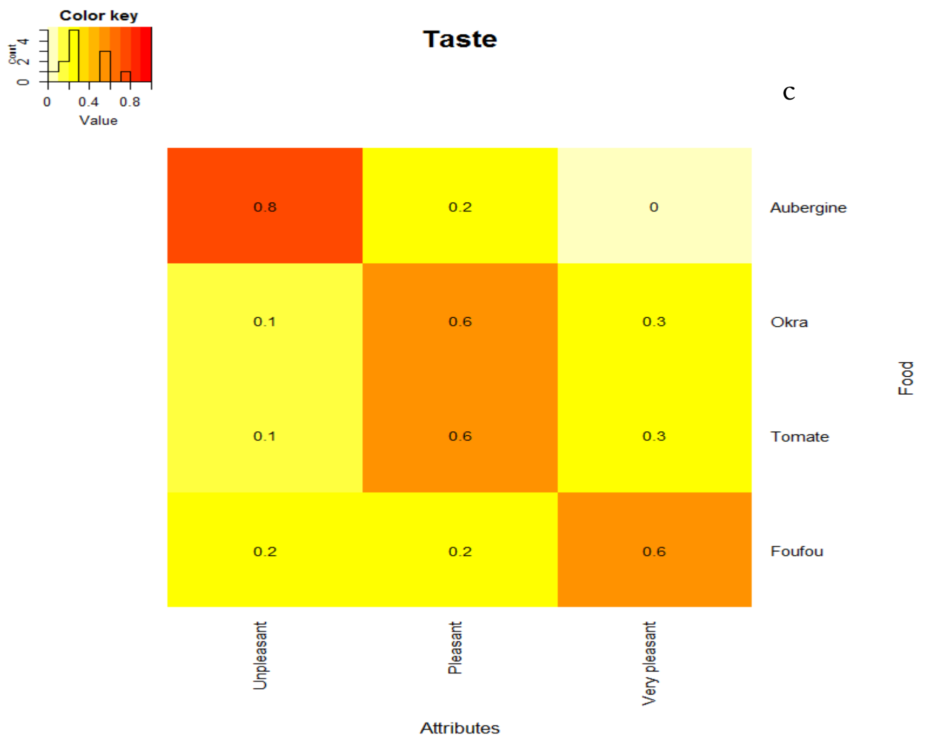


Figure 5.9 Outcomes of sensory analysis of aubergine, Okra, tomato and foufou on appearance (a) and olfactory intensity (b) expressed in percentage of the number of panellists who reported the judgement for each sensory attribute and for each grilled vegetable and foufou



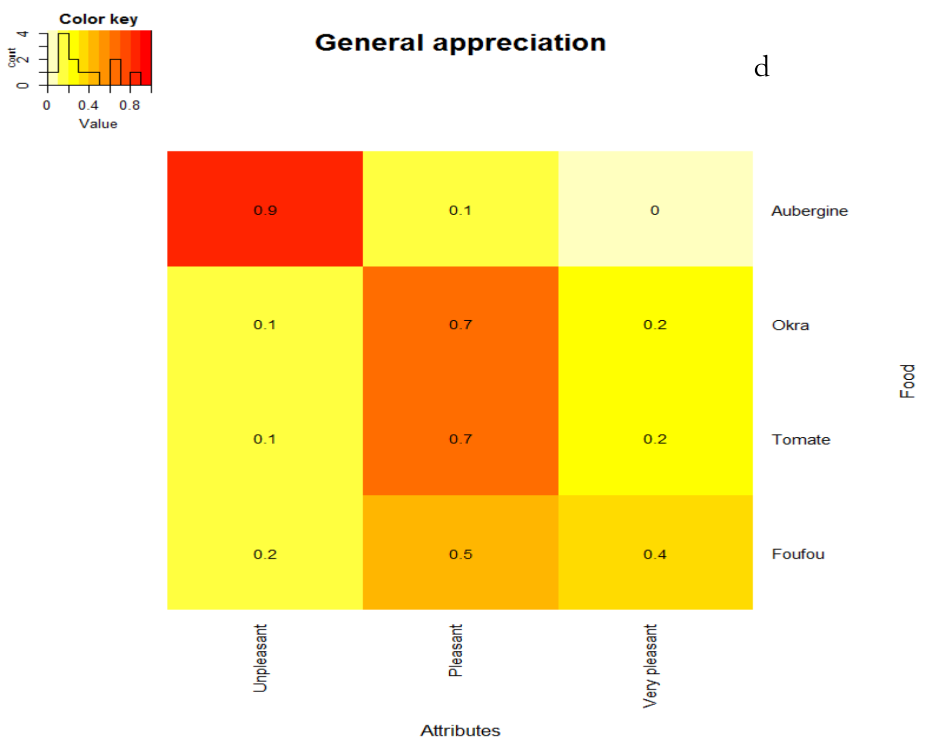


Figure 5.9 (continue): Outcomes of sensory analysis of aubergine, Okra, tomato and foufou on taste(c) and general appreciation (d) expressed in percentage of the number of panellists who reported the judgement for each sensory attribute and for each grilled vegetable and foufou.

5.5 Conclusions

In this chapter, the general and specific food based practices to improve nutritional status in developing countries through reduction of foodborne diseases and better diets have been illustrated. Our results showed that some improvement is possible; mainly:

- by the introduction of filters for drinking water helps significantly to improve the health of families and consequently the general welfare; reducing at the same time, the problems of malnutrition and the costs of health care, especially for children. Indeed the introduction of the use of filters makes probably more conscious the families about the health problems. Despite the availability of these simple to use filters on the local market, they are not yet widespread in rural families where other forms of drinking water supply of adequate quality are not available. The filters acquisition however, is not enough; information efforts on their importance and their maintenance are therefore essential;
- using Solar Water Disinfection (SODIS) technique which could be applied in rural D R Congo (where filters are not yet available). In our trials, the reduction of pathogenic bacteria have been confirmed by microbiological tests. However, SODIS shall be applied accurately according the real water features of local source used for drinking within a given community;
- as regard the use of a macronutrient balanced food for complementary feeding of breastfed babies in developing coutries in general and R D Congo in particular, although World Health Organization (WHO) suggests the exclusive breastfeeding at least until 6th month, many mothers cannot adequately breastfeed for several reasons, especially work 3 months after delivery and work for long hours. In this case, our results showed that appropriate complement of breastfeeding in the age between 3rd and 6th months is necessary for better growth of babies. However, other researches are needed to evaluate the integration to macronutrients balanced complement with micronutrients, especially mineral microelements and vitamins (possibly with local foods) combined with other approaches such as

health assessment, mothers education on food and drinking water hygiene, etc;

- preservation of local ingredients that may be used to produce a homemade snack in developing countries have been successful because earthenware could be used as utensil for homemade plantain chips'preservation (at least for 3 months without evident deterioration risks). However other general food hygiene issues shall be always considered. Indeed, peanuts, plantain and maize could be used in combination to produce a macronutrient well-balanced snack that could contribute for a better diet especially for schooling children of rural areas of developing countries. Further applied researches on this topic are needed to better understand which quality of the peanuts, especially from lipids oxidation point of view, may be used. Finally, the costs of the ingredients in the snack formulation may be carefully studied case by case according to the targeted community;
- in the last part, grilling of vegetables with a modified grid to be used in household food preparation in developing countries has been studied and proposed as contribution to improve diet allowing reduction of pathogenic bacteria while preserving the content of vitamins and the sensory quality of some vegetables. Nevertheless, more researches are necessary for a better understanding of the magnitude of the presence of pathogenic bacteria and intestinal parasites - in specific available vegetables - to improve this technics considering also the availability of such a grid in rural areas.

5.6 References

Acho-Chi, C. 2002. "The Mobile Street Food Service Practice in the Urban Economy of Kumba, Cameroon." *Singapore Journal of Tropical Geography* 23 (2): 131–48.

Adelakun, O E, O J Oyelade, B I O Ade-Omowaye, I a Adeyemi, and M Van De Venter. 2009. "Chemical Composition and the Antioxidative Properties of Nigerian Okra Seed (Abelmoschus Esculentus Moench) Flour." *Food and Chemical Toxicology : An International Journal Published for the British Industrial Biological Research Association* 47 (6).

Aidam, Ba, R Pérez-Escamilla, A Lartey, and J Aidam. 2005. "Factors Associated with Exclusive Breastfeeding in Accra, Ghana." *European Journal of Clinical Nutrition* 59 (6): 789–96.

Alzaheb, Riyadh A. 2016. "Factors Associated with the Early Introduction of Complementary Feeding in Saudi Arabia." *International Journal of Environmental Research and Public Health* 13 (7).

Am, Agence, Charles Mbutamuntu Lwanga, and Marie-jeanne Offosse. 2012. "Analyse Du Financement De L ' Eau , L ' Hygiene Et L ' Assainissement En RDC 2007-2009. Bethesda, MD: Projet 'Système de Santé' Abt Associates Inc."

Arifeen, Shams, Robert E Black, Gretchen Antelman, Abdullah Baqui, Laura Caulfield, and Stan Becker. 2001. "Exclusive Breastfeeding Reduces Acute Respiratory Infection and Diarrhea Deaths among Infants in Dhaka Slums." *Pediatrics* 108 (4): E67

Bendech, M. Ag, M. Chauliac, and D.J.M. Malvy. 1998. Assessment of Dietary Intake at Home and Outside the Home in Bamako (Mali). *Ecology of Food and Nutrition* 37 (2).

Bisimwa, Ghislain, Victor O Owino, Paluku Bahwere, Michele Dramaix, Philippe Donnen, Filippo Dibari, and Steve Collins. 2012. "Randomized Controlled Trial of the Effectiveness of a Soybean-Maize-Sorghum – Based Ready-to-Use Complementary Food Paste on Infant Growth in South Kivu , Democratic." *American Journal of Clinical Nutrition* 95.

CE. 1998. "Council Directive 98/83/EC of 3 November 1998 on the Quality of Water Intended for Human Consumption." *Official Journal of the European Communities* L330: 32–54.

Choi, Y., S. M. Lee, J. Chun, H. B. Lee, and J. Lee. 2006. "Influence of Heat Treatment on the Antioxidant Activities and Polyphenolic Compounds of Shiitake (Lentinus Edodes) Mushroom." *Food Chemistry* 99 (2): 381–87.

Chumyam, Athiwat, Kanda Whangchai, Jarunee Jungklang, Bualuang Faiyue, and Kobkiat Saengnil. 2013. "Effects of Heat Treatments on Antioxidant Capacity and Total Phenolic Content of Four Cultivars of Purple Skin Eggplants." *ScienceAsia* 39 (3): 246–51.

Clasen, Thomas F, Ian G Roberts, Taber Rabie, Wolf-Peter Schmidt, and Sandy Cairncross. 2006. "Interventions to Improve Water Quality for Preventing Diarrhoea." *Cochrane Database of Systematic Reviews*, no. 224: 1–78.

Dejung, Simon, Ivan Fuentes, Gabriela Almanza, Ruth Jarro, Lizeth Navarro, Gina Arias, Evelin Urquieta, et al. 2007. "Effect of Solar Water Disinfection (SODIS) on Model Microorganisms under Improved and Field SODIS Conditions." *Journal of Water Supply: Research and Technology - AQUA* 56 (4): 245–56.

EC. 2005. "Commission Regulation (EC) No 2073/2005 of 15 November 2005 on Microbiological Criteria for Foodstuffs." *Official Journal of the European Union* L322 (2073): 1–19.

Ekerette and Olukemi. 2016. "Complementary Feeding Practices among Mothers and Nutritional Status of Infants in Akpabuyo Area, Cross River State Nigeria." *SpringerPlus* 5 (1). Springer International Publishing: 2073.

Emmanuel K. Marfo, Benjamin K. Simpson, Johnson S. Idowu, and Olusegun L. Oke. 1990. "Effect of Local Food Processing on Phytate Levels in Cassava, Cocoyam, Yam, Maize, Sorghum, Rice, Cowpea, and Soybean" 38 (7): 1990..

FAO 2016. Street Food Vending in Accra, Ghana. Field Survey Report

Fewtrell, Lorna, Rachel B Kaufmann, David Kay, Wayne Enanoria, Laurence Haller, and John M

Colford. 2005. "Water, Sanitation, and Hygiene Interventions to Reduce Diarrhoea in Less Developed Countries: A Systematic Review and Meta-Analysis." *The Lancet. Infectious Diseases* 5 (1): 42–52.

Ficano, Tancredi. 2017. *Cibo Di Strada*. Matiolli 1885.

Fisher, Michael B., Mercedes Iriarte, and Kara L. Nelson. 2012. Solar Water Disinfection (SODIS) of Escherichia Coli, Enterococcus Spp., and MS2 Coliphage: Effects of Additives and Alternative Container Materials." *Water Research* 46 (6).

Gemede, Habtamu Fekadu, Negussie Ratta, Gulelat Desse Haki, Ashagrie Z Woldegiorgis, and Fekadu Beyene. 2015. "Nutritional Quality and Health Benefits of Okra (Abelmoschus Esculentus): A Review." *Global Journal Of Medical Reseacrh: K Interdisciplinary* 14 (5): 29–37.

Grace, D. 2015a. "Food Safety in Developing Countries : An Overview," no. October: 1–83.

Grace, D. 2015b. Food Safety in Low and Middle Income Countries. *International Journal of Environmental Research and Public Health* 12 (9).

Jeddi, Maryam Zare, Masud Yunesian, Mohamad Es haghi Gorji, Negin Noori, Mohammad Reza Pourmand, and Gholam Reza Jahed Khaniki. 2014. "Microbial Evaluation of Fresh, Minimally-Processed Vegetables and Bagged Sprouts from Chain Supermarkets." *Journal of Health, Population and Nutrition* 32 (3): 391–99.

KÄFERSTEIN, F. 2003. "Foodborne Diseases in Developing Countries: Aetiology, Epidemiology and Strategies for Prevention." *International Journal of Environmental Health Research* 13 (sup1): S161–68.

Kamiloglu, Senem, Melek Demirci, Saniye Selen, Gamze Toydemir, Dilek Boyacioglu, and Esra Capanoglu. 2014. "Home Processing of Tomatoes (Solanum Lycopersicum): Effects on in Vitro Bioaccessibility of Total Lycopene, Phenolics, Flavonoids, and Antioxidant Capacity." *Journal of the Science of Food and Agriculture* 94 (11): 2225–33.

Kathryn and Kenneth. 2003. "Update on Technical Issues Concerning Complementary Feeding of Young Children in Developing Countries and Implications for Intervention Programs." *Food and Nutrition Bulletin* 24 (1): 5.

Kimani-Murage, Elizabeth W., Frederick Wekesah, Milka Wanjohi, Catherine Kyobutungi, Alex C. Ezeh, Rachel N. Musoke, Shane A. Norris, Nyovani J. Madise, and Paula Griffiths. 2015. "Factors Affecting Actualisation of the WHO Breastfeeding Recommendations in Urban Poor Settings in Kenya." *Maternal and Child Nutrition* 11 (3): 314–32.

Levin, Carol E, Marie T Ruel, Saul S Morris, Daniel G Maxwell, Margaret Armar-Klemesu, and Clement Ahiadeke. 1999. "Working Women in an Urban Setting: Traders, Vendors and Food Security in Accra." *World Development* 27 (11): 1977–91.

Lindsay H. Allen. 2003. "Animal Source Foods to Improve Micronutrient Nutrition and Human Function in Developing Countries." *J. Nutr* 133: 3965–71.

Lingua, Mariana S., María P. Fabani, Daniel A. Wunderlin, and María V. Baroni. 2016. "From Grape to Wine: Changes in Phenolic Composition and Its Influence on Antioxidant Activity." *Food Chemistry*

208. Elsevier Ltd: 228–38.

Martí, Raúl, Salvador Roselló, and Jaime Cebolla-Cornejo. 2016. "Tomato as a Source of Carotenoids and Polyphenols Targeted to Cancer Prevention." *Cancers* 8 (6): 1–28.

McGowan, L L, C R Jackson, J B Barrett, L M Hiott, and P J Fedorka-Cray. 2006. "Prevalence and Antimicrobial Resistance of Enterococci Isolated from Retail Fruits, Vegetables, and Meats." *J Food Prot* 69 (12): 2976–82.

McGuigan, Kevin G., Ronán M. Conroy, Hans Joachim Mosler, Martella du Preez, Eunice Ubomba-Jaswa, and Pilar Fernandez-Ibañez. 2012. "Solar Water Disinfection (SODIS): A Review from Bench-Top to Roof-Top." *Journal of Hazardous Materials* 235–236: 29–46. d

Mckenzie, David, and Isha Ray. 2005. "Household Water Delivery Options in Urban and Rural India," no. 224: 59.

Motarjemi, Y, F Käferstein, G Moy, and F Quevedo. 1993. "Contaminated Weaning Food: A Major Risk Factor for Diarrhoea and Associated Malnutrition." *Bulletin of the World Health Organization* 71 (1): 79–92.

Muinde, Ok, and E Kuria. 2005. "Hygienic and Sanitary Practices of Vendors of Street Foods." *African Journal of Food Agriculture Nutrition and Development* 5 (7): 1–15.

Mwabi, J. K., F. E. Adeyemo, T. O. Mahlangu, B. B. Mamba, B. M. Brouckaert, C. D. Swartz, G. Offringa, L. Mpenyana-Monyatsi, and M. N B Momba. 2011. "Household Water Treatment Systems: A Solution to the Production of Safe Drinking Water by the Low-Income Communities of Southern Africa." *Physics and Chemistry of the Earth* 36 (14–15). Elsevier Ltd: 1120–28.

Mwangi, Alice Mboganie, Adel P. den Hartog, R. K N Mwadime, Wija A. van Staveren, and D. W J Foeken. 2002. "Do Street Food Vendors Sell a Sufficient Variety of Foods for a Healthful Diet? The Case of Nairobi." *Food and Nutrition Bulletin* 23 (1): 48–56.

Nago, Eunice S, Carl K Lachat, Lieven Huybregts, Dominique Roberfroid, Romain A Dossa, and Patrick W Kolsteren. 2010. "Food , Energy and Macronutrient Contribution of out-of-Home Foods in School-Going Adolescents in Cotonou , Benin British Journal of Nutrition." *British Journal of Nutrition* 103 (2010): 281–88.

Acho-Chi, C. (2002) The mobile street food service practice in the urban economy of Kumba, Cameroon. *Singapore Journal of Tropical Geography* 23 (2): 131–48.

Adelakun, O E, O J Oyelade, B I O Ade-Omowaye, I a Adeyemi, and M Van De Venter. 2009. "Chemical Composition and the Antioxidative Properties of Nigerian Okra Seed (Abelmoschus Esculentus Moench) Flour." *Food and Chemical Toxicology : An International Journal Published for the British Industrial Biological Research Association* 47 (6).

Aidam, Ba, R Pérez-Escamilla, A Lartey, and J Aidam. 2005. "Factors Associated with Exclusive Breastfeeding in Accra, Ghana." *European Journal of Clinical Nutrition* 59 (6).

Alzaheb, Riyadh A. 2016. "Factors Associated with the Early Introduction of Complementary Feeding in Saudi Arabia." *International Journal of Environmental Research and Public Health* 13 (7).

Am, Agence, Charles Mbutamuntu Lwanga, and Marie-jeanne Offosse 2012. "Analyse Du Financement De L ' Eau , L ' Hygiene Et L ' Assainissement En RDC 2007-2009. Bethesda, MD: Projet 'Système de Santé' Abt Associates Inc."

Arifeen, Shams, Robert E Black, Gretchen Antelman, Abdullah Baqui, Laura Caulfield, and Stan Becker 2001. "Exclusive Breastfeeding Reduces Acute Respiratory Infection and Diarrhea Deaths among Infants in Dhaka Slums." *Pediatrics* 108 (4).

Bendech, M. Ag, M. Chauliac, and D.J.M. Malvy. 1998. Assessment of Dietary Intake at Home and Outside the Home in Bamako (Mali). *Ecology of Food and Nutrition* 37 (2).

Bisimwa, Ghislain, Victor O Owino, Paluku Bahwere, Michele Dramaix, Philippe Donnen, Filippo Dibari, and Steve Collins. 2012. "Randomized Controlled Trial of the Effectiveness of a Soybean-Maize-Sorghum – Based Ready-to-Use Complementary Food Paste on Infant Growth in South Kivu , Democratic." *American Journal of Clinical Nutrition* 95.

CE. 1998. "Council Directive 98/83/EC of 3 November 1998 on the Quality of Water Intended for Human Consumption." *Official Journal of the European Communities* L330: 32–54.

Choi, Y., S. M. Lee, J. Chun, H. B. Lee, and J. Lee. 2006. Influence of Heat Treatment on the Antioxidant Activities and Polyphenolic Compounds of Shiitake (Lentinus Edodes) Mushroom. *Food Chemistry* 99 (2): 381–387

Chumyam, Athiwat, Kanda Whangchai, Jarunee Jungklang, Bualuang Faiyue, and Kobkiat Saengnil. 2013. Effects of Heat Treatments on Antioxidant Capacity and Total Phenolic Content of Four Cultivars of Purple Skin Eggplants. *ScienceAsia* 39 (3): 246–51.

Clasen, Thomas F, Ian G Roberts, Taber Rabie, Wolf-Peter Schmidt, and Sandy Cairncross 2006. Interventions to Improve Water Quality for Preventing Diarrhoea." *Cochrane Database of Systematic Reviews*, no. 224: 1–78.

Dejung, Simon, Ivan Fuentes, Gabriela Almanza, Ruth Jarro, Lizeth Navarro, Gina Arias, Evelin Urquieta, et al. 2007. Effect of Solar Water Disinfection (SODIS) on Model Microorganisms under Improved and Field SODIS Conditions. *Journal of Water Supply: Research and Technology - AQUA* 56 (4): 245–256.

EC. 2005. "Commission Regulation (EC) No 2073/2005 of 15 November 2005 on Microbiological Criteria for Foodstuffs." *Official Journal of the European Union* L322 (2073): 1–19.

Ekerette and Olukemi 2016. Complementary Feeding Practices among Mothers and Nutritional Status of Infants in Akpabuyo Area, Cross River State Nigeria. *SpringerPlus* 5 (1). Springer International Publishing.

Emmanuel K. Marfo, Benjamin K. Simpson, Johnson S. Idowu, and Olusegun L. Oke. 1990. Effect of Local Food Processing on Phytate Levels in Cassava, Cocoyam, Yam, Maize, Sorghum, Rice, Cowpea, and Soybean" 38

FAO 2016. Street Food Vending in Accra, Ghana. Field Survey Report.

Fewtrell, Lorna, Rachel B Kaufmann, David Kay, Wayne Enanoria, Laurence Haller, and John M

Colford. 2005. Water, Sanitation, and Hygiene Interventions to Reduce Diarrhoea in Less Developed Countries: A Systematic Review and Meta-Analysis." *The Lancet. Infectious Diseases* 5 (1): 42–52.

Ficano, T. (2017). *Cibo Di Strada*. Matiolli 1885.

Fisher, Michael B., Mercedes Iriarte, and Kara L. Nelson. 2012. Solar Water Disinfection (SODIS) of Escherichia Coli, Enterococcus Spp., and MS2 Coliphage: Effects of Additives and Alternative Container Materials." *Water Research* 46 (6).

Gemede, Habtamu Fekadu, Negussie Ratta, Gulelat Desse Haki, Ashagrie Z Woldegiorgis, and Fekadu Beyene. 2015. "Nutritional Quality and Health Benefits of Okra (Abelmoschus Esculentus): A Review." *Global Journal Of Medical Reseacrh: K Interdisciplinary* 14 (5).

Grace, Delia. 2015a. Food Safety in Developing Countries : An Overview.

Grace, Delia. 2015b. Food Safety in Low and Middle Income Countries. *International Journal of Environmental Research and Public Health* 12 (9).

Jalan, Jyotsna, E Somanathan, and Saraswata Chaudhari 2003. Awareness and the Demand for Environmental Quality: Drinking Water in Urban India: Discussion Papers in Economics. *Discussion Papers in Economics*, no 33.

Jeddi, Maryam Zare, Masud Yunesian, Mohamad Es haghi Gorji, Negin Noori, Mohammad Reza Pourmand, and Gholam Reza Jahed Khaniki. 2014. Microbial Evaluation of Fresh, Minimally-Processed Vegetables and Bagged Sprouts from Chain Supermarkets." *Journal of Health, Population and Nutrition* 32 (3): 391–99.

KÄFERSTEIN, F. 2003. "Foodborne Diseases in Developing Countries: Aetiology, Epidemiology and Strategies for Prevention." *International Journal of Environmental Health Research* 13 (sup1): S161–68.

Kamiloglu, Senem, Melek Demirci, Saniye Selen, Gamze Toydemir, Dilek Boyacioglu, and Esra Capanoglu. 2014. "Home Processing of Tomatoes (Solanum Lycopersicum): Effects on in Vitro Bioaccessibility of Total Lycopene, Phenolics, Flavonoids, and Antioxidant Capacity." *Journal of the Science of Food and Agriculture* 94 (11): 2225–33.

Kathryn and Kenneth. 2003. Update on Technical Issues Concerning Complementary Feeding of Young Children in Developing Countries and Implications for Intervention Programs. *Food and Nutrition Bulletin* 24 (1).

Kimani-Murage, Elizabeth W., Frederick Wekesah, Milka Wanjohi, Catherine Kyobutungi, Alex C. Ezeh, Rachel N. Musoke, Shane A. Norris, Nyovani J. Madise, and Paula Griffiths. 2015. Factors Affecting Actualisation of the WHO Breastfeeding Recommendations in Urban Poor Settings in Kenya. *Maternal and Child Nutrition* 11 (3)

Levin, Carol E, Marie T Ruel, Saul S Morris, Daniel G Maxwell, Margaret Armar-Klemesu, and Clement Ahiadeke. 1999. "Working Women in an Urban Setting: Traders, Vendors and Food Security in Accra." *World Development* 27 (11): 1977–91.

Lindsay H. Allen. 2003. Animal Source Foods to Improve Micronutrient Nutrition and Human Function in Developing Countries." *J. Nutr* 133: 3965–71.

Lingua, Mariana S., María P. Fabani, Daniel A. Wunderlin, and María V. Baroni. 2016. From Grape to Wine: Changes in Phenolic Composition and Its Influence on Antioxidant Activity." *Food Chemistry* 208. Elsevier Ltd: 228–38.

Martí, Raúl, Salvador Roselló, and Jaime Cebolla-Cornejo. 2016. Tomato as a Source of Carotenoids and Polyphenols Targeted to Cancer Prevention." *Cancers* 8 (6): 1–28.

McGowan, L L, C R Jackson, J B Barrett, L M Hiott, and P J Fedorka-Cray. 2006. Prevalence and Antimicrobial Resistance of Enterococci Isolated from Retail Fruits, Vegetables, and Meats." *J Food Prot* 69 (12): 2976–82.

McGuigan, Kevin G., Ronán M. Conroy, Hans Joachim Mosler, Martella du Preez, Eunice Ubomba-Jaswa, and Pilar Fernandez-Ibañez. 2012. Solar Water Disinfection (SODIS): A Review from Bench-Top to Roof-Top. *Journal of Hazardous Materials* 235–236: 29–46.

Mckenzie, David, and Isha Ray. 2005. Household Water Delivery Options in Urban and Rural India. no. 224: 59.

Motarjemi, Y, F Käferstein, G Moy, and F Quevedo. 1993. Contaminated Weaning Food: A Major Risk Factor for Diarrhoea and Associated Malnutrition." *Bulletin of the World Health Organization* 71 (1): 79–92.

Muinde, Ok, and E Kuria. 2005. Hygienic and Sanitary Practices of Vendors of Street Foods. *African Journal of Food Agriculture Nutrition and Development* 5 (7): 1–15.

Mwabi, J. K., F. E. Adeyemo, T. O. Mahlangu, B. B. Mamba, B. M. Brouckaert, C. D. Swartz, G. Offringa, L. Mpenyana-Monyatsi, and M. N B Momba. 2011. Household Water Treatment Systems: A Solution to the Production of Safe Drinking Water by the Low-Income Communities of Southern Africa." *Physics and Chemistry of the Earth* 36 (14–15).

Mwangi, Alice Mboganie, Adel P. den Hartog, R. K N Mwadime, Wija A. van Staveren, and D. W J Foeken. 2002. Do Street Food Vendors Sell a Sufficient Variety of Foods for a Healthful Diet? The Case of Nairobi." *Food and Nutrition Bulletin* 23 (1).

Nago, Eunice S, Carl K Lachat, Lieven Huybregts, Dominique Roberfroid, Romain A Dossa, and Patrick W Kolsteren. 2010. Food , Energy and Macronutrient Contribution of out-of-Home Foods in School-Going Adolescents in Cotonou , Benin British Journal of Nutrition." *British Journal of Nutrition* 103(2010): 281–88.

Nalwanga, R., B. Quilty, C. Muyanja, P. Fernandez-Ibañez, and K. G. McGuigan. 2014. Evaluation of Solar Disinfection of E. Coli under Sub-Saharan Field Conditions Using a 25L Borosilicate Glass Batch Reactor Fitted with a Compound Parabolic Collector. *Solar Energy.*

New South Wales (NSW Food Authority). 2009. Microbiological Quality Guide for Ready to-Eat Foods. A Guide to Interpreting Microbiological Results. *NSW Food Authority.*

Ngure, Francis M., Brianna M. Reid, Jean H. Humphrey, Mduduzi N. Mbuya, Gretel Pelto, and Rebecca J. Stoltzfus. 2014. Water, Sanitation, and Hygiene (WASH), Environmental Enteropathy, Nutrition, and Early Child Development: Making the Links." *Annals of the New York Academy of Sciences* 1308 (1): 118–28.

Ntuli, Victor, Peter Chatanga, Raphael Kwiri, Henry Tendekayi Gadaga, Jephris Gere, Taole Matsepo, and Rethabile Portia Potloane. 2017. Microbiological Quality of Selected Dried Fruits and Vegetables in Maseru, Lesotho. *African Journal of Microbiology Research* 11 (5).

Onabolu, B., O. D. Jimoh, S. B. Igboro, M. K C Sridhar, G. Onyilo, A. Gege, and R. Ilya. 2011. Source to Point of Use Drinking Water Changes and Knowledge, Attitude and Practices in Katsina State, Northern Nigeria. *Physics and Chemistry of the Earth* 36 (14–15).

PNUE. 2011. Problématique de L ' Eau En République Démocratique Du Congo Défis et Opportunités.

Robillard, PY, and R Christon. 1993. Lipid intake During Pregnancy in Developing Countries : Possible Effect of Essential Fatty Acid Deficiency on Fetal Growth.

Sabbithi Alekhya, R. Naveen Kumar, L. Kashinath, V. Bhaskar, and V. Sudershan. 2014. Microbiological Quality of Salads Served along with Street Foods of Hyderabad, India. *International Journal of Microbiology*.

Scalzo Roberto , Marta Fibiani, Giuseppe Mennella, Giuseppe L. Rotino, Monica Dal Sasso, Maria Culici, Alessandra Spallino and Pier Carlo Braga. 2010. Thermal Treatment of Eggplant (Solanum Melongena L.) increases the Antioxidant Content and the Inhibitory Effect on Human Neutrophil Burst. *Journal of Agricultural and Food Chemistry* 58 (6).

Scalzo Roberto , Marta Fibiani, Gianluca Francese, Antonietta D'Alessandro, Giuseppe L. Rotino, Pellegrino Conte, and Giuseppe Mennella. 2016. Cooking influence on physico-Chemical fruit characteristics of eggplant (Solanum Melongena L.). *Food Chemistry* 194 (September).Elsevier Ltd.

Scott Elizabeth.2003. Food fafety and foodborne disease in 21st Century Homes. *The Canadian Journal of Infectious Diseases* 14 (5): 277–80.

Sibhatu Kibrom T, Vijesh V Krishna, and Matin Qaim. 2015. Production Diversity and Dietary Diversity in Smallholder Farm Households. *Proceedings of the National Academy of Sciences* 112 (34): 10657–62.

Simonis, Jean Jacques, and Albertus Koetzee Basson 2011. Evaluation of a Low-Cost Ceramic Micro-Porous Filter for Elimination of Common Disease Microorganisms. *Physics and Chemistry of the Earth* 36 (14–15).

Shashikantha Sk., Sheethal Mp, Vishma Bk. 2016. Dietary Diversity among Women in the Reproductive Age Group in a Rural Field Practice Area of a Medical College in Mandya District , Karnataka , India" 3 (3): 746–49.

Slimestad, Rune, and Michel Verheul. 2011. Properties of Chalconaringenin and Rutin Isolated from Cherry Tomatoes." *Journal of Agricultural and Food Chemistry* 59 (7).

Steyn Nelia Patricia, Zandile McHiza, Jillian Hill, Yul Derek Davids, Irma Venter, Enid Hinrichsen, Maretha Opperman, Julien Rumbelow and Peter Jacobs 2013. Nutritional contribution of street foods to the Diet of People in Developing Countries: A Systematic Review." *Public Health Nutrition* 17 (6): 1363–74.

Tontisirin Kraisid, Guy Nantel and Lalita Bhattacharjeef 2002. Food-based strategies to meet the challenges of micronutrient malnutrition in the developing world.

UN WWDR 2016 Water and Jobs: Facts and figures. *The United Nations World Water Development Report*.

UNWWAP 2017. *WWAP United Nations World Water Developement Report*.

Velioglu, Y. S., G. Mazza, L. Gao, B. D. Oomah. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *Journal of Agricultural and Food Chemistry* 46 (10):

Vescovo Marisa, Gianluigi Scolari and Carla Zacconi 2006. Inhibition of Listeria innocua growth by antimicrobial-producing lactic acid cultures in vacuum-packed cold-smoked Salmon. Food Microbiology. Vol. 23.

Wegelin, M., Canonica, S., Mechsner, K., Fleischmann, T., Pesaro, F., Metzler, A. 1994. Solar Water Disinfection: Scope of the process and analysis of radiation experiments. *Journal of Water Supply Research and Technology* 43(3): 154–69.

WHO and FAO. 2006. Guidelines on food fortification with micronutrients.

Who and Unicef 2014. Progress on sanitation and drinking-water. *Monitoring Programme for Water Supply and Sanitation.*

WHO. 2001. The optimal duration of exclusive breastfeeding - report of an expert consultation.

6 GENERAL CONCLUSIONS

In this doctoral thesis, the main objectives were the assessment of nutritional status as a prodromic step to improve it. This approach is very important because the nutritional status and health are tightly related issues both in industrialized and developing countries. The nutritional status has been here defined as the physiological status of an individual that results from the relationship between nutrient requirements and the body's ability to digest, absorb and use the nutrients from foods.

As regard industrialized countries, our study has evaluated the consumption of some groups of foods, of animal and plant origin, in central Italy population through intake estimation and some biomarkers. The results showed that in dietary controls, especially aiming to ascertain the long-term effects on health, consumption measurements by different questionnaires cannot be exclusive, but other indexes (e.g. pigments, certain vitamins, creatinine, etc. in blood) and anthropometric ones could be also useful. Our research has made possible to find a new index based on the 5 major plasma pigments: β-carotene, β-cryptoxanthin, lutein + zeaxanthin and lycopene, whose values seem to be well correlated with the ingestion of fruits and vegetables.

As regard developing countries, it has been confirmed that in both case studies of rural India and D R Congo, the malnutrition is a serious issue. Namely, the children particularly between 3 and 5 years are more prone to that problem: 26% of severe chronic malnutrition in India and more than 60% in D R Congo. To face such problems, we analyzed possible pathways to improve small farmers' livelihood through multisectorial activities including appropriate food production for a better diet, new income generating activities, like different crafts, etc. within a given community. In fact, in the two countries our results on nutritional status assessment have confirmed the interconnection with different livelihood conditions; therefore, the better general condition of India is accompanied with a better nutritional status. Moreover, it emerged that a multifactorial empowerment particularly for mothers (mainly by education) is likely a key factor of integrated development through a proper nutrition in the strategic life age of 3-5 years when good nutrition is critical also for cognitive capacity development as well. A further objective of this empowerment, also strategic for proper physical and cognitive capacities of children is the hygiene and particularly potable drinking water to avoid gastro-intestinal diseases which are an important component of malnutrition (impairing nutrients'absorption). In this respect, field practices have been carried out to improve nutritional status in developing countries through reduction of foodborne diseases and better diet; interesting results have been obtained:

1) the introduction of filters for drinking water in Garo population (in India) helped significantly to improve the health of families and consequently the general welfare and could reduce at the same time, the problems of malnutrition and the costs of health care;
2) again, as regard to water sanitation, Solar Water Disinfection (SODIS) resulted to be a possible solution in rural D R Congo although it shall be accurately done according the real drinking water source used in each community to fulfil an appropriate sanitation according to the magnitude of pathogenic bacteria burden;
3) although World Health Organization (WHO) suggests the exclusive breastfeeding at least until 6th month, our results in R D Congo showed that several mothers are forced to work away from home after 3 months from delivery; therefore, the introduction of macronutrient balanced foods for complementary feeding of breastfed babies is necessary for their better growth;
4) the evaluation of the grilling of vegetables with a modified grid to be used in household food preparation in developing countries showed that such technique can be proposed as contribution to improve diet allowing reduction of pathogenic bacteria while preserving the content of vitamins and sensory quality. In these communities, where vegetables are in fact eaten always cooked, especially for hygienic reason but losing some of their specific nutritional values (vitamins and antioxidants), this technique could be useful. Nevertheless, more researches are necessary for a better understanding the features of pathogenic bacteria and other intestinal parasites, and therefore to improve these practice considering also the availability of such a grid in rural areas.

To conclude, nutritional status remains a large topic of research to achieve more healthy and nutritious diets appropriate for each given specific community. Holistic studies, considering also agriculture, nutrition and hygiene, could contribute to improve the real situation case by case, both in industrialized and developing countries.