Health enhancing traditional foods in Brazil: an interdisciplinary approach to food and nutritional security

Thesis presented in fulfillment of requirement for the degree of Doctor of Philosophy in Natural Sciences (*Dr. rer. nat.)*

Faculty of Natural Sciences

University of Hohenheim

Institute for Biological Chemistry and Nutrition

Chair of Nutritional Biochemistry

Prof. *Dr. rer. nat*. Lutz Graeve

Presented by

Fernanda Dias Bartolomeu Abadio Finco

From Rio de Janeiro, Brazil.

2012

Health enhancing traditional foods in Brazil: an interdisciplinary approach to food and nutritional security

Dissertation zur Erlangung des Doktorgrades der Naturwissenschaften
(*Dr. rer. nat.)*

Fakultät Naturwissenschaften

Universität Hohenheim

Institut für Biologische Chemie und Ernährungswissenschaft
Lehrstuhl Biochemie der Ernährung
Prof. *Dr. rer. nat*. Lutz Graeve

vorgelegt von

Fernanda Dias Bartolomeu Abadio Finco

aus Rio de Janeiro, Brazil.

2012

Dean of Faculty: Prof. Dr. rer. nat. Heinz Breer

1. Supervisor: Prof. Dr. rer. nat. Lutz Graeve
2. Co-supervisor: Prof. Dr. med. Hans Konrad Biesalski
3. Examiner: Prof. Dr. Anne Camilla Bellows (*Ph.D*.)

Date of oral examination: November 5th, 2012.

“Imagination is more important than knowledge. Knowledge is limited. Imagination encircles the world”.

Albert Einstein (1879 - 1955)

I dedicate this work

To the love of my life,

My husband Marcus

and

My dad Pedro Abadio (*in memorium*).

Acknowledgments

To Prof. Lutz Graeve for his friendly and competent supervision, for the great opportunity to develop my own ideas during my PhD project which, surely enhanced and boosted my professional skills. For always supporting me on all initiatives, ideas, experiments etc. and for his ever open mind and ears. Also for the pleasant lab meetings, the great talks, and for the great humanistic and exemplary conducting of the academic work and “Arbeitsgruppe”, I extend my gratitude.

To Prof. Biesalski for accepting to be my second supervisor and for keeping the Institute of Biological Chemistry at University of Hohenheim’s doors permanently open to foreign talents.

To Prof. Anne Bellows for being my third examiner, for the support during grant applications.

To Eiselen Foundation, Ulm; International Foundation for the Promotion of Nutrition Research and Nutrition Education (I*nternationale Stiftung für Ernährungsforschung und Ernährungsaufklärung*) Switzerland. and to the *Landesgraduiertenförderungsgesetz* (LGFG) for the financial support.

To the Institute of Plant Foodstuff Technology (150d) on the persons of Prof. Dr. Carle and Dr. Kammerer for the valuable and professional assistance with the chromatographic analysis.

To the Institute of Nutritional medicine (180) for the access to the FACS facilities.

To the students Larissa Borges, Juliane Guimarães, Sabrina Böser, Wen-Hsin Tseng, Anne Volkert, Lorraine Kloss for their participation and contribution to this work.

To Dagmar Mvondo, Monika Schumacher and Suzana Cvek for their technical assistance.

To all staff from Institute 140 and all working groups, which were very supportive and open during my project performance and stay at Hohenheim.

To Instituto Sócio Ambiental Missão Verde, on the persons of Mr. William and Mr. Deusimar for the help with the field study.

To the two rural communities and all participants of the field study, for opening their houses and lives to science and to share the amazing rural world with me.

To all friends I met in German, for the moments of happiness and sadness, for the conversations and for introducing me to the German culture.

And to my friends in Brazil, for the virtual laughs and supportive talks, keeping my Brazilian soul warm and alive.

To my family in Brazil, for the love and support.

And last, but not least, to my husband Marcus, for his everlasting love and for his support during the entire time we spent in Germany.

Preliminary remarks:

Origin and Development of the work:

The current work is the result of the original research project entitled: Community Based Nutrition Strategies: Assessing Savannah fruits as a health promotion strategy in Tocantins, Brazil. The project was authored and coordinated by Fernanda Abadio Finco under the supervision of Prof. Dr. Graeve as first Brazilian-German cooperation initiative between the Food and Nutrition Security lab of the Federal University of Tocantins, Brazil and the Institute of Biological chemistry and Nutrition of the University of Hohenheim, Germany.

The idea was conceived based on the preliminary experience of the Mrs. Abadio Finco in Food and Nutrition Security topics in Brazil, especially in the Tocantins State, which belongs to the Legal Amazon Area. The previous activity in the food and nutritional security discussions combined with the holistic profile of the author lead the research to an interdisciplinary approach. Therefore, the first part of the work comprises fieldwork with rural communities in Tocantins State and was financially supported by Eiselen Foundation, Ulm, Germany. The second part covered the biofunctional properties of the Brazilian fruit bacaba (*Oenocarpus Bacaba*) and had the financial support of the International Foundation for the Promotion of Nutrition Research and Nutrition Education (*Internationale Stiftung für Ernährungsforschung und Ernährungsaufklärung*) Switzerland.

The present work has in part been published or presented as follows:

Accepted full papers:

1. Abadio Finco, F.D.B.; Böser, S.; Graeve, L. Antiproliferative activity of bacaba (*Oenocarpus bacaba*) and jenipapo (*Genipa americana L.*) phenolic extracts: a comparison of assays. Nutrition & Food Science, v. 43 n.2
2. Abadio Finco, F.D.B.; Kammerer, D. R.; Carle, R.; Tseng, W-H.; Böser, S.; Graeve, L. Antioxidant activity and characterization of phenolic compounds from bacaba (*Oenocarpus bacaba* Mart.) fruit by HPLC-DAD-MS^n. Journal of Agriculture and Food Chemistry. **2012**, *60* (31), pp 7665–7673. DOI: 10.1021/jf3007689

Submitted full papers:

3. Abadio Finco, F.D.B.; Tseng, W-H.; Böser, S.; Kammerer, D. R.; Carle, R.; Graeve, L.Determination of phenolic contents and antioxidant capacity of extracts from *Genipa americana* L. ("Jenipapo") fruit.

Invited talks:

4. Abadio Finco, F.D.B. Health Enhancing Local Foods: an interdisciplinary approach to Gender and Nutritional Security. Summer 2009 Speaker series: New Perspectives in Food and Gender. Kompetenzzentrum Gender und Ernährung, University of Hohenheim, Stuttgart, Germany, July, 1st 2009.

5. Abadio Finco, F.D.B. Chemopreventive effect of bacaba (*Oenocarpus bacaba*) on MCF-7 cells. 9th SLACA Simpósio Latino Americano de Ciência e Tecnologia de Alimentos (Latin American Symposium of Food Science and Technology) November 5th to 8 th, 2011 Campinas, Brazil).

Poster presentations:

6. Abadio Finco, F.D.B. Chemopreventive effect of bacaba (*Oenocarpus bacaba*) on MCF-7 cells. 9th SLACA Simpósio Latino Americano de Ciência e Tecnologia de Alimentos (Latin American Symposium of Food Science and Technology) November 5th to 8 th, 2011 Campinas, Brazil).

7. Kloss, L. Abadio Finco, F.D.B.; Graeve, L. The apoptotic effect of bacaba (*Oenocarpus bacaba*) phenolic extract on MCF-7 breast cancer cells. Scientific Student Conference 2010, Wageningen University, The Netherlands, 20-21 October, 2011.

8. Volkert, A. Abadio Finco, F.D.B.; Graeve, L. The effect of bacaba phenolic extract (*Oenocarpus bacaba*) on the differentiation of 3T3-L1 preadipocytes into adipocytes. Scientific Student Conference 2010,Wageningen University, The Netherlands, 20-21 October, 2011.

9. Böser, S.; Abadio Finco, F.D.B.; Graeve, L. Antioxidant and Antiproliferative Effects of Phenolic Extracts from Bacaba (*Oenocarpus bacaba*) and Jenipapo (*Genipa americana*) on HepG2 Cancer Cell Line. ELLS Scientific Student Conference 2010, November, 11-12, Copenhagen, Denmark.

10. Abadio Finco, F.D.B.; Graeve, L. Apoptotic effect of the Brazilian berry Bacaba *(Oenocarpus bacaba* Martius*)* on MCF-7 breast cancer cells. SFRR Europe: Society for Free Radical Research Meeting. September 12 - 15, 2010, Oslo, Norway.

11. Abadio Finco, F.D.B.; Graeve, L. Apoptotic effect of the Brazilian berry Bacaba (*Oenocarpus bacaba* Martius) on MCF-7 breast cancer cells. Metabolomics 2010 27th June to 1st July Amsterdam, The Netherlands

Collaborators:

During the project evolution a work team of students and researchers contributed to its development and therefore are co-authors in the respective publications. Sabrina Böser (2010); Wen-Hsin-Tseng (2011); Loreen Kloss (2011) and Anne Volkert (2011) were involved and developed their respective bachelor or master theses under my co-supervision in the Institute of Biological Chemistry and Nutrition at the University of Hohenheim. Therefore, their contributions also take part in this doctoral thesis. Dr. Kammerer and Prof. Carle (Institute of Food Technology, Section Plant Foodstuff Technology, Hohenheim University) contributed valuably to the extraction optimization and chromatography analysis and Dr. Marcus Finco (Food and Nutritional Security Lab, Federal University of Tocantins) collaborated on the interpretations of the regression analysis and socioeconomic data.

Table of Contents

List of Tables

List of Figures

1. Introduction

Industrialization, rapid urbanization and market globalization have affected countries all over the world in different fields such as economy, social systems, health and environment. In this context, countries like Brazil suffer impacts of all natures, which also have consequences on people's lifestyle and health conditions. The latter are characterized by an increasing prevalence of non-communicable diseases (NCD), such as obesity, cardiovascular disease and cancer, for instance (*2*).

Currently, the big challenge for Brazil is to keep the potential food production within the sustainability framework. Therefore, small scale agriculture, especially focused on traditional communities has received some attention and support, although the food production in Brazil is mostly performed by large scale farming. It is important to note that the country is ranked as one of the main food producers in the world. On the other hand, Brazil is also recognized by its richness in biodiversity especially when one considers the Amazon Rainforest.

More recently, traditional communities are also recognized for their role in environmental protection. As these people are immersed in the natural environment and possess traditional knowledge, they are enabled to use native species available in the forest in a sustainable fashion. Moreover, traditional products are becoming more popular in NCD prevention and therefore, the positive health benefit of plant extracts is being increasingly investigated. Last but not least, the consumer market is also facing a growing demand for traditional products that improve health conditions.

In this context, the debate about native and underutilized plants arises with great importance. From the nutritional aspects of native species, different issues can be improved regarding income generation, health and nutrition of traditional communities in the Amazon Region. Considering nutritional and health properties, traditional communities can also improve their diets with fruits and vegetables and at the same time add value to the artisan and traditional food produced by them.

As the utilization of the native species is performed by gathering them directly from the natural environment through suitable forest management, improving and establishing products from native species and traditional communities can be an important strategy for forest preservation. Furthermore, based on the positive health benefit of fruit and vegetable consumption, more research about protective ingredients is necessary in developing countries in order to show the possible functional properties of native fruits. When investigated properly, some of these fruits could be very strategic from a physiological, pharmacological and economic standpoint and therefore, more studies about traditional fruits in the Amazon region are absolutely important and required. In addition, nutrient density needs to be analyzed to obtain knowledge about the importance of different food related to adequate micronutrient supply. Until recently, there has been little data about the nutrient and non-nutrient composition of native foods from Latin America, especially from the Brazilian Amazon region (*1*, *3*).

Based on the discussion above and aiming at supporting the food chain of traditional food and communities in the Brazilian Amazon, actions should be carried out to bring together different fields of knowledge and scientific disciplines. For this purpose, issues regarding environmental, economic, nutritional and health aspects should be integrated towards a main and unique initiative. Thus, to better comprehend this panorama in Brazil, different issues regarding socioeconomic, environmental, nutritional and political aspects are described hereunder.

1.1. The Brazilian Amazon Rainforest

Brazil is considered a tropical country endowed with great and rich biodiversity within different biomes, such as Savannah, Semi-arid, Atlantic forest, Pampa, Caatinga and Rainforest. However, when the discussion goes towards biodiversity and environmental protection, it is unavoidable to immediately associate the topic with the Amazon Rainforest.

The Amazon Rainforest is considered the biggest rainforest in the world and comprises an area from the Atlantic Ocean to the eastern hills of the Andes, covering a large territory belonging to nine different countries in South America. The Brazilian Amazon Rainforest covers 69% of the total Amazon Rainforest (*4*). In Brazil, the Amazon region has an area of 4,871,000 km^2 and a population of 20 million of people (*5*). In addition, it possesses a colossal flora and fauna. It is estimated that the highest biodiversity worldwide is present in this biome, which therefore, has the biggest genetic bank of animal and vegetal species in the world.

The Brazilian Amazon is politically defined as “Legal Amazon” and includes different states (Pará, Amazonas, Maranhão, Tocantins, Mato Grosso, Acre, Amapá, Rondônia and Roraima) (Figure 1). The Brazilian Legal Amazon corresponds to 60% of the national territory, and is characterized by a large diversity when considering socio-cultural issues (*6, 7*).

Figure 1: Brazilian Legal Amazon.

The traditional model of the Legal Amazon occupation has being taken vis-à-vis an intense process of deforestation, which can be defined as a complex and multifaceted phenomenon (*8*). When one looks into the historical process of colonization motivated by the Brazilian government during the 1960's, for instance, one realizes that this process was promoted via exploitation of the forest without any thought of sustainability. The process of colonization boosted the agriculture and livestock production at the expense of the Amazon deforestation – a process that remains until today (*4*, *8, 9*). In addition, economic activities such as timber extraction started to play an important role as a catalyst in the exhaustion of the Amazon biome (*9, 10*).

Tropical forests are continuing to disappear at an alarming rate: between 1990 and 2005, the rate of deforestation averaged about 13 million hectares a year, occurring mostly in tropical countries (*3*). In the Brazilian Legal Amazon the area of deforestation reached approximately 653 thousand of km^2 in 2003. However, this deforestation is not based on a homogeneous dynamic. By contrast, the deforestation is highly concentrated in the southern-eastern Amazon, whose boundaries comprise the southern part of Maranhão state, northern part of Tocantins state, southern part of Pará state, northern part of Mato Grosso state, Rondônia, southern part of Amazonas state and southeast part of Acre state, forming the so-called "Arch of Deforestation" (Figure 2) (*11-14*).

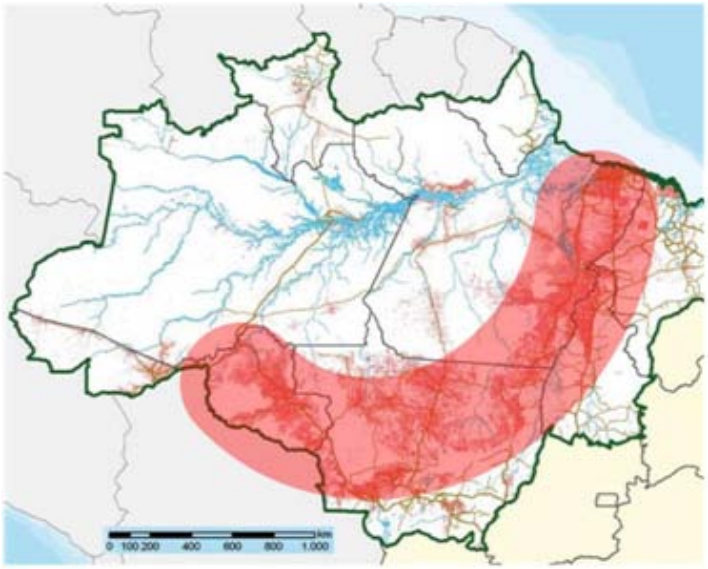


Figure 2: Boundaries of Legal Amazon and the spatial distribution of deforestation with highlights on the "Arc of deforestation" (adapted from Brasil 2008 (*6*) by Lui and Molina, 2009) (*14*).

As one can see in Figure 2, the "Arc of deforestation" defines a wide area of the Brazilian territory that covers the mid-western and northern of Brazil, where the Ecotone zone, i.e. a transition region between Rainforest and Savannah biome is located.

The process of deforestation is also linked to soil erosion, changes in the groundwater and biodiversity loss. The process also contributes to global climate instability, since the forest develops a key role in carbon sequestration preventing global warming and its secondary effects. An Ecotone zone, for instance, includes species from both Savannah and Amazon rain forest and, therefore, has more capacity to store carbon when compared to Savannah '*stricto sensu*'. The range of carbon sequestration values reflects the fact that the entire process depends on several natural conditions, such as humidity, quantity of rain and sun, which are negative impacted by forest degradation (*3*). In addition, traditional communities also have their access to fruits and other forest-based foods diminished due to natural resources exhaustion.

The Amazon Rainforest is characterized by a high complexity of ecological and social relationships, and can be considered a “hotspot study field” regarding different disciplines and point of views. For this reason and based on the need of forest preservation, efforts should be integrated aiming at promoting the sustainable development in the region. Albeit the significant role of the Amazon Rainforest to the global climate and the richness of biodiversity, deforestation is presently being considered as a threat to the biome and traditional communities. The combination of roads overture and the political tax incentives given to agribusiness led to a condition of predatory occupation, where the native forest is considered by farmers as an obstacle to be overcome. Therefore, the great current challenge is to merge the production profitability of the large-farming activity with the forest preservation and the small-scale (traditional communities) well-being as well.

Despite the importance of large-scale agriculture to the country’s economy especially regarding the Gross Domestic Product (GDP)[1], the small-scale farming system has its economic, social and environmental relevancy to Brazil. The family agriculture sector is always mentioned by its social reproduction and its capacity to supply the national and international markets as well. However, it is important to highlight some other factors that are extremely important. The family agriculture plays an important role in rural population fixation, decreasing therefore, the rural exodus, especially in the Amazon region. In addition, family agriculture and its pluri-activity profile is responsible for income generation and pushes some small-scale farmers out of rural poverty. Therefore, a social approach is usually focused on the economic characteristics of this sector, that corresponds about 10% of the GDP, on average (*15*). Also, family agriculture in Brazil is characterized by regional differences. Despite these characteristics, the great feature of the family agriculture model considers sustainable development and forest preservation.

[1] GDP - Refers to the market value of all final goods and services produced in a country in a given period

Based on the Brazilian agriculture profile mentioned above, it is undeniable that up-to-date technology-based farming is indispensable for producing food for the world population. However, interests in crop quality and production rarely include nutritional sciences and their links to traditional community activities performed by those living in the forest. Albeit, some interventional actions and research have focused on providing micronutrients through processes such as biofortification, more concern has to be given to the use of native species containing nutritional assets such as micronutrients, fibers and phytochemicals with antioxidant power, especially within the family agriculture system (*6, 16, 17*).

1.2. The Brazilian nutritional situation

Until the beginning of the 1940s, infectious diseases in Brazil were the main cause of death, being responsible for more than 40% of the deaths. However, in 1986, cardiovascular diseases took over as the main cause of deaths (33.5%) in the country. In 2005, at least 35 millions of people died from NCD (non-communicable diseases) all over the world. This figure corresponds to the double of deaths related to infectious diseases and shows the nutritional transition experienced in Brazil up to now (*18*). In addition, NCD caused the majority of deaths in the last decades. In 2004, for instance, it reached 74.3% of proportional mortality in Brazil (*6*).

Surely, NCD are considered as a serious public health problem in both developed and developing countries. However, developing countries suffer from it at higher levels due to a reduced ability to implement public policies that aim to positively change the social determinants. Also, NCD are important causes of morbidity, mortality and invalid status and are responsible for major economic costs for families, health systems and society over all. The costs can occur directly (costs related to internment, medicines, clinical treatment) or indirectly (loss of production associated with these diseases, early retirement, etc.). In this way, preventing NCD can also be related not only to social and public health but also to economic benefits (*19*).

The multiple aetiology of NCD does not allow its causes to be comprehended or defined clearly. However, biomedical investigations made it possible to identify many risk factors involved. As obesity and inadequate diet are closely related - both are considered as risk factors. Diet, however, is a risk factor that has as an advantage the possibility to be improved and changed (*20*).

Obesity has taken hold as a nutritional problem associated with a high incidence of cardiovascular diseases, cancer, and diabetes, thereby influencing the morbidity and mortality profile of populations. At least 40% of the adult Brazilian population are presently overweight (BMI $>$ 25Kg/m^2) and at the same time, obesity (BMI $>$ 30 Kg/m^2) is found in 8.9% of adult men and 13.1% of adult women (*7*).

It is important to highlight that NCD are present in all strata of population and have a high prevalence even in poor rural areas. Vellasquez-Meléndez *et al*. have found that 33.6% of women had a Metabolic Syndrome in rural settlements of Vale do Jequitinhonha (a very poor region of Brazil). Obesity risk factors can be controlled. Individual factors (for example diet and lifestyle) are associated with several diseases. The reduction of one of these risk factors can result in the prevention and control of several diseases at the same time (obesity, atherosclerotic cardiovascular disease, lipidemic disorders, hypertension and diabetes mellitus) (*21, 22*).

As previoulsy reported (*22*), the scientific base for prevention is based on two components: the first is the knowledge of the biological and epidemic processes underlying the emergence of the diseases; and the second is the effectiveness of its prevention. In this context, a healthy and affordable diet for the prevention of NCD should be designed, and the increase of fruit consumption can be considered as an important element that fulfills these requirements. This illustrates the importance to study the relation between the increased consumption of fruits (including new species of fruits) and nutritional status and health (*22*).

Fruits and vegetables, in general, have played an important role in the prevention of NCD. Evidence-based studies suggest a role for oxidative stress on the physiopathology of obesity and several chronic diseases, such as atherosclerosis, cancer and degenerative diseases (*23*). Therefore, the frequent use of foods with antioxidant capacity and phytochemical protectors can be relevant for the prevention of diseases related to increased oxidative stress (*23*). The protective effect of fruits and vegetables against chronic diseases is attributed to their content of phytochemicals with corresponding antioxidant activity and is leading researchers world-wide to investigate this functional role of foods.

Many studies have shown that “berries” (e.g. strawberries, blackberries) play a role in NCD prevention and others have been done with fruits which have an established high consumption pattern all over the world, such as apple, mango, peaches and pear (*24*). However, until now few researchers are engaged to investigate the preventive role of wild fruits that are consumed and accessed locally in Brazil.

The urgent need to better demonstrate and document the association between biological diversity, dietary diversification and improved nutritional situation requires an integrative research at the laboratory, community and food system levels. Therefore, it is important to examine the relationships between dietary antioxidant intakes, oxidative stress and inflammation in populations that are at risk for future disease (*25*). Prevalence data of NCD are still insufficient in the Legal Amazon, especially in Tocantins State (Brazil) and those obtained by the Government information systems are often incomplete (*26*). Despite the absence of nutritional information in Tocantins State, a similar epidemiological and nutritional trend can be presumed (like in northern region and Brazil as a whole) with a high prevalence and incidence of overweight/obesity and their associated diseases. Also, there is a lack of information in the scientific literature on dietary intake and nutritional status of rural populations and their relationship with the consumption of local fruits from Brazilian traditional food.

1.3. The Brazilian public policies towards food and nutrition security

Internationally, the loss of biodiversity and how other environmental changes affect diet and health are usually perceived as a food security issue, regarding mainly food supply and access. However, Brazil can be considered in the vanguard of food and nutritional security, as its perception and comprehension on food security includes a holistic approach to the topic. Based on this, Brazil does not consider the terminology "food security" alone, but "food and nutritional security" in issues regarding this topic.

Recently, Brazil significantly advanced the topic of Food and Nutrition Security (FNS) during the II and III CNSAN (National Conference of Food and Nutrition Security) in 2004 and 2007, respectively. Through a participatory process during the II CNSAN a definition of "Food and Nutrition Security" was established and also a specific policy for the country was designed. Both were in accordance with the Brazilian reality (which comprises an enormous diversity of people, culture, environment and so on), as follows:

"Food and Nutrition Security is the achievement of the right of all people to access food regularly and permanently, with quality and enough quantity, without compromising the access to other basic needs, based on food practices to promote health, with respect to cultural differences and being social, economic and environmentally sustainable" (*27*).

According to the definition above, Food and Nutrition Security is focused not only on hunger eradication, but also on the assurance of the exercise of the Right to Adequate Food, which means that people have access to a diet containing not only an adequate quantity of calories but also all micronutrients (vitamins, minerals) and any other compounds that are important to health and nutrition. Moreover, this diet should consider the culture factor and the environment into which each individual is emersed. In fact, the nutritional value of a food has to be considered but its social

and anthropological role cannot be forgotten (*27*). The components of the Brazilian FNS policy can be seen in Figure 3.

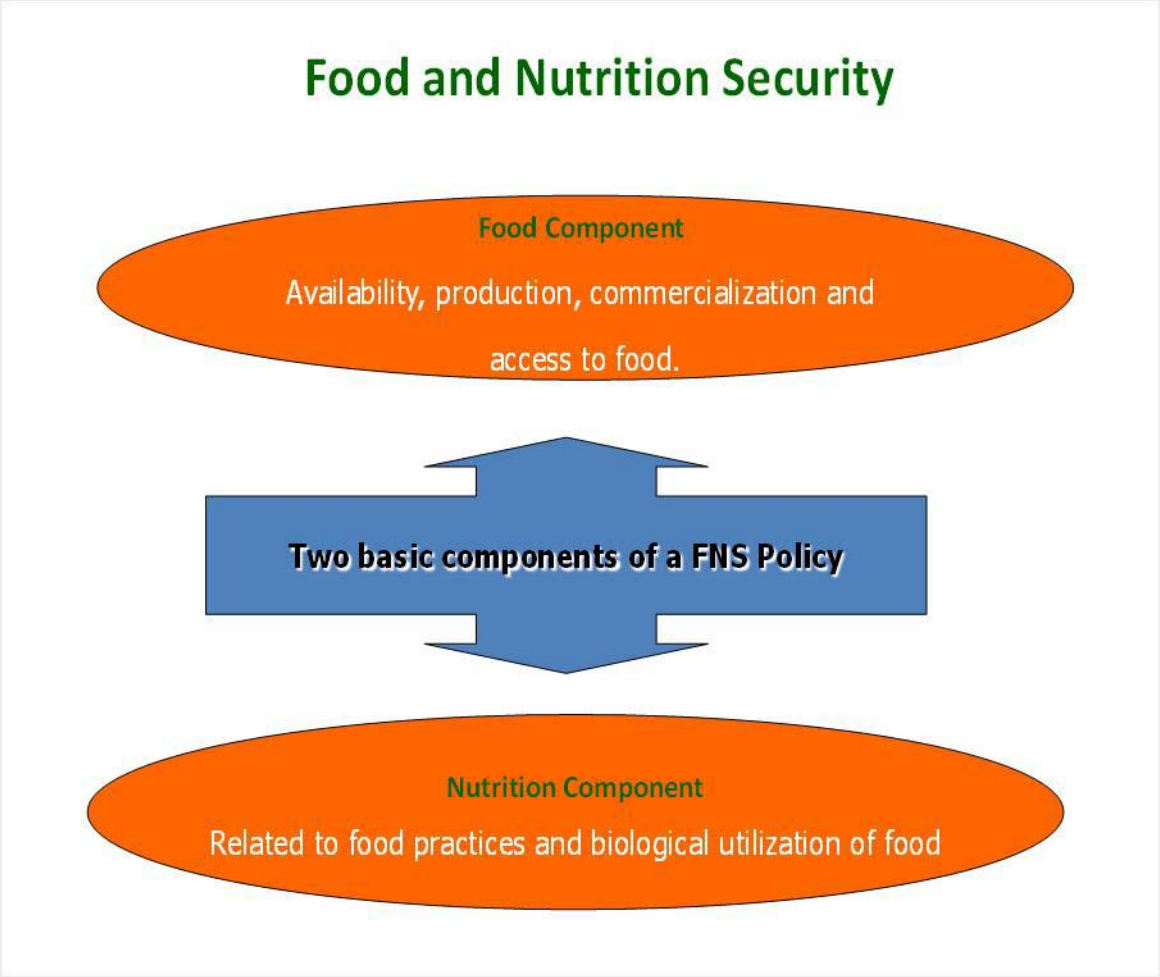


Figure 3: Components of Brazilian Food and Nutritional Security Concept.

Surely, to fully reach the FNS of the population, initiatives involving various fields of knowledge and science should be established. However, not only the mix of knowledge is necessary, but also the interrelationship between the various sectors and institutions at different levels is necessary in order to outline their FNS programs and policies together. It is worth mentioning that any policy of social inclusion may resort to emergency activities in order to solve an urgent situation.

However, structural actions shall be executed in parallel in order to keep the situation provided for emergency action and ensure the perfect social inclusion. In this sense,

actions that promote sustainable development generate income and social justice have been designed and executed by the Brazilian government. These actions have a transversal nature and include the civil society and the academic sector.

In the Brazilian FNS context, which includes among other things, production, culture and environment, family farmers and traditional communities have an extremely important role for the achievement of the FNS goal.

Therefore, one can realize the perfect integration between the policies of FNS and another Brazilian public policy (*28*): the National Policy for Sustainable Development of Traditional Peoples and Communities which aims at "... include support and ensure productive inclusion with the promotion of sustainable technologies, respecting the system of social organization of peoples and traditional communities, valuing natural resources and local practices, knowledge and traditional technologies."

Among other issues, this policy aims at achieving food security and nutrition of the rural population, and respect for cultural issues. Thus, special attention has been given to the culture and traditions, the richness of biodiversity and the integration of policies and programs.

In line with the policy of FNS, and in order to articulate government policies aimed at sustainable development, income generation and social inclusion, in 2009 the National Plan for Promotion of Socio-Biodiversity Product Chains (PNPSB) was established (*29*). PNPSB is a joint coordination effort by the Brazilian Ministry of Agrarian Development, Ministry of the Environment, Ministry of Social Development and Fight Against Hunger and the National Supply Company. PNPSB is a policy strategy supported by relevant public authorities, civil society, private sector, financial institutions and the German Agency for International Cooperation (GIZ) in Brazil[2]

[2] MDA – Ministry of Agrarian Development, Brazil.

The PNPSB was developed through a participatory process, being guided in the Brazilian scene of great biological diversity, social and cultural. Therefore, the plan considers the insertion of biodiversity into the socio-cultural panorama exercised by traditional communities, family farmers and extractive farmers. This raises the concept of sociobiodiversity.

The aim of this plan is to develop integrated actions for promoting and strengthening the role of socio-biodiversity product chains in building sustainable markets. Moreover, it aims to ensure environmental conservation and income generation by means of the productive inclusion of traditional peoples and communities, as well as family farmers. Its major actions include the promotion of public policies, such as credit delivery mechanisms, technical assistance and agricultural extension, markets and marketing tools, social and environmental added value and income-generating activities, food security, enhancement of social and productive organization, and business management.

Altogether the Brazilian national policy for Food and Nutrition Security merged to a current policy will motivate researchers to organize themselves into an interdisciplinary arrangement in order to meet the country's aims regarding the scientific contribution towards sustainable development. As a result, actions concerning food and nutrition in Brazil should take health, environmental and economic issues in combination with sustainability. Therefore, this thesis considers, among other references, the Brazilian perspective on Food and Nutrition Security at present, conducting the discussion in an interdisciplinary and holistic approach.

1.3.2. Health enhancing local foods: a food and nutrition-relevant action that offers a sustainable alternative and solutions to forestry protection.

According to Lajolo (*1*) and Melissa Williams (*30*) there are many opportunities to develop traditional plant markets in rural areas with indigenous knowledge and with

lower costs due to lower labor costs. Some species can be mentioned as an example of this statement as described in Figure 4.

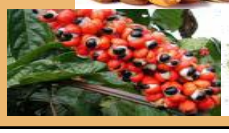
	Source	Component/function of interest
	acai	Lycopene, anthocyanins
	Camu-Camu fruit	Ascorbic acid
	Brazil nuts	Selenium
 	Guarana, Mate	Energy giving properties

Figure 4: Amazonian species with known functional properties. Source: Lajolo (*1*)

In summary, health enhancing local foods, also defined as functional foods, can contribute to health promotion; many Brazilian species have no commercial value and are locally consumed in an artisan manner. Nothing is known about their biofunctional properties and getting to know the health properties of these native fruits from the Amazon adds commercial and environmental values to underutilized species. In addition, health enhancing local foods would contribute to health promotion of local communities, going along with the aforementioned FNS policy.

The hypotheses, assumed to address the relationship of biodiversity and nutrition, should consider the access and consumption revealing the food component of FNS through socioeconomic and dietetic inquiry. However, research considering physiological, and biochemical investigation meets the nutritional factor of FNS. In addition, research aiming at providing data in contribution to food and nutrition security advances, should combine both aspects sharing a transdisciplinary approach. For this, aiming at the effective contribution of research initiatives on sustainability, scientific investigation should be performed within a transdisciplinary

approach and therefore, connected to policy makers, executioners and practitioners. Most societies recognize that food, medicine and health are interconnected. Ingested plant and animal products offer functional benefits to health in addition to essential nutrition (*31*).

The concept of functional foods was raised in Japan in the early 1980s and the first legislation for functional foods was first established as “Foods for Specified Health Use” (FOSHU) and in the early 1990s a first discussion for legislation was implemented. In addition, due to high costs of health care, especially because of the increased rates of non-communicable diseases and prolonged life spans, the country fostered a special research programme funded by the Japanese Ministry of Education, when the term “food function” appeared for the first time(*32*). Since, 2001 FOSHU products in Japan can also take the form of pills and tablets, even though most products are still in more conventional forms (*33*).

In Europe, The European Commission Concerted Action on Functional Food Science in Europe (FUFOSE), coordinated by International Life Science Institute (ILSI) defined functional food as: ‘‘a food product can only be considered functional if together with the basic nutritional impact it has beneficial effects on one or more functions of the human organism thus either improving the general and physical conditions or/and decreasing the risk of the evolution of diseases. The amount of intake and form of the functional food should be as it is normally expected for dietary issues. Thus, according to this, a functional food could not be in the form of pill or capsule, but just in the normal food form’’ (*34*). The European legislation however, does not contemplate functional foods as specific food categories, but somewhat as a concept (*35*). Based on this, the rules to be applied are numerous and depend on the nature of the foodstuff (*36, 37*).

Currently, a worldwide consensus defines functional foods as “foods expected to have specific healthy effects due to relevant constituents or foods from which allergens have been removed and foods where the effects of such an additional or

removal has been significantly evaluated, permission has been granted to make claims regarding the specific beneficial effect on health expected from their consumption". In another shorter definition it can be defined "food that may provide benefits beyond basic nutrition" (*38, 39*).

In Latin America, being at the vanguard in this issue, Brazil was the first country to establish a committee and develop legislation concerning functional foods in line with the international discussions, especially to the Codex Alimentarius Commission (*40*). In the late 1990s, a legal structure was developed concerning the biofunctionality properties of foods and ingredients.

In line with the European trend, the Brazilian legislation does not define properly the functional or health food, being more focused on the regulation of functional or health claims. However, it establishes that those foods that claim to be health and functional food are foods or ingredients that beyond their basic nutritional function, with regard to a nutrient, can produce metabolically or physiological effects and/or health benefits, being safe for consumption without medical supervision (*41*).

Firstly, the Brazilian legislation defines and establishes the guidelines for the analysis and proof of the functional property claims of a food (*41*). Based on this, a food should have its functional property claim proven and recognized by means of previously consumption or recommended by the producer, function, conditions of use and nutritional value, if relevant. Applied scientific evidence such as, nutritional, chemical and molecular composition; biochemical, nutritional, physiological and toxicological *in vivo* assays; epidemiological studies and clinical trials should also be a basis for the claim. Furthermore, wide evidence in scientific literature, institution of health and legislation internationally renown are also taken into account. Importantly, the legislation also mentions that the traditional use observed in the population without any association with health damage, is also considered in the proof basis requirements (*41*).

In addition, the food health and functional claims are also recommended as follow (*41*):

-Functional property claim - regarding the metabolic or physiological role of a nutrient or non-nutrient compound in the growth, development, maintenance and other normal functions of human organism.

-Health property claim - regarding the statement, suggestion or implication of the existence of a relationship between the food or ingredient and the disease or the condition related to health.

Health or functional claims can deal with the general maintenance of health, to the physiological role of nutrients and non-nutrients compounds or to the risk reduction to diseases. However, curative, therapeutic or preventive claims are not allowed (*41*).
Secondly, the Brazilian legislation regulates the procedures to register a food with health or functional properties. Every food which contains any health or functional claims should be submitted to health authorities and get registered identification (*42*).

With regard to the use of isolated bioactive compounds and probiotics, those have also been regulated since 2002 (*43*). The isolated bioactive compounds are defined as any compound extracted from its original source *e.*g. carotenoids, flavonoids and polyphenols. Considering the isolated bioactive compounds, any health or functional claim should follow the suitable regulation (*41*). Moreover, the product should also have safe quality for the recommended consumption and consider the use as a isolated bioactive compound within the food practices of the population. This type of product can be presented in solid or liquid forms such as, tablets, pills, granules, solutions or suspensions (*43*).
The Brazilian legislation defines the functional foods based on the nutritional concept of it and does not consider functional food as a pharmacological issue (*1, 41, 42*).
The legislation for food claims is necessary to protect consumers with regard to public health issues. Also, having the legal issue well established is an encouragement to the technological development, since it formalizes the food product category and establishes a new market for it (*44*).

However, it is undeniable that although the Brazilian legislation is considered at the vanguard with regard to Latin America, it still has to be improved since there is some overlapping in the regulations concerning food with functional and health claims, new food or ingredients, and bioactive substance or probiotics with functional properties and/or health claim yet (*45, 46*). As reported previously (*45*) lycopene, chitosan and garlic could be classified differently according to the current Brazilian regulations. For instance, lycopene could either be: a bioactive substance or a probiotic, a food with functional properties and/or health claims a new food or ingredient, or a medicinal plant, according to the current Brazilian legislation (*45*).

In addition to the health benefits, functional foods present new economic opportunities for many developing countries endowed with a rich biodiversity and traditional knowledge of the health effects of certain indigenous species. In a market-oriented economy, biodiversity including wild and agricultural ecosystems provides a significant portion of food consumed by either urban or rural populations (*31, 47, 48*). Currently, the demand for natural products, including functional foods, is continuously increasing worldwide. As reported previously (*30*) functional foods entered the global markets with force in the past decade and rapidly gained market shares conservatively estimated to exceed those for organic food.

Many plants used as ethnic foods are getting consumer's attention and are welcome in the marketing of nutraceuticals extracted from them (*49, 50*). In developing countries, the market for functional food is also emerging e.g. in China and Latin American (*51, 52*). Despite the health consciousness and the interest in well-being, consumers are not so open to excessive novelty (*53*). According to Barcelos *et al*. (2009) Brazilian consumers are expected to demonstrate a more traditional eating behavior due to strong regional and cultural roots of conservative consumers (*54*). Based on this, the development of functional food which takes the cultural aspects of food and nutrition could be an advantage. Although the eating culture is very diverse, health enhancing local foods could be not only a forestry preservation strategy but also an encouragement to new market frontiers.

With the population care and awareness of health and the growing market of functional and traditional foods, recently there have been many researchers engaged in discovering and studying the healthy functional compounds of vegetables, fruits and plants. Previous research was focused on studying the healthy effects such as anti-oxidant and anti-inflammatory effects of fruits and vegetables. Currently, much research is also focused on the anti-cancer effects of functional foods. The methods could apply qualitative or quantitative research to search for new molecules or content of beneficial elements existing in samples and applying *in vivo* or *in vitro* model testing to examine cell pathways.

For developing countries, the market of functional foods is important not only for its economic return but also in helping local communities to develop and/or improve their livelihoods. During the development of a functional food market, many job opportunities can be provided for local communities e.g. in raw material planting, food processing factories and wholesalers and retailers. According to Lajolo (*1*) and Melissa Williams (*30*) there are many opportunities to develop traditional plant markets in rural areas with indigenous knowledge and with lower costs due to lower labor costs. Some species can be mentioned as an example of this statement as described in Figure 4.

Besides the opportunity for diversified and high-value production, farming for the functional foods industry can benefit primary producers and rural communities in other ways. Poorer communities can benefit from growing functional food markets through domestication of wild plant species. Moreover, some of the crops with health-enhancing features may be native to marginal areas, where more traditional farming is difficult and returns are low. Also, functional properties can increase the value of otherwise rare plant species, which can aid in biodiversity conservation if their sustainable use is carefully managed (Figure 5).

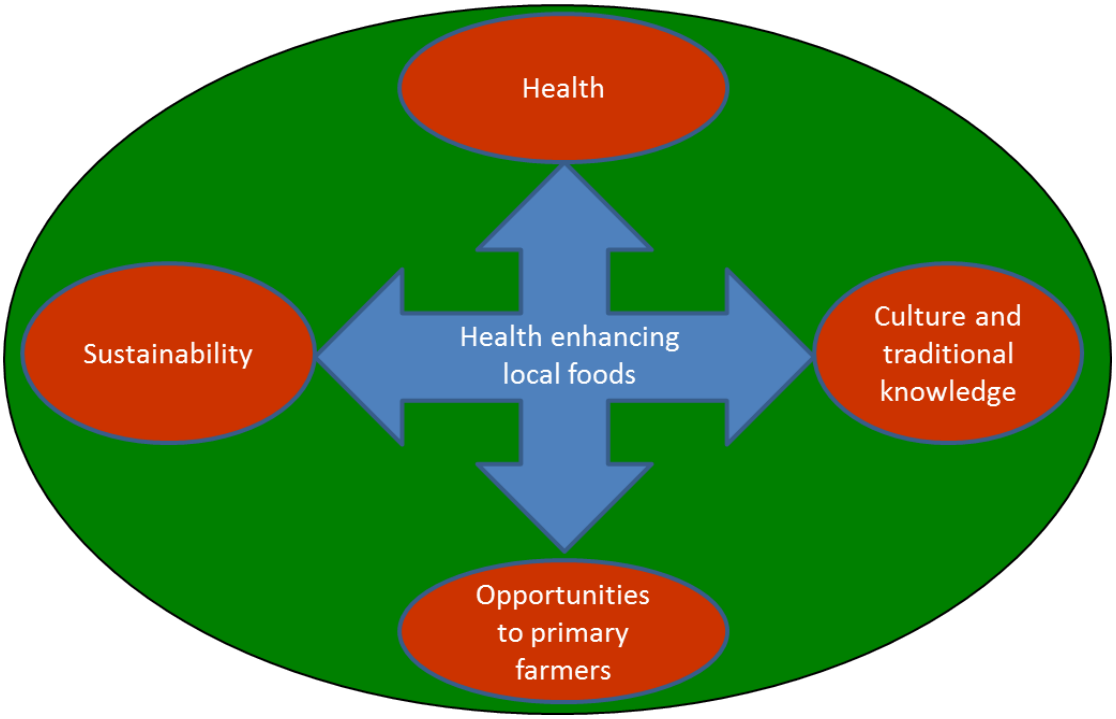


Figure 5: Overall Impact of Health enhancing local foods in Brazil. Source: Authors.

Figure 5 reflects some of the fields affected by health enhancing traditional foods. As aforementioned, the valorization of traditional species by the investigation of health properties could provide opportunities to primary farmers though extractivism activity. Extractivism is a sustainable activity since it preserves the forest. In addition, by knowing the health properties of neglected species, those could have their consumption reinforced, having an impact on rural community health without excluding their food culture practices.

As previously report (*55*) there is a growing movement of corporations which are perceiving environmental sustainability as simple and good business. Furthermore, the need for an improved debate about cohesive interactions among stakeholders such as industrial, governmental, large and small farmers, non-governmental organizations, scientific and conservation interests are of great importance.

The hypothesis assumes to address the relationship of biodiversity and nutrition, and should consider the access and consumption revealing the food component of FNS through socioeconomic and dietetic inquiry. However, research considering physiological, and biochemical investigation meets the nutritional factor of FNS. In addition, research aiming at providing data in contribution to food and nutrition security advances, should combine both aspects sharing a transdisciplinary approach. For this, aiming at the effective contribution of research initiatives in sustainability, the scientific investigation should be performed within a transdisciplinary approach and therefore, connected to policy makers, executioners and practitioners.

1.4 The potential preventive role of traditional fruits in health and nutrition through bioactive compounds

According to epidemiological studies, an increased intake of fruits, and hence of phytochemicals, can reduce the risk of non-communicable diseases such as cardiovascular diseases and cancer, which are still the leading causes of death all over the world. Most of NCDs are linked to aging and have been associated with oxidative damage caused by free radicals formed in a constant process occurring in aerobic organisms (*56*).

Free radicals are very reactive chemical species having an unpaired electron and are produced continuously in cells either as accidental by-products of metabolism or deliberately during e.g. phagocytosis. In cells, the most relevant are oxygen and its radical derivatives i.e. superoxide and hydroxyl radical, hydrogen peroxide and transition metals.

Reactive free radicals generated within cells can oxidize biomolecules and lead to cell death and tissue injury. However, cells possess several mechanisms of antioxidant defenses to prevent free radical formation or to at least counteract their harmful effects. Among enzymes that decompose peroxides and proteins that

sequester transition metals there are a great variety of compounds to “scavenge” free radicals (*57*)

According to Halliwell and Gutteridge (*58*) the biological antioxidant defenses are classified in radical chain–breaking antioxidants and preventive antioxidants. The first act by converting reactive free radicals to stable and non-aggressive molecules through hydrogen atom transfer reactions; the second act either by converting reactive oxidative precursors to unreactive species or by inhibiting the oxidation reaction to occur.

Antioxidants contribute to our biological antioxidant defense system and help to protect cells from oxidative species. Antioxidant systems for example scavenge reactive oxygen and nitrogen-free radical species, metabolize peroxides to non-radical products and prevent the generation of oxidizing species by chelating metal ions (*59*). Antioxidants can be of endogenous as well as of exogenous origin. Endogenous antioxidants are e.g. enzymatic systems like mitochondrial superoxide dismutase, glutathione peroxidase or catalase (*23*). Proteins that minimize the availability of pro-oxidants such as iron ions, copper ions and haem are also important (*e.g.* transferrin) (*58*). Most important exogenous antioxidants come from the diet.

An imbalance between oxidants and antioxidants and the overproduction of oxidants can lead to cell damage, which is called “oxidative stress”. It is widely believed that oxidative stress contributes crucially to the development of NCDs (*60*). Human cells are permanently exposed to various oxidizing agents. Some of them are necessary for life such as normal endogenous metabolic processes. Chronic inflammations are also important sources of free radicals. Moreover, external sources such as pollutants, cigarette smoke and sunlight have to be taken into account (*56, 61*).

Oxidative damage affects all types of biomolecules, including lipids, proteins and DNA (*23*). Oxidative stress is a highly relevant factor causing oxidative DNA damage

that can eventually lead to mutations if left unrepaired. However, the impact on the development of cancer is not only based on direct effects on the DNA but also on processes like signal transduction, cell proliferation and cell death. Since injury mechanisms can overlap, it is unclear in many situations which one is the major target. The primary cellular target of oxidative stress varies depending on the cell, the type of stress imposed and how severe the stress is.

The search for antioxidants from natural sources is ongoing and efforts have been made to identify compounds which could help to prevent oxidative damage from occurring in the body. Dietary phytochemicals in fruits and vegetables have recently been made responsible for decreasing cancer risk by reducing oxidative stress and modulating signal transduction pathways involved in cell proliferation and survival.

1.4.1. Phytochemicals

Phytochemicals are chemical compounds found in plants and defined as bioactive compounds. They have been associated with a reduced risk of major chronic diseases e.g. cancer and cardiovascular disease due their antioxidant abilities (*62*). Until recently, more than 5000 individual phytochemicals have been identified in fruits, vegetables and grains. But a large part of them is still not characterized and needs to be investigated (*63*). Phytochemicals are classified into five different groups namely carotenoids, phenolics, alkaloids, nitrogen-containing compounds and organosulfur compounds. Most of the phytochemicals belong to the “phenolics” group. This group can be further divided into five sub-groups: phenolic acids, flavonoids, stilbenes, coumarins and tannins (*62*). Figure 6 shows a classification schema of dietary phytochemicals.

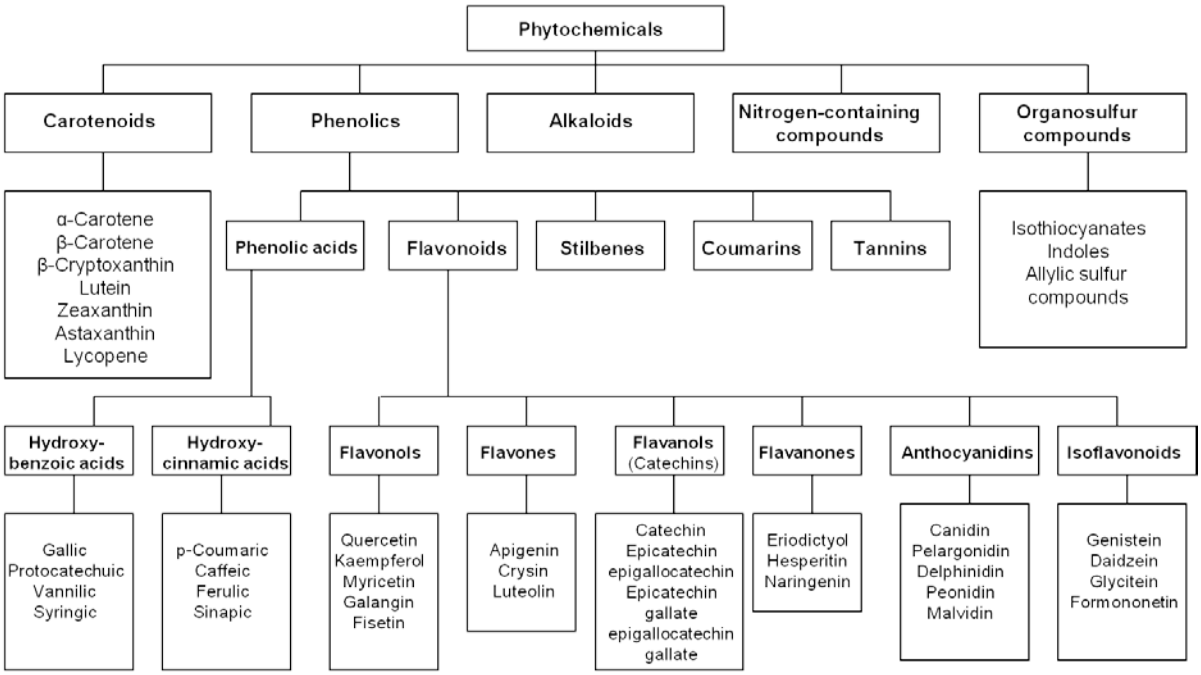


Figure 6: Classification chema of dietary phytochemicals: Classification schema of dietary phytochemicals (63).

Phytochemicals can improve the immune system, regulate gene expression in cell proliferation and apoptosis, regulate the hormone metabolism, and have antibacterial effects (*61*). Studies with phytochemicals from several common fruits and vegetables have already shown an antioxidant and antiproliferative effect on different cell lines (*61, 64, 65*). Proteggente *et al*. (*66*) showed that fruits and vegetables rich in anthocyanins (e.g. strawberry, raspberry and red plum) displayed the highest antioxidant activities, followed by those rich in flavonones (e.g. orange and grapefruit) and flavonols (e.g. onions, leek, spinach), while the hydroxycinnamate rich fruits (*e.g*. apple, tomato, peach) had lower antioxidant activities. Numerous studies point out that the antioxidant and anticancer activities of fruits and vegetables are based on the additive and synergistic effects of their phytochemicals (*63, 65, 67, 68*). Thus, the benefit of a diet rich in a variety of fruits and vegetables goes back to the complex mixture of phytochemicals which are present in whole fruits (*63*).

1.4.1.1. Phenolic compounds

Phenolics are important products of the secondary plant metabolism characterized by possessing one or more aromatic groups with one or more hydroxyl groups. They can be conjugated to mono- and disaccharides or even form a complex with oligosaccharides, lipids, amines and carboxylic and organic acids (*59*). In plants, they affect physiological functions, such as reproduction and growth; they are also part of the defense mechanisms against pathogens, parasites and predator. In addition to these crucial functional aspects in plant life, phenolics also play structural roles in different supporting and protective tissues and have signaling properties which are especially important for the interactions between plants and their environment (*69*); finally, phenolics also contribute to plant color.

The important antioxidant activities of phenolic compounds can be used in processed food as a natural antioxidant. However, in addition to their major roles in plants, phenolics play a significant protective role concerning health benefits for humans.

Currently, many studies are conducted with phenolic compounds with antioxidant ability or anti-cancer activity in vegetables, fruits, whole grains and other plants; this can also help make phenolics healthy food additives in order to prevent chronic diseases. Due to their antioxidant activity, phytochemicals can scavenge free radicals and modulate enzyme activities of detoxification, oxidation and reduction (*70*).

The antioxidant capacity of phenolics is mainly due to their redox properties. For example they are able to donate a hydrogen atom from an aromatic hydroxyl (OH) group to a free radical and to support unpaired electrons by delocalization (*59*). They can act as singlet oxygen quenchers and reducing agents (*71, 72*). Most of the phenolics can be found in berry, fruits, tea and coffee. The best sources of berries are chokeberry (96 mg/100g), blueberry (85 mg/100g), sweet rowanberry (75mg/100g). The content in teas range from 30 to 36 mg/100g and coffee has an amount of 96mg/100g (*62, 73, 74*).

1.4.1.2. The chemopreventive effect of phenolic compounds on cancer

There are many oxidizing agents existing in the environment. The agents appear in food, water, air or can be activated by metabolism through the cell itself. Overproduction of oxidants can cause an imbalance of physiological conditions of the human body. Oxidative stress can cause damage of DNA, proteins and lipids leading to diseases in human body such as cancer (*75*). Cancer is a disease in which cells of the body start to grow out of control and finally spread all over the body forming metastases. Many types of cancer form a lump or a tumor which derives its name from the part of the body where it initially grows, e.g. hepatocellular carcinoma (*76*).

There are three main steps involved in the process of cancer formation: tumor initiation, promotion and progression. The initiation stage is fast and irreversible and a chain reaction of extracellular and intracellular events. In this stage, uptake or exposure to a carcinogenic agent happens. The carcinogenic agent can be transported to the target tissue or organ where it is either detoxified or reacts with the target DNA leading to genotoxic damage. The promotion stage is a relatively long and reversible process; pre-neoplastic cells can proliferate and accumulate during this stage. In the final progression stages, transformation of neoplastic cells to fast growing tumor cells occurs with aggressive and lethal potential to the human body (*77*).

Dietary phytochemicals in fruits and vegetables are likely responsible for a decreased cancer risk by reducing oxidative stress and modulating signal transduction pathways involved in cell proliferation and survival. Numerous phenolic plant compounds have been found to exhibit an antiproliferative activity (*61, 73, 78, 79*). Liu *et al*. (*80*) observed a direct relationship between phenolic compounds and antiproliferative activity in raspberries. Strawberries' phenolic extracts inhibited hepatoma HepG2 cell proliferation significantly and in a dose-dependent manner (*81*).

1.5. Objective

The absence of information about the health situation and dietary intake spawned a nutritional survey as a first step in this research; based on these preliminary results, a second phase was developed to determine the biochemical profile of two Brazilian traditional fruits.

Based on the Food and Nutrition Security concept aforementioned, the work comprised an interdisciplinary profile and aimed at studying the potential of traditional Brazilian fruits as a possible strategy in health promotion in NCD prevention, especially for the rural people of Tocantins State by the investigation of these fruits as a functional food in a community-based study.

2. Methodology

2.1 Research Design

Based on the principle of Sustainable Food and Nutrition Security discussed in the introduction topic, this study was performed in two parts. The first part consists of a nutrition survey (field study) involving two different communities in the territory of Cantão, Tocantins. The food and nutritional status of families and the nutritional status of adults were assessed in this part, based on realistic data of the health and nutritional situation of the communities. Also based on the contact with the rural people, two fruits were selected for the second part of the study. Then, laboratory assays aiming at investigating the functional properties of the selected fruits were performed.

2.2. Field study

2.2.1. Research area and Sampling

The research was carried out in the Tocantins State, located in the northern Brazil, in a region well-known as Brazilian Legal Amazon Region. The State is situated in a transition area, having climate and vegetation from the Amazon Rainforest (15%) and Cerrado (85%) - or Brazilian savannah This transition area, so-called Ecotone zone, comprises traditional communities (family agriculture, indigenous as well as quilombolas) and a rich biodiversity, which is responsible for several environmental services. For this reason, scientific efforts, studies and research in the area are extremely important aiming at understanding the different farming systems and their linkages to the local economy and environment.

The region also comprises an Environmental Preservation Area of Bananal Island/Cantão[3] (APA-Cantão), which was created by a law of Tocantins State in 1997, and occupies around 1,687,000 hectares, and is characterized as a "Conservation Unit of Direct Use" which means that exploitation and economic use are allowed if done in a sustainable manner. It is also defined as a "Unit of Sustainable Use", where the preservation of biodiversity and natural resources must be in harmony and equilibrium with the exploitation of these resources. In addition, the biological value of Cantão is based on its formation as a Delta of the Javaés River with more than 800 lakes and channels, and it is considered a complex ecotone area with elements of Savannah, Amazon Rainforest, Pantanal and Atlantic Forest (Figure 7). Although APA-Cantão is considered a local priority for, conservation, it also involves different stakeholders due to the increasing human occupation and agricultural activities in this area.

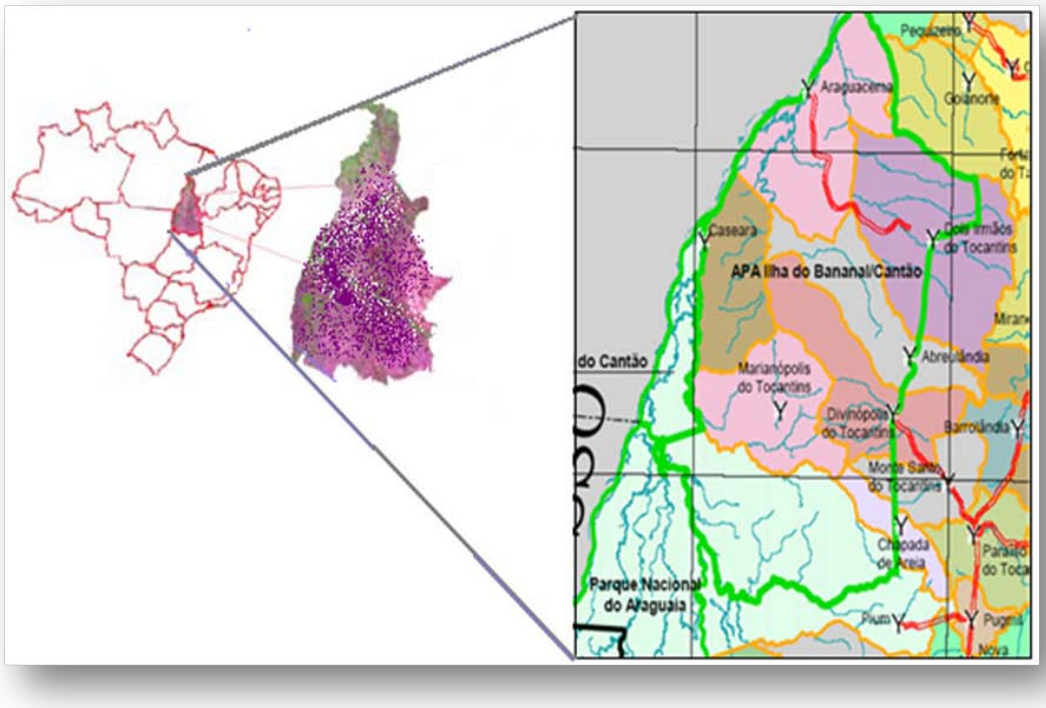


Figure 7: APA-Cantão - (with the green borderline).

[3] The APA-Cantão (Environmental preservation Area – Cantão) comprises the following municipalities: Abreulândia, Araguacema, Caseara, Chapada de Areia, Divinópolis, Dois Irmãos, Marianópolis, Monte Santo e Pium.

2.2.2. Socioeconomic status of Households

Aiming at doing the data collection and therefore, form the database, a cross-sectional study with men and women (above 18 years old) was carried out at two rural settlements (so-called União and Califórnia) located at the municipality of Caseara, which belongs to the Environmental Preservation Area of Bananal Island/Cantão (APA-Cantão). As the settlement origin process is very diversified, it might influence food and nutrition security issues. Thus, both rural settlements chosen were located nearby each other, and the communities have similar characteristics.

The rural settlements were identified and the sampling consisted of a mini-census where all families were visited (n=90), informed about the objective of the study, as well as invited to take part in it (*82*). All households of rural settlements were visited (57 families interviewed, 3 refuses, 31 absents), with a response rate of 62.2%. The household heads were invited to answer a household questionnaire, and adults (above 18 years old) were invited to answer an individual level questionnaire (Figure 8 and Figure 9). A total of 108 individuals (58 women and 50 men) agreed to take part to the study; 3 were excluded due to pregnancy, and 3 refused to participate. In addition, the results presented later on in the chapter aim to support future extension projects in the communities as a feedback for their participation and involvement in the research.

Figure 8: Participant being interviewed during field research. Source: author (2008).

Information concerning socioeconomic, health and food security were accessed by the household questionnaires. Socioeconomic data is very important and widely used for its contribution to the knowledge of the public under study and helping the interpretation of other variables. The household food security level was evaluated through the household questionnaire and measured by the Brazilian Scale on Food and Nutrition Security (EBIA), which was validated in a national study involving rural and urban populations (*83*). For the purpose of the research, the software package SPSS version 16 (2007; SPSS Inc., Chicago, IL, USA) was used to compute all statistical analysis.

Figure 9: Participant family. Source: author (2008)

2.2.3. Analysis of Nutritional situation of adults from the two rural communities

2.2.3.1. Anthropometric measures of subjects

Anthropometric measures were carried out in triplicate, by well-trained anthropometrists and the mean values were recorded. At the home visit, subjects (adults) were weighed with light clothes but without shoes or socks, using an electronic portable scale with an accuracy of 100g (Plena®, São Paulo, Brazil) and height was obtained by a stadiometer with an accuracy of 0.1cm (Altura Exata®,

Minas Gerais, Brazil), in standing position, feet together and head placed according to the Frankfurt plan. Waist circumference (WC) was determined with a flexible non-elastic measuring tap, and taken exactly halfway between the margin of the lowest rib and above the iliac crest. The used cut-off point for WC was identified for the Brazilian population by (*84*) which indicates 88cm for men and 83 cm for women.
Body mass index (BMI) was calculated as Kg/m^2 as the following expression:

$$BMI = \text{weight (Kg)}/\text{height}^2 \text{ (m)}$$

BMI was analyzed in accordance to World Health Organization (WHO) cut-off point as described in Table 1.

Table 1: Classification for Nutritional Status according to BMI

Classification	BMI (kg/m^2)
underweight	<18.50
eutrophic	18.50 - 24.99
overweight	≥25.00
obese	≥30.00

Source: WHO, 2004 (*18*).

2.2.3.2. Dietary intake and Energy requirements

During home visit subjects were interviewed about their 24-hour recall and food frequency questionnaire (FFQ) to achieve dietary intake and food patterns. Dietary data from 24-hour recall were analyzed for food composition using AvaNutri Software (food database: TACO) (2008; Avanutri, Rio de Janeiro, Brazil).

Food frequency questionnaires (FFQ) for the previous last two months were used to measure dietary patterns. Consumption frequency of each group of food was divided into six categories and the consumption of each food was quantified, receiving the weights presented in Table 1 The food consumption was specified based on weekly

frequency The codification used in the research was adapted from those used by (*85*)(*85*)(*85*)Liu *et al*. (*85, 86*).

Table 2: Codification of weekly food consumption

No consumption	1 to 3 times /month	Once a week	2 to 4 times/ week	5 to 6 times/week	Once a day	2 or more times/day
0	0	1	3	5.5	7	14

Source: adapted from Liu *et al*. (*85*).

Basal Metabolic Rate (BMR) was calculated using the equation of Schofield for the specific age as described in Table 3.

Table 3: Equations for estimating BMR from body weight*:

Age (years)	BMR: MJ/day	see[a]	BMR: kcal/day	see[a]
Males				
< 3	0.249kg – 0.127	0.292	59.512kg – 30.4	70
3–10	0.095kg + 2.110	0.280	22.706kg + 504.3	67
10–18	0.074kg + 2.754	0.441	17.686kg + 658.2	105
18–30	0.063kg + 2.896	0.641	15.057kg + 692.2	153
30–60	0.048kg + 3.653	0.700	11.472kg + 873.1	167
≥60	0.049kg + 2.459	0.686	11.711kg + 587.7	164
Females				
< 3	0.244kg – 0.130	0.246	58.317kg – 31.1	59
3–10	0.085kg + 2.033	0.292	20.315kg+ 485.9	70
10–18	0.056kg + 2.898	0.466	13.384kg + 692.6	111
18–30	0.062kg + 2.036	0.497	14.818kg + 486.6	119
30–60	0.034kg + 3.538	0.465	8.126kg + 845.6	111
≥ 60	0.038kg + 2.755	0.451	9.082kg + 658.5	108

*Weight is expressed in Kg. Source: *Schofield (87)* FAO, (*88*).

[a] see = standard error of estimate.

Total energy expenditure (TEE) was calculated by totaling Basal Metabolic Rate (BMR) multiplied by the physical activity level factor (PAL) which, is equivalent to the energy requirement. Minimum limits for PAL internationally suggested by FAO (2004)(*88*) were applied. Mild PAL (1.4) was used for those subjects involved in light activity lifestyle (domestic work, teaching, office work, driving cars). Moderate PAL (1.7) was used to those with active lifestyle and high PAL (2.0) was applied to subjects with vigorous lifestyle and occupation e.g. non-mechanized rural workers. Energy Balance (EB) was calculated from energy intake minus total energy expenditure.

The Nutrition Survey data was collected during the months of June, July and August of 2008. The study was approved by the Ethics Committee of the Federal University of Tocantins and conducted in accordance with the Brazilian resolution CNS 196/96.

2.2.3.3. Statistical Analysis

Data is expressed as means $\pm$ standard deviation (SD), and the statistical differences between eutrophic and overweight/obese of the same sex were determined with an unpaired student's *t* test, after checking for normality of distribution of the dependent variable with Kolmogorov-Smirnov test. Otherwise, a Mann-Whitney test was performed. $\chi 2$ (Chi-square) test was carried out for the proportions.

2.2.4.1. The Linear model

In the linear probability model, the estimated coefficients express the effect of unit variations in the independent variables about the probability of the dependent variable takes value equal to one. Linear regression was carried out as the equation n. 1 and variables description is illustrated in Table 4.

$$Ln\ (\text{BMI})_i = \beta_0 + \beta_1(\text{male})_i + \beta_2(\text{PAL moderate})_i + \beta_3(\text{PAL high})_i + \beta_4 \ln(\text{Kcal/body weight})_i + \beta_5 \ln(\text{protein/body weight})_i + \beta_6 \ln(\text{carbohydrate/body weight})_i + \beta_7 \ln(\text{lipids/body weight})_i + \varepsilon_i \quad \text{eq. 1}$$

Table 4: Variables description of linear regression

Name	Description	Unit of measurement
GENDER	Dummy for gender 1 = female 0 = male	
PAL MOD	Dummy for Moderate Physical activity level 1 = moderate 0 = otherwise	
PAL HIGH	Dummy for High Physical activity level 1= high 0 = otherwise	
KCALBW	kcal/bodyweight	(Kcal)/(Kg)
PTNBW	protein consumed/kg body weight	(g)/(Kg)
CHOBW	carbohydrate consumed/body weight	(g)/(Kg)
LIPBW	lipid consumed/bodyweight	(g)/(Kg)

Each variable was transformed into logarithms (*Ln*), partly to reduce heterocedasticity (*89*). The coefficients of the variables expressed in logarithms were thus the "elasticities" which means that for the % change in the dependent variable resulting from a 1% change in the independent variables) (*90*).

The software package SPSS version 16 (2007; SPSS Inc., Chicago, IL, USA) was used to compute all statistical analysis and the level of statistical significance was set at $p < .05$.

2.3. Biofunctional properties of traditional Brazilian fruits *in vitro*

2.3.1. Chemicals

All chemicals used in the study were of analytical or HPLC grade. Gallic acid, aluminum chloride ($AlCl_3$), (+)-catechin, cyanidin–3-glucoside, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid (Trolox), DCFH-DA (2'-7'-dichlorofluoresceindi-acetate); 2,2'-azinobis (3-ethyl-benzothiazoline-6-sulfonate) (ABTS), MTT (3,-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-tetrazolium bromide), MUH (4-methylumbelliferyl heptanoate), methylene blue were obtained from *Sigma*–Aldrich (*Steinheim*, *Germany*). Folin-Ciocalteau reagent; *2*,*4*,*6*-tris(*2-pyridyl*)-s-triazine (TPTZ), quercetin dihydrate and 2,2`-azobis(2-amidino-propane) dihydrochloride (AAPH), fluorescein in CAPS solution (20 mM) and Trypan blue solution were obtained from *Fluka-Chemie* **(***Buchs*, Switzerland). ABAP (2,2'-azobis (2-amidipropane)) (WakoChemicals, USA); DMEM/Ham's F-12 was from PromoCell GmbH (Heidelberg, Germany). Fetal calf serum (FCS), penicillin/streptomycin, trypsin/EDTA and HBSS (Hanks´ Balanced Salt Solution (with Ca^{++}, Mg^{+}, without phenol red)) were obtained from PAA Laboratories (Pasching, Austria). Water used in all analyses was ultra-pure water produced through a Milli-Q system (Millipore Corporation, France).

2.3.2 Plant Materials and Sample preparation

2.3.2.1. Bacaba (*Oenocarpus Bacaba* Mart.) and Jenipapo (*Genipa Americana* L.)

Bacaba and jenipapo are edible native fruits from Brazil originating from the Amazon and Cerrado (Brazilian Savannah). The fruits locally consumed are processed into drinks, jelly, ice cream etc. In folk medicine, these fruits are believed to possess functional properties; however, there are no studies investigating the assumed functional properties such as antioxidant activity until recently.

Fruit samples of bacaba (*Oenocarpus bacaba* Mart.) and jenipapo (*Genipa americana* L.) were acquired from small farmers and gathered in Tocantins State, Brazil, during November and December of 2008, washed with water to remove debris and dried manually (Figure 1). Fruits were selected and damaged units were excluded. For bacaba fruit only seeds were discarded. Subsequently, samples were lyophilized (LS 3000, Terroni, Brazil), milled in a knife mill (8500 rpm; 12 s) (Retsch, Grindomix GM200, Germany), sealed under vacuum and stored in the dark in a refrigerator (4 °C) until use (Figure 1). Additionally, water contents of original samples were determined by drying at 105 °C ± 5 °C to constant weight to allow calculations based on fresh weights of fruits (FW) (*91*). Consequently, all results are expressed and referred to fresh weight contents of fruits.

2.3.2.2. Plant materials

Fruit samples of bacaba (*Oenocarpus bacaba Mart*.) and jenipapo (*Genipa americana L.*) were acquired from small farmers and gathered in Tocantins State, Brazil, during November and December of 2008. Fruits were washed with water to remove debris and dried manually. Afterwards, fruits were selected and damaged units were excluded. Shell and seeds were removed from jenipapo, but for bacaba only seeds were discarded. Then, samples were lyophilized (LS 3000, Terroni, Brazil) and milled in a knife mill (Grindomix GM200, Retsch, Germany) at 8500 rpm for 12 s. Powders were sealed under vacuum and stored in the dark and at 4°C until further use.

2.3.2.3. Fruit Extract Preparation for HPLC analysis

On the basis of methodology tests to establish optimal conditions for the quantitative recovery of phenolic compounds (data not shown), aqueous acetone (80%; v:v) was used for the extraction of phenolic compounds from bacaba fruit. Extracts were prepared as described by Kammerer *et al*. (*92*). Briefly, aliquots of 50 mg were weighed into conical flasks and extracted with 5 mL of solvent for 2 h under stirring

after flushing with argon in order to prevent oxidation during extraction. The extracts were centrifuged (10 min, 4000g). The combined supernatants were evaporated to dryness in vacuum at 30 °C. For the HPLC analysis the residue was dissolved in 10 mL of acidified water (final pH= 2.25), whereas to the additional analyses the residue was resuspended in PBS^- (final pH = 6.35).

2.3.2.4. Fruit Extract Preparation for biological assays with cells

Fruit extracts were prepared as described by Kammerer *et al*. (*92*) with slight modifications. Aliquots of 20 g were weighted into conical flasks and extracted with 400 mL of solvent (80% acetone) for 2 h under stirring after flushing with argon in order to prevent oxidation during extraction. The extracts were filtered and centrifuged for 10 min at 4000 g. The supernatant was evaporated to dryness in vacuum at 30°C and the residue dissolved in 100mL of PBS^- (Phosphate saline buffer), resulting in a stock solution of 200 mg of fruit dry weight (DW)/mL. The final solution was sterile-filtered aliquoted and kept in -80 °C until use.

2.3.3. Total phenolic contents, antioxidant and antiproliferative activities of bacaba and jenipapo phenolic extracts.

2.3.3.1. Total phenolics

The quantification of polyphenols was performed using the Folin-Ciocalteau (FC) assay (*93*). Total phenolic contents were expressed as milligram of gallic acid equivalents (GAE) per gram of fruit fresh weight (FW).

2.3.3.2. Total flavonoids

The content of total flavonoids was assessed by the colorimetric aluminum chloride assay described by Sariburun *et al*. (*94*) and adapted to microplate measurements in our lab. Briefly, 20 µL of $NaNO_2$ solution (10%) was added to 50 µL of catechin or

sample dilutions into 96 well plates. After 5 min, 20 µL of $AlCl_3$ (5%, m/v) was added to each well and left for 6 min. Subsequently, 100 µL of 1M NaOH solution was added, and absorbance was read at 510 nm with a microplate reader Synergy MX (Biotek, USA). Results were expressed as mg of catechin equivalents (CTEq)/100 g FW.

2.3.3.3. Total anthocyanins

Total monomeric anthocyanin content was assessed by the pH-differential method using two buffer systems - potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M) as described by Sellappan *et al*. (*95*) and adapted to microplate volumes in our lab. Briefly, 20 µL of standard or sample solution were pipetted into two different 96 well-plates and mixed with the respective buffers (180 µL). Subsequently, each plate was read at 510mn and 700 nm of absorbance. Wells containing buffer without sample solution were used as blanks. Cyanidin-3-glucoside was used as standard, and therefore results were expressed as mg of cyanidin-3-glucoside equivalents (cyn-3-glcEq)/100 g FW. Absorbance (A) was calculated according to the following formula (eq.2):

$$A = (A_{510\ nm} - A_{700\ nm})_{\ pH\ 1.0} - (A_{510\ nm} - A_{700\ nm})_{\ pH\ 4.5} \qquad \text{eq.2}$$

2.3.3.4. Oxygen radical absorbance capacity (ORAC) assay

The ORAC assay procedure applying fluorescein as a fluorescence probe was performed according to the methodology developed by Cao and Prior (*96*). Briefly, fluorescein in 20 mM CAPS solution, pH10 (Fluka, St. Louis, USA) was used as stock solution and diluted in phosphate buffer solution pH 7.4 (PBS) to obtain a working solution of 4 nM. AAPH (peroxyl radical was generated using 2,2`-azobis(2-amidino-propane) dihydrochloride) was freshly prepared for each run at a concentration of 15 mM and kept in ice until automatic injection. Trolox (6 - hydroxy - 2, 5, 7, 8 - tetramethylchroman-2-carboxylic acid) solutions were used for establishing calibration curves (0, 6.25, 12.5, 25, 50, 100 µM) and were prepared daily from a 100

µM stock solution stored at -80 °C. For each set of measurements, a standard curve with trolox as reference was plotted. Analyses were performed using clear bottom black 96-well plates (Greiner Bio-one Cellstar, Frickenhausen) and an automated plate reader (Synergy MX, Biotek, USA). The exterior wells of the plate were filled with 300 µL of water and pre-heated to 37 °C to avoid oscillation of temperature during the analysis. Sample aliquots (25 µL) were mixed with 200 µL of fluorescein solution (4 nM) and incubated for 30 min at 37 °C in the microplate. AAPH (25 µL) was automatically injected, and the microplate was shaken for 20 s at fast mode. Fluorescence readings ($\lambda_{excitation}$ = 485 $\pm$ 20 nm and $\lambda_{emission}$ = 520 $\pm$ 20 nm) were registered each minute over 60 min. All samples were analyzed in three different experiments with five wells for each dilution. Antioxidant activity was expressed as µM of trolox equivalents (TE)/100 g fresh weight (FW) based on the calculation of the area under the curve (AUC; eq. 3) and interpolation to the regression analysis of trolox calibration curve.

$$AUC = 0.5 + (f_2/f_1) + (f_3/f_1) + (f_4/f_1) + \ldots\ldots\ldots + 0.5(f_{60}/f_1) \qquad \text{eq. 3}$$

2.3.3.5. Trolox equivalent antioxidant capacity (TEAC) assay

This assay was performed as previously described by Re and Pellegrini (*97*). Briefly, the radical was formed by mixing 0.5 mL of 20 mM ABTS in phosphate buffer (pH 7.4) with 95 mL of a 2.5 mM ABAP (2, 2'-azobis (2-amidinopropane hydrochloride) solution. The solution was heated for 15 min at 60 °C and protected from light. Subsequently, 190 µL of the radical solution was added to 10 µL of different dilutions of fruit extracts (0.01-0.6 mg/mL) and trolox (5-140 µM) with an automatic injector. Absorbance was monitored at 734 nm for 30 min. All samples were analyzed in triplicate for each dilution. Antioxidant activity was expressed as µM TE/100 g FW based on the calculation of AUC values and interpolation to the regression analysis of trolox calibration curve (eq. 4).

$$AUC = 0.5 + (f_2/f_1) + (f_3/f_1) + (f_4/f_1) + \ldots\ldots\ldots + 0.5(f_{60}/f_1) \quad \text{eq. 4}$$

2.3.3.6. Ferric reducing ability of plasma (FRAP) assay

The measurement of the reduction of the ferric 2, 4 ,6-tripyridyl-s-triazine complex (Fe^{3+}-TPTZ) to its ferrous form (Fe^{2+} -TPTZ) in the presence of antioxidants was carried out as previously described (*98*),(*99*) Briefly, FRAP solution was prepared by mixing 30 mM acetate buffer (pH 3.6), 10 mM TPTZ and 20 mM $FeCl_3x6\ H_2O$ with a ratio of 10:1:1 (v/v/v). $FeSO_4x7\ H_2O$ and trolox were used as standards to allow further comparisons. A 1.0 mM stock solution of $FeSO_4x7\ H_2O$ and 1.5 mM stock solution of Trolox were used for preparing different dilutions (0.02-0.14 µM and 15-50 µM, respectively) for the standard curves, and different dilutions of bacaba (0.5-2.5 mg/mL) were also performed in order to build a curve allowing the quantification in a linear range of detection. A total of 150 µL of FRAP reagent was added to each well. Additionally, 20 µL of standard or sample solutions were added. Absorbance was read at 593 nm, and a well containing FRAP reagent without sample was used as blank. FRAP values were calculated by the interpolation of trolox and $FeSO_4x7\ H_2O$ calibration curves

2.3.3.7. Free radical scavenging capacity (DPPH assay)

The free radical scavenging capacity was analyzed by the DPPH assay in accordance with the method described by Al-Duais *et al*. (*100*) and adapted to microplate volumes in our lab. 100 µL of standard or diluted sample solution of bacaba (0.01-0.5 mg/mL were mixed with 100 µL of DPPH (0.3 mM, ethanolic solution). Methanol (200 µL) was used as control, and 100 µL of ethanol with 100 µL of DPPH was used as a blank. A calibration curve was prepared with different dilutions of trolox (10, 20, 40, 60, 80, 100, 120, and 140 µM). The absorbance was measured at 510 nm for 30 minutes (*101*). Absorbance was converted to antioxidant

activity (AA %). The radical scavenging activity (DPPH •) was calculated according to equation 5:

$$AA\% = 100-[(A_{Sample}- A_{Blank})*100/A_{Control}] \quad \text{eq. 5}$$

Where:

A_{Sample} = sample absorbance after 30 min of reaction;

A_{Blank} = blank absorbance;

$A_{Control}$ = control absorbance

Results of DPPH reduction from samples were linearized and interpolated to the standard trolox curve. Results were obtained from three independent experiments and expressed in mM of trolox equivalents (TE)/100 g of fruit (FW).

2.3.3.8. Cell culture of HepG2 cells

HepG2 cells were cultured in DMEM/Ham's F12 with stable glutamine supplemented with 10 % FCS and 1 % Penicilin/Streptomicin at 37 °C in a water-saturated atmosphere with 5 % CO_2. Media was changed every two or three days and cells were sub-cultured before reaching 80% confluency.

2.3.3.9. Cellular antioxidant activity assay (CAA)

The cellular antioxidant activity assay was performed as described by Wolfe and Liu (*102*). Briefly, HepG2 cells were seeded into a black 96-well-plate (Nunc, Denmark) at a density of 6 x 10^4 cells/well in 100 µL of growth media without phenol red to avoid undesired background fluorescence. The outer wells of the plates were filled with water to favor temperature stability during the assay. After incubation for 24 h (37 °C; 5% CO_2), growth media was removed by gently turning the plate upside-down onto a towel paper soaked in 70% ethanol, and cells were washed with 100 µL of Hanks´ Balanced Salt Solution (HBSS). Afterwards, cells were treated for 1 h with

100 µL of fruit samples (37 °C; 5% CO_2). Different concentrations of fruit extracts were prepared in media containing 25 µM of DCFH-DA and being devoid of phenol red and FCS. Quercetin was used as a standard compound in different dilutions in each plate as suggested by Wolfe and Liu (*102*). After 1 h of treatment, media were removed, and cells were washed again with 100 µL of HBSS. Subsequently, 600 µM ABAP was applied to the cells in 100 µL HBSS. Control triplicates were treated with DCFH-DA and ABAP but did not contain fruit extracts, and blank triplicates were treated only with HBSS without ABAP. The outer wells, which were not used in the beginning, were filled with 300 µL of sterile pre-warmed water (37 °C). Fluorescence was read at 37 °C in a microplate reader (Synergy MX, Biotek, Bad Friedrichshall, Germany) ($\lambda_{excitation}$ = 485 $\pm$ 20 nm and $\lambda_{emission}$ = 538 $\pm$ 20 nm) in 5 min intervals for 90 min. Wells were treated in triplicate for each extract concentration, and control and blank wells were included containing only media plus DCFH-DA without fruit extracts. At least three independent experiments were performed to determine cellular antioxidant activity. Triplicates were used in each experiment for each extract concentration. After blank subtraction the AUC values for the fluorescence *versus* time plot were determined to calculate the CAA units for each fruit concentration according to equation 6:

$$\text{CAA unit} = 1 - (\int SA / \int CA) \qquad \text{eq. 6}$$

with:

$\int$ SA = area under the sample fluorescence *versus* time curve
$\int$ CA = area under the control curve.

The median effective dose (EC_{50}) was determined from the median effect plot of $\log_{(fa/fu)}$ *versus* $\log_{(dose)}$, where f_a is the fraction affected (CAA unit) and f_u is the fraction unaffected (1-CAA unit) by the treatment. EC_{50} is the concentration at which f_a/f_u = 1, as calculated from the linear regression of the median effect curve. EC_{50} values are expressed as mean ± SD for triplicate data obtained from the same experiment. In each experiment, quercetin was used as standard, and cellular

antioxidant activities for fruit extracts were expressed as µM of quercetin equivalent (QE) per 100 g of fruit (FW) and µM QE/100 µmol of phenolics.

2.3.3.10. MTT assay

HepG2 cells were seeded at a density of 2 x 10^4 cells/well into 96-well-plates and incubated for 4 h at 37°C in a 5 % CO_2 atmosphere. Afterwards, media with fruit extracts in different concentrations (400, 600, 800, 1000, 1200, 1400 µg/mL) were added and the plate was further incubated for 96 h. As a negative control, culture media without extracts was added and as a positive control, 2 % DMSO in culture media was used in order to kill cells.

After incubation, the culture media including the extracts was removed by gently turning the plate upside-down. Then the cells were washed with 100 µL of PBS^- before being incubated with 20 µL of MTT solution and 100 µL of culture media without FCS. During 1 h of incubation, mitochondrial enzymes of viable cells reduce the water-soluble yellow tetrazolium salt into violet formazan. As the formazan product is generated in a solid form, a so-called “stop mix solution” (10% SDS (w/v), 0.6% acetic acid (v/v) DMSO (pH=4.5)) was added to the reaction in order to solubilize the formazan crystals. Specifically, 100 µL of stop mix solution was added and the plate was rocked at room temperature for 30 min. The absorbance was read at 550 nm as a test wavelength and 630 nm as a reference wavelength. At least three independent experiments with six replicates each were performed for each fruit extract.

2.3.3.11. MUH assay

Cells were seeded at a density of 2 x 10^4 cells/well in 100 µL of growth media without phenol red in a black 96-well-plate. The plate was incubated for 4 h at 37 °C in a 5 % CO_2 atmosphere. Then 100 µL of growth media including the fruit extracts in different concentrations were added. As a negative control, culture medium without extract

dilution was added and as a positive control 2 % DMSO in culture media was used. After incubation for 96 h, the culture media including the extracts was removed as described above. Cells were washed with 100 µL of PBS^- and treated with 100 µL of MUH solution. For this, a stock solution of MUH (20 mg/mL) was diluted in PBS^- to a final concentration of 100 µg/mL. After 30 min of incubation the emission was measured at 460 nm with excitation at 355 nm.

2.3.3.12. MTT and MUH assay of cell-free fruit extracts

Fruit extracts were tested for undesired reactions between the reagents used in the MTT and MUH assays and phenolic compounds of the extracts. To this end, the same extract concentrations were prepared as for the MTT and MUH assay, however, cells were excluded from the experiment. 100 µL of extracts in media without FCS and 20 µL of MTT solution were added to 96-well plates. The plate was incubated for 1 h (37 °C; 5 % CO_2), 100 µL of stop mix solution was added, the plate was rocked for 30 min at room temperature and absorbance read at the same conditions as for the original assay.

For the MUH test the same extract concentrations were prepared as for the original assay. Different extract concentrations were prepared in PBS^- and 50 µL of these extract dilutions were pipetted in a dark 96-well-plate. 100 µL of MUH solution (100 µg/mL) were added in each well and fluorescence was read after 30 min of incubation with the same conditions as for the original assay.

2.3.3.13. Trypan blue assay

Cells were seeded in tissue culture dishes (60 x 15 mm) with 4 mL of culture media. After 4 h of incubation at 37 °C in 5 % CO_2, culture media was removed and replaced for media including the fruit extracts in different concentrations. Negative control plates for each fruit were included, containing only media without extracts and plates containing 2 % DMSO in media were used as positive control. The plates were then

incubated for 96 h. Afterwards cells were harvested by trypsination, centrifuged at 900 g and re-suspended in 1mL of PBS^-. Then, 40 µL of the cell suspension was added to 40 µL of trypan blue solution (4%) and incubated for 2 min. Cells were visualized under optical microscope (Telaval 31, Zeiss, Göttingen, Germany) where blue and colorless cells were counted separately with a hematocytometer (Neubauer chamber).

2.3.3.14. Methylene blue assay

HepG2 cells were plated at a density of 2 x 10^4 cells/well in 100 µL of growth media in each well of a transparent 96-well-plate. The plate was incubated for 4 h at 37 °C in a 5 % CO_2 atmosphere. Afterwards, media were replaced by growth media with fruit extracts in different concentrations. As a negative control, culture media without extract dilution was included and 2 % DMSO in culture media was used as a positive control. After incubation for 96 h, the culture media including the extracts was removed. Cells were washed with PBS^- and stained with 50 µL of fresh methylene blue solution (1 % glutaraldehyde, 0,6 % methylene blue in Hanks balanced salt solution, w/v) and incubated for 1 h. The plate was washed three times in a bath with ultrapure water and dried overnight. On the following day, 100 µL of a elution solution (49% PBS^-, 50% Ethanol, 1% Acetic Acid) was added to each well and the plate was shaken at room temperature for 15 min. Absorbance was read at 570 nm. The resulting absorbance directly correlates with the number of viable cells.

2.3.4. Characterization of phenolic compounds of bacaba extract by HPLC-DAD-MS^n

Because the availability of phenolic reference substances is limited and the identification of structurally related plant secondary metabolites solely on the basis of their UV/Vis spectra is impossible, and as HPLC coupled to mass spectrometry has proven to be extremely helpful for peak assignment and further characterization of individual substances, preliminary mass spectrometric analyses were performed with

a Esquire 3000+ ion trap system (Bruker, Bremen, Germany) coupled to the HPLC system described below (*103*). For this purpose, the residue obtained by freeze drying was dissolved in 10 mL of water acidified with diluted HCl solution (pH 3.0). Prior to phenolic compound enrichment, the solution was adjusted to pH 7.0 and applied to SPE C18 cartridges. Subsequently, phenolic acids and flavonoids were recovered by elution with methanol. Preliminary analyses were carried out using an Agilent HPLC series 1100 equipped with ChemStation software, a model G1322A degasser, a model G1312 binary gradient pump, a model G1329/G1330A thermoautosampler, a model G1316A column oven, and a model G1315A diode array detector (Agilent, Waldbronn, Germany). Different LC (liquid chromatography) systems for phenolic acid and anthocyanin separation were tested, and analyses were performed according to a method described by Kammerer *et al*. (*92*). Different injection volumes (ranging from 20 to 40 µL) and concentrations of the samples (ranging from 20 to 200 mg/mL) were tested in order to optimize separation conditions for further HPLC analysis using the systems described below.

System I (anthocyanins): The mobile phase consisted of water/formic acid (95:5, v/v; eluent A) and water/formic acid/methanol (10:10:80, v/v/v, eluent B) using a gradient system. Monitoring was performed at 520 nm at a flow rate of 0.4 mL/min. Total run time was 78 min.

System II (colorless phenolics): The mobile phase consisted of 2% (v/v) acetic acid in water (eluent A) and 0.5% acetic acid in water and methanol (50:50, v/v, eluent B). Total run time was 72 min. Simultaneous monitoring was performed at 280 nm and at 320 nm at a flow rate of 1.0 mL/min.

2.3.5. Chemopreventive effect of bacaba Phenolic Extract on MCF-7 cells

2.3.5.1. Cell culture of MCF-7 cells

MCF-7 cells were cultivated in tissue culture flasks at 37 °C in 5% CO_2 water-saturated atmosphere. Cells were cultured and passaged in DMEM high glucose supplemented with 10% of FCS, 1% Penicilin/Streptomicin (PAA, Pasching, Austria) and 10 µg/mL insulin. Cells were passaged at pre-confluent densities using 0.25% Trypsin-EDTA solution (PAA, Pasching, Austria).

2.3.5.2. Measurement of cell proliferation

Cell proliferation was measured as previously described (*104*). Briefly, MCF-7 cells were seeded at a density of 2 x 10^4 cells/well into 96-well-plates and incubated for 4 h at 37°C. Afterwards, media with fruit extracts (final concentration 0-1000 µg/mL) were added and the plate was incubated for 96 h at 37 °. Culture media including the extracts were removed and cells were washed with 100 µL of $PBS^{-\cdot}$ 20 µL of MTT solution and 100 µL of culture media without FCS was added and incubated for 1 h (37 °C; 5 % CO_2). Then, 100 µL of stop mix solution, an acidified solution of DMSO, was added and the plate was rocked at room temperature for 30 min. Absorbance was read at 550 nm as a test wavelength and 630 nm as a reference wavelength. At least three independent experiments with six replicates were performed to determine cell proliferation.

2.3.5.3. Morphology assessment

Cells were seeded at 60 mm dishes. After 24 h medium was replaced by bacaba extracts diluted in growth medium (final concentration 0 - 800µg/mL). After 72h of incubation cells were visualized in an optical microscopy with 10x and 32x objectives.

2.3.5.4. Ethidium Bromide/Acridine Orange Assay

A stock solution was prepared by dissolving 50 mg Ethidium Bromide and 15 mg of Acridine Orange (EB/AO) in 1 ml 95% ethanol and 49 ml distilled water. This solution was aliquoted and stored frozen at -20°C. A working solution was prepared diluting the stock solution (1:1000 in PBS). For each 500 µl of cell suspension tested, 25 µl of the dyes mixture was added. 5 x 10^4 MCF-7 cells were seeded on coverslips in 24 well-plates. After 24h cells were incubated with bacaba extract (250 µg/mL) for 72 h. Then, cells were washed twice with PBS and stained with 300 µL of EB/AO working solution. Cells were washed three times with PBS and mounted on glass slides with Fluoromount G. Cell visualization was performed in a fluorescent microscope using the Axioplan microscope with 20x, 40x and 100x objectives.

2.3.5.5. DNA fragmentation assay

DNA fragmentation was assessed by agarose gel electrophoresis and Ethidium Bromide staining as previously described by Gong (*105*). MCF-7 cells were plated at 60-mm plates. After 24h, different concentrations (0 - 500µg/mL) of bacaba extracts in growth medium were added and incubated for 72 hours. Then, cells were harvested by trypsinization, centrifuged at 200 g for 5 min and resuspended in 1mL Hank's Buffered Salt Solution (HBSS). Cells were transferred into 10mL of ice-cold 70% ethanol and store at -20 °C for 24 hours. Afterwards, samples were centrifuged at 800 g for 5 min and ethanol was removed. The cell pellet was resuspended in 40µL of phosphate-citrate buffer (PCB), consisting of 192 parts of 0.2M Na_2HPO_4 and 8 parts of 0.1M citric acid (pH 7.8) and incubated for 30 min. Then, cells were centrifuged at 1000 g for 5 min and supernatant transferred to new test tubes. After 3 µL of 0.25% of the non-ionic detergent IGEPAL CA-630 and 3 µL of RNase (1mg/mL in water) were added to the samples and incubated for 30 min at 37°C. Thereafter, 3 µL of proteinase K (Qyagen) was added to the samples and incubated for another 30 min at 37°C. Loading dye (Fermentas) was added to the samples. Samples were loaded into 1.5% agarose gel (w/v) and ran at 60V for 4h. MassRuler™ DNA Ladder

Mix (Fermentas) was used as ladder and DNA was detected via Ethidium Bromide under UV light.

2.3.5.6. Annexin V and PI double staining assay

3 x 10^5 cells were seeded into 60mm dishes and incubated for 24h. Afterwards, cells were treated with different concentrations of bacaba extracts (0 - 1000µg/mL). Staurosporin (STS) 2µM was used as a positive control. After 24 h of incubation, cells were harvested with Accutase (including dead cells), washed and transferred into test tubes. Cells were centrifuged for 5 min at 900 g. The cell pellet was washed with 1 mL FACS Cell Wash. Cells were resuspended in 1 mL of 1x binding buffer and passed through a cell strainer (0.7µm) to exclude cell clumps. Then, cells were stained with 7 µL Annexin V-FITC (BD Biosciences, San Diego, California) and 10 µL PI (BD Biosciences) for 15 min at room temperature in the dark and subsequently analyzed by flow cytometry (FACS Canto, Benton Dickson) using FACS Diva software.

2.3.5.7. Estimation of cell volume

Relative cell volume was estimated by flow cytometry using forward side scatter (FSC) of the flow cytometry analysis. Cells were incubated with different concentrations of bacaba (0-1000µg/mL) for 24 h and submitted to flow cytometry analysis (FACS Canto, Benton Dickson).

2.3.5.8. Measurement of mitochondrial membrane potential (ΔΨm)

Mitochondrial membrane potential ($\Delta\Psi_m$) was assessed by the mitochondrial voltage-sensitive dye 5,5' ,6,6'-tetrachloro-1,1' , 3,3' – tetraethylbenzimidazole carbocyanide iodide (JC-1, Biotium, USA). Mitochondria with integral membrane potential concentrate JC-1 into aggregates that show red fluorescence. De-energized mitochondria cannot concentrate JC-1 and show green fluorescence. Briefly, 3 x 10^5

cells were seeded into dishes and incubated for 24 h. Then, cells were washed once with PBS and stimulated with different concentrations of bacaba extract (0 - 1000µg/mL) for different time points (0, 3, 6 h). Cells were washed twice with PBS/ FACS CellWash (BD Biosciences), respectively, and incubated with 2mL JC-1 working solution (1.6 µM) for 20 minutes. Subsequently, cells were washed again twice with FACS CellWash, harvested with Trypsin/ EDTA and resuspended in 2 mL media. Cell pellet was obtained through centrifugation (900 g; 5min) and washed with FACS Cell Wash solution and resuspended in 500 µL phenol red free medium. Samples were protected from light exposure and analyzed by flow cytometry (FACS Canto) using FACS Diva software.

2.3.5.9. Determination of caspase-9 activity

3 x 10^5 cells were seeded into 60mm petri dishes and incubated for 24 h. Afterwards cells were treated with different concentrations of bacaba extracts (0 - 1000µg/mL). Staurosporin (STS) 2µM was used as positive control. After 24 h of incubation, cells were harvested, washed and transferred into test tubes. Cells were centrifuged for 5 min at 900g. The cell pellet was washed with 1 mL FACS Cell Wash. Cells were stained with 0.5 µL of FITC-LEHD-FMK (Biocat, Heidelberg, Germany). Additionally, 500 µL pre-warmed phenol red free media without FCS was added and cells were incubated at 37 °C for at least 45 min. Afterwards cells were washed with 500 µL Wash Buffer, centrifuged at 900 g for 5 min. The supernatant was removed and cells were resuspended in 300 µL of Wash Buffer. Samples were analyzed by flow cytometry (FACS Canto) (FACSCanto) using FACS Diva software.

2.3.5.10. Determination of caspases-6 and -8 activities

Cells at 70% of confluence were stimulated with bacaba extract (0 -1000µg/mL) for 24 h. Cells incubated with thapsigargin (2µM) for 24h or TNF- alpha (100ng/mL) for 8h were used as positive, unstimulated cells as negative controls. After exposition, cells were washed twice with PBS and harvested with a cell scraper. Cell pellet was

obtained through centrifugation at 900 g 5 min and the supernatant was replaced by 70 µl of chilled/ ice cold cell lysis buffer. The suspension was transferred to a test tube and incubated on ice for 30 min. Afterwards, the cell lysate was centrifuged at /2000 g at 4°C. The supernatant containing cytoplasmic proteins was transferred into new test tubes. Samples were stored at -20°C. Protein determination was performed with Bio Rad DC. The enzymatic activity of the caspases -6 and -8 induced by bacaba extract was assessed using a fluorometric assay kit (Biocat) based on detection of cleavage of the specific substrate, according to the manufacturer's protocol. 10 µg of the protein was incubated with 20 µL of Reaction Buffer containing DTT (10mM) and and 1.6 µL substrate (Ac-VEID-AFC for caspase-6 or Z-IETD-AFC for caspase-8) at 37°C for 2 h. The fluorescence of the reaction mixture was quantified using a microplate reader with a 400 nm excitation filter and a 505 nm emission filter.

2.3.5.11. Assessment of PARP (Poly (ADP-ribose) polymerase) cleavage

Cells were seeded into small tissue culture plates (60 mm) with normal culture media for 24 h. When cells were approximately 70 % confluent, the plates were incubated with the extracts diluted in media without FCS. As a positive control staurosporine (1µM) was included. After 24 h and 48 h of incubation with fruit extracts, plates were washed twice with 2 mL of ice-cold PBS^-. Then 200 µL of lysis buffer (20mM Tris-HCL (pH 7.5), 150mM NaCl, 10mM NaF, 1mM EDTA, 1% Triton X -100; 0.4mM PMSF, 10µg/mL aprotinin, 10µg/mL leupeptin, 10µg/mL pepstatin) was added to each plate, cells were scrapped off and transferred into 1.5 mL test tubes and incubated on ice for 30 minutes on ice. Then, cells were centrifuged at 13.000 g for 5 min at 4 °C. The supernatant was collected and stored at -80°C for later use. PARP-cleavage was assessed by western blotting. In some experiments the specific caspase-9 inhibitor Z-LEHD-VAD (BD Biosciences, San Diego, USA) was included.

2.3.5.12. Western Blotting

50-100 µg of protein for each sample was loaded onto 10% SDS-polyacrylamide gel together with a prestained molecular weight marker (ColorPlus Prestained Protein Ladder, New England, Biolabs). Proteins were separated at 100V for 85min, then transferred to a polyvinylidene membrane (Roti-PVDF, Carl Roth, Germany) which was afterwards blocked with 5% (w/v) skim milk powder in TBS-Tween (TBST) for 1 hour. The membrane was incubated with primary anti- PARP antibodies (Cell Signaling Technology, Beverly, USA) (1:1000) overnight. Subsequently, blots were washed extensively with TBST and incubated with horseradish peroxidase-linked goat anti-rabbit IgG (1:2000) (Amersham) in TBST containing 5% (w/v) of skim milk at room temperature for 1 hour. Bands were visualized by enhanced chemiluminescence (ECL, Thermo scientific).

2.3.5.13. Statistical analysis

All analyses were carried out at least in three independent experiments with six replicates each and data was reported as mean ± SD. Means were compared with ANOVA followed by Tukey's test ($p < .05$) using XLSTAT software. Correlation analyses were performed using SPSS version 18.Curve fitting and calculation of IC_{50} values were done using a nonlinear regression model using Graphpad prism version 5.

3. Results

3.1. Nutrition Survey

3.1.1 Socioeconomic, health and food security assessment of two rural communities in the region of APA/Cantão, Tocantins state (Brazilian Amazon Region)

The socioeconomic, health and food security situation of families was assessed by the methodology of Living Standard (*106*). According to Doppler (1993) living standard is a multi-dimensional phenomenon and it is determined by criteria, such as family income, possession of goods, supply of water, housing and sanitary equipment, health conditions, as well as food supply and food security. Therefore, this section presents the living standard of farm families in the study area aiming to better understand their current situation. The most relevant resources in farm families can be separated into natural and man-made resources, of which the former are basic and limited and therefore play an extremely important role in the farm sector. Based on this, the objective of the present section is to identify and analyse the resource endowment and therefore provide information about the families' options for decision making.

Table 5 presents the main results regarding the family size and its composition in the study area. The family size averages around 4.74 persons per family, which is slightly lower than the mean for rural areas in the northern Brazil (4.9 persons per family) (*107*).

Table 5: Family size and its composition, Tocantins, 2008

Item	Family farmers (n=57)	
	Mean	SE
Family size	4.74	0.28
N. people under 18 years old	1.89	0.25
N. years living in the settlement	7.6	0.42

In Figure 10 is displayed the women's share as a household head. It is interesting to point out that the results from the study area are higher (24.6%) if compared to those of other rural areas in the northern Brazil where only 9.2% of households are commanded by women. Even when one compares the figures to Brazil as whole, Tocantins has a larger participation of women as household heads, since just 12.8% of households in rural areas of Brazil have a woman as the family head (*107*).

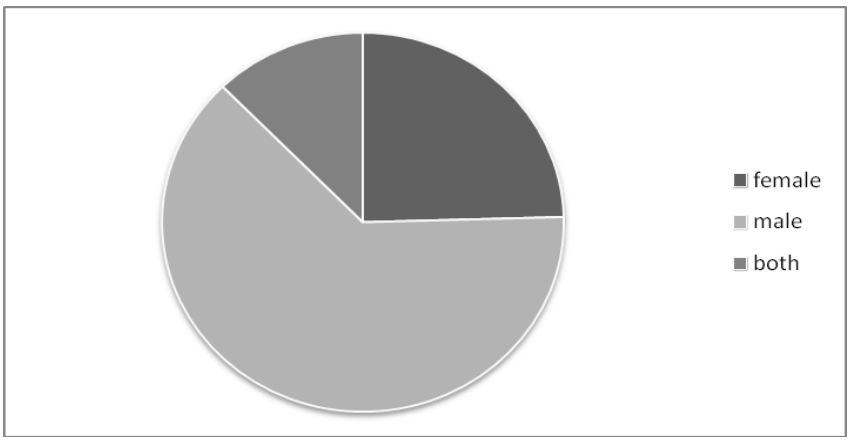


Figure 10: Gender distribution of household head , Tocantins, 2008. Source: author. Notes: female (24.6%), male (63.2%), both (12.3%).

With regard to land resources, when inquired about the deforestation of native forests, around 30% of families responded positively, i.e. they have cut down the forest in order to start crop cultivation or pasture formation. This figure depicts the

opportunity costs of native biomes in the region in question, since family farmers do not receive any economic incentive to protect or conserve the forests. A possible economic alternative for that is to enable the farmers to receive a fund for environmental services provided by forests. An example is the REDD project (Reducing Emissions from Deforestation and Forest Degradation) implemented in Amazonas state, called “Bolsa Floresta”, where family farmers receive a certain amount not to cut down the forests.

Table 6: Land use change, Tocantins, 2008

Item	**Family farmers (n=57)**	
	Mean	SE
Initial natural forest (ha)	6.75	0.81
Current natural forest (ha)	4.21	0.57

The capital resource base is considered an important pillar for the welfare and living standard of families and a *proxy* for the investment’s ability of the farm families in the medium and long run as well as the ability of farmers to buy necessary inputs for production. According to Tai (2004) ensuring capital availability is very important for farm families to carry out their activities successfully and within the required time. In addition, the lack of access to capital tends to decrease the well-being of families and therefore might lead to a vicious circle of difficulties where the family faces several problems to overcome (*108*). The composition of families’ capital assets in the study area is shown in Table 7.

Table 7: Possession of goods by farm families, Tocantins, 2008

	Family farmers (n=57)	
Possession of goods	Absolute Frequency (n)	Relative Frequency (%)
Fridge		
No	12	21.1
Yes	45	78.9
Television		
No	5	8.8
Yes	52	91.2
Radio		
No	23	40.4
Yes	34	59.6
Small wash machine		
No	17	29.8
Yes	40	70.2
Motorcycle		
No	34	59.6
Yes	23	40.4
Car		
No	43	75.4
Yes	14	24.6

As one can see in Table 7, the major part of farm families have access to basic goods such as fridge, television, radio, as well as a small washing machine. It is important to note that the currently low number of households deprived of those goods and services can be explained, at least in part, by the easy access to formal credit which was fostered by the federal government during the last years. However, regarding means of transport, one can notice that a significant part of family farmers still possesses neither a motorcycle nor a car, and therefore has to rely on public

transportation in order to sell their products at the market or to reach a hospital, for instance. It is noteworthy that television is considered an important good for families, since owing this device (91.2%) is more relevant than owing a fridge (78.9%).With regard to credit, the percentage of farm families who received formal credit in the last 24 months is considerably high, 73.7% on average, showing that this kind of financial support is usual and common in the study area (Table 8). However, although many farm families report that the formal access to credit lines, such as PRONAF (National Program for the Family Agriculture), is not difficult, many of them default since they cannot access further credits until they have paid back the amount already borrowed and interest rates.

Table 8: Credit access by families, Tocantins, 2008

	Family farmers (n=57)	
Item	Absolute Frequency (n)	Relative Frequency (%)
Access Credit		
No	15	26.3
Yes	42	73.7

The farm and off-farm revenues and therefore the family income were assessed and analyzed; they are presented in Table 9 In the present study, farm income was calculated for the year of 2007. Off-farm income, on the other hand, is the income generated by all activities exerted by members of the family inside (non-agricultural activity) or outside the rural property (agricultural activity and/or non-agricultural activity) as well as from pensions and governmental transfers. Based on that, Marini and Pierroni (*109*) show that the family as an active unit presents three basic characteristics: (i) linkages between the production and reproductive spheres: (ii) internal relationship among members defined by gender, age and labor capacity; and (iii) the social position in the family life cycle. So the family is transformed in a multi-

dimensional unit where both agricultural and non-agricultural activities are practiced and generate different sorts of revenues. Based on this, the concept of pluri-activity emerges which is related with the operation of multiple activities by members of the same family (*109*). In addition, according to Berdegue, Reardon and Escobar, the rural non-agricultural job is responsible for 40% of the rural inhabitants' income in Latin America (*110*).

As one can see in Table 9, off-farm income is higher than farm income among farm families in the region. These figures corroborate a study carried out by Reardon and Vosti (*111*), which shows that the non-agricultural incomes are extremely important to rural communities and in many cases are the only source of income for those families. With regard to social cash transfer programs, more than 60% of families are depending on Federal and/or state programs of income transfer, as shown in Figure 11.

Table 9: Composition of family income, Tocantins, 2008

Income and revenue (R$/year)	**Family farmers (n=57)**	
	Mean	SE
Farm income	4427.49	1072.32
Off-farm income	6017.90	863.27
Family income	10090.08	1256.72

With regard to social assistance, 61.4% of families declared to be included in social assistance programs i.e., to receive money from federal (Bolsa Família Program) or State (Pioneiros mirins) programs Figure 11.

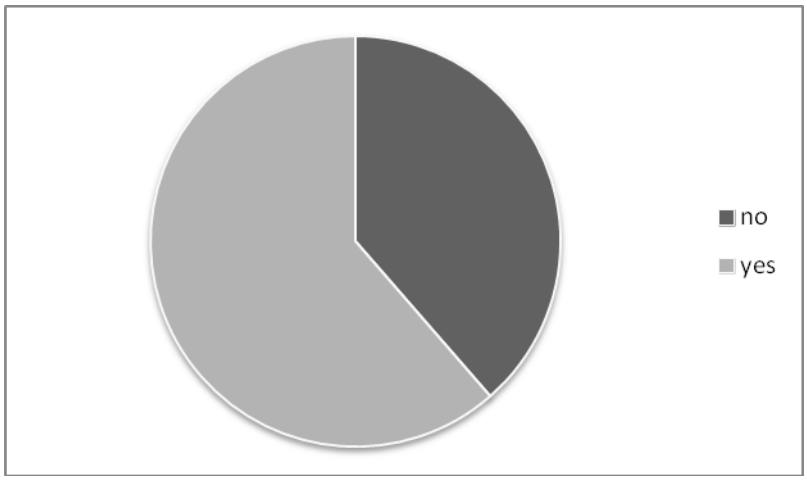


Figure 11: Social assistance from federal and/or state government.Notes: no (31.6%), yes (61.4%)

The focus of the following data are some living standard criteria that are related to the living standard of families such as: food supply and food security, supply of water, housing and energy, health conditions and social security. These criteria include the supply of basic goods and services that are the minimum for the farm family's survival and therefore must be satisfied.

Table 10 shows the basic infrastructure present (or not) inside the farm family's house. As one can observe, although almost 90% of families have access to treated water, only 24.6% of them possess toilet facilities inside the house. And, regarding the quality of water, just 1 smallholder (or 1.8%) reported that his water has a bad quality whereas 52,6% reported that the water has a very good quality. Regarding the garbage, since there is no collection, the most part of families burn it (93%) or leave it exposed outdoor (7%) what can be a source of different types of diseases.

Table 10: Housing, water, garbage and energy facilities, Tocantins, 2008Housing, water, garbage and energy facilities, Tocantins, 2008

Item	Family farmers (n=57)	
	Absolute Frequency (n)	Relative Frequency (%)
Toilet facilities at home		
No	43	75.4
Yes	14	24.6
Energy used for cooking		
Wood	46	80.7
Gas	11	19.3
Quality of water		
Very Good	30	52.6
Good	19	33.3
Regular	7	12.3
Bad	1	1.8
Treated water		
Yes	51	89.5
No	6	10.5
Garbage		
Burn	53	93
Outdoor exposed	4	7

As shown in Figure 12, food self-production is not sufficient to feed the family in almost 60% of family farms i.e. they are considered net food buyers and therefore need to acquire food from the local markets.

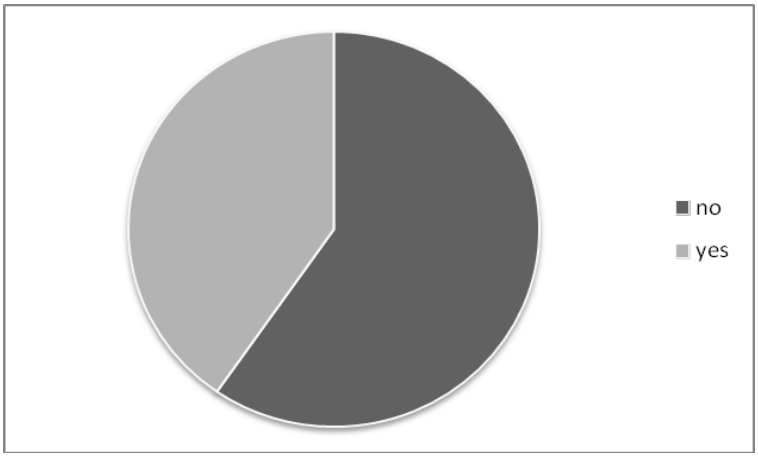


Figure 12: Self-production is enough to feed the family. Tocantins, 2008

The food and nutrition security condition was measured by the Brazilian Scale of Food Insecurity (EBIA), a validated instrument to measure food security in Brazilian studies (*83*). The majority of families were afflicted with food insecurity (84.2%), mostly (40.3%) expressed as a mild food insecurity situation (Table 11).

Table 11: Food and nutrition security condition of families, Tocantins, 2008

Food security	**Family farmers (n=57)**	
	n	%
With Food security	9	15.5
With Food insecurity	48	84.2
Level of Food Insecurity		
Mild Food Insecurity	23	40.3
Moderate Food Insecurity	16	28.1
Severe Food Insecurity	9	15.5

According to Kitchaicharoen (*112*), social security is defined as security of the families in the future (*113*). Moreover, social security may comprise the social capital that farm families have built up with the aim to support them and therefore minimize risks, uncertainty and vulnerability whenever facing natural and/or man-made catastrophes. In addition, social capital is related to norms, institutions and organizations that promote the trust, reciprocity and cooperation among its members. Thus, social security is hereby expressed through the farm families' social capital. On

the other hand, health condition is a crucial indicator of farm families' living standard and thus some factors were selected in order to assess the health status of families in the study area (*114*).

Based on this, most farmers have the religion as the main social capital. They attend services regularly and have the church as an important symbol of support and help in difficult moments. The settlement association also plays an important role; about 74.1% of the village population takes part in it. The rural trade union appears to be another important organization, since 68.5% of the people consider this organization a crucial supporter aiming to diminish transaction costs and risks as well.

When asked about their perception regarding the health condition of the family, around 26.3% of the farmers reported that health is good, and 12.3% that heath is very good. Bad and very bad health status was reported by 3.5% and 1.7% of families, respectively (Table 12).

Table 12: Heath condition of farm families, Tocantins, 2008

Item	Family farmers (n=57)	
	Absolute Frequency (n)	Relative Frequency (%)
Health status		
Very good	7	12.3
Good	15	26.3
Regular	32	56.1
Bad	2	3.5
Very bad	1	1.7
Use of traditional methods for health care		
Yes	56	98.3
No	1	1.7
Health assistance		
Public	54	93.1
Private	3	5.2
Evaluation on Local Health Public Service		
Very good	2	3.5
Good	10	17.5
Regular	25	43.9
Bad	6	10.5
Very bad	14	24.6

As one can deduce from Table 12, except for 1 family (1.7%), all other farm families interviewed responded positively towards the use of traditional methods for health care (98.3%). Regarding the type of health assistance (public or private), 93.1% of families have access to the public service (SUS) since they cannot afford a private health service. When inquired about the quality of the public health service, 43.9% of families reported that it is regular. Nevertheless, a significant part of families found it to be bad (10.5%) or very bad (24.67%), respectively. Thus, one can note that a substantial percentage of families were not satisfied with the quality of public health services, which in some cases may aggravate the health condition of a family member.

3.1.2. Nutritional situation of adults of two rural communities in the region of APA-Cantão, Tocantins state (Brazilian Amazon Region)

The results of the nutritional status of the adults of the two rural settlements studied are shown in Table 13. The mean (± standard deviation) age of the study population was 44.0 ± 12 years for women and 47.0 ± 15.0 year for men. The mean length of education was 4.8 ± 4.3 years for men and 3.7 ± 3.6 years for women which corresponds to a basic level of education for both genders. Family income/year (Brazilian Real) was R$ 8599.57 ± 7193.84 for males and R$ 10130.34 ± 10058.09 for females. Although females had a slightly higher family income, there was no significant difference between them.

Men were significantly taller than women ($p < .05$), 1.65 ± 0.07 *vs.* 1.52 ± 0.06. The mean BMI of females was higher (26.6 ± 4.31) than the BMI of males (24.4 ± 3.58) ($p < .05$). Waist circumference (WC) was 86.4 ±10.24 for females and 85.79 ± 10.24 for males. Results with main characteristics of the sample are shown in Table 13.

Table 13: Subjects Characteristics

	Gender		
	Male	**Female**	
	(*n*=58)	(*n*= 50)	
Characteristics	Mean ± SD	Mean ± SD	*p-value*
Age (years)	47.0 ± 15.0	44.0 ± 12.0	.27
Education (years)	3.7 ± 3.6	4.8 ± 4.3	.19
Family income/year (Brazilian Real)	8599.57 ± 7193.84	10130.34 ± 10058.09	.54
Weight (Kg)	66.60 ± 11.07	61.97 ± 10.85	.31
Height (m)	1.65 ± 0.07	1.52 ± 0.06	<.001
BMI (kg/m²)	24.4 ± 3.58	26.6 ± 4.31	.04
Waist circumference (cm)	85.79 ± 10.24	86.4 ± 10.8	.73

*p < .05

The health profile of the study population is shown in Table 14. Most people lived with partners (76.9%). The overall consumption of alcohol and tobacco were 32.4%

and 38.9%, respectively. Men were more often smokers (50.0%) and consumed more alcohol (42.0%) than women (29.3% and 24.1%, respectively). The prevalence of NCD for the whole sample was 27.8% showing the same trend pointed out by another study (*21*).

Table 14: Health Profile of the study population

	Gender				Combined		
	Female		Male				$\chi 2$
Sample profile	Absolute Frequency (n)	Relative Frequency (%)	Absolute Frequency (n)	Relative Frequency (%)	Absolute Frequency (n)	Relative Frequency (%)	*p*-value
Gender	58	53.7	50	46.0	108	100	
Living with partner							
Yes	45	77.6	38	76.0	83	76.9	.84
No	13	22.4	12	24.0	25	23.1	
Smoking							
Yes	17	29.3	25	50.0	42	38.9	.02
No	41	70.7	25	50.0	66	61.1	
Alcohol Consumption							
Yes	14	24.1	21	42.0	35	32.4	.02
No	44	75.9	29	58.0	73	67.6	
Non Communicable Diseases							
Yes	16	27.6	14	28.0	30	27.8	<.001
No	42	72.4	36	72.0	78	72.1	
Diabetes							
Yes	3	5.2	1.0	2.0	4	3.7	.12
No	55	94.8	49.0	100	104	96.3	
Hypertension							
Yes	15	25.9	14	28.0	29	26.9	.19
No	43	74.1	36	72.0	79	73.1	
Cancer							
Yes	0	0	0	0	0	0	
No	58	100	50.0	100	108	100	
Communicable Diseases							
Yes	2	3.4	6	12.0	8	7.4	.14
No	56	96.6	44	88.0	100	92.6	
Nutritional Profile (BMI)							
Underweight	1	1.7	0	0.0	1	0.9	.01
Eutrophic	18	31.0	31	62.0	49	45.4	
Overweight	28	48.3	14	28.0	42	38.9	
Obesity	11	19.0	5	10.0	16	14.8	

*p < .05

3.7% of the study population presented with diabetes and 26.9% had hypertension. Despite the prevalence of NCD, communicable diseases such as leishmaniasis and hanseniasis were also prevalent among participants (7.8%). This result reflects the epidemiological transition characteristics of developing countries which is still experienced in Brazil (*21*). In the same context, the nutrition transition was also recognized as only one participant was underweight and the prevalence of overweight was 38.9% for the whole sample, being even higher among women (48.3%).

Overweight and obese adults together make up 60.2% of the study sample and were considered as one group named as “overweight”. Anthropometric profiles of the eutrophic and overweight adults are compared in Table 15.

Weight, BMI and WC were different between eutrophic and overweight groups for both gender. The mean weight for eutrophic men was 61.15 ± 7.73 kg whereas for overweight men it was 76.31± 9.41. The BMI for eutrophic men was 22.16 ± 1.57 whereas for the overweight group was 28.33 ± 2.58 kg. Similar results were obtained for women whose mean BMI values were 22.67± 2.02 kg for eutrophic and 29.02 ± 3.22 for overweight persons. Also, overweight subjects had higher WC values than eutrophic ones.

Table 15: Anthropometric profile of eutrophic and overweight adults

	Eutrophic		Overweight	
	Men (n=32)	Women (n=20)	Men (n=18)	Women (n=37)
Anthropometric data	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Age (years)	46.00 ± 15.14	42.10 ± 15.53	48.00 ± 15.40	45.64 ± 10.91
Weight (Kg)	61.15 ± 7.73*	53.72 ± 7.14*	76.31 ± 9.41	66.97 ± 9.22
Height (m)	1.65 ± 0.07	1.53 ± 0.07	1.64 ± 0.07	1.51 ± 0.05
BMI (kg/m²)	22.16 ± 1.57*	22.67 + 2.02*	28.33 ± 2.58	29.02 ± 3.22
Waist circumference (cm)	79.75 ± 5.75*	78.32 ± 7.71*	96.52 ± 7.17	91.51 ± 8.86

*$p < .05$

Table 16 describes the average daily energy intake and expenditure of subjects. There was no difference between eutrophic and overweight adults. Eutrophic individuals had higher mean values of PAL than overweight persons for both gender. TEE was significantly lower between eutrophic and overweight women, showing no difference in men. There was no difference between energy intakes of eutrophic or overweight groups. Eutrophic men energy intake was 1466.32 ± 901.78 kcal/d and that of overweight men was 1411.75 ± 565.44 kcal/d. Similar results were found for women, eutrophic women consumed 1889.07 ± 910.07 kcal/d and overweight 1957.77 ± 1170.35 kcal/d.

Table 16: Dietary variables (Energy) and Physical activity levels (PAL) of eutrophic and overweight adults*

	Eutrophic		***Overweight***	
	Men (*n*=32)	**Women (*n*=20)**	**Men (*n*=18)**	**Women (*n*=37)**
Daily energy intake	*Mean±SD*	*Mean±SD*	*Mean±SD*	*Mean±SD*
Energy intake (kcal)	1466.32 ± 901.78	1889.07 ± 910.07	1411.75 ± 565.44	1957.77 ± 1170.35
Kcal/kg body weight	24.95 ± 17.35	36.28 ± 20.33	18.89 ± 7.89	29.89 ± 19.53
Basal metabolic rate (BMR)	1525.08 ± 149.09*	1269.88 ± 116.16*	1675.64 ± 179.16	1454.21 ± 145.97
Physical activity factor (PAL)	1.88 ± 0.19*	1.50 ± 0.20*	1.68 ± 0.28	1.42 ± 0.10
Total Energy expenditure (TEE)	2873.82 ± 901.78	1913.16 ± 324.54*	2831.95 ± 615.34	2072.15 ± 269.26
EB (EI – TEE) (Kcal)	-1407.49 ± 956.41	-24.09 ± 920.60	-1420.20 ± 968.46	-114.38 ± 1181.81

*$p < .05$

There was no statistical difference between the groups with regard to the intake of protein, kilocalories from protein and percentage of energy from protein. The daily intake of carbohydrate in grams and energy (Kcal) was higher in the eutrophic groups. However, there was only a statistically significant difference ($p < .05$) between eutrophic and overweight for both genders when carbohydrate uptake was expressed as g/Kg body weight. Eutrophic men had a higher intake of fat than overweight ($p < .05$). Eutrophic women had higher daily intake of fat than overweight when it was expressed as g/kg body weight. Table 17 also shows the fiber intake. There was no difference in fiber consumption between the groups.

Table 17: Macronutrients daily intake of eutrophic and overweight adults*

Macronutrients	*Eutrophic*		*Overweight*	
	Men (*n*=32)	**Women (*n*=20)**	**Men (*n*=18)**	**Women (*n*=37)**
	Mean±SD	*Mean±SD*	*Mean±SD*	*Mean±SD*
Protein (g)	103.07 ± 81.56	67.32 ± 32.83	96.04 ± 47.27	75.71 ± 54.44
Protein (Kcal)	412.30 ± 327.46	269.28 ± 131.33	384.19 ± 189	302.83 ± 217.73
Protein (%energy)	22.04 ± 7.45	21.83 ± 11.10	21.49 ± 8.93	17.29 ± 8.36
g/kg body weight	1.65 ± 1.22*	1.25 ± 0.60	1.24 ± 0.55	1.14 ± 0.80
Carbohydrate (g)	318.05 ± 204.05	205.60 ± 106.48	222.21 ± 126.26	159.28 ± 82.35
Carbohydrate (Kcal)	1272.20 ± 816.21	822.41 ± 425.95	888.86 ± 505.06	637.12 ± 329.42
Carbohydrate (%energy)	51.31 ± 13.34	49.37 ± 15.55	53.51 ± 11.94	55.74 ± 14.08
g/Kg body weight	5.17 ± 3.30*	3.94 ± 2.30*	2.92 ± 1.64	2.42 ± 1.30
Fat (g)	59.09 ± 44.42*	51.80 ± 30.02	62.28 ± 48.00	42.36 ± 24.85
g/Kg body weight	0.95 ± 0.65	0.96 ± 0.54*	0.79 ± 0.58	0.63 ± 0.37
Fat (Kcal)	531.86 ± 399.81	466.22 ± 270.18	560.53 ± 440.90	381.31 ± 223.70
Fat (%energy)	26.63 ± 8.56	28.80 ± 9.39	25.00 ± 8.15	26.95 ± 9.57
Fiber (g)	19.94 ± 15.21	8.06 ± 5.77	16.95 ± 14.21	9.97 ± 5.92

*p < .05

The physical activity level of participants describes a sedentary profile for women in the rural communities for both groups, since 75% of eutrophic women and 94.6% of overweight women have a mild PAL, as depicted in Figure 13. This is explained by the fact that men are responsible for the agriculture activity in the field whereas women are more concerned with domestic duties.

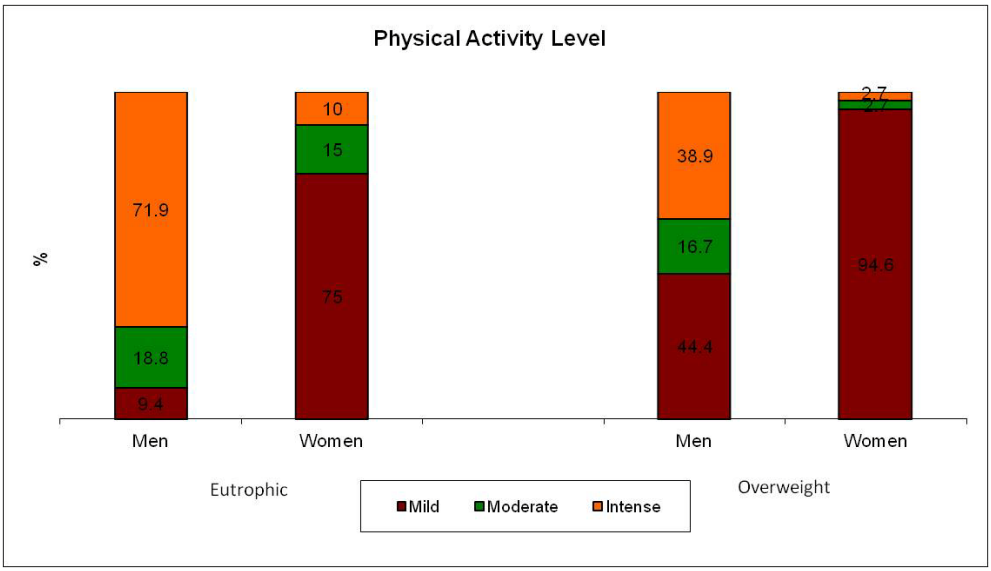


Figure 13: Physical activity levels of eutrophic and overweight adults. Tocantins 2008.

With regard to the linear model applied, when one observes the explanatory variable behavior, one can notice that all of them exert significant influence on the dependent variable, except for the variable gender and g of protein consumed/kg of body weight (Table 18).

Table 18: Regression coefficients and *p*-values for correlates of BMI

	Coefficients	**SE***	***t***	***p*-value**
CONSTANT	3.560	0.080	44.462	.00
GENDER	-0.029	0.037	-0.792	.43
PAL MOD (Moderate physical activity level)	-0.094	0.045	-2.111	.04
PAL HIGH (High physical activity level)	-0.095	0.040	-2.375	.02
KCALBW (kcal/bodyweight)	-0.064	0.023	-2.769	.00
PTNBW (g of protein consumed/kg body weight)	-0.003	0.028	-0.009	.93
CHOBW (g of carbohydrate consumed/kg of body weight)	-0.066	0.027	-2.464	.02
LIPBW (g of lipid consumed/kg of bodyweight)	0.001	0.031	0.037	.97
R^2	0.31			
F Statistic	6.53			.000

*Standard error

Regarding the *dummy* variables, the coefficients of PALMOD and PALHIGH were significant and the sign (negative) goes hand in hand with the nutritional science theory, i.e. the higher PAL performed the lower is the BMI. The elasticity provided by the model indicates that people performing either PALMOD or PALHIGH have 9% lower BMI than those who are under PALMILD, *ceteris paribus*. In other words, in the studied sample, the gender has no influence on the BMI fluctuation, but the physical activity level of participants is significantly relevant for their BMI (Table 18).

Regarding the variables KCALBW and CHOBW, one observes that the coefficient is significant and the sign is negative, i.e. more Kcal/Body weight and more Carbohydrate/Body weight will decrease the BMI. The elasticities of both variables are 0.06% i.e. an increase of 10% in Kcal/Body weight or Carbohydrate/Body weight decreases the BMI by 0.6%, *ceteris paribus*. When one considers the variables PTNBW and LIPBW, one observes that the coefficient is not significant. The coefficient of determination (R^2) was 0.31, i.e. 31% of the variation of BMI is explained by the variation of independent variables in the model. The low value of R^2 was as expected once the data is cross-sectional (*115, 116*). The F-test pointed out that the overall model was significant (Table 18). The equation developed in the present work was (eq. 7):

Log-log model:

$$Ln\,(BMI)_i = 3.560 - 0.029\,Gender_i - 0.094\,PALMod_i - 0.095\,PAL\,Hig_i - 0.064\,KcalBW_i - 0.003\,PTNBW_i - 0.066\,CHOBW_i + 0.001\,LIPBW_i \quad \text{eq.7}$$

The food frequency questionnaire (FFQ) is the most widely used method for recording past diet, specifically due to its ability to classify individuals according to their usual eating patterns. Besides, it is also an easy management tool and

inexpensive, therefore, enabling its usage in population studies (*117*). The results obtained by the FFQ represent the food consumption practiced by adults in their usual diets. A quantitative approach of food consumption is presented in Table 19, which contains a comparison of the average consumption of the same food for both groups reflecting the food patterns practiced by participants in their daily lives and the analysis of the average consumption of foods**.**

Table 19: Quantitative analysis of food consumption of eutrophic and overweight adults

	Eutrophic (*n*=52)	Overweight (*n*=55)
	Mean ±SD	Mean ±SD
Acerola berry	0.72 ± 1.80	0.48 ±2.04
Apple	0.40 ± 1.41	0.16 ±0.68
Artificial juice	2.07 ± 3.27	1.85 ±3.08
Bacon, pork fat	0.83 ± 2.84	0.84 ±2.83
Banana	2.05 ± 2.88	2.51 ±3.87
Beans	9.96 ± 5.49	10.10 ±4.96
Bread	1.50 ± 2.96	1.46 ±2.27
Butter	0.38 ± 1.41	0.54 ±1.61
Cake	2.17 ± 3.38	1.37 ±2.07
Cara	0.25 ± 0.73	0.62 ±1.74
Cheese	0.95 ± 1.61	1.08 ±2.55
Chicken	1.61 ± 2.32	1.71 ±2.93
Chocolate	1.42 ± 3.25	0.68 ±1.84
Coffe with sugar	10.93 ± 4.88	9.69 ±5.76
Corn products	1.99 ± 3.11	1.56 ±1.97
Egg	2.45 ± 3,55	1,65 ±2.54
Fish	1.21 ± 1,52	1,75 ±2.56
Honey	1.15 ± 2,88	1,25 ±2.88
Ice cream	0.15 ± 0.50	0.18 ± 0.61
Jerked beef	0.67 ± 1.27	0.80 ±1.43
Leafy vegetables	2.54 ± 3.92	1.97 ±3.05

Mayonnaise	0.19 ± 0.52	0.16 ±0.60
Manioc	1.78 ± 1.92	2.56 ±2.81
Margarine	4.58 ± 4.73	3.10 ±3.57
Meat (beef)	7.50 ± 5.41	7.55 ±5.64
Milk	5.56 ± 5.37	5.91 ±5.64
Natural juice	2.90 ± 3.79	2.64 ±3.45
Orange	2.42 ± 3.03	2.07 ±3.25
Papaya	1.93 ± 3.57	2.05 ±3.24
Passion fruit	0.87 ± 2.29	1.30 ±2.69
Pasta	1.94 ± 2.74	1.48 ±1.97
Pineapple	1.72 ± 3.60	1.31 ±3.05
Pork meat	0.80 ± 1.54	1.32 ±2.85
Potato	1.57 ± 2.82	1.20 ±2.53
Rice	13.60 ± 2.13	12.60 ±3.33
Salted cookie	1.85 ± 2.73	1.73 ±2.61
Sausages	0.26 ± 0.74	0.48 ±1.35
Soft drinks	0.81 ± 1.57	1.20 ±2.53
Sweet cookie	1.07 ± 2.49	1.90 ±1.85
Sweets	2.13 ± 3.26	1.35 ±2.39
Vegetables	2.85 ± 3.48	3.38 ±3.13
Vegetable oil	11.68 ± 4.18	11.02 ±5.21
Viscera	0.34 ± 0.90	0.29 ±1.04
Watermelon	0.64 ± 2.31	0.64 ±2.32
Yam	0. 71 ± 1.52	0.50 ±1.22

From the quantitative analysis of food consumption, foods were grouped according to their values of relative frequency of consumption (0 < 1 = no consumption≥1 to < 3 = once a week; ≥ 3 to < 5.5 = 2 to 4 times a week; ≥ 5.5 -7 = 5 to 6 times a week; ≥ 7 to < 14 = once a day; 14 = 2 or more times a day). As a result, this classification provided the food pattern of the two groups in Table 20.

Table 20: Food pattern of surveyed people based on quantitative analysis

Eutrophic	Overweight
No consumption	
Acerola berry, apple, bacon, pork fat, butter, cake, cará, ice cream, jerked beef, mayonnaise, passion fruit, pork meat, sausages, soft drinks, viscera, watermelon, yam,	Acerola berry, apple, pork fat, butter, cake, cará, chocolate, ice cream, jerked beef, mayonnaise, passion fruit, sausages, viscera, watermelon, yam,
Once a week	
Artificial juice, Banana, bread, cheese, chicken, chocolate, corn products, eggs, fish, honey, leafy vegetables, manioc, natural juice, orange, papaya, pasta, pineapple, potato, salted cookie, sweet cookies, sweets, vegetables,	Artificial juice, Banana, bread, cheese, chicken, corn products, eggs, fish, honey, leafy vegetables, manioc, natural juice, orange, papaya, pasta, pineapple, pork meat, potato, salted cookie, soft drinks, sweet cookies, sweets,
2 to 4 times a week	
Margarine	Margarine, vegetables
5-6 times/week	
Beans, milk	Beans, milk
1 or 2 more times a day	
Coffee with sugar, meat (beef), rice, vegetable oil	Coffee with sugar, meet (beef), rice, vegetable oil

Basically, the food pattern of surveyed people is based on rice, beans and meat as daily food. Coffee is also highly consumed with added sugar.

3.2. Biofunctional properties of traditional fruits

Brazilian native fruits bacaba and jenipapo were investigated for their phenolic compounds, antioxidant and antiproliferative effect on HepG2 cells. Therefore, fruits were acquired, selected and prepared for experiments. As a first step and aiming at allowing further calculations expressing results in fresh weight (FW) the water content of fruit samples was assessed. The moisture content was 69.86 ± 1.13 and 43.10 ± 1.53 % for jenipapo and bacaba, respectively (Table 21). The yield of pulp was also assessed during the plant material preparation by weighing residues and obtained pulp as shown in Table 21.

Table 21: Water content and yield of fruit samples (n=3)

Sample	Water content (%)	Yield (%)
Bacaba	43.10 ± 1.53	29.13
Jenipapo	69.86±1.13	37.13

3.2.1. Optimization of Phenolics Extraction

Commonly solvents used for phenolic extraction are methanol, acetone and water. Therefore, in order to optimize extraction procedure, total phenolic content of samples was assessed by Folin-Ciocalteau assay after extraction with different dilutions of methanol and acetone. As shown in Figure 14 bacaba phenolics were significantly better extracted with 80% acetone and for jenipapo fruit only 100% acetone was significantly different to the others solvents, as can be seen in Figure 14B ($p < .05$).

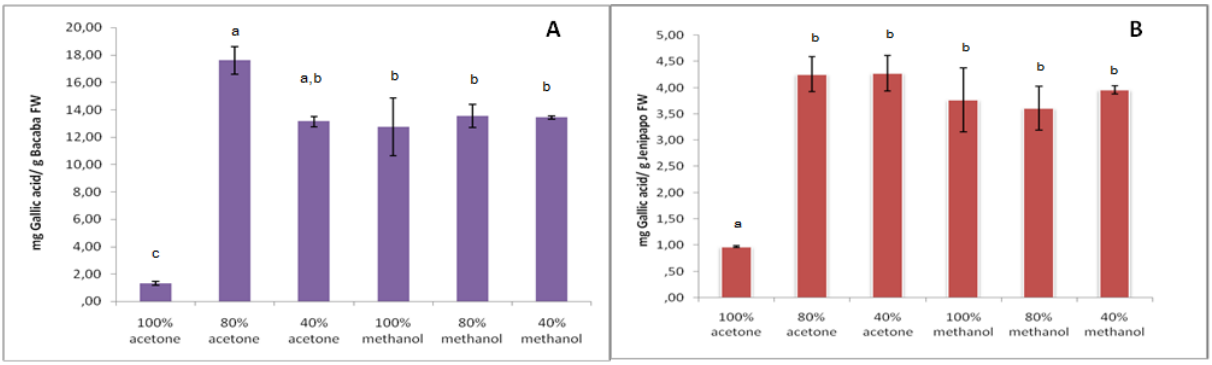


Figure 14: Extraction optimization for bacaba (A) and jenipapo (B) samples. Different letters mean significant difference by ANOVA and Tukey's test of triplicates ($p < .05$) (n=3).

3.2.2. Phenolics Content of bacaba and jenipapo fruits

Fruits were examined for total phenolics, flavonoids and anthocyanins (Table 22). bacaba fruit had higher values for all those parameters when compared to jenipapo. Regarding total phenolic content, bacaba fruit had 1759.27 ± 1.01 mg GAEq/100g and jenipapo 426.53 ± 0.33 mg GAEq/100g. Flavonoids results were 1134.32 ± 0.03 mg CTEq/100g and 7.12 ± 0.00 mg CTEq/100g for bacaba and jenipapo, respectively. Anthocyanins were not detected in jenipapo extracts whereas bacaba had 34.69 ± 0.00 mg cyn-3-glu/100g.

Table 22: Total phenol, total flavonoid and total anthocyanin content of bacaba and jenipapo fruits (n=3).

Fruit	Total phenolic (mg GAEq/100g (FW))	Total flavonoid (mg CTEq/100g FW))	Total anthocyanin (mg cyn-3-glu/100g (FW))
Bacaba	1759.27 ± 1.01	1134.32 ± 0.03	34.69 ± 0.00
Jenipapo	426.53± 0.33	7.12 ± 0.00	0.0 ± 0.00

3.2.3. Antioxidant activity of bacaba and jenipapo fruits.

Antioxidant methods differ regarding their assay principle as multiple reactions mechanisms, characteristics and experimental conditions (*118*). To this respect a

sole assay doesn't express accurately all antioxidants groups, especially in a mixed or complex system as fruits matrices. Therefore, for having a better comprehension about the antioxidant ability of the fruits studied here, different antioxidant assays were applied (ORAC, TEAC, FRAP, DPPH, CAA) to allow broader comparison with other fruits already described in the literature. The results of the antioxidants assays are reported in Table 23.

Table 23: Antioxidant activity of bacaba and jenipapo fruits (n=3).

Sample	ORAC (µmol of TEq /100g (FW))	TEAC (µmol TEq / 100g (FW))	FRAP 1 (mmol $FeSO4.7H_2O$ Eq/ 100g (FW))	FRAP 2 (mmol TEq/ 100g (FW))	DPPH (mmol TEq/100g (FW))
Bacaba	10750.71 ± 1496.51	3294.55 ± 301.55	23.60 ± 0.53	13.44 ± 0.20	34.25 ± 0.20
Jenipapo	4655.60 ± 562.83	257.62 ± 30.46	0.36 ± 0.00	0.21 ± 0.00	0.56 ± 0.02

Both fruits expressed a considerable antioxidant effect. However, as expected from the results obtained for the phenolics quantification bacaba showed higher antioxidant ability than jenipapo in all antioxidant assays performed. The ORAC value for bacaba was 10750.71 ± 1496.51 µmol of TEq/100 g of fruit and for jenipapo 4655.6 ± 562.83 µM of TEq/100 g of fruit. Bacaba had a TEAC value of 3294.55 ± 301.55 whereas jenipapo had 257.62 ± 30.46 µmol TE /100 g of fruit. For the FRAP assay, in order to allow comparisons to other studies $FeSO_4.7H_2O$ and trolox were applied as standards as both chemicals are commonly applied to calibration curves in this test. FRAP values for bacaba were 23.60 ± 0.53 mmol $FeSO_4.7H_2O$ /100 g of fruit and 13.442 ± 0.206 mmol TEq/100 g of fruit. FRAP values for jenipapo were 0.364 ± 0.003 $FeSO_4.7H_2O$ (mmol/100 g of fruit) and 0.214±0.005 mmol TEq/100 g of fruit. In the DPPH assay the % of inhibition was calculated for different concentrations of samples and compared to a calibration curve having Trolox as standard. The DPPH antioxidant ability of bacaba was 34.25 ± 0.20 mmol TEq/100 g of fruit whereas jenipapo had 0.561 ± 0.028 mmol TEq/100 g of fruit.

The antioxidant activity of the phenolic extracts from bacaba and jenipapo was also measured using Cellular Antioxidant Activity (CAA) assay, recently designed and

proposed by Wolfe and Liu (*102*). For assessing the antioxidant activity, cells were exposed to different fruit extract concentrations. On the basis of methodology optimization tests (data not shown), concentrations ranging from 0.1 to 5 mg/mL and 0.5 to 25 mg/mL were used for bacaba and jenipapo, respectively. Concentrations chosen for quercetin ranged from 1 to 10 µM. Each experiment ran at least three times independently. Following the proposed methodology, EC_{50} was calculated from the dose–response curves for the inhibition of peroxyl radical-induced DCFH oxidation by standard and samples as illustrated by Figure 15.

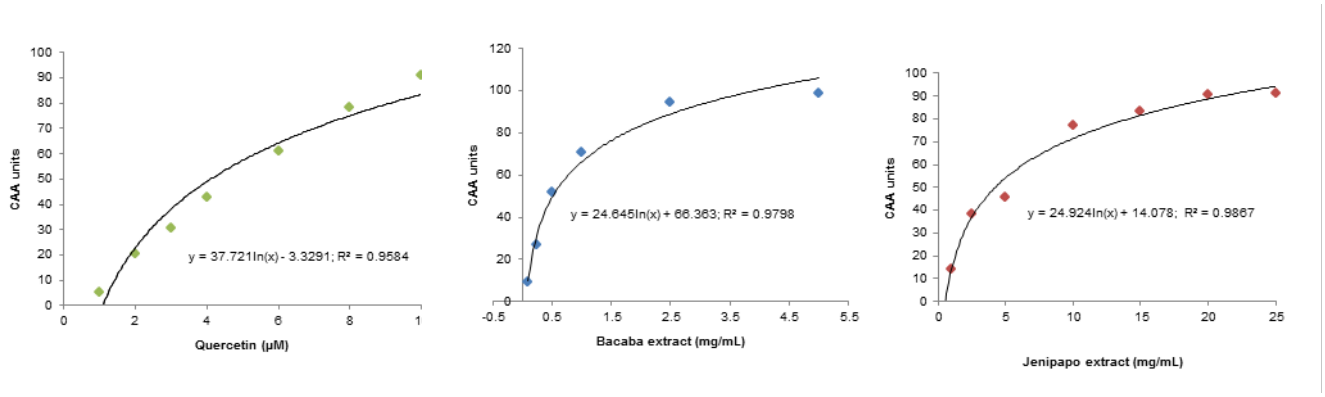


Figure 15: Dose-response curves for the inhibition of peroxyl radical-induced DCFH oxidation by Quercetin, Bacaba and Jenipapo. Graphs are an illustration from one single and representative experiment (n=3).

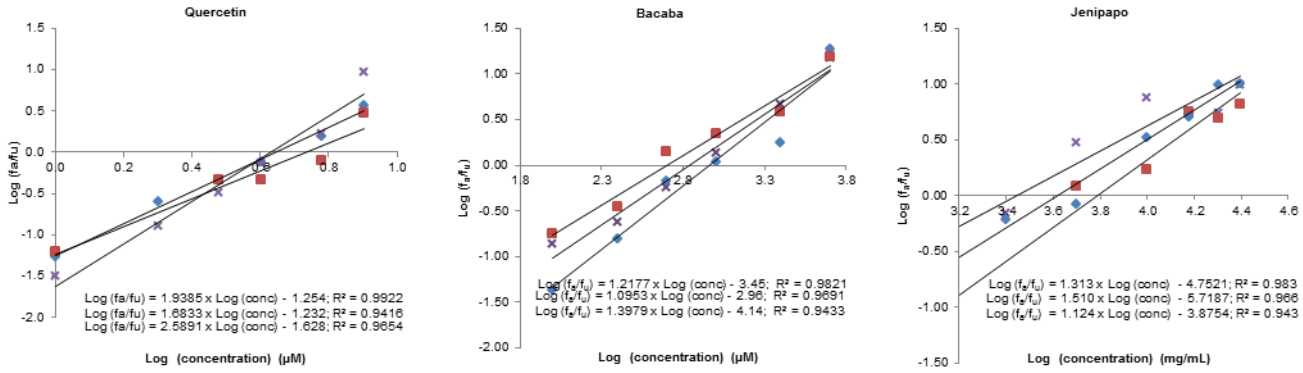


Figure 16: Median effect plots inhibition of peroxyl radical-induced DCFH oxidation by Quercetin, Bacaba and Jenipapo (n=3).

Furthermore the EC_{50} values were calculated from the linear regression of the median effect was curve plotted by data generated from dose response curves. The EC_{50} values express the concentrations of the fruit extracts which have half of the

maximum antioxidant activity achieved by samples (Figure 16). Additionally to the EC_{50} values the results are expressed as CAA values which describe the antioxidant activity of the fruit extracts as µM of Quercetin Equivalent (QE) / 100 g of fruit (FW) and to address the Cellular antioxidant quality (CAQ) results were also expressed in µM QE/100µmol of phenolics. For calculation of QE's, quercetin, a pure phytochemical compound which has a high antioxidant activity, was always run as a standard. All values are listed in
Table **24** and expressed as mean ± SD.

Table 24: EC50 and CAA values for the inhibition of peroxyl radical-induced DCFH oxidation by Quercetin, Bacaba and Jenipapo (n=3).

Sample	**EC_{50}**	**CAA values (µM QE/100g fruit FW)**	**CAQ values (µM QE/100µmol of phenolics)**
	Mean ± SD	Mean ± SD	Mean ± SD
Quercetin	4.66 ± 0.61		
Bacaba	0.7 ± 0.21	305.2 ± 90.8	2.95 ± 0.88
Jenipapo	4. 3± 1.61	83.0 ± 31.3	0.80 ± 0.30

The dose-response curve for each phenolic extract shows that all phenolic fruit extracts express potent antioxidant activities in a dose-dependent manner. Bacaba had EC_{50} value of 0.7 ± 0.61 mg/mL and jenipapo 4.3 ± 1.61 mg/mL. Quercetin, the standard, had an EC_{50} value of 4.66 ± 0.61 µM. CAA value for bacaba was 305.2 ± 90.8 µM of QE/100 g of fruit and 2.95 ± 0.88 µM QE/100µmol of phenolics. Jenipapo had a CAA value of 83.0 ± 31.3 µM of QE/100 g of fruit and 0.80 ± 0.30 µM QE/100µmol of phenolics. When investigating antioxidants, not only the amount is needed but the quality of the antioxidants should also be regarded. Cellular antioxidant quality measures the cellular antioxidant activity provided by 100µmol of phenolics found in the fruit. The CAQ value of bacaba was 2.95 ± 0.88 µM QE/100µmol of phenolics whereas jenipapo had a value of 0.80 ± 0.30 µM QE/100µmol of phenolics.

3.2.4. Inhibition of Cell Proliferation

In order to assess a possible direct effect of the fruit extracts from bacaba and jenipapo on the MTT and MUH chemicals, a cell-free experiment was performed. The fruit extracts were incubated with MTT or MUH solution and the absorbance or fluorescence was measured, respectively. The results from the cell-free tests showed that the bacaba fruit extract had a reducing influence on the MTT solution, i.e. it led to a dose-dependent increase in the absorbance measured (Figure 17A). Jenipapo fruit extracts showed no increasing absorbance in the MTT cell-free test and consequently had no effect on the chemical (Figure 17A).

In the MUH cell-free test, jenipapo extracts in a dose-dependent manner led to an increasing fluorescence (Figure 17B). In contrast, bacaba fruit extracts showed no effect. Therefore, it can be concluded that this fruit had no effect on MUH hydrolysis alone (Figure 17B).

In the next group of experiments, MTT, MUH, methylene blue and trypan blue assays were carried out in order to assess the effect of the fruit extracts on living cells. HepG2 cells were chosen for this as this cell line is a good model to address liver cell metabolism and since these cells usually give consistent results in such assays (*119*). In each assay, HepG2 cell proliferation was measured after cells had been incubated with bacaba and jenipapo extracts with concentrations ranging from 0 to 1400 µg/mL for 96 h. Cells incubated with 2% DMSO were used as a positive control for maximal cell proliferation inhibition. Absolute values recorded for non-treated controls (100%) were an optical density of 0.97 $\pm$ 0.25 for MTT and a fluorescence value of 10813.38 $\pm$ 394.10 relative fluorescence units for MUH, respectively.

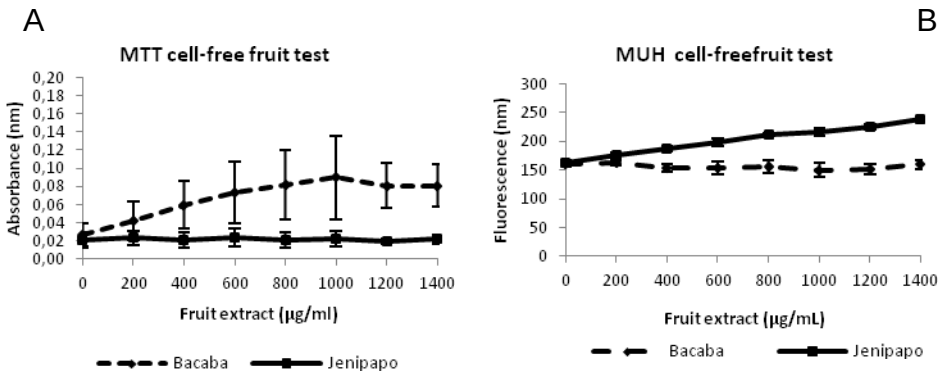


Figure 17: MTT and MUH cell-free test of both fruit extracts, bacaba and jenipapo (n=3). MTT and MUH chemicals were mixed with fruit extracts and medium as for the original assay on a cell free-system.

Both phenolic fruit extracts significantly inhibited HepG2 cell proliferation in a dose-dependent manner in all assays performed (Figures 18 and 19). Results were fitted into a nonlinear regression model and the IC_{50} values shown in Table 25 were calculated.

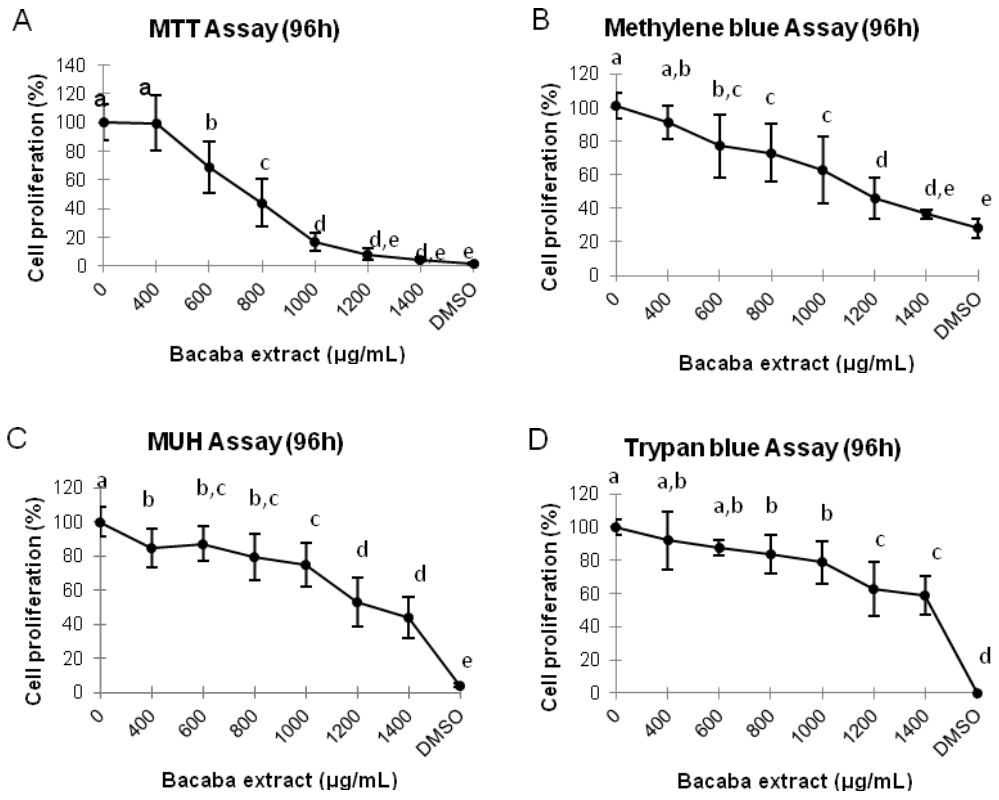


Figure 18: Percentage inhibition of HepG2 cell proliferation by bacaba extracts (n=3). Cells were stimulated with bacaba extracts and incubated for 96h. DMSO (2%) was used as positive control. Points with no letters in common are significantly different ($p < .05$).

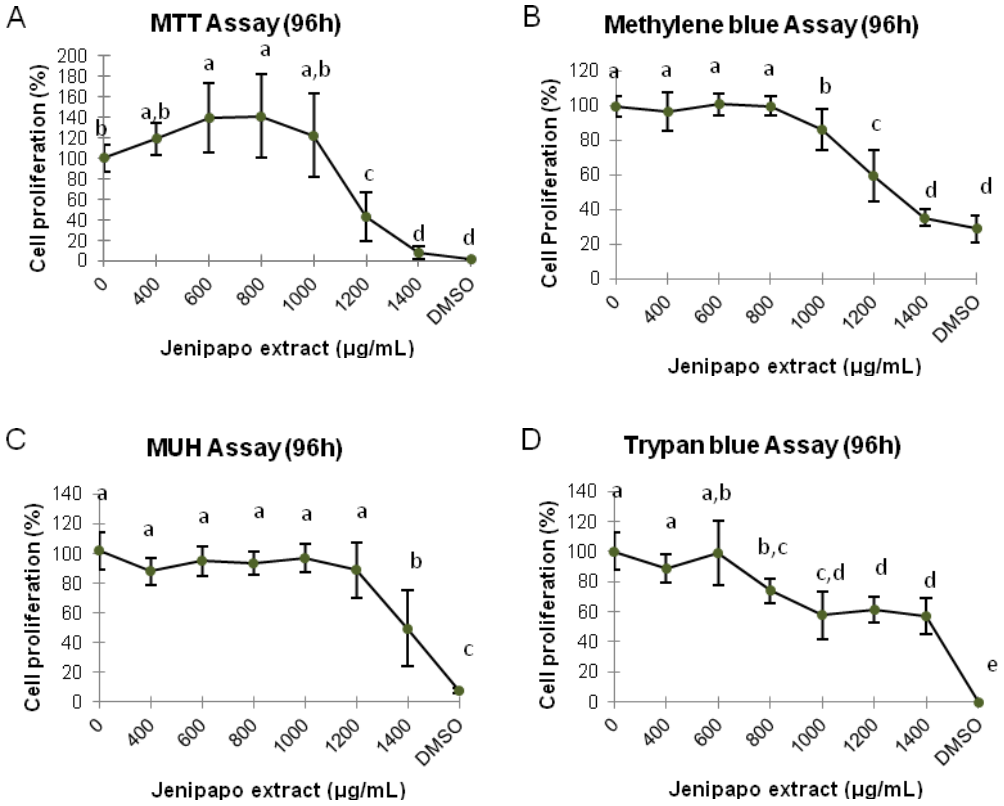


Figure 19: Percentage inhibition of HepG2 cell proliferation by jenipapo extracts (n=3).Cells were stimulated with jenipapo extracts and incubated for 96h. DMSO (2%) was used as positive control. Points with no letters in common are significantly different ($p < .05$).

Bacaba fruit showed the highest antiproliferative capacity with an IC_{50} of 649.6 ± 90.3 µg/mL in the MTT test and an IC_{50} of 1080.97 ± 0.7 µg/mL in the MUH assay, respectively. The Methylene blue and Trypan blue assays did not fit in the nonlinear regression model for bacaba. For jenipapo, the Methylene blue assay indicated an IC_{50} of 1076.7 ± 53 µg/mL, and of 1194.33 ± 96.2 µg/mL in the MTT and 1386 ± 60.32 µg/mL in the MUH assay, respectively. For jenipapo the Trypan blue assay also did not fit in the nonlinear regression model. Indicated by the IC_{50} values it can be seen that bacaba has a higher antiproliferative capacity than jenipapo. Jenipapo's lower antiproliferative capacity also can be seen when fitted into a nonlinear regression model (Table 25).

Table 25: IC_{50} values and regression coefficients (R2) for bacaba and jenipapo extracts in MTT, MUH, Methylene blue (MB) and Trypan blue (TB) assay (n=3).

	Bacaba		**Jenipapo**	
	IC $_{50}$ (µg/mL) Mean ± SD	**R^2 Mean ± SD**	**IC $_{50}$ (µg/mL) Mean ± SD**	**R^2 Mean ± SD**
Assay				
MTT	649.6 ± 90.3	0.97 ± 0.02	1194.33 ± 96.2	0.88 ± 0.03
MUH	1080.97 ± 0.7	0.7 ± 0.15	1386.0 ± 60.32	0.71 ± 0.08
Methylene blue	Not converged		1076.7 ± 53.0	0.71 ± 0.08
Trypan blue	Not converged		Not converged	

* Results were obtained from three different experiments and fit into a nonlinear model.

Correlation analysis was performed to ascertain the association among the applied assays as described in Table 26. The MTT test was highly correlated with all other assays.

Table 26: Spearman's Correlation coefficients among different assays (MTT, MUH, MB, TB)*

	Bacaba		**Jenipapo**	
	MTT	MUH	MTT	MUH
MTT	-	-	-	-
MUH	0.806	-	0.807	-
MB	0.858	0.705	0.866	0.692
TB	0.808	0.759	0.808	0.759

*All values were significant ($p < .05$)

3.3.5 Characterization of phenolic compounds of bacaba by HPLC-DAD-MS^n

Phenolic compounds were analyzed by RP-HPLC in cooperation with Dr. Dietmar Kammerer and Prof. Dr. Reinhold Carle at the Institute of Plant Food Technology of the University of Hohenheim. Ten major compounds were eluted from the HPLC column and were well separated using the chromatographic system applied. The HPLC chromatogram demonstrates the presence of 27 compounds absorbing at 370

nm. The separation of phenolic components of a bacaba extract is presented in Figure 20.

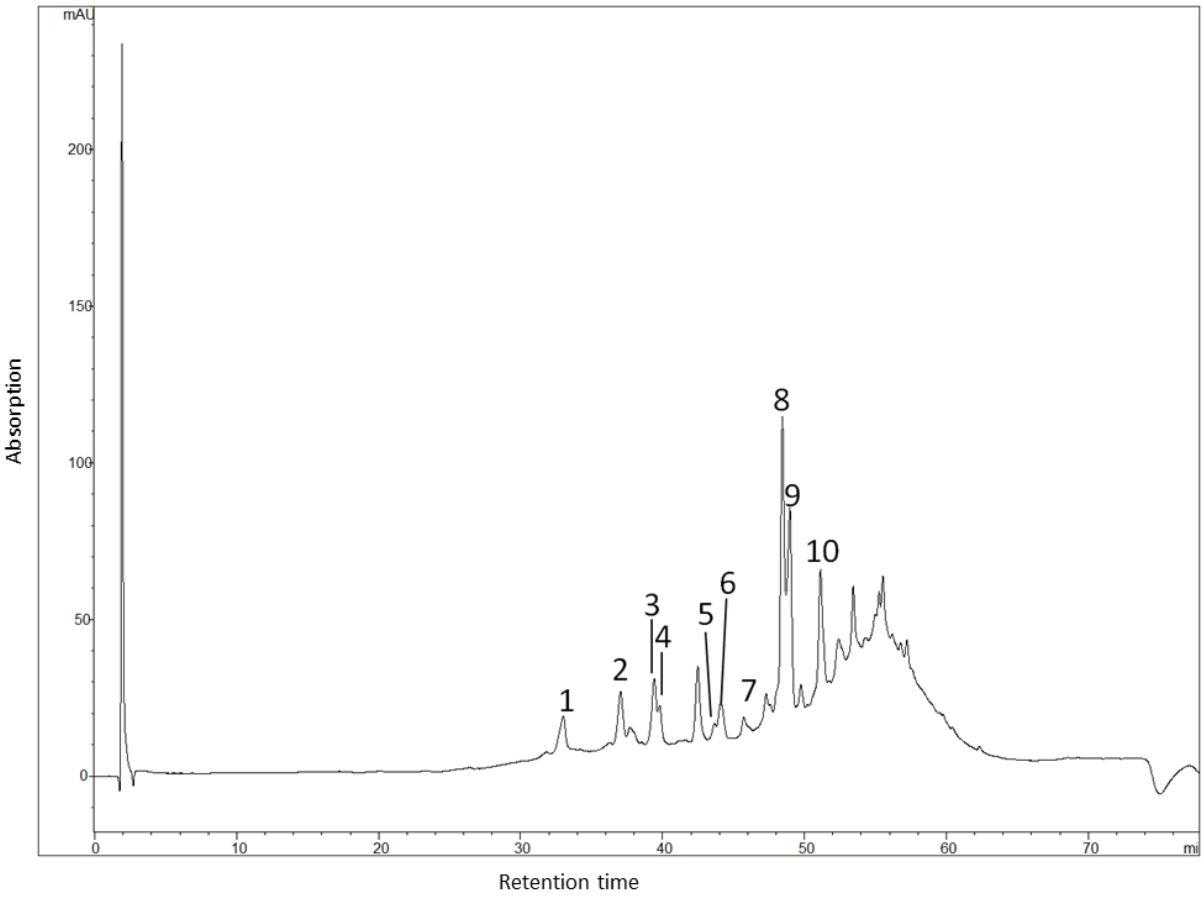


Figure 20: HLPC chromatogram of the phenolics present in a bacaba (*Oenocarpus bacaba* Mart.) extract at a detection wavelength of 370 nm.

The phenolic fractions were characterized by ESI-MS. The assignment of individual non-anthocyanin compounds was based on their main $[M - H]^-$ ions together with the interpretation of their fragments produced in collision-induced dissociation (CID) experiments. Based on the analysis of pseudomolecular ions and on the fragments released in MS^2 and MS^3 experiments, some compounds could be tentatively identified. MS^n spectra are very useful for identifying the aglycone of flavonoids, and the analysis of fragmentation patterns is highly diagnostic, allowing to elucidate structures by comparison with literature data (*120*). MS spectra of flavonoid

glycosides reveal characteristic patterns, depending mainly on the number and nature of the saccharides bound to the aglycon and their linkage type, i.e. C- or O-glycosidic linkage (*121*). Many flavonoids reveal low sensitivity in positive ionization mode MS analyses and are therefore, preferably detected in negative ionization mode (*122*). Mass spectrometric data obtained in the present study using negative ionization mode, are presented in Table 27.

Table 27: Characterization of individual phenolic compounds in bacaba extracts by HPLC/DAD/ESI-MSn

Peak	$t_{(R)}$	Compound class	Compound (tentative identification)	HPLC/DAD UV	$[M-H]^-$	HPLC / ESI (-)-MS^n experiment
	(min)			λ_{max} (nm)	*m/z*	*m/z* (% base peak) [a]
1	33.0	C-glycoside	Vicenin 2 (apigenin-6,8-di-C-glycoside 5,7,4'-trihydroxyflavone-6,8-di-C-glycoside)	235, 268, 337	593	MS^2 [593]: 473 (100), 353 (39), 503 (37), 383 (20), 354 (17), 285 (15) MS^3 [593 → 473]: 353 (100), 354 (19), 383 (15)
2	37.0	Flavonoid, C-hexoside	Orientin (luteolin-8-C-glucoside) or isorientin (luteolin-6-C-glucoside)	348	447	MS^2 [447]: 327 (100), 357 (56), 328 (29)
3	39.4	C-glycosylflavone	Apigenin-8-C-glucoside (vitexin)	340	431	MS^2 [431]: 311 (100), 312 (17), 283 (17), 341 (6) MS^3 [431 → 311]: 283 (100), 312 (37), 118 (27)
4	39.8	Isorhamnetin hexoside	isorhamnetin-dihexoside or rhamnetin-dihexoside	310	639	MS^2 [639]: 315 (100), 477 (53) MS^3 [639 → 315]: 300 (100), 270 (67)
5	43.7	Quercetin hexoside	Quercetin-3-*O*-hexoside	355	463	MS^2 [463]: 301 (100), 302 (25)
6	44.1	Quercetin diglycoside	Rutin (quercetin 3-*O*-rutinoside)	265	609	MS^2 [609]: 301 (100), 302 (27), 300 (20), 271 (14), 343 (14)
7	45.7	Quercetin glycoside	Quercetin-3-O-hexoside	260	505	MS^2 [505]: 301 (100), 300 (37), 463 (37)
8	48.5	Flavonoid	Hexose + deoxyhexose attached to methoxyapigenin methoxyluteolin diosmetin	345	608	MS^2 [607]: 299 (100), 284 (61), 285 (19), 300 (14) MS^3 [607 → 299]: 284 (100), 285 (36)
9	48.9	Hexose + deoxyhexose methoxyl function		345	637	MS^2 [637]: 329 (100), 314 (25), 330 (23) MS^3 [637 → 329]: 314 (100), 315 (44), 329 (22)
10	51.1	Isorhamnetin glucoside	Isorhamnetin acetyl-hexoside	355	519	MS^2 [519]: 315 (100), 300 (18), 316 (16) MS^3 [519 → 315]: 300 (100), 255 (17)

[a] Based on fragmentation pattern in mass spectrometric experiments

[b] Tentative identification was obtained by comparing the elution order and pseudomolecular ions $(M-H)^-$ with data available in the literature (*123*).

Peak 1 - The wavelength of maximum absorption for band Ï (300 – 380 nm) was below 355 nm and, thus, is indicative of hydroxyl substitution at the 3-position (*124*). UV spectra showing a λ_{max} at 267 nm and 338 nm are characteristic of apigenin. In the MS^2 experiment losses 120 Da and 90 Da were observed, thus producing fragments at *m/z* 285, 474 and 503. The fragmentation of C-glycosides typically produces $[M–H–90]^-$ and $[M–H–120]^-$ ions (*124*). MS^2 analyses of the predominant ion at *m/z* 593 detected for compound 1 provided the typical fragmentation of a di-C-glycosylflavone. Particularly, the ions at *m/z* 503 ($[M - H - 90]^-$) and 473 ([M - H - 120], base peak) suggest the occurrence of a 6,8-di-C-hexoside. The ions at *m/z* 353 ([aglycone + 83 Da]) and 383 ([aglycone + 113 Da]) indicated the aglycone to be a trihydroxyflavone as similarly described by Gil-Izquierdo, Riquelme, Porras, & Ferreres (*125*). The precursor ion and major fragment ions corresponding to peak 1 have previously been described in other studies, and by comparison of these mass spectrometric data with those obtained in the present study, this compound was tentatively identified as vicenin-2 (*125, 126,127*).

Peak 2 - Losses of 120 and 90 Da were observed in the MS^2 experiment, corresponding to cross-ring cleavages in the sugar moiety (*128*). This fragmentation of the glycoside was responsible for product ions observed at *m/z* 357 ($[M–H–90]^-$) and *m/z* 327 ($[M–H–120]^-$). The evaluation of the fragmentation behavior indicates compound 2 to correspond to a flavone *C*-glycoside such as isoorientin (luteolin-6-*C*-glucoside) and orientin (luteolin-8-*C*-glucoside), respectively. Although the fragment ions of the parent ion allow the differentiation between *C*-glycosylation at the 6- and 8-positions, it was impossible in the present study to perform a more detailed characterization of this compound due to low signal intensities in the fragmentation experiments (*128, 129*).

Peak 3 - Among the compounds with UV spectra characteristic of flavones, two groups of compounds were differentiated on the basis of their MS^n fragmentation: C-glycosylflavones and O-glycosylflavones. In the MS^2 experiments of the studied C-glycosylflavones, losses of 90 and 120 Da were observed, being characteristic of a

C-glycosylated compound with a hexose linked to the flavone nucleus. The absence of an [M - H - 18]$^-$ ion presumably indicates the position of the C-glycosylation at carbon 8 (*130*). Additionally, compound 3 revealed an [M−H]$^-$ ion at *m*/*z* 431, and its MS/MS spectrum yielded ions at *m*/*z* 341 ([M−H−90]$^-$) and 311 ([M−H−120]$^-$). Consequently, this component was tentatively assigned to apigenin-8-*C*-glucoside, also named vitexin (*131*,*132,133*).

Peak 4 - Mass spectrometric experiments of compound 4 in the negative ionization mode produced an [M-H]$^-$ ion at *m/z* 639 and fragment ions at *m/z* 477 ([M-H-hexose]$^-$), and *m/z* 315 ([M-H-2xhexose]$^-$). The ion at *m/z* 315 is characteristic of rhamnetin and isorhamnetin, respectively, and the fragmentation pattern indicated this compound to contain two hexose moieties (*122, 134*). Thus, this compound was tentatively identified either as isorhamnetin-dihexoside or rhamnetin-dihexoside.

Peak 5 - The fragmentation pattern indicates this compound to be a quercetin-hexoside (*135*, *136*). In ESI experiments in the negative ionization mode, quercetin mono- and diglycosides are known to produce the quercetin aglycone at *m/z* 301 as a result of the loss of the glycosyl moieties (*137*). CID experiments with compound 5 generated the aglycon ion at *m/z* 301 revealing a loss of 162 Da together with a fragment ion at *m/z* 179, which is characteristic of quercetin-3-*O*-glucoside (*138*). The same findings were also previously described for quercetin hexoside with an [M–H]$^-$ ion at *m/z* 463 showing a typical loss of 162 Da resulting in the characteristic fragment ion at *m/z* 301 (*139*).

Peak 6 - Compound 6 exhibited a pseudomolecular ion at *m*/*z* 609 and the loss of the glycosidic moiety, releasing the quercetin aglycon ion at *m*/*z* 301 and being equivalent to the loss of a hexose moiety (162 Da) and deoxyhexose moiety (146 Da). Further fragmentation of the fragment ion at *m/z* 301 produced further fragments at *m/z* 179 and 151, which are characteristic of quercetin. Based on similar mass spectral studies reported in the literature, this compound was tentatively identified as rutin (quercetin 3-*O*-rutinoside) (*140*,*141*).

Peak 7 - The mass spectra indicated the presence of a quercetin derivative (aglycone fragment at *m/z* 301) glycosylated with a hexose and a acetyl moiety attached to it (*142*). The peak was tentative identified as Quercetin-3-O-hexoside (*143*).

Peak 8 - The compound with a pseudomolecular ion at *m/z* 607 revealed a fragment ion at *m/z* 299 ($[M - H - 308]^-$) in the MS^2 spectrum, suggesting the loss of a rutinose moiety (flavonoid *O*-rutinoside). Shi *et al*. (*144*) found a similar fragmentation pattern for diosmin (*145*).

Peak 9 - Compound 9 showed a UV spectrum typical of a flavonoid. In the mass spectrometric analyses, a characteristic loss of 308 Da was observed in the MS^2 experiment, being indicative of a diglycoside composed of a hexose and a deoxyhexose moiety.

Peak 10 - The HPLC–ESI–MS/MS experiments brought about the loss of 204 Da characteristic of an acetylhexoside (162 Da + 42 Da). Further fragmentation in the MS^3 experiment showed a further loss of a methoxyl function (15 Da), and an ion at *m/z* 300 characteristic of an isorhamnetin or rhamnetin backbone unit. A similar spectrum has previously been described for isorhamnetin acetyl-glucoside (*139, 146, 147*).

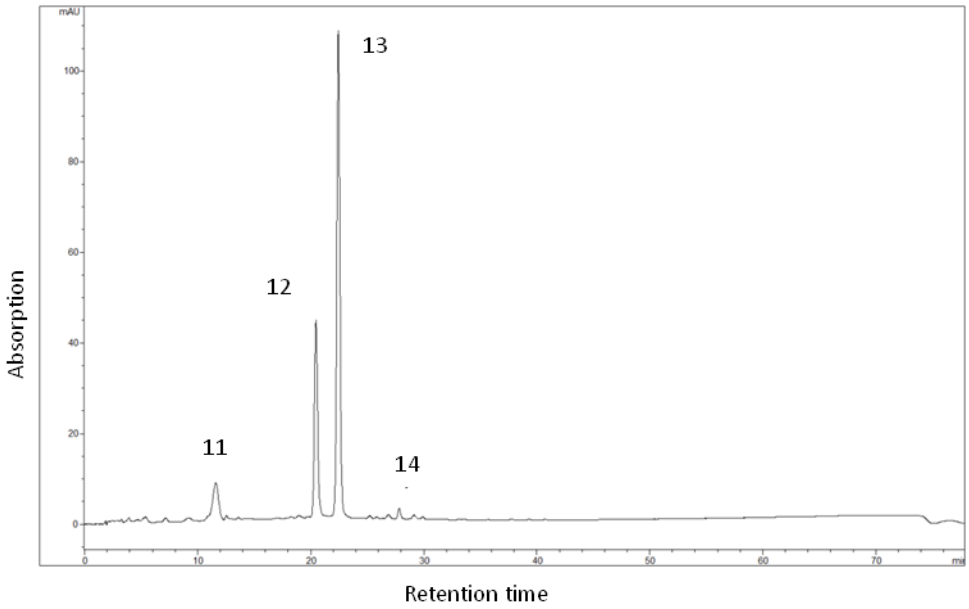


Figure 21: HPLC chromatogram of anthocyanins from a bacaba (*Oenocarpus bacaba* Mart.) sample at a detection wavelength of 520 nm.

The separation of anthocyanins extracted from a bacaba sample is presented in Figure 21. As can be seen, baseline separation was achieved for all compounds, and four major peaks were observed in the chromatogram. UV/Vis spectroscopic and mass spectrometric data obtained in the positive ionization mode of anthocyanins are presented in Table 4. All compounds were found to be cyanidin glycosides due to the release of the characteristic fragment ion at *m/z* 287. Mass spectrometric analyses of the four peaks yielded molecular ions M^+ at *m/z* 595, 758, 449, 595, 963, respectively, as specified in Table 28.

Table 28: UV/Vis and mass spectrometric characterization of individual anthocyanins in bacaba extracts by HPLC/DAD/ESI-MSn

Peak	$t_{(R)}$ (min)	Compound characterization	Tentative identification	HPLC/DAD UV λ_{max} (nm)	$[M]^+$ *m/z*	HPLC / ESI (+)-MS^n experiment [a] (% base peak)
11	11.6	Cyanidin glycoside		270, 505	758	MS^2 [758]: 596 MS^3 [758 →596]: 449, 287
12	20.5	Cyanidin hexoside	Cyanidin 3-*O*-glucoside	270, 505	449	MS^2 [449]: 287
13	22.5	Cyanidin glycoside	Cyanidin-3-*O*-rutinoside	260, 510	595	MS^2 [595]: 449, 287
14	27.9	Cyanidin glycoside		305, 520	963	MS^2 [963→]: 449, 287 MS^3 [967 →595]: 449, 287

[a] Based on HPLC–MS mass fragmentation pattern

Peak 11 - Compound 1 was assigned to a cyanidin hexoside due to the loss of 162 Da in the MS^2 experiment and the release of an ion at *m/z* 287 upon further fragmentation.

Peak 12 - The fragmentation of compound 2 was characteristic of a cyanidin (*m/z* 287) aglycon glycosylated with a hexose (162 Da) moiety.Compound 2 was tentatively identified as cyanidin 3-*O*-glucoside.

Peaks 13 and 14 - Compounds 3 and 4 revealed fragmentation patterns characteristic of anthocyanins containing hexose and deoxyhexose moieties attached to the cyanidin aglycon, respectively. Compound 3 was tentatively identified as cyanidin-3-*O*-rutinoside (*129*).

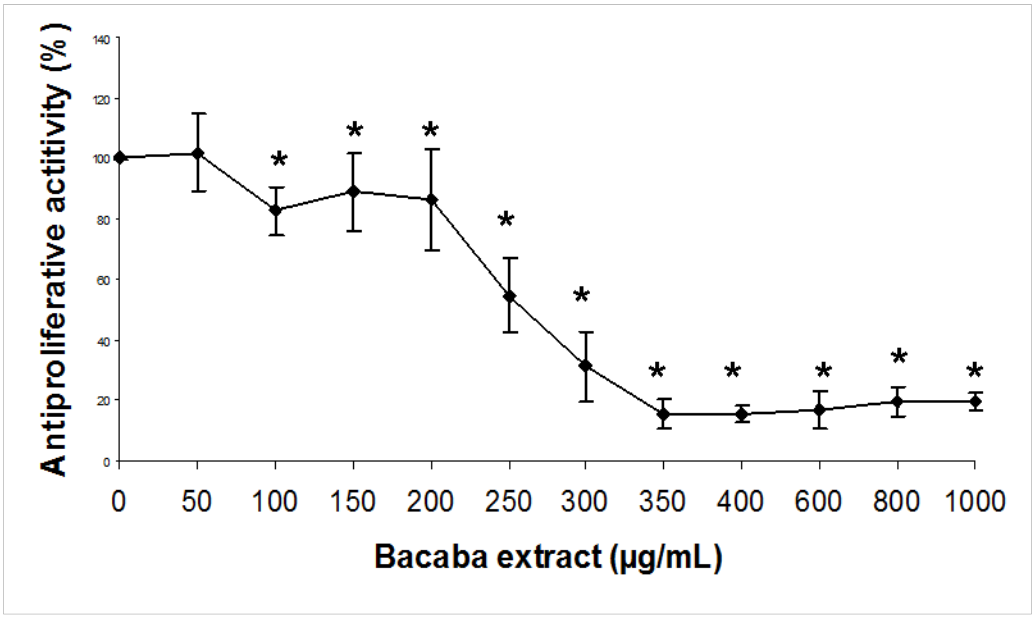
The characterization of phenolic compounds is important as these compounds are associated with a range of different health properties. HPLC with diode array and mass spectrometric detection has been proven to be very useful for the characterization of individual phenolic compounds. Ten polyphenols were detected and characterized in bacaba extracts in the present study. Based on their UV spectral data, most of these compounds were characterized as quercetin and rhamnetin derivatives besides a range of further flavonoids. So far, *Oenocarpus bacaba* extracts have not been systematically analyzed. To the best of our knowledge, the present study represents the first chemical investigation of this plant material. Due to the limited availability of reference compounds, the similarity of spectral characteristics and the chromatographic behavior allow tentative peak assignment. For this purpose, mass spectrometric analyses were also performed (*92*). Nine compounds were tentatively identified based on the comparison with results previously described in the literature. Although some compounds were not identified, they are relevant to comprehensively characterize the chromatographic fingerprint which is a plant-specific trait. Taking together the outcome of the present study, it becomes apparent that bacaba is a potential source of pigments and natural antioxidants which should be considered for future exploitation.

3.3.6. Chemopreventive effect of bacaba extract on MCF -7 cells

3.3.6.1. Effects of bacaba extract on MCF-7 cell proliferation and morphology

A phenolic extract of the bacaba fruit was prepared as described in materials and methods and its total phenolic content was determined using the Folin-Ciocalteau reagent. Bacaba fruit had 1759.27 $\pm$ 1.01 mg GAEq/100g of phenolic content.

In order to test for an antiproliferative effect of the bacaba extract, MCF-7 breast cancer cells were incubated with different concentrations of the phenolic extract for 96 h and quantified by the MTT assay. The proliferation of MCF-7 cells was significantly inhibited by bacaba extract after 96 h of incubation at concentrations of 100µg/mL or above (Figure 22A). The IC_{50} was 252.10µg/mL with a confidence interval range from 242.8 - 261.7. Morphological changes in MCF-7 cells treated with bacaba extract were observed by contrast microscopy (Figure 22B). A difference in cell morphology can be clearly observed when cells were incubated with 400µg/mL bacaba extract or more (Figure 22B). Control cells showed a typical intact appearance whereas cells incubated with the bacaba extract displayed morphological alterations such as cellular shrinkage and reduction of the cell monolayer area (400µg/mL), rounding and poor adherence as well as floating shapes (600 and 800 µh/mL). These observations suggest that bacaba extracts may induce apoptosis in MCF-7 cells.



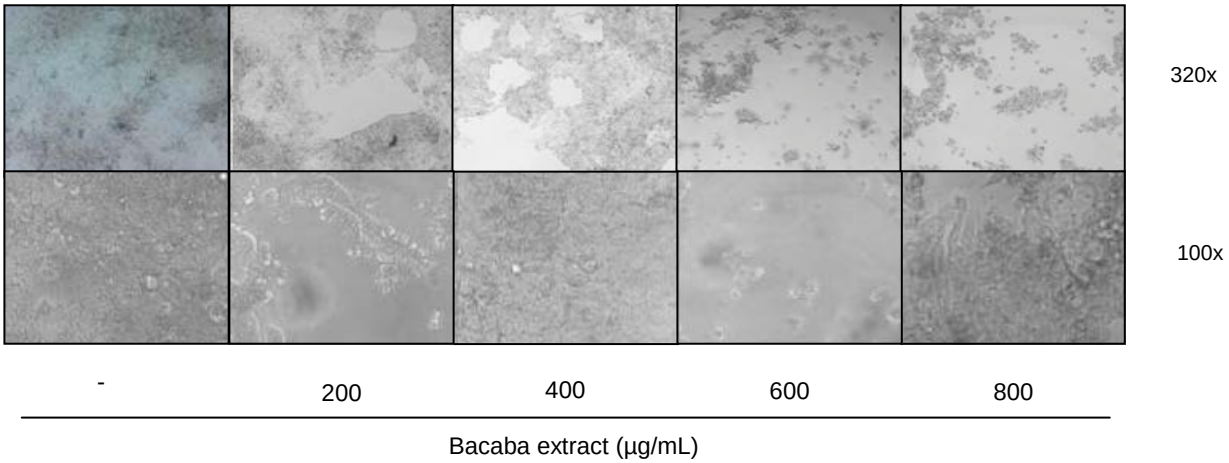


Figure 22: Antiproliferative effect of bacaba extract on MCF-7 cells**.** A. Effect of bacaba extract on cell proliferation. MCF-7 cells were treated with or without different concentrations of bacaba extract for 96h to assess cell proliferation. Data are presented as means ± standard deviation from at least three independent experiments. Each experiment was conducted in six replicates (* $p < .05$). B. Morphological changes in MCF-7 Cells incubated with bacaba extract for 72h. Cells were observed by contrast optical microscopy (magnification 100x and 320x) in controls and after treatment with bacaba extracts (200 – 800µg/mL). Pictures are representative of at least three independent experiments.

Apoptosis is defined by a characteristic set of changes in cell morphology during cell death. Cell alterations include loss of focal adhesions, formation of cell membrane buds or blebs and decrease in total cell volume. Recent studies suggest that the morphological changes are an early prerequisite to apoptosis and precede key biochemical time-points (*148*). It has been suggested that as a rule, classification of cell death in a given model should always include morphological examination coupled with at least one other assay (*149*). Following this recommendation, morphological observation was performed together with two other preliminary assays to check for a possible apoptotic effect of bacaba extract on MCF-7 cells (ethidium bromide/acridine orange (EB/AO) staining and DNA laddering) (Figure 23).

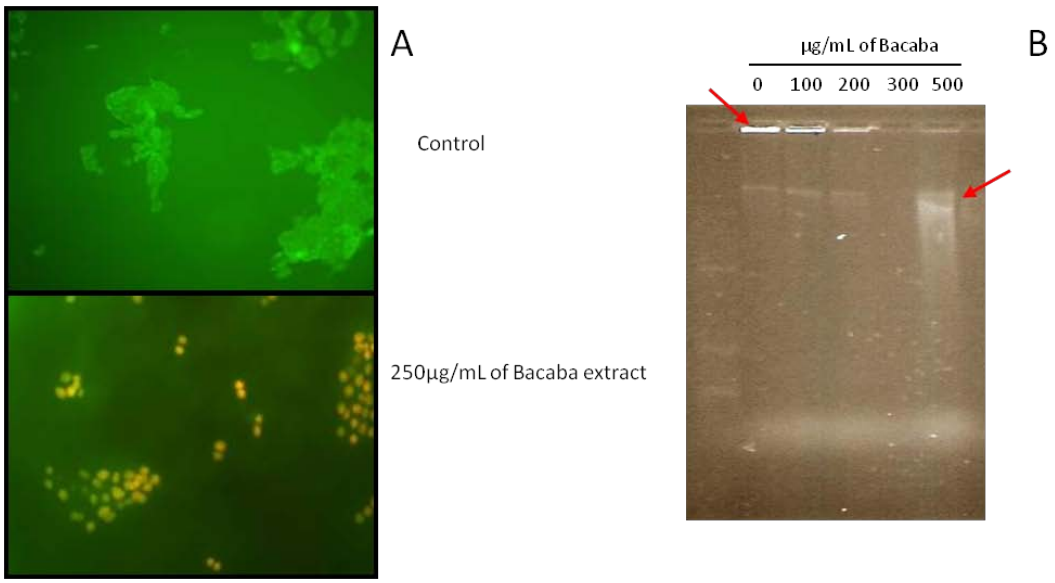


Figure 23: EB/AO staining of MCF-7 cells treated with 0 and 250 µg/mL of bacaba extract after 72h of incubation. A. Cells stained in green are viable cells whereas orange cells indicate apoptotic cells (magnification 200x). Pictures are representative of at least three independent experiments. **B**. DNA fragmentation for MCF-7 cells. Cells were treated with bacaba extract (0,100,200,300,500µg/mL) for 72 h and DNA was analyzed by agarose gel electrophoresis.

Fluorescence light microscopy with differential uptake of fluorescent DNA binding dyes such as ethidium bromide/acridine orange staining (EB/AO) is a suitable method for the evaluation of cellular viability. During early stages of apoptosis, cells are impermeable to ethidium bromide and their nuclei stain green, during the later stages, nuclei stain orange/red. EB also dominates over AO. Representative cellular morphology of control and treated cells was assessed following staining with AO/EB. Viable cells were impermeable to ethidium bromide and had normal round nuclei that stained green (Figure 23A). Cells incubated with 250µg/mL of bacaba extract for 72 h showed characteristics of late apoptosis, i.e. their nuclei stain mostly orange. Indeed, this concentration is a value close to that found for the IC_{50} to the antiproliferative activity (Figure 23).

3.3.7.2. DNA fragmentation and PARP cleavage

DNA fragmentation and the cleavage of the abundant nuclear enzyme poly(ADP-ribose) polymerase (PARP) are typical hallmarks of apoptosis and therefore, were investigated next (*150*). To assess DNA fragmentation, MCF-7 cells were treated with bacaba extract for 72 h. Genomic DNA was isolated and run through an agarose gel. Figure 23 shows that cells exposed to 500µg/mL of bacaba extract demonstrated a DNA laddering indicating the fragmentation of DNA when compared to control (red arrows). In later stages of apoptosis, the 116 kD PARP protein is cleaved by caspase-3 into an 85 kD fragment, whose presence indicates that cells are undergoing apoptosis (*151*). To assess PARP cleavage, cells were incubated with bacaba extract for 24 and 48h. A considerable PARP proteolysis was observed in cells exposed to bacaba extracts for 48 h in a dose dependent-manner indicating a significant induction of apoptosis. The control showed no PARP-cleavage whereas incubation with staurosporin - a known inducer of apoptosis - resulted in complete PARP cleavage (Figure 24). No PARP cleavage was observed during the incubation of cells with bacaba extracts for 24 h (Figure 24).

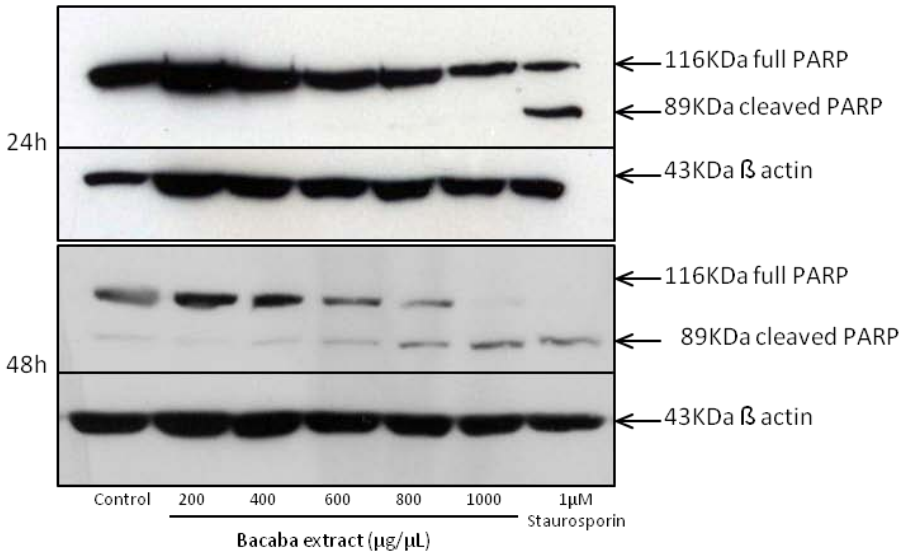
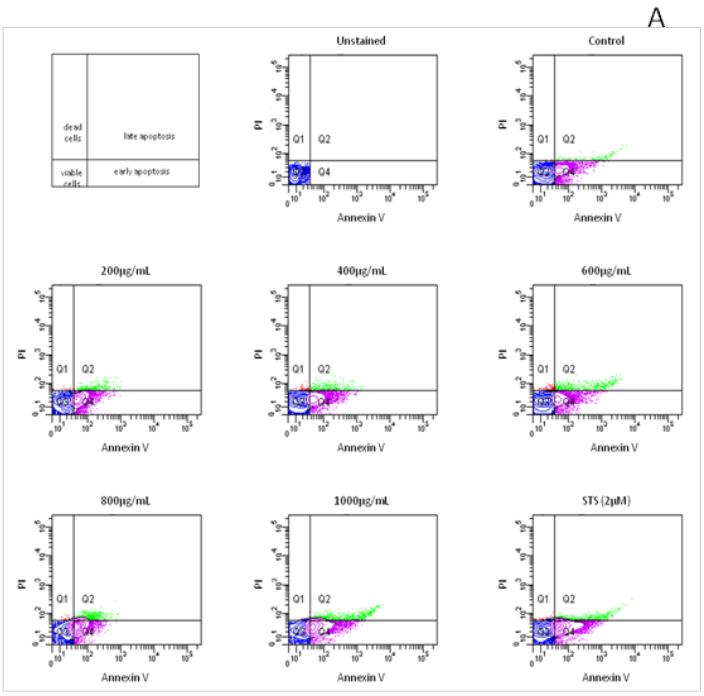
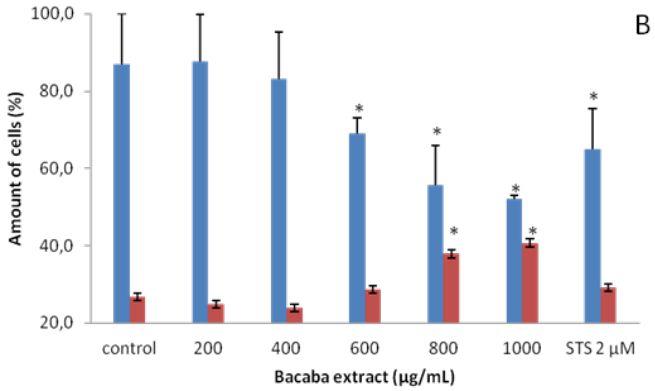


Figure 24: PARP – cleavage by bacaba extract. Cells were incubated with bacaba extract (0-1000µg/mL) for 24 h and 48 h. Cell lysates were prepared and analyzed by SDS-PAGE and western blotting.

3.3.7.3 Bacaba induced apoptosis as measured by annexin V/propidium iodide (PI) flow cytometry

DNA fragmentation, DNA staining and PARP cleavage are important methods to characterize apoptosis. However, these methods only detect an increase of apoptotic signals, and cannot easily quantify percentage of live, apoptotic or dead cells. Consequently, a quantitative approach was also carried out to characterize the apoptosis induced by bacaba extract. Therefore, cells were incubated with bacaba extract for 24h and stained with annexin V-FITC/propidium iodide and analyzed by flow cytometry (Figure 25).


A
Unstained
Control
200µg/mL
400µg/mL
600µg/mL
800µg/mL
1000µg/mL
STS (2µM)
dead cells
late apoptosis
viable cells
early apoptosis
PI
Annexin V
Q1
Q2
Q3
Q4


B
Amount of cells (%)
100,0
80,0
60,0
40,0
20,0
control
200
400
600
800
1000
STS 2 µM
Bacaba extract (µg/mL)
viable cells
early apoptosis

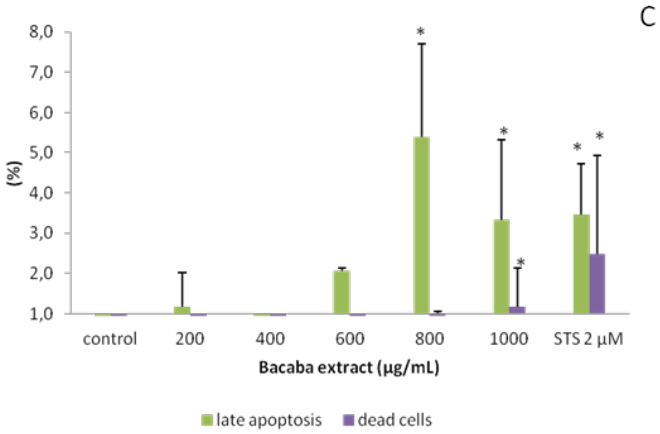

C
(%)
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
control
200
400
600
800
1000
STS 2 µM
Bacaba extract (µg/mL)
late apoptosis
dead cells

Figure 25: Annexin V/PI staining of cells treated with bacaba extract (0-1000µg/mL).A. Induction of apoptosis by bacaba extract on MCF-7 cells. Representative flow cytometric analysis observed through annexin V-FITC/propidium iodide (PI) staining. Viable cells are negative for annexin V and PI (- Annexin V / - PI). In early apoptosis stages cells are positive for annexin V as represented at the down-right quadrant (+ Annexin V/ -PI). Late apoptosis cells are annexin V and PI positive (+ Annexin V/ +PI) (upper right). Dead cells are solely stained by PI (- annexin V/ +PI) (upper-left). 2µM staurosporin (STS) was used as positive control. **B**. Quantitation of viable and early apoptotic cells treated with bacaba extract (0-1000µg/mL) and stained with annexin V/PI. * ($p < .05$). **C**. Quantitation of cells incubated with bacaba extract (0-1000µg/mL), stained with annexin V/PI and analyzed by FACS. * ($p < .05$).

Loss of plasma membrane asymmetry and consequently translocation of phosphatidylserine (PS) to the outer leaflet is an early event of apoptosis and can be assessed using annexin V, a protein that binds to exposed PS. When conjugated with FITC (fluoresceinisothiocyanat), annexin V can be detected by flow cytometry. To differentiate apoptotic cells from dead cells, annexin V is usually used conjoined with a vital dye such as propidium iodide. PI is a nucleic acid intercalator, which penetrates the plasma membrane of dead cells. However, it cannot enter integral membranes of viable cells. Flow cytometry assay showed noticeable changes in MCF-7 cell profiles after incubation with bacaba extract for 24 h indicating that bacaba extract could induce apoptosis on MCF-7 cells (Figure 25A). After 24 h of incubation a significant decrease in cell viability (- annexin V-FITC/ - PI) was observed with concentrations of 600µg/mL or more of bacaba extract. Cell viability ranged from 87.05 $\pm$ 12.0 % (control) to 52.06 $\pm$ 1.0% (1000µg/mL). Early apoptotic cells (+ annexin V-FITC/ - PI) ranged from 23.9 $\pm$ 2.20 to 40.7 + 4.2 and a significant difference was observed at concentrations above 800 µg/mL ($p < .05$) (Figure 25B). Late apoptotic (+annexin V / +PI) cells were significantly observed upon treatment with concentrations higher than 800 µg/mL (37.9 $\pm$ 11.1 %) and dead cells (- annexin V / + PI) were present at 1000 µg/mL ($p < .05$; Figure 25C). Thus, an effect of bacaba extract on phosphatidylserine exposure by early apoptotic cells as well as on the decrease of cell viability was observed in a dose-dependent fashion.

3.3.7.4. Reduction of cell volume

Variation in forward side scatter (FSC) of treated MCF-7 cells indicates a decrease in cell size. A shift from right to left can be seen after 24 h of incubation with different concentrations of bacaba extract (0-1000 µg/mL) in Figure 25 A. These data point to the typical reduction of cell volume during the apoptotic process. Staurosporin (2µM) was used as a positive control. Cells were quantified and classified as apoptotic and non-apoptotic accordingly to their size (Figure 25 B). An increase in the number of cells with reduced size (apoptotic) was observed in a dose-dependent manner after incubation with bacaba extracts for 24h (Figure 26). Similarly, a decrease of the number of non-apoptotic cells was observed in the same fashion ($p < .05$).

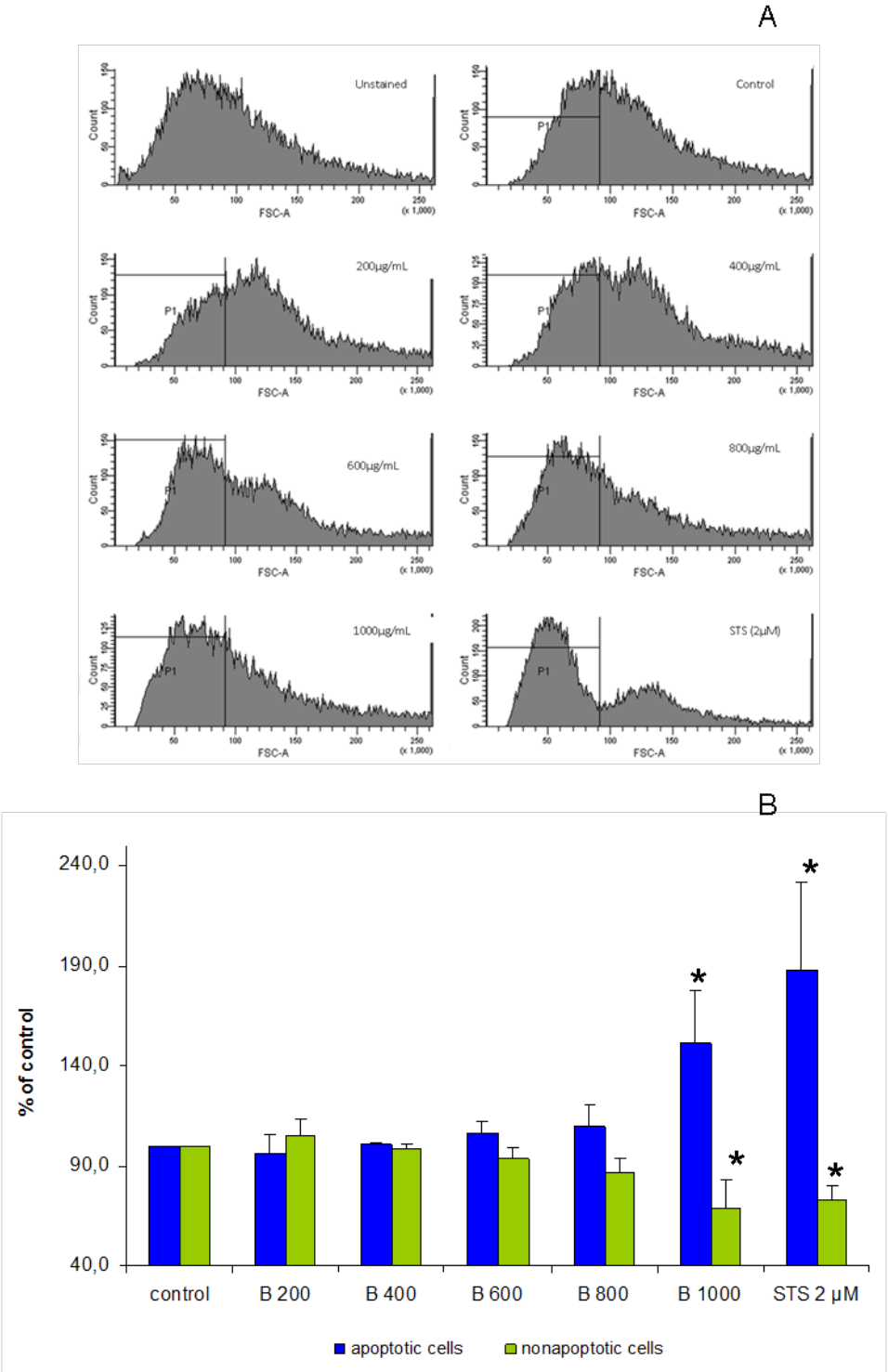


Figure 26: Decrease of cell volume in MCF-7 cells exposed to different concentrations of bacaba extract. **A**. Decrease of cell volume in MCF-7 cells exposed to different concentrations of bacaba extract. Volume was characterized by FACS analysis using forward side scatter (FSC) of untreated control cells and cells treated with bacaba extract for 24 h. Staurosporin (2µM) was used as positive control. P1 represents the gated area for apoptotic cells. Data depicted are representative of at least

three independent experiments. **B**. Quantitation of Light scatter properties (cell size, forward light scatter (FSC)). Cells were gated according to the controls and values of FSC for apoptotic and nonapoptotic cells were recorded and analyzed. Cell size distribution differs between apoptotic and nonapoptotic cells * ($p < .05$).

3.3.7.5. Bacaba extract reduces the mitochondrial membrane potential ($\Delta\Psi_m$) in MCF-7 cells

To investigate whether bacaba extract-induced apoptosis involved the mitochondrial membrane potential ($\Delta\Psi_m$) the cationic fluorescent dye JC-1 was used to detect the $\Delta\Psi_m$ when cells were treated with or without 500 and 1000µg/mL of the extract for 1, 3 or 6h. Samples incubated with 1000µg/mL showed a significant decrease in the $\Delta\Psi_m$ at all time points (Figure 27).

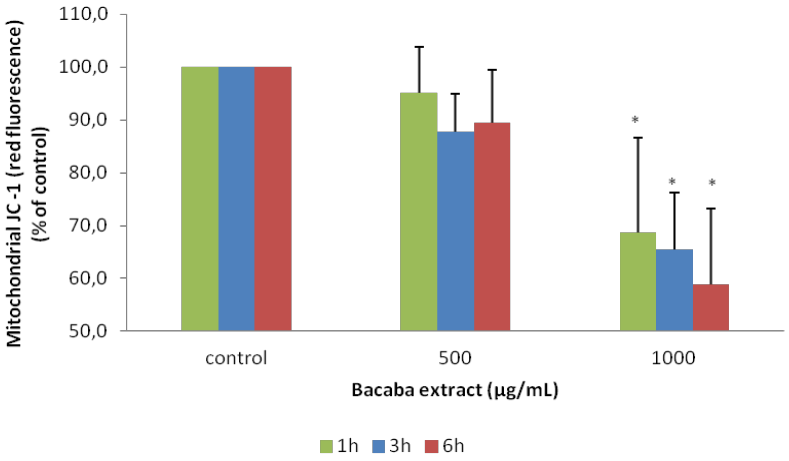


Figure 27: Mitochondral membrane potential of MCF-7 cells. Cells were incubated with different concentrations of bacaba extract (500 and 1000µg/mL) for different time points (1, 3 and 6h). Afterwards, cells were stained with JC-1 and analyzed by flow cytometry. The loss of percentage indicates the reduction of $\Delta\Psi_{m.}$ * ($p < .05$).

3.3.7.6. Apoptosis induced by bacaba extract is caspase-dependent: activation of caspases -6, -8 and -9

The induction of apoptosis by many cytotoxic agents is highly dependent on the activation of caspases (*152*). To characterize the apoptotic process triggered by bacaba extract, activation of caspases was investigated. Bacaba extract was found to induce the activation of caspases -6, -8 and 9 using three different fluorogenic substrates (VEID-AFC, IETD-AFC and FITC-LEHD-FMK) for caspase-6, -8 and -9, respectively (Figure 28 A, B, C).

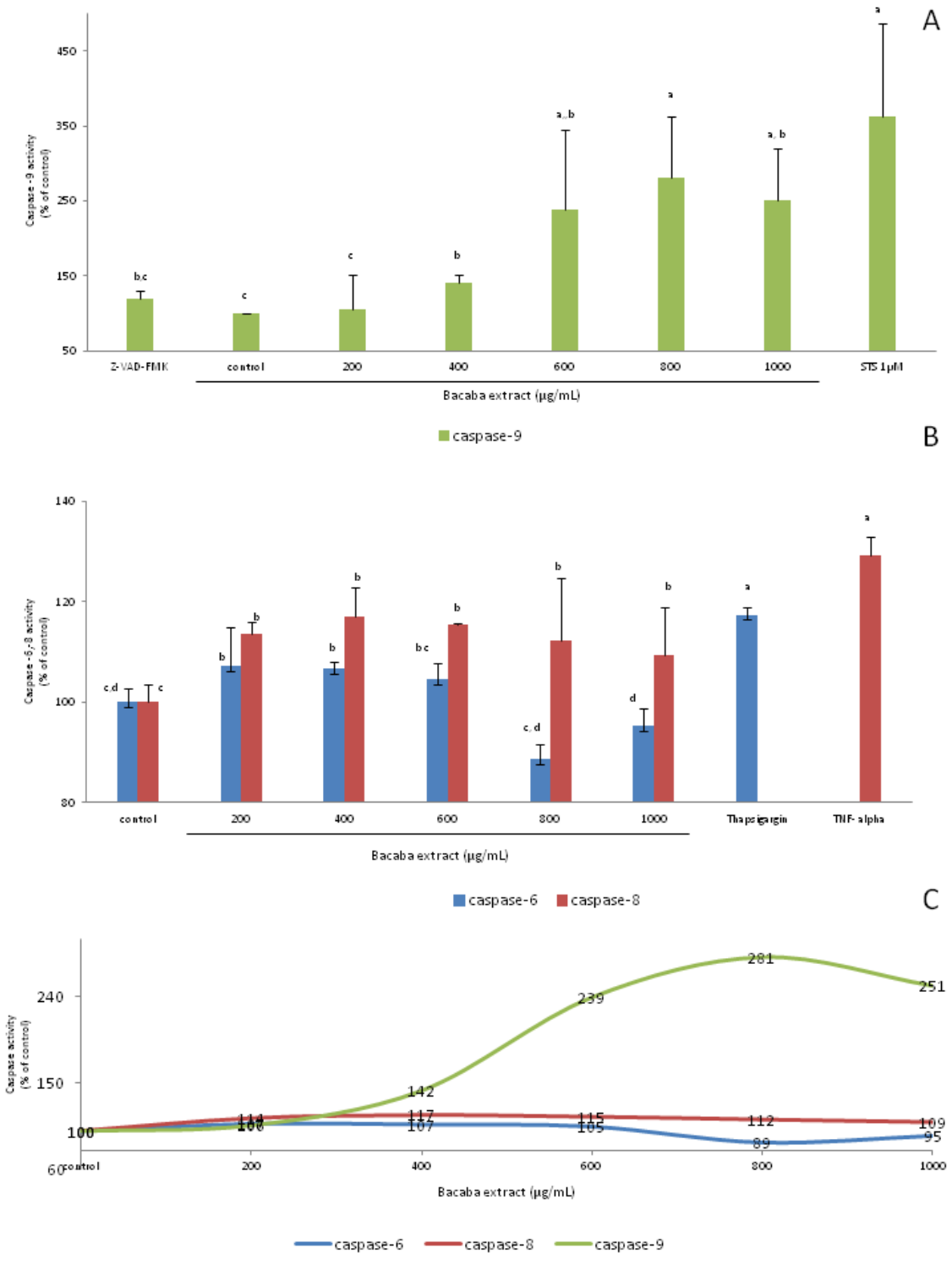


Figure 28: Caspases activation by bacaba extracts.Caspases activation by bacaba extracts. **A**. Caspase-9 activation after incubation with bacaba extracts (0-1000µg/mL) for 24h. **B**. Caspases-6 and -8 activation after incubation with bacaba extract (0-1000µg/mL) for 24h. **C**. Comparison of the level of activation for caspases-6, -8 and -9.

Caspase-9 was activated after 24 h of incubation with bacaba extract (400 – 1000µg/mL) in a dose-dependent manner (Figure 28. A). Staurosporin (1µM) was used as positive control. Also, caspase-8 was activated by all concentrations of bacaba extract applied (200-1000µg/mL) (Figure 28 B). However, there was no significant difference among the different doses of extracts, and the response to the different concentrations did not reach the level of activation of the positive control used (TNF-alpha 100ng/mL), as can be seen in Figure 28 B. ($p < .05$). Caspase-6 was also active after 24h of incubation with bacaba extracts (Figure 28). Again, there was no difference in the level of activation when cells were incubated with 200, 400 and 600µg/mL of bacaba extract, though a significant decrease was observed after incubation with 800 and 1000µg/mL ($p < .05$). Thapsigargin (2µM) was used as positive control in this case. Although all investigated caspases (6-, -8 and -9) showed a significant higher level of activation from the non-stimulated control cells ($p < .05$), the activation of caspase -9 was much more pronounced than the one of caspase -6 and -8. In comparison to the non-stimulated control cells, caspase-9 reached a maximum of 280.90 $\pm$ 81.8% of activation (800µg/mL of bacaba extract) whereas caspase -6 and -8 reached a maximum of 107.2 + 7.59 % (200 µg/mL) and 117.2 $\pm$ 5.8 (400µg/mL), respectively.

3.3.7.7. PARP-cleavage by caspase – 9 in MCF-7 cells

Initiator caspase-3 is usually located upstream of caspase-8 and -9. Due to a genetic defect (a deletion mutation in exon 3 of the gene**)**, MCF-7 cells do not express caspase-3 (*153*). Therefore, the role of caspase-9 on PARP-cleavage was assessed. Cells at 70 % of confluence were incubated with 1000µg/mL of bacaba extract for 48 h in the absence or presence of the specific caspase-9 inhibitor Z-LEHD (20µM). After 48 h of incubation with bacaba extract, a PARP cleavage was observed in control cells. In contrast, cells incubated with Z-LEHD and bacaba phenolic extract did not show the characteristic 85KDa band of cleaved PARP (Figure 29) indicating that caspase-9 is involved in PARP cleavage.

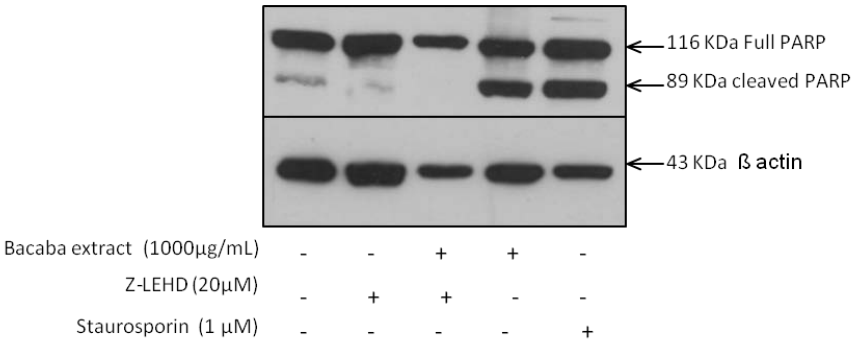


Figure 29: PARP–cleavage by Caspase-9. MCF-7 cells were incubated for 48h with or without bacaba extract and the specific caspase 9 inhibitor Z-LEHD. Cells lysates were prepared and analyzed by western blotting.

4. Discussion

4.1 Nutrition Survey

4.1.1. Socioeconomic, health and food security assessment of two rural communities in the region of APA/Cantão, Tocantins state (Brazilian Amazon Region)

Human beings play a central role in farming systems. Every relationship among the resource usage and its allocation to the farm, household and off-farm sector is exerted by a human being and its behaviour is crucial to lead the family to a better off (or worse off) situation. Herein, a family is defined as all members of a household who share a common kitchen, contribute to family income and/or utilise benefits from family income permanently. The family size of the two rural communities studied is similar to (4.7 persons/family) the average for rural areas in northern Brazil (4.9 persons/family) (*107*). It is noteworthy to mention that the farm families in the study area are rural settlers who are on average less than 10 years on the land. Thus they still have relatives, personal contacts and professional relations in urban areas. In this sense, the rural exodus especially of adolescent male and female members might be higher compared to other regions within the Brazilian Amazon region.

In the study area, the land use is dictated mainly by its soil quality, availability of water as well as access to credit. It is important to note that farmers are located in a transition area between Cerrado and Amazon rainforest, the so-called ecotone biome, which plays an important role as a source of fauna and flora.

At first glance, the forest deforestation does not seem impressive, since roughly 70% of the remaining families have not deforested the biome. However, when taking into account the environmental services provided by native forests, this perspective might change. It is worth mentioning that forests also provide a range of ecosystem services, such as water storage, increased rainfall, nutrient recycling, biodiversity and

soil stabilization. They can help with flood control and boost agricultural productivity, which surely would contribute to a positive impact on families' living standards. Thus, the deforestation of native biomes generates many negative environmental consequences, up to the risk of species extinction since the entire chain of environmental services is compromised. Table 6 shows the amount of the native biome which was deforested for agriculture or livestock production.

With regard to the families' own assets, results give an overview of the family possessions and briefly indicate their living standard. The absence or presence of these assets is therefore inextricably linked to the socioeconomic well being of farm households (*154*). Based on the families' own assets, food storage and access to information seem to be not a problem faced by the families in the study area. However, means of transportation are still an obstacle, since only a small part of families possesses a motorcycle or car, i.e. the majority of farmers still rely on bicycle and/or public transportation. Considering the long distances from the rural settlements to the city (15- 25Km), and also that the public transportation is not regular, one can conclude that most farmers do not have a good access to markets, either to purchase or sell the agricultural surpluses, which can lead to a vicious circle of rural poverty, for instance (*3, 155, 156*).

Credit is an important driver for farm activities in the study area since farm families are very sensitive to this external source of capital (*3, 155, 156*). The credit provides the cash and the liquidity to fulfill the objectives and needs of the farm families. Once without it, many of farm families do not plant nor have livestock on their properties. In this context, the analysis of credit amount and availability is crucial to better understand the choices made by the farm families in the study area. The main objective of the credit received by the farm families is to invest in livestock production. Crop purchase appears to be the second reason for credit demand together with other activities such as construction of fences, purchase of specific equipment, etc. It is interesting to note that in Brazil, 69% of credit for family

agriculture is related to crop and 31% to livestock activities (*107*). So, the figures of the study area in question show the importance of livestock activity there.

The strategic role of the pluri-activity in the rural development process is to contribute to the generation of social inclusion, to reduce poverty and to overcome inequalities. Despite the fact that social assistance (federal and state income transfer) has a small but not negligible impact on family income, the number of families that receive social assistance is high, i.e. more than 60% of families are participating in a federal and/or state program of income transfer (Figure 11).

Although the majority of farm families have access to drinking water through wells, only part of them has access to tap water. Another problem is that some farm families suffer from a drinking water deficit in the dry season (May up to October). The wells no longer supply water and some farm families have to walk 1, 2 or even 4 km to get drinking water. Irrigation systems are very rare in the study region due to the costs of acquisition and maintenance. During the rainy season (November up to April), all the water for crop production comes from the rain and therefore the irrigation system if present is not used. Regarding the energy, all farm families have access to it, but the wood stove is still the facility used most widely.

The majority of families were subject to a mild food and nutrition insecurity. According to the Brazilian Scale of Food and Nutrition Security (EBIA), mild food and nutrition insecurity occurs when there is a worry regarding lack of food in the near future, expressing the psychological component of the insecurity. In this case domestic arrangements are made with food or meals to extend their availability. A substantial number of families experienced moderate food insecurity, i.e. a situation in which the food and meal's quality is compromised in order to maintain the amount of food available. At this level, there is a reduction of the amount of food available to the family (food shortage). Although not in high percentage, the severe food insecurity was also present in the studied communities (15.5%), which mean that these families had quantitative food deficiencies leading to hunger among adults and children. This

data are comparable to the Brazilian National Household Survey (PNAD) which identified 56.5% of households as standing at any level of food insecurity (*157*).

Additionally, food and nutrition security was assessed through a quantitative approach focusing on the food supply, where food security comprises the amount and quality of food supply from the farm as well as from the market (*158*). This amount is influenced by family size, access to the market, diversification within the property and resources for production. In the study area, the market orientation is regular, i.e. even though farm families consume part of their own production, in some cases a surplus is offered in local markets. However, as one can see in Figure 12, self-production is not sufficient to feed the family in the majority of family farms. This demonstrates that families depend on local markets to fulfill their needs, i.e., families in the region in question can be considered as food net buyers.

The health condition is a crucial indicator of farm families' living standards and thus some factors were selected in order to assess the health status of families in the study area. The use of traditional methods for health care is almost a consensus to the families. Similarly, the public service (SUS) is the main health service used by the families, since they cannot afford a private health service. But it is worth to mention that the public health service was not well graded by the users, being classified as regular or worse, reflecting the dissatisfaction towards the quality of the public health service, which in some cases can aggravate the health condition of a family member.

4.1.2. Nutritional Situation of adults in two rural settlements of APA – Cantão

It has been suggested that excessive energy intake is the main cause of obesity. However, the role of diet composition in weight control and obesity remains controversial (*159*). In the current study, the prevalence of overweight and obesity altogether was as high as found in other studies in Brazilian rural areas (60.2%) (*160, 161*). In a study in poor communities in the State of São Paulo, the standardized prevalence of obesity was significantly higher in the rural area in comparison to the urban area. In the same study, the researchers also observed that the obesity is

fundamentally a problem to the women's health since in all age groups women had a higher prevalence of obesity than men (*162*). Similarly, the results of the Brazilian Household Budget Survey (2002-2003) showed that women of rural areas of different regions in Brazil had a prevalence of 40.7% for overweightness and 12.7% for obesity (*7*).

Taking the study of Velasquez-Melendez *et al*. (*163*) as reference, in which the subjects were categorized into those of short stature (women < 150 cm, men < 162cm) and those of normal stature (women > or = 150 cm, men > or = 162 cm), the population of the current study can be considered as a short one. Indeed, the height of the participants also suggests a previous undernutrition, especially of women. The subject's characteristics also confirmed the vulnerability of women's health. The abdominal obesity was shown by the waist circumference of women which exceeded the usual cut-off point for this variable (*164*). It is well known that excessive abdominal fat is a determinant factor for insulin resistance and metabolic syndrome (*165, 166*).

With regard to the dietetic assessment, the results found for energy expenditure depicts a significant difference between eutrophic and overweight women. This could be explained by the fact that this variable is calculated by taking into account body weight and physical activity and therefore its results could be influenced by these other variables, as physical activity requests more energy as does the body weight (*167*). Possibly, these variables compensate each other in men explaining why there is no difference observed here. Thus, obese men could have their energy requirements increased by the higher body weight and eutrophic men by a larger physical activity factor.

The observed absence of differences between the energy intake for both eutrophic and overweight groups was also reported by other studies (*163*). Although, the intrinsic errors inherent to the dietetic assessment methods (e.g. underreporting) are well documented, especially concerning the semi-quantitative food frequency

questionnaire, the instrument approached here (24-hour recall) was shown to minimize underreporting, which gives more confidence for the analysis here presented (*168*). In addition, it is noteworthy to mention that individual organisms can adapt to low energy intake levels through diet-associated metabolic changes explaining the similarity for energy intake for both groups (*169*).

In many studies, gender was shown to be an important factor for obesity in rural areas. In a study in four rural communities in Mexico, women also had a higher prevalence for obesity (33.0%) in comparison to men (9.0%) (*170*). However, in our study, according to the regression model the gender itself did not have an influence on the BMI. Actually, physical activity was shown to be an expressive determinant factor for overweightness in the two rural communities. Data from the linear regression model confirmed the role of physical activity level (PAL) on body weight management in the study population; since according to the results, individuals performing high and moderate PAL have lower BMI than those who practice mild PAL. This also could explain the fact that the highest prevalence was seen in women which performed the lowest level of activity due to domestic duties.

Carbohydrate quality and quantity has received considerable attention lately. However, the hypothesized link between carbohydrate quality and quantity and either obesity or disease risk is still controversial (*171*). Dietary fat has been related to obesity since it has good palatability, high energy density, low effect on satiety and provides more efficient conversion to fat tissue (*167*). Most of epidemiologic studies show an inverse relation between carbohydrate consumption and BMI (*172-174*). However, in our cross-sectional study opposite results were obtained from the data acquired and modeled. According to the regression model, an increase in the values of the variables KCALBW (Kcal/kg of body weight) and CHOBW (g of carbohydrate consumed/kg of body weight) would contribute to a reduction of the BMI values in the two rural communities here investigated. According to the results of the regression analysis, it could be implied that this population practices an imbalanced diet, explaining the result found with the variable CHOBW. Indeed, the carbohydrate

intake of the overweight group is lower than that of the eutrophic one. In the study population, the physical activity seems to be the main determinant of the observed overweightness. Similar results were found in a very-low-income population in the Northeast of Brazil, where the food consumed does not account for the higher prevalence of obesity among stunted adults (*175*). Gazzaniga and Burns (*176*) also found that overweight individuals consumed less carbohydrate in comparison to their lean counterparts. Additionally, Miller *et al*. (*177*) suggested that body fat is related to diet composition and exercise activity rather than to energy intake. Randomized controlled trials with proper controls for confounding dietary variables and behavioral factors are needed to elucidate the independent effect of carbohydrates on BMI outcomes. Also, further research is needed to help to explain possible gender and environmental differences. For instance, some studies point out that the small short period between pregnancies could also be a determinant factor for overweightness in women; this was not analyzed in our study.

With regard to lipid consumption, Matos and Ladeia (*178*) mentioned that in rural areas the use of saturated fat in food preparation is common. However, in the present study population, mostly vegetable oil is used for cooking. In addition, the variable LIPBW (g of lipid consumed/kg of body weight) was not significant, i.e. the amount of lipid per body weight ingested does not influence the BMI variation in the study population. Nevertheless, there is some evidence that fat in the diet might not predispose for obesity (*179-181*). It is worth mentioning that the modeling applied here does not have any virtual use for the aim of prediction, since researchers working with the food consumption surely also possess the information about body mass and height of individuals, being able to calculate the BMI directly from the data. Nevertheless, the relevance of the estimated model is to show the existence, the level and the functional form of the association between BMI and the other analyzed variables (*182*).

The food pattern of the two communities expresses an expected monotonous diet, with the traditional Brazilian combination of rice, beans and beef. Indeed the low

consumption of vegetables in both groups is typical for the culture of Northern Brazil, where leafy vegetables and vegetables, in general, are not so common (*86*). The added sugar is also observed in the food pattern, especially to coffee and other beverages, which calls the attention to the glycemic index of the diet practiced by the studied rural communities. A diet high in fiber-rich, low-glycemic-index foods may result in less central adiposity, since it may affect the satiety through decreased gastric emptying, increased colonic transit and decreased insulin response, which consequently would also decreases hunger and energy intake (*183-186*). Also, an imbalance of macronutrient intake can interfere in the biochemical pathways leading to obesity.

The current study did not address social or demographic issues that may affect dietary intake. Nevertheless, the genetic factor is a well know component of obesity (*187*). However, the role of hereditary factors which may influence the relation between diet and adiposity was not investigated in the current cross-sectional study. Prospective studies are needed to address these determinants of obesity especially in rural and poor areas.

4.2. Biofunctional properties of bacaba phenolic extract

4.3.1. Total phenol, total flavonoid and total anthocyanin contents in bacaba and jenipapo

Total phenolics, total flavonoids and total anthocyanins contents were addressed to bacaba and jenipapo fruits. Both fruits show good phenolics quality by its content. However, the results of bacaba qualify this fruit as a good source of phenolics, flavonoids and even anthocyanins.

The total phenolics contents of bacaba were higher than those found to other native fruits in a previous study from our lab where cagaita and murici had 32.26 ± 0.051 and 298.26 ± 8.06 mg GAEq/100 g, respectively. A rank of comparison between

bacaba and jenipapo and fruits already described in literature is depicted in Table Table 29.

Table 29: Comparison between bacaba and jenipapo phenolic, flavonoid and anthocyanin total contents and other fruits described in the literature.

Fruit	Scientific name of the fruit and cultivar (cv.)	Total phenolic (mg GAEq/100 g)	Total flavonoid (mg CTEq/100 g)	Total anthocyanin (mg cyn-3-glu/100 g)	Reference
Açaí	*Euterpe oleracea* Mart.				
Bacaba	*Oenocarpus bacaba* Mart	1759.27 ± 1.01	1134.32 ± 0.03	34.69 ± 0.00	
Bacuri	*Platonia insignis* Mart.	266.80 + 3.3	103.8 ± 0.3	--	(*188*)
Buriti	*Mauritia vinifera* Mart.	108.1 +6.8	71.3 ± 3.6	-	(*188*)
Cagaita	*Eugenia Dysenterica*	32.26 ± 0.051			(*189*)
Jenipapo	*Genipa americana* L.	426.53± 0.33	7.12 ± 0.00	0.0 ± 0.00	
Guava	*Psidium guajava* L.	344.9 + 33.6			(*190*)
Murici	*Byrsonima crassifolia* (L.) Rich	298.26 ± 8.06			(*189*)
Pequi	*Caryocar villosum (*Aubl.) Pers.	4623.4 ± 102.4	741.2 ± 36.6	-	(*188*)
Rasperry methanolic extract	*Rubus idaeus* L. cv*. Hollanda Boduru*	2062.3 ± 4.1	41.1 ± 0.9	24.3 ± 0.3	(*94*)
Rasperry methanolic extract	*Rubus idaeus* L. cv*. Hollanda Boduru*	1822.0 ± 11.9	21.1 ± 0.1	45.6 ± 0.3	(*94*)
Blackberry water extract	*Rubus fruticosus* L. cv. *Jumbo*	2445.9 ± 14.8	42.8 ± 0.3	87.1 ± 1.0	(*94*)
Blackberry methanolic extract		2786.8 ± 21.9	82.2 ± 1.3	52.9 ± 1.1	(*94*)
Blueberry	*Vaccinium myrtilus*	670.9	190.3	-	(*191*)
Tucumã	*Astrocaryum aculeatum* G. May	456.8 ± 5.2	433.2 ± 10.4	-	(*188*)

Bacaba had a flavonoid content of 1134 ± 0.03 CTE mg/100 g FW *versu*s 7.12 ± 0.00 CTE mg/100 g FW of jenipapo. When comparing flavonoids content of pulp from two native fruits from Brazil which belongs to the same family of bacaba (*Arecaceae)* content of buriti (*Mauritia vinifera* Mart.) and tucumã (*Astrocaryum aculeatum* G. May), both had lower values than bacaba (71.3 + 3.6 CTE mg/100 g FW and 433.2 ± 10.4CTE mg/100g FW, respectively). Indeed, regarding the flavonoid contents for pequi (*Caryocar villosumpers* Pers.) (741.2 + 36.6 CTE mg/100 g FW), another Brazilian native fruit, bacaba had higher flavonoid contents (*188*).

Even when compared to commonly commercial berries which are actually recognized by its high amounts of phenolics, bacaba results expresses that this fruit can be included to those berries group. For example, raspberry cultivars, such as *Hollanda Boduru* showed a total flavonoid content of 41.1 + 0.9 CTE mg/100g FW. In comparison to the higher flavonoid content blackberry cv. Jumbo (82.2 + 1.3 CTE mg/100g FW), bacaba still shows extreme high content (*192*).

Anthocyanins belong to a class of flavonoid compound which presents the color of orange, red, purple and blue of fruits and vegetables. From the obtained results, no anthocyanins were detected in jenipapo fruit. However, bacaba expressed a high anthocyanin content (34.69 ± 0.00 cyn-3-glu mg/100g FW) when compared to the raspberry cv. *Hollanda Boduru* (24.3 ± 0.3 cyn-3-glu mg/100g FW) (*192*). Therefore, this fruit can be considered a good source of anthocyanins. In addition, several studies have described the biofunctional properties of foods rich in anthocyanins. For example, in Peru, people consume as a colored infusion made from purple corn (*Zea mays* L.), known as “china morada”, to improve health. Actually, purple corn is one of the plants with higher anthocyanins content and researches have indicated that purple corn inhibited colorectal carcinogenesis in rats. Purple corn indicated 46 times more (1.642 cyn-3-glu g/100g FW) and sweet potato showed 5 times more (0.182 cyn-3-glu g/100g FW) of anthocyanin compared to bacaba (*193*).

4.3.2. Antioxidant Activity of bacaba and jenipapo extracts

Antioxidant has been defined by the Institute of Medicine of the National Academy of Sciences as follows: “a substance in foods that significantly decreases the adverse effects of reactive species, such as reactive oxygen and nitrogen species, on normal physiologic function in humans”.

According to Wolfe and Liu (*102*) the measurement of antioxidant activity is an important screening method to compare the oxidation/reduction potentials of fruits and vegetables and their phytochemicals in various systems. The antioxidant assays can deactivate radicals by two main different mechanisms: either by hydrogen atom transfer (HAT) or single electron transfer (SET). HAT-based methods measure the classical ability of an antioxidant to quench free radicals by hydrogen donation. SET-based methods detect the ability of a potential antioxidant to transfer one electron to reduce any compound, including metals, carbonyls and radicals. In biological samples, SET and HAT mechanisms occurs simultaneously, with the balance determined by antioxidant structure and pH (*194*).

Many chemical methods with different approaches are in wide use to measure the antioxidant activity as for example the ORAC, TEAC and FRAP assay (*195*,*175*,*181,184*). ORAC assay is currently the most recommended and used method. However, even for ORAC assay some misleading are raised as this test being a unique antioxidant assay to be applied on fruits and vegetables antioxidant activities. Ou *et al*. (*196*) states that ORAC assay covers solely the peroxyl radical absorbance capacity.

As antioxidants respond to different reactive species in different tests, which is partially attributed to multiple reaction mechanisms and reaction phases, many authors highlight that no single antioxidant assay can accurately reflect the antioxidant potency of food matrix and the need of carrying out more than one type of

antioxidant test in order to consider the different mechanisms of antioxidant properties (*194, 197, 198*).

Seeram *et al*., found different antioxidant rankings for fruit juices when comparing different antioxidant assays (ORAC, FRAP, DPPH, LDL oxidation), reinforcing the need to apply different of assays to cover all aspects of antioxidant activity. Considering the actual need of applying more than one method for antioxidant screening investigations, bacaba and jenipapo phenolic extracts were tested for different antioxidants assays in order to allow comparisons with other food or plant materials already described in the literature. As most of antioxidant activity features attributed to food products are based on *in vitro* assays based solely on chemical reactions which hardly reflect cellular physiological conditions and do not consider bioavailability and metabolic issues, the CAA assay was also included as this test possess a nearer approach to biological systems by having a biological factor (viable cells) added as a variable (*118*). Therefore, it is expected that results obtained with this assay could offer relevant results.

The ORAC assay depicted relevant antioxidant activity for both fruits. Although bacaba had a higher antioxidant assay, both fruits are among other fruits of well-known antioxidant activity as blueberry, cranberries and sour cherry as shown in Table 21 (*191, 199*). However, none of the samples here analyzed had higher ORAC value than the Brazilian amazon berry açaí (*Euterpe oleracea* Mart.) which belongs to the same family of bacaba (*Arecaceae*) and is known by the media as a “superfruit” by its extremely high antioxidant activity. Schauss (*200*) estimated a ORAC value of 630µmol TEq/g of açaí, in the same units bacaba would have 107.5 µmol TEq/g.

Similarly to ORAC, TEAC value showed higher antioxidant activity for bacaba when compared to jenipapo. However, in this assay, bacaba did not have such a high value in comparison to other fruits (e.g. guava) as depicted in Table 30.

.

Table 30: Antioxidant values of bacaba, jenipapo and other fruits. *

Sample	Scientific name of the fruit and cultivar (cv.)	ORAC (µmol of TEeq/100g)	TEAC (µmol TEeq /100g)	FRAP 1 (mmolFeSO4.7 H_2O Eq/100g)	FRAP 2 (mmol TEeq/ 100g)	DPPH (mmol TEeq/100g)	Reference
Açaí (FW)	*Euterpe oleracea* Mart. *cv. allahabad*	63000	-	-	-	-	(*200*)
Bacaba (FW)	*Oenocarpus Bacaba*	10750.71 ± 1496.51	3294.55 ± 301.55	23.60 ± 0.53	13.44 ± 0.20	34.25 ± 0.20	
Blackberry	*Rubus fruticosus* L.*cv Jumbo*				17.7		(*94*)
Blueberry (FW)	*Vaccinium*	6184 ± 775	-	-	-	-	(*191*)
Cranberry (FW)	*Vaccinium*	9256 ± 138	-	-	-	-	(*191*)
Date palm (DW)	*Phoenix dactylifera* L.,	-	-	-	3.28	-	(*201*)
Guava (FW)	*Psidium guajava* L.	2550 + 160	3790 + 340	3333 + 1.4	-	3200 + 510	(*190*)
Jenipapo (FW)	*Genipa americana* L	4655.60 ± 562.83	257.62 ± 30.46	0.36 ± 0.00	0.21 ± 0.00	0.56 ± 0.02	
Raspberry (FW)	*Rubus idaeus*	4765 ± 718			12.7		(*94, 191*)
Sour Cherry (FW)	*Prunus cerasus L*	1145 to 1916	2000-2600				(*199*)
Walnuts (DW)	*Juglans regia*					6.1	(*202*)

*FW- fresh weight; DW – dry weight

Two standard compounds ($FeSO_4 \cdot 7H_2O$ and trolox) were applied for FRAP assay. bacaba indicated higher FRAP values than jenipapo (23.60 ± 0.54 mmol FeSO4.7H_2O Eq/100g (FW) *vs*. 0.364 ± 0.00 mmol FeSO4.7H_2O Eq/100g (FW). Khanavi *et al*. (*203*) indicated that the pulp of date palms (cv. *Khenizi*), which belongs to the same family as bacaba *(Arecaceae*), and is usually applied in the folk medicine in Iran, had 3.28 mmol FeSO4.7H_2O Eq/100g (FW) with DMSO extract of dry plant which is lower than bacaba 23.60 ± 0.53 mmol FeSO4.7H_2O Eq/100g (FW). Berry fruits are known by their usually high antioxidant values when assessed by different assays. Bacaba had higher FRAP values than the methanol extract raspberry *cv*. *Hollanda Boduru* (12.7 TEq mmol/100g FW) or methanol extract from blackberry *cv*. Jumbo (17.7 TEq mmol/100g FW) assessed by Sariburun *et al*.(*94*). From DPPH method, bacaba indicated higher antioxidant power compared to jenipapo (34.251±0.205 mmol TEq /100g FW and 0.561 ± 0.028 mmol TEq /100g FW). Bacaba has a high DPPH value when compared to guava (*Psidium guajava*) (3.6 TEq mmol/100g FW) and walnuts (*Juglans regia*) (6.1 TEq mmol/100g DW) (*202*).

Regarding the CAA, bacaba and jenipapo phenolic extracts had relatively potent antioxidant activities with an EC_{50} value of 0.7 ± 0.61 mg/mL and 4.3 ± 1.61 mg/mL, respectively. Quercetin, a pure phytochemical, had an EC_{50} value of 4.66 ± 0.61 µM. Compared to other fruits assessed by Wolfe *et al*. with EC_{50}'s of 3.21 ± 0.14 mg/mL for blackberry, 6.77 ± 1.05 mg/mL for wild blueberry, 11.8 ± 0.9 mg/mL for strawberry and 14.7 ± 0.8 mg/mL for cranberry (*119*), jenipapo fruit seems to have a comparable EC_{50} value. However, bacaba with its EC_{50} of 0.7 ± 0.61 mg/mL has the lowest EC_{50} value of all of these fruits and therefore, seems to have the better antioxidant activity. Wolfe *et al*. (*119*) showed the highest antioxidant activity for blackberry with a CAA value of 154 ± 6.8 mg/mL. Blackberry is followed by wild blueberry with a CAA value of 74.1 ± 12.5 mg/mL, cranberry with 33.6 ± 2.0 mg/mL and strawberry with 42.4 ± 3.3 mg/mL. Bacaba and jenipapo reached CAA values of 305.2 ± 90.8 mg/mL and 83.0 ± 31.3 mg/mL, respectively. Compared to the CAA values named before, it can be seen that bacaba has the highest CAA value of all the fruits indicating the highest

antioxidant activity for bacaba fruit. For jenipapo only blackberry has a higher CAA value and all the other fruits also have a lower value indicating for this fruit also a very high antioxidant capacity. Wolfe and Liu (2007) concluded in their work that the CAA values for berries (e.g. wild blueberry, blackberry, strawberry, cranberry) tend to be the highest ones and that berries tend to be rich in anthocyanins(*119*). According to Wolfe *et al*. (*119*) the cellular antioxidant quality measure the cellular antioxidant activity provided by 100µmol of phenolics found in the fruit. The CAQ value for bacaba is comparable to those found for wild blueberry (2.9) and strawberry (3.0) (*119*).

A high antioxidant activity is correlated with a high concentration of phenolics and therefore higher antioxidant and anticancer activity (*61, 204*). Song *et al*. (*205*) reported a positive correlation between total phenolics in different vegetables and CAA values. Fruits rich in anthocyanins have been shown to have the highest antioxidant activities (*66*). This could possibly explain the very high antioxidant activity of bacaba fruit, which, by the results found is an anthocyanin source.

The difference of antioxidant activity values found by different assays for the same samples, even when using the same standard compound, is based on the reaction mechanisms from the methods themselves. Either, the fruit extracts could have different antioxidant values than those stated to their compounds when those are analyzed purely. Although correlations analyses were not performed, it is implied that the high antioxidant abilities found in bacaba could be based on its high total phenolics, flavonoid and anthocyanin contents. Surely, regarding analytical assays of fruits and vegetables, some factors inherent to the scientific research can influence the results *e.g.* environmental characteristics, period of harvesting, cultivar variability, fruit maturity and extraction solvent procedures. However, this issue doesn't nullify the comparison between results already stated in the literature. Indeed, comparing the results with the other fruits is valuable and gives a clear idea about the status of bacaba and jenipapo on the discussion of biofunctionality of native fruits in developing countries.

4.3.3. Antiproliferative activities of bacaba and jenipapo fruits

Colorimetric assays are extensively used to assess cellular proliferation and viability. The main advantage of the colorimetric assay besides its high precision is that the assay can be performed very fast, especially when using a 96 well plate, where a large number of samples can be measured with a high degree of precision in multiwell scanning spectrophotometers. Additionally, these assays are non-radioactive and economic (*206, 207*). However, regarding the screening of compounds with intrinsic reductive potential, some precautions have to be taken into account.

The MTT assay is a test widely used to assess cell viability and proliferation in cell models, especially for chemical compounds screening. The MTT salt is reduced to formazan by enzymes in the mitochondria, endosomes and lysosomes and can be quantified by spectrophotometry (*208*). However, some authors have argued the efficacy of MTT test on the purposes aforementioned (*209-214*). Sims and Plattner (*215*) assessed the effect of STI571, an Abl kinase inhibitor that leads melanoma and breast cancer cells to apoptosis, by the MTT assay. When comparing a non-reduction assay (Cell titer glo) and MTT, authors observed that in a range of 1-10µM the MTT test indicated a rise in cell proliferation while cells were actually dead. Bruggisser *et al*. (*207*) reported a possible reducing effect of some screening compounds (e.g. phytoestrogens, antioxidant substances and dried plant extracts) on the MTT salt to its violet formazan through cell-independent chemical reaction. All antioxidant substances they screened reduced the MTT tetrazolium ring. According to Hanauske (*216*) the MTT assay is a valuable method but one should be aware of its limitations and create clever strategies to overcome those and reach confident results. Based on this discussion and research findings, the antiproliferative effects of the phenolic extracts from bacaba and jenipapo were assessed by additional assays.

The MUH assay is based on the ability of 4-methylumbelliferyl esters to determine cell viability. The fluorogenic substrate 4-methylumbelliferyl heptanoate (MUH) was

shown to be the best substance in providing sufficient activity and low background (*217*). The substrate diffuses through the cell membrane and where it is hydrolyzed by intracellular esterases or sulfatases leading to the highly fluorescent 4-methylumbelliferone. The MUH has rapidity and simplicity as important advantages, which are based on a fast uptake and cleavage of the fluorogenic substrate by living cells which only takes 30 min. According to Virág *et al*. (*217*) MUH combines the well-established MTT assay with the very high sensitivity of fluorescent assays and can be even accurate when no washing steps are done. However, to prevent a possible influence of the extracts, a washing step was included. As the production of a fluorescent compound from a fluorogen substrate in the MUH assay is similar to the MTT assay, where the tetrazolium salt is cleaved to the blue formazan, these two assays are completely comparable.

The Methylene blue solution is a redox indicator which turns colorless when reduced by cellular enzymes. Trypan blue staining is based on the membrane integrity of living cells since this dye only permeates cellular membranes and stains dead cells.

The MTT and MUH cell-free test indicated that there could be a possible influence of the fruit extracts themselves on the test chemical. In MTT cell-free test, bacaba fruit showed a dose-dependent increasing absorbance indicating a reducing effect on MTT solution whereas jenipapo fruit had no effect on MTT solution. In comparison to that, jenipapo showed an influencing effect on MUH solution as increasing jenipapo concentrations lead to increased fluorescence whereas bacaba did not show any influence on MUH solution.

The plant extracts also had a reducing effect, but for some of them the effect was only weak. Bruggisser *et al*. (*207*) reported that the MTT reduction by the test compounds could be reduced when a washing step is included (*207*). When natural products are assessed, they recommend including a pre-screening in cell-free systems before any cell culture experiment is performed. Furthermore they recommend, when reduction occurs, to include a washing procedure and to interpret

the results carefully to avoid false positive results. It is suggested, that with an adequate washing the potentially interfering agents can be removed (*207*). Therefore, a washing step was included before the chemicals application to all the assays performed. As relatively potent antiproliferative activities not only in MTT and MUH assay, but also in Methylene blue and Trypan blue assay could be recorded, it can be concluded that the results obtained are reliable and not influenced by the fruit extracts.

The phenolic extracts from bacaba and jenipapo were added to HepG2 human liver cancer cells to determine whether tumor cell proliferation could be inhibited. The results gained in MTT, Methylene blue and Trypan blue assay showed that for bacaba fruit all concentrations ≥ 600 µg/mL and in MUH assay concentrations ≥ 400 µg/mL had stronger inhibiting activity than the control ($p < .05$). Cell proliferation was inhibited in a dose-dependent manner when exposed to bacaba concentrations > 400 µg/mL.

However, jenipapo extracts had a weaker inhibiting ability and were less effective than bacaba extracts. This could also be related to the lower antioxidant capacity of jenipapo in comparison to bacaba, as shown by the antioxidant activities test. For jenipapo fruit in MUH assay concentrations ≥ 1400 µg/mL, in MTT assay ≥ 1200 µg/mL, in Methylene blue assay ≥ 1000 µg/mL and in Trypan blue ≥ 800 µg/mL showed a stronger inhibiting activity than the control ($p < .05$). Hence, jenipapo fruit also had more variances between the different assays than bacaba fruit. Jenipapo's lower antiproliferative capacity also can be seen when fitted into a non-linear regression model. The IC_{50}'s calculated with this model are lower for bacaba than for jenipapo with a lower IC_{50} indicating higher antiproliferative capacity. IC_{50} for bacaba were 649.6 ± 90.3 µg/mL in MTT assay and 1080.97 ± 0.7 µg/mL in MUH assay. Methylene blue and Trypan blue did not converge in the non-linear regression model. In comparison to that, IC_{50}'s for jenipapo were 1194.33 ± 96.2 µg/mL in MTT assay, 1386 ± 60.32 µg/mL in MUH assay and 1076.7 ± 53 µg/mL in Methylene blue assay. Trypan blue also did not fit in the model here.

Sun *et al*. (*61*) assessed the antioxidant and antiproliferative effects of a variety of common fruits. Highest antiproliferative activity was reached for cranberry and lemon with an IC_{50} of 14.50 mg/mL and 30.56 mg/mL, respectively. Apple and strawberry had an IC_{50} of 49.37 mg/mL and 56.33 mg/mL, respectively Compared to these fruits, bacaba and jenipapo have competent antiproliferative activity.

The strong inhibition of tumor cell proliferation *in vitro* could also be explained by bacaba's and jenipapo's combination of phytochemicals, as it was already suggested for the antiproliferative effects of apples. These phytochemicals are natural antioxidants which help to provide an antioxidant balance needed to quench oxygen species which in turn are associated with tumorigenesis. For example phytochemicals in apples seem to significantly enhance the antioxidant quality and their capacity to inhibit tumor cell proliferation *in vitro* when using HepG2 cells (*68*). The inhibition of cancer cell proliferation is – at the moment – attributed to some unknown phenolic compounds present in bacaba and jenipapo fruits. Probably it is the additive and synergistic role of phytochemicals present in these fruits which contributes significantly to the potent ability to inhibit tumor cell proliferation *in vitro*.

Furthermore, the higher antiproliferative effect of bacaba could also be assigned to the anthocyanin content of this fruit, as jenipapo does not have detectable content of these compounds. Multiplicity studies have been showing that plants containing high content of anthocyanins also depict an antiproliferative effect to cancer cells using *in vitro* or *in vivo* models (*218, 219*).

Sun *et al*. (*61*) found no obvious linear relation between total phenolic content and inhibition of HepG2 cell proliferation. For the fruits tested in this thesis it has to be further investigated whether there is a positive correlation or not. Also a significant relation between total antioxidant activity and antiproliferative activity of the fruits tested has to be investigated.

Both assays, MTT and MUH, respectively, give very similar quantitative results which can be compared (*217*). IC_{50} values for bacaba were different for MTT and MUH assay with 649.6 ± 90.3 µg/mL in MTT and 1080.97 ± 0.7 µg/mL in MUH assay.

Although the cell-free test indicated an interaction between jenipapo extract and the MUH reagent by the observed increase in the fluorescence this behavior was not reproduced in any of the tests with cells. Indeed, jenipapo had antiproliferative effect in all assays and the IC_{50} values for jenipapo showed comparable results to MTT (1194.33 ± 96.2 µg/mL) and MUH assay (1386 ± 60.32 µg/mL).

The modified Methylene blue was also comparable therefore this staining can be recommended. Absorbance is read with a microplate reader and reflects the number of surviving cells. When multiple samples have to be counted simultaneously this method is faster and more accurate and especially useful. However, it has to be considered that cells treated with Methylene blue are necessarily killed by the addition of glutaraldehyde and the method. Therefore it can only be used when counting is the final act (*220*).

The accurate cell counts are essential in both quantitative and qualitative experiments. To date, the trypan blue assays is commonly used for cell counting and to determine cell proliferation or cytotoxicity effects through blue staining followed by microscopic quantification using a hemacytometer. Results of Trypan blue assay did not converge in the nonlinear model. However, the cell proliferation rates achieved in this assay for bacaba or jenipapo, were lower than the other three methods assessed. This difference could be associated to the loss of cells due to trypsinization before the counting which could lead to underestimation of the cell number. In addition, trypan blue staining is reliable only when a free cells suspension is used and cell clumps could influence the accuracy of this assay. Moreover, cell counting with a hemacytometer can be very subjective and suffer an analyst effect as it can vary from researcher to researcher as observed during this work (*220*). In

addition, the counting of cells included in the Trypan blue assay is a very time consuming way to assess cell proliferation.

Virág *et al*. (*217*) suggested that the differences gained between the assays could not be due to a difference in the detection technique (e.g. fluorimetric or colorimetric), but to a difference in the assay principle itself (e.g. different enzymes acting to assess metabolic functions, loss of membrane integrity). Traditional cell counting based on staining methods as for example the Trypan blue and Methylene blue staining are good methods to prove the results gained in other assays. These two assays are not influenced by natural extracts as it is possible for MTT and MUH assay when no washing step is included. Most times, these assays are measured directly on the plate in which the cells were grown and stimulated with the compound of interest and therefore eliminate some disadvantages which can be seen by Trypan blue cell counting.

Albeit the test fruit implied a reduction of MTT salt by bacaba extract, results of cell proliferation was very similar to MUH where the extract did not interfere. Also, as pointed out by Liu *et al*. (*162,167,170,177*,*176, 208, 209*). The MTT assay is not only based on the salt reduction but also in the salt endocytosis giving another factor to the cell viability measurement. Thereby, the MTT assay is valid as an endocytosis which is a fundamental feature of most living cells. Also, the PBS washing step could remove the surplus of extracts compounds located extracellularly and thus contributing to the similarity between results of MTT and the other non-reduction assays used. Moreover, MTT was highly and positively correlated with all other assays in a significant manner ($p < .05$), indicating the application of this assay to assess cell proliferation in bacaba and jenipapo extracts. Therefore, based on our findings, MTT method was established with confidence to further anti-proliferative effects in the following experiments with bacaba and jenipapo extracts in our lab.

4.3.4. Characterization of phenolic compounds of bacaba extract by HPLC-DAD-MS^n

The characterization of phenolic compounds is important as these compounds are associated with a range of different health properties. HPLC with diode array and mass spectrometric detection has been proven to be very useful for the characterization of individual phenolic compounds. Ten polyphenols were detected and characterized in bacaba extracts in the present study. Based on their UV spectral data, most of these compounds were characterized as quercetin and rhamnetin derivatives besides a range of further flavonoids. So far, *Oenocarpus bacaba* Mart. extracts have not been systematically analyzed. Consequently, the present study represents the first chemical investigation of this plant material. Due to the limited availability of reference compounds, the similarity of spectral characteristics and of the chromatographic behavior allow tentative peak assignment. For this purpose, mass spectrometric analyses were also performed (*92*). Nine compounds were tentatively identified based on the comparison with results previously described in the literature. Although some compounds were not identified, they are relevant to comprehensively characterize the so-called chromatographic fingerprint which is a plant-specific characteristic. Taking together the outcome of the present study, it becomes apparent that Bacaba is a potential source of pigments and natural antioxidants which should be considered for future exploitation.

4.3.5. Chemopreventive effect of bacaba extract on MCF -7 cells

Diet-derived compounds have shown modulation of tumor growth through modification of gene expression, post-translational events and apoptosis (*221*). Unlimited cell proliferation and resistance to apoptosis is a typical hallmark of the carcinogenic process as is the resistance to chemotherapeutic drugs. As a consequence, researchers are engaged to investigate biofunctional properties of foods as a strategy to cancer prevention. According to epidemiological evidence, phytochemicals such as phenolic compounds may play an important role in cancer

prevention (*222*). Their efficacy and mechanisms against cancer is a key target to unfold the evidences shown by epidemiological studies and reinforce the relevant role of diet and nutrition on the prevention of such disease.

The Brazilian biodiversity offers a great variety of fruit species endowed with an infinite number of phytochemicals with potential preventive or therapeutic properties for non-communicable diseases. Our study indicates that a phenolic extract from bacaba shows antiproliferative properties on MCF-7 breast cancers cells in a dose-dependent manner after 96 h of incubation (Figure 22).

These results led to the investigation of the mechanisms involved in cell death, especially whether the effect of bacaba extracts on MCF-7 cells is due an apoptotic effect. Apoptosis is a physiological and complex process of a regulated destruction of the cell as a result of a cascade of events involving various families of proteins influenced by the activity of many genes (*223*). Opposition to apoptosis or defects in its regulation can lead to the proliferation and accumulation of immortal cells and consequently be a sign of cancer. For that reason the selective induction of apoptosis by bacaba phenolic extract in cancer cells is an important possibility to highlight the potential effect of this fruit on cancer prevention (*224*). Indeed, the investigation of apoptotic mechanisms of bioactive compounds in cancer cells can help to elucidate the possible preventive role of these compounds evidenced by epidemiological studies.

The antiproliferative activity and apoptotic induction of bacaba phenolics on MCF-7 cells were clearly demonstrated in this study. According to previous reports, caspase-3 has been reported to initiate DNA fragmentation by proteolytically inactivating ICAD (inhibitor of caspase-activated deoxyribonuclease), which releases the active endonuclease CAD (caspase-activated deoxyribonuclease), allowing CAD to enter the nucleus and degrade chromosomal DNA (*225*). Albeit MCF-7 cells are caspase-3-deficient, some reports have shown that these cells undergo apoptosis even without DNA fragmentation (*226*). However, in the current study a mild DNA

fragmentation was observed after 72h of incubation with 500µg/mL of bacaba extract corroborating the hypothesis that other caspases or even other endonucleases can be involved on this event (Figure 23). For instance, endonuclease G results in DNA fragmentation in murine embryonic fibroblast cells following UV irradiation, independently of caspase activation (*227*).

There are two main signaling pathways with respect to apoptosis; the intrinsic or mitochondrial pathway and the extrinsic or death receptor pathway. Apoptosis triggered by activation of the mitochondria-dependent caspases pathway can be an important mechanism of chemoprevention. Mitochondria can play a critical role during apoptotic cell death by different mechanisms such as production of oxygen free radicals, control of Ca2+ ions, and extrusion of apoptogenic molecules such as cytochrome c or AIF (apoptosis inducing factor). The impairment of mitochondrial function and the resulting collapse in the mitochondrial membrane potential ($\Delta\Psi_m$) symbolizes a great stimulus to cell death being a hallmark of permeability transition and many studies have shown that apoptosis is associated with the decrease of the $\Delta\Psi_m$ (*228, 229*). Dissipation of $\Delta\Psi_m$ occurs early during apoptosis and leads to the permeabilization of the mitochondrial membrane which facilitates the efflux of cytochrome c and its interaction with APAF-1(Apoptotic protease activating factor 1) and caspase-9 to form the apoptosome and initiate the caspase cascade, consequently leading to the activation of caspase-3, -6, -7.(*229*).

The cell death receptor pathway, mediated particularly by active/cleaved caspase-8, has an elementary role in the maintenance of tissue homeostasis and is initiated by binding of cell death inducing ligands to death receptors and consequently, activation of caspase-8, -3, -6, -7 (*230*).

The caspases are a family of aspartate-specific cysteine proteases which play an important role in the apoptotic process. Caspases are synthesized as zymogens (procaspases) that need to be cleaved at aspartate residues to become active. Caspases can be classified according to their functionality as initiator and effector

caspases. The initiator caspases, such as, caspase-8, -9 and 10, contain a long pro-domain that is used to recruit enzymes to high molecular weight activation platforms such as the apoptosome in the intrinsic mitochondrial pathway or death-induced signaling complex in the death receptor pathway. In contrast, effector caspases, such as caspase-3, -6 and -7 cleave cellular substrates e.g. PARP and lamin A/C (*229, 231*). In the current study, the activation of caspases -6, -8 and -9 was observed after 24 h of incubation with bacaba extracts (1-1000 µg/mL), however with different efficiencies. The results for caspase-6 activation indicate a dose-dependent response but in a different fashion than caspase-9, suggesting different kinetics of activation between these two caspases (Figure 28). Fluorometric results also showed an activation of caspase-8, however the level of activation was the same for all doses of bacaba extract applied. Caspase-8 is an initiator caspase involved in the apoptotic signals triggered by death receptors (*232*). But, recent studies have shown that caspases-8 is not only activated in the death receptor pathway, but can also be activated independently, e.g. by direct cleavage through other proteases (granzyme B). Chung *et al*. (*229*) found caspase-8 activation in HeLa cells induced by the flavonoid eupafolin without the participation of the death receptor signaling. While testing caspase inhibitors the researchers observed that caspase-8 became active after the activation of caspases -3 and -9 and therefore hypothesized that caspase-3 is required for the activation of caspase-8 during eupafolin-induced apoptosis. In the death receptor-independent activation pathway it is suggested that caspase-8 act as an executioner caspases. It was also suggested that caspases-8 activation is mediated in a post-mitochondrial event by caspases-6 and -3 (*233*). Our observation that caspase-8 was activated only to a low extent in a dose-independent manner suggests that caspase-8 cleavage in MCF-7 cells is due to a cross talk between the two apoptotic pathways and not directly related to an activation of the death-receptor pathway. Since MCF-7 cells do not express caspase-3 due to a genetic defect (*226, 234*), the current results suggest that caspases 6- or even caspase-9 could activate caspase-8 directly in a cross-talk between the intrinsic and extrinsic apoptotic pathways (*234, 235*).

Currently, caspase-6 is considered to be an executioner caspase that is activated downstream of caspase-3 during apoptosis (*236*). However, recent evidence suggests that caspase-6 can be the initiator caspase after treatment with resveratrol (*237*). According to Ekert *et al*. (*237*, *236*, *238*, *239*) the activation of caspase-6 occurs prior to the activation of caspase-3, -2 and 7 (*238*). Therefore, the authors considered caspase-6 not only to be an executioner but also an initiator caspase (*238*).

No difference on the activity of caspase-6 was observed for cells incubated with 200, 400 and 600µg/mL of bacaba extract. However, a significant decrease on caspase-6 activation occurred to the treatment with 800 and 1000µg/mL ($p < .05$). Probably, under these concentrations caspase-6 is already degraded due to an advanced apoptotic stage. A kinetic study (time dependent) could elucidate and confirm this hypothesis.

Although our results depicted activation for caspase -6 and -8, the most important activation, proportionally, is attributed to caspase-9 (Figure 28). Results indicate that apoptosis induced by bacaba extract on MCF-cells was mainly mediated by caspases-9 activation. Kinetic studies in a time-dependent manner would be helpful to clarify the sequence of caspase activation events and apoptotic features triggered by bacaba extracts.

Caspase -3 is recognized as the main executioner caspase responsible for the PARP cleavage. However, as aforementioned, MCF-7 cells do not express caspase-3, a result confirmed by western blotting (data not shown) (*234*). Therefore, as PARP cleavage was observed after 48 h of incubation with bacaba extract, it was assumed that another caspase could cleave PARP, in the absence of caspase – 3. Since in cells incubated with 1000µg/mL of bacaba extract and the specific caspase-9 inhibitor Z-LEHD-VAD, no PARP cleavage was observed we propose that in the absence of caspase -3, caspase -9 become the main responsible enzyme for the cleavage of PARP (Figure 29). Albeit PARP is a recognized substrate for caspase-3,

studies *in vitro* has shown that many caspases, including caspase-9 can cleave PARP when added at high concentrations (*239*). Moreover, other studies have shown that in the absence of caspase-3 another caspases may take over the role of this caspase (*235*).

In summary, the induction of apoptosis in MCF-7 cells by bacaba extract is caspase-3 independent and seems to be mediated in part by caspase-6 and caspase-9. Apoptosis is accompanied by PARP cleavage, suggesting that in these caspase-3-deficient cells caspase-9 may mediate this apoptotic hallmark.

These results, to the best of our knowledge are the first to report on the biofunctional properties of bacaba and to characterize its effect on cell proliferation. They indicate that bacaba extract exert their action on cell proliferation inhibition mainly through the induction of apoptosis. Based on these findings we suggest that bacaba may be considered as a potential chemopreventive fruit. With all the caution called for concluding from *in vitro* cell line experiments with regard to the responses in *in vivo* studies of cancer cells, the dietary bacaba phenolics may be claimed to be an important preventive agent in cancer disease. Therefore, a deeper comprehension on how bacaba compounds exert their anticancer effect is important to allow claims for its consumption for cancer prevention to be made.

5. Summary

The Brazilian nutritional profile is currently characterized by the so-called "nutrition transition process" i.e. the population presents nutritional status characteristics of both developing and developed countries. Therefore, malnutrition is present not only in the form of undernutrition but increasingly also presents as overweight and obesity. Some studies suggest that this is not only a particular problem of urban societies but also of rural communities.

Recently, Brazil has impressively advanced on issues which address nutrition, agriculture and health within a sustainable framework. One of the recent initiatives encompasses the Brazilian Food and Nutrition Security Policy, which could be considered as the vanguard of this theme by covering different dimensions of nutritional issues, as defined hereunder: “Food and Nutrition Security is the achievement of the right of all people to access food regularly and permanently, with quality and enough quantity, without compromising the access to other basic needs, based on food practices to promote health, with respect to cultural differences and being social, economic and environmentally sustainable”.

Since the Brazilian Food and Nutritional Policy is characterized by a broad view on food and nutrition, different components related to food and nutrition have to be considered. Therefore, the health side of the food, in a pluralistic vision has to be taken into account. Thus, food and their consumers are unavoidably connected.

Beyond classical nutrients, much attention has recently been focused on bioactive compounds and their preventive role on non-communicable diseases such as diabetes, cardiovascular diseases and cancer. Therefore researchers are increasingly interested to unfold the preventive biochemical processes of these compounds.

Hence, the current research aimed to investigate health enhancing properties of traditional Brazilian fruits within the Food and Nutrition Security definition of the country. Given the interdisciplinary feature of the topic Food and Nutrition Security, the work was performed in two stages. The first one encompasses a nutritional survey with two rural communities in APA – Cantão, Tocantins State, Brazil and the second part comprises experimental laboratory research.

The outcome from the nutrition survey showed a high level of food insecurity among the families (84.2%). The nutritional profile of the study population expressed a high prevalence of overweight for the adults (53.7%). Regression analysis showed that the high Body Mass Index (BMI) is influenced by the consumption of an imbalanced diet and the physical activity level. Furthermore, women had a higher prevalence of overweight and obesity in comparison to men. Another observation is that rural communities have a monotonous diet with very low consumption of fruits and vegetables. Besides the negative effect on their body composition, this last result points to the risk of developing micronutrient deficiencies, i.e. hidden hunger. Based on the outcome and the demand presented by the participants in the nutrition survey, two fruits available in the region were chosen to investigate their possible biofunctional properties.

Different assays were performed with Bacaba *(Oenocarpus bacaba* Mart.*)* and Jenipapo (*Genipa americana* L.) phenolic extracts. Extracts from both fruits showed antioxidant and antiproliferative capacities. Since bacaba displayed higher activities than Jenipapo, this fruit was chosen for a more detailed investigation of the biochemical mechanisms involved. The results showed that bacaba phenolic extracts induced apoptosis in MCF-7 breast cancer cells through the mitochondrial pathway. Caspase-6, -8 and -9 were activated when compared to the untreated control in a dose dependent manner ($p<.05$). However, caspase-9 showed the highest activation. Since MCF-7 cells do not express caspase-3 and based on additional investigations on PARP (Poly (ADP-ribose) polymerase) - cleavage, the experiments suggest that caspase-9 plays an important role in the observed apoptotic effect. The laboratory

work thus emphasizes the potential healthy properties of traditional fruits from the Brazilian biodiversity with high antioxidant activities.

Altogether, the results indicate the need of a better nutritional education with the involved communities in order to promote healthy eating practices and to increase the consumption of fruit and vegetables. Based on this, it is suggested for government and policy makers to take action in rural communities.

Indeed, it is undeniable that the biodiversity available in Brazil is a huge treasure and source of novel “superfruits”. Therefore, the current work reinforces the development of research in this area in order of identify health enhancing neglected traditional fruits and to promote their consumption, add value and generate income to small farmers and traditional communities with not only the improvement of their economic power, but also of their diets and health respecting their tradition and culture. Not to mention the contribution to biodiversity preservation since plants that were merely discarded could now have a multifactor value in line with the Brazilian Food and Nutritional Security policy.

6. Zusammenfassung

Derzeit ist das brazilianische Ernährungsprofil vom so genannten „nutrition transition process“ geprägt, das heißt, die Landbevölkerung weist Ernährungscharakteristika von Industrie- und Entwicklungsländern auf. Deshalb ist Fehlernährung in Brazilien nicht nur als Unterernährung vorzufinden, sondern auch in Form von Übergewicht und Adipositas. Manche Studien belegen, dass dies nicht nur ein Problem in Städten sondern auch inländlichen Gebieten ist.

In letzter Zeit hat sich Brasilien in eindrucksvoller Weise auf verschiedenen Gebieten wie z.B. Ernährung, Landwirtschaft und Gesundheit weiterentwickelt und diese in ein Nachhaltigkeitskonzept integriert. Eine der neuesten Initiativen ist die brasilianische Nahrungs- und Ernährungssicherheitsstrategie. Die Grundidee der Initiative ist Vorreiter in diesem Themengebiet, indem sie verschiedene Dimensionen von Ernährungsfragen verbindet und sind wie folgt definiert: “Die Nahrungs- und Ernährungssicherheit ist das Erreichen des Rechts aller Menschen, regelmäßigen und permanenten Zugang zu qualitativ hochwertiger Nahrung in ausreichender Menge zu haben, ohne Gefährdung anderer grundlegender menschlicher Bedürfnisse, basierend auf Ernährungsweisen, die unter Einbeziehung kultureller Hintergründe und unter Berücksichtigung sozialer, ökonomischer und ökologischer Faktoren die Gesundheit fördern“.

Die brasilianische Nahrungs- und Ernährungsinitiative beinhaltet eine holistische Betrachtung von Lebensmittel und Ernährung. Aus diesem Grund müssen verschiedene Aspekte, die mit Lebensmitteln und Ernährung zu tun haben, ganzheitlich gesehen werden. Daraus folgt, dass Nahrung und ihre Konsumenten unvermeidbar miteinander verbunden sind.

In der Wissenschaft wird derzeit bioaktiven Substanzen und ihrer präventiven Rolle bei nicht übertragbaren Krankheiten, wie z.B. Diabetes, Herz-Kreislauferkrankungen und Krebserkrankungen, viel Aufmerksamkeit geschenkt. Deshalb steigt das

Interesse der Wissenschaftler, diesen Vorgang auf biochemischer Ebene zu entschlüsseln.

Aus diesem Grund war es Ziel der vorliegenden Arbeit, die gesundheitsfördernden Eigenschaften von traditionellen brasilianischen Früchten auf Basis der Nahrungs- und Ernährungssicherheitsinitiative Brasiliens zu untersuchen. Da dieses Konzept interdisziplinäre Aspekte aufweist, wurde die Studie in zwei Abschnitten durchgeführt. Im ersten Teil wurde in zwei ländlichen Gebieten des APA-Cantão, Tocantins State, Brasilien, eine Ernährungserhebung durchgeführt. Der zweite Teil beinhalte die Untersuchung von traditionellen brasilianischen Früchten im Labor.

Die Fragebögen zur Ernährungssituation zeigten, dass bei den 84,2% der Familien eine große Ernährungsunsicherheit herrscht. Der Ernährungsstatus der befragten Bevölkerungsgruppe weist darauf hin, dass es eine sehr hohe Prävalenz (53,7%) von Übergewicht bei den Erwachsenen existiert. Des Weiteren bestätigte die Regressionsanalyse, dass ein hoher Body Mass Index (BMI) von Faktoren wie z.B. einer unausgewogenen Ernährung und dem Grad der körperlichen Aktivität beeinflusst wird. Darüber hinaus zeigten die Frauen im Vergleich zu den Männern eine höhere Prävalenz für Übergewicht und Adipositas. Zusätzlich wurde beobachtet, dass im ländlichen Gebiet die Bevölkerung eine recht einseitige Ernährungsweise aufweist und Früchte sowie auch Gemüse sehr wenig konsumiert werden. Neben den Einflüssen auf den Körperbau zeigt dieses Ergebnis eine hohe Gefahr des Risikos für Mikronährstoffmangel (versteckter Hunger), auf. Auf Grundlage der Ergebnisse und Wunsch der Teilnehmer der Studie wurden zwei Früchte aus der Region ausgewählt, um sie auf potentiell biofunktionelle Eigenschaften hin zu untersuchen.

Verschiedene biochemische Assays wurden mit phenolischen Extrakten aus Bacaba (*Oenocarpus bacaba* Mart.) und Jenipapo (*Genipa americana* L.) durchgeführt. Beide Fruchtextrakte zeigten sehr hohe antioxidative und antiproliferative Eigenschaften. Da Bacaba eine höhere Aktivität aufwies als Jenipapo, wurde es für die weiteren

Experimente ausgewählt. Die antioxidative Aktivität vom Bacaba-Extrakt wurde an der MCF-7 Brustkrebs-Zelllinie untersucht. Die Ergebnisse zeigten, dass der phenolischen Extrakt aus Bacaba eine pro-apoptotische Wirkung aufwies, die durch eine Aktivierung des mitochondrialen Signalweges zu erklären ist. Im Gegensatz zur Kontrollprobe hatte Bacaba eine dosis-abhängige Wirkung auf die Aktivierung von den Caspasen -6, -8, und -9. Caspase-9 zeigte dabei die höchste Aktivierung. Die Tatsache, dass MCF-7 Zellen nicht Caspase-3 exprimieren und die Ergebnisse weiterer Untersuchungen an der PARP (poly(ADP-ribose)-Polymerase) -Spaltung deuten darauf hin, dass Caspase-9 eine wichtige Rolle bei der Apoptose spielt. Insgesamt unterstreichen die Laborergebnisse die potentiellen gesundheitsfördernden Eigenschaften und hohen antioxidativen Wirkungen der traditionellen Früchte aus Brasilien.

Zusammenfassend deuten die Ergebnisse darauf hin, dass es wichtig ist, den beteiligten Dörfern Ernährungsbildung anzubieten, um gesunde Ernährungsweisen und einen höheren Konsum von Früchten und Gemüse zu fördern. Darauf basierend sollten die Regierung und die Gesetzgeber neue Inititativen zur Förderung einer gesunden Ernährung in den ländlichen Gebieten initiieren.

Es ist unbestreitbar, dass die verfügbare Artenvielfalt eine der größte Schätze Brasiliens ist und eine Quelle vieler neuer „Superfrüchte“. In dieser Arbeit wurde gezeigt, wie wichtig es ist, die Forschung in diesem Bereich mit dem Ziel zu intensivieren, um vernachlässigte Früchte mit gesundheitsfördernden Eigenschaften zu identifizieren und ihren Verzehr zu fördern. Dies würde neben der Möglichkeit für Kleinbauern und traditionelle Gemeinschaften, zusätzliches Einkommen zu erzielen, auch unter Berücksichtigung der Tradition und Kultur die Ernährungsweise und Gesundheit fördern. Doch nicht nur der wirtschaftliche Vorteil, sondern auch der Erhalt der biologischen Vielfalt im brasilianischen Regenwald wäre gewährleistet, womit diese Früchte, gemäß der neuen brasilianischen Nahrungs- und Ernährungssicherheitsinitiative, einen multifaktoriellen Wert für die Menschen dieser Gebiete aufweisen würden.

7. Resumo

O perfil nutricional brasileiro atualmente é caracterizado pelo processo conhecido com transição nutricional, isto é, a população apresenta simultaneamente estado nutriticional característico de países desenvolvidos e em desenvolvimento. Por tal motivo, a má nutrição está presente não somente na forma de desnutrição, mas também de sobrepeso e obesidade. Alguns estudos sugerem que este quadro pode não ser apenas um problema particular de sociedades urbanas mas também de comunidades rurais.

Recentemente, o Brasil tem avançado expressivamente nos assuntos relacionados às questões nutricionais, agrícolas e de saúde, dentro do contexto da sustentabilidade. Umas das iniciativas recentes abrange a Política Nacional de Segurança Alimentar e Nutricional, a qual pode ser considerada na vanguarda deste tema por abranger as diferentes dimensões da nutrição, como descrito a seguir: “A segurança alimentar e nutricional consiste na realização do direito de todos ao acesso regular e permanente a alimentos de qualidade, em quantidade suficiente, sem comprometer o acesso a outras necessidades essenciais, tendo como base práticas alimentares promotoras de saúde que respeitem a diversidade cultural e que sejam ambiental, cultural, econômica e socialmente sustentáveis.”

Em virtude na Política Nacional de Segurança Alimentar e Nutricional possuir uma visão abrangente da alimentação e nutrição, diferentes componentes envovidos com este tema devem ser considerados. Desta forma, o aspecto de saúde deve ser visto dentro de um contexto pluralístico. Estando o alimento e seus consumidores inevitavelmente conectados.Além dos nutrientes clássicos, compostos bioativos, tais como substâncias fenólicas, têm chamado a atenção por seu papel na prevenção de doenças crônicas não transmissíveis, tais como diabetes, doenças cardiovasculares e câncer. Assim, pesquisadores estão progressivamente interessados em desdobrar os processos bioquímicos envolvidos no poder preventivo destas substâncias.

Assim, a presente pesquisa tem como objetivo investigar propriedades de saúde em frutas tradicionais brasileiras com base no conceito de segurança alimentar e nutricional. Em virtude da característica interdisciplinar inerente à temática segurança alimentar e nutricional, o trabalho foi desenvolvido em duas etapas e considerou as diferentes dimensões da nutrição. A primeira etapa incluiu um inquérito nutricional com duas comunidades rurais na APA – Cantão, Tocantins; e a segunda etapa compreendeu um pesquisa experimental em laboratório.

Os resultados do inquérito nutricional demonstram elevado nível de insegurança alimentar entre as famílias (84.2%). O perfil nutricional da população estudada expressa a elevada prevalência de sobrepeso e obesidade (53.7%) em adultos. A análise de regressão demonstrou que o Índice de Massa Corporal (IMC) é influenciado pelo consumo de uma dieta desbalanceada e pelo nível de atividade física. Aqueles que desenvolvem elevado nível de atividade física tiveram menores valores de IMC. Ademais, é importante ressaltar que as mulheres tiveram elevada prevalência de sobrepeso e obesidade em comparação aos homens. Outra observação diz respeito à ingestão dietética das comunidades rurais que demonstra uma dieta monótona com baixo consumo de frutas e verduras. Adicionalmente ao estado nutricional encontrado, este último resultado chama a atenção para a exposição ao risco de carências de micronutrientes (ex. fome oculta). Com base nos resultados da presente pesquisa e na demanda apresentada pelos participantes do inquérito nutricional, duas frutas disponíveis na região e sugeridas pelos participantes foram escolhidas para trabalho experimental em laboratório com objetivo de investigar suas possíveis propriedades funcionais.

Differentes ensaios químicos e bioquímicos foram realizados com extrato fenólico de bacaba *(Oenocarpus bacaba* Mart.*)* e jenipapo (*Genipa americana* L.). Ambos extratos tiveram expressiva atividade antioxidante e antiproliferativa de maneira dose-dependente. Entretanto, como a bacaba demonstrou maior potencial antioxidante e antiproliferativo que o jenipapo, esta fruta foi escolhida para investigação mais detalhada no que tange os processos bioquímicos envolvidos na

atividade antiproliverativa do extrato de bacaba na linha celular de câncer de mama MCF-7. Os resultados demonstram que o extrato fenólico de bacaba induziu apoptose por meio da via mitocondrial com ativação de caspases. Caspase-6, -8 e -9 foram significativamente ativadas em comparação ao controle não estimulado de forma dose-dependente ($p<.05$). Entretanto, caspase-9 teve o maior nível de ativação. Em virtude da linha de célula MCF-7 não expressar caspase-3 e com base em investigação adicional quanto à clivagem da proteína PARP (polimerase poli (do ADP-ribose)) , os experimentos sugerem que caspase-9 possui importante papel no efeito apoptótico observado. A pesquisa laboratorial aqui relatada enfatiza o potencial de saúde de frutas tradicionais da biodiversidade brasileira com elevados níveis de atividade antioxidante.

De forma geral, os resultados apontam a necessidade de atividades de educação nutricional com as comunidades envolvidas de forma a promover práticas alimentares saudáveis e aumentar o consumo de frutas e verduras. Com base nos dados obtidos, sugere-se às instituições governamentais e elaboradores e executores de políticas públicas que ações sejam desenvolvidas com atenção às comunidades rurais.

Ainda, é inegável que a biodiversidade disponível no Brasil é um grande tesouro e fonte de novas “superfrutas”. Por tal motivo, este trabalho reforça o desenvolvimento de pesquisa nesta área de forma a realçar as propriedades de saúde de frutas negligenciadas, promover o consumo, agregar valor e gerar renda à pequenos agricultores e comunidades tradicionais, não somente para aprimorar seu poder econômico, mas também suas dietas e saúde, respeitando a tradição e a cultura local. Cabe ainda mencionar a contribuição para a preservação da biodiversidade, já que à espécies que seriam meramente descartadas pode ser atribuído um valor plural (social, ecônomico, de saúde, nutricional etc.) em conssonância com a Política Nacional de Segurança Alimentar e Nutricional brasileira.

8. References

1. Lajolo, F. M., Functional foods: Latin American perspectives. *British journal of nutrition* **2002,** 88, (SupplementS2), S145-S150.

2. Popkin, B. M.; Adair, L. S.; Ng, S. W., Global nutrition transition and the pandemic of obesity in developing countries. *Nutrition reviews* **2012,** 70, (1), 3-21.

3. Finco, M. V. A.; Doppler, W., Bioenergy and sustainable development: The dilemma of food security and climate change in the Brazilian savannah. *Energy for sustainable development* **2010,** 14, (3), 194-199.

4. Ab'Saber, A. N., Os domínios morfoclimáticos da América do Sul. Primeira aproximação. *Geomorfologia* **1977,** 52, (121).

5. INPE *Monitoramento da floresta.*; São José dos Campos, 2004.

6. BRAZIL, Plano Amazônia Sustentável: Diretrizes para o desenvolvimento sustentável da Amazônia Brasília In MMA (Brazilian Ministry of the Environment): 2008.

7. IBGE, Pesquisa de orçamentos familiares (POF) 2002-2003: análise da disponibilidade domiciliar de alimentos e do estado nutricional no Brasil. In Brazilian Institute of Geography and Statistics (IBGE): Rio de Janeiro, 2004; p 76.

8. Alencar, A.; Nepstad, N.; McGrath, D.; Moutinho, P.; Pacheco, P.; Dias, M. D. C. V.; Filho, B. S., *Desmatamento na Amazônia: indo além da emergência crônica*. Brazilian National Institute for Research in Amazonia (INPA): Manaus, 2004.

9. Margulis, S. *Causes of Deforestation of the Brazilian Amazon*; 2003.

10. Fearniside, P. M. *A floresta Amazônia nas mudanças globais*; Instituto Nacional de Pesquisas Amazônicas (INPA): Manaus, 2003; p 134.

11. Fearniside, P. M., Soybean cultivation as a threat to the environment in Brazil. *Environmental conservation* **2001,** 28, 23-28.

12. INPE *Monitoramento da floresta amazônica brasileira por satélite: relatório 2000-2001.*; 2002.

13. IBGE, Indicadores de desenvolvimento sustentável: Brasil 2004. In IBGE: Rio de Janeiro, 2004.

14. Lui, G. H.; Molina, S. M. G., Ocupação humana e transformação das paisagens na Amazônia brasileira. *Amazônica - Revista de antropologia* **2009,** 1, (1).

15. Guilhoto, J. J. M.; Ichiara, S. M.; Silveira, F. G.; Diniz, B. P. C.; Azzoni, C. R.; Moreira, G. R. C., A importância da agricultura familiar no Brasil e em seus estados. In *ANPEC*, 2007.

16. Nestel, P.; Bouis, H. E.; Meenakshi, J. V.; Pfeiffer, W., Biofortification of staple food crops. *The journal of nutrition* **2006,** 136, (4), 1064-1067.

17. Johns, T.; Eyzaguirre, P. B. *Nutrition and the environment*; Geneva, 2002.

18. WHO *Preventing chronic diseases: a vital investiments.*; Geneva, 2005; p 182.

19. Popkin, B. M., The nutrition transition in the developing world. *Development Policy Review* **2003,** 21, (5-6), 581-597.

20. Comissão Intergestora Tripartite (CIT) Diretrizes e Recomendações. Cuidado Integral de Doenças Crônicas Não-Transmissíveis. Promoção da Saúde, Vigilância, Prevenção e Assistência. In Brazilian Ministry of Health: Brasília, 2007.

21. Velásquez-Meléndez, G.; Gazzinelli, A.; Côrrea-Oliveira, R.; Pimenta, A. M.; Kac, G., Prevalence of metabolic syndrome in a rural area of Brazil. *Sao Paulo Medical Journal* **2007,** 125, 155-162.

22. Sichieri, R.; Castro Joelma Ferreira, G.; Moura Aníbal, S., Fatores associados ao padrão de consumo alimentar da população brasileira urbana. *Cadernos de saúde pública* **2003**.

23. Ames, B. N.; Shigenaga, M. K.; Hagen, T. M., Oxidants, antioxidants, and the degenerative diseases of aging. *Procedigs of the national academy of science USA* **1993**, (90), 7915-7922.

24. Liu, R. H., Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *American Journal of Clinical Nutrition* **2003,** 78, (3 Suppl), 517S-520S.

25. Daryani, A.; Basu, S.; Becker, W.; Larsson, A.; Riserus, U., Antioxidant intake, oxidative stress and inflammation among immigrant women from the Middle East living in Sweden: associations with cardiovascular risk factors. *Nutrition, metabolism, and cardiovascular diseases : NMCD* **2007,** 17, (10), 748-56.

26. Health Situation. In Health, S. S. o., Ed. Government of Tocantins State: Palmas, 2004; p 89.

27. BRAZIL, Lei orgânica de Segurança Alimentar e Nutricional. Federal law n 11346, September 15th, 2006. In 2006.

28. BRAZIL, Federal decree nº 6.040, February 7th, 2007. Institui a Política Nacional de Desenvolvimento Sustentável dos Povos e Comunidades Tradicionais. In Diário Oficial da União; Poder executivo, February 8th, 2007.: 2007.

29. BRAZIL, Inter-ministerial Administrative Order n. 239, July 21st, 2009. Estabelece orientações para a implementação do Plano Nacional de Promoção das Cadeias de Produtos da Sociobiodiversidade, e dá outras providências. In Diário Oficial da União, July 22nd, 2009.: Brasília, 2009.

30. Williams, M.; Pehu, E.; Ragasa, C. *Health enhancing foods: opportunities for strengthening the sector in developing countries*; The International Bank for Reconstruction and Development/The World Bank: Washington, DC, 2006.

31. Johns, T.; Sthapit, B. R., Biocultural diversity in the sustainability of developing-country food systems. *Food and nutrition bulletin* **2004,** 25, (2), 143-55.

32. Nutraingredients Functional foods in Japan. http://www.nutraingredients.com/Research/Functional-foods-in-Japan (1st march),

33. Ohama, H.; Ikeda, H.; Moriyama, H., Health foods and foods with health claims in Japan. *Toxicology* **2006,** 221, (1), 95-111.

34. Scientific concepts of functional foods in Europe. Consensus document. *The british journal of nutrition* **1999,** 81 Suppl 1, S1-27.

35. Coppens, P.; da Silva, M. F.; Pettman, S., European regulations on nutraceuticals, dietary supplements and functional foods: A framework based on safety. *Toxicology* **2006,** 221, (1), 59-74.

36. Siro, I.; Kapolna, E.; Kapolna, B.; Lugasi, A., Functional food. Product development, marketing and consumer acceptance--a review. *Appetite* **2008,** 51, (3), 456-67.

37. Palou, A.; Serra, F.; Pico, C., General aspects on the assessment of functional foods in the European Union. *European journal of clinical nutrition* **2003,** 57 Suppl 1, S12-7.

38. Williams, G. R. G. a. C. M., *Functional foods-concept to products*. Wooodhead Publishing Limited Abington Hall: Cambrige, England, 2003.

39. Lawrence, M.; Rayner, M., Functional foods and health claims: a public health policy perspective. *Public health nutrition* **1998,** 1, (2), 75-82.

40. Codex Alimentarius Commission. Appendix 1: Draft guidelines for use of health and nutrition claims. . In Food and Agriculture Organization of the United Nations, World Health Organization, CL1995/26-FL: 1995.

41. BRAZIL, Brazilian Health Surveillance Agency (ANVISA). Resolução n° 18, April 30th, 1999. Aprova o regulamento técnico que estabelece as diretrizes básicas para análise e comprovação de propriedades funcionais e ou de saúde alegadas em rotulagem de alimentos, constante do anexo desta portaria. *In* Diário Oficial da União; Poder executivo, de May 3rd,1999.: Brasília, 1999.

42. BRAZIL, Brazilian Health Surveillance Agency (ANVISA). Resolução n° 19, April 30th, 1999. Regulamento técnico para procedimento de registro de alimentos com alegações de propriedades funcionais e ou de saúde em sua rotulagem. *In* Diário Oficial da União; Poder executivo, May 3rd, 1999.: Brasília, 1999.

43. BRAZIL, Brazilian Health Surveillance Agency (ANVISA). Resolução RDC n° 2, January 7th, 2002. Aprova o regulamento técnico de substâncias bioativas e probióticos isolados com alegação de propriedades funcional e ou de saúde. *In* Diário Oficial da União; Poder executivo, January 9th, 2002.: Brasília, 2002.

44. Jew, S.; Vanstone, C. A.; Antoine, J. M.; Jones, P. J., Generic and product-specific health claim processes for functional foods across global jurisdictions. *The journal of nutrition* **2008,** 138, (6), 1228S-36S.

45. Silveira, T. F. V. d.; Vianna, C. M. d. M.; Mosegui, G. B. G., Brazilian legislation for functional foods and the interface with the legislation for other food and medicine classes: contradictions and omissions. *Physis: revista de saúde coletiva* **2009,** 19, 1189-1202.

46. Stringheta, P. C.; Oliveira, T. T. d.; Gomes, R. C.; Amaral, M. d. P. H. d.; Carvalho, A. F. d.; Vilela, M. A. P., Políticas de saúde e alegações de propriedades funcionais e de saúde para alimentos no Brasil. *Revista brasileira de ciências farmacêuticas* **2007,** 43, 181-194.

47. Johns, T.; Smith, I. F.; Eyzaguirre, P. B., Understanding the links between agriculture and health. In IFPRI (International Food Policy Research Institute): Washington, 2006; Vol. Focus 13.

48. Bélanger, J.; Johns, T., Biological diversity, dietary diversity, and eye health in developing country populations: establishing the evidence-base. *Ecohealth* **2008,** 5, (3), 244-256.

49. Sabbe, S.; Verbeke, W.; Deliza, R.; Matta, V.; Van Damme, P., Effect of a health claim and personal characteristics on consumer acceptance of fruit juices with different concentrations of açaí (Euterpe oleracea Mart.). *Appetite* **2009,** 53, (1), 84-92.

50. Pochettino, M. L.; Puentes, J. P.; Buet Costantino, F.; Arenas, P. M.; Ulibarri, E. A.; Hurrell, J. A., Functional foods and nutraceuticals in a market of bolivian immigrants in Buenos Aires (Argentina). *Evidence-based complementary and alternative medicine : eCAM* **2012,** 2012, 320193.

51. Yang, Y., Scientific substantiation of functional food health claims in China. *The journal of nutrition* **2008,** 138, (6), 1199S-205S.

52. Arai, S., Global view on functional foods: asian perspectives. *The british journal of nutrition* **2002,** 88 Suppl 2, S139-43.

53. Tuorila, H.; Lähteenmäki, L.; Pohjalainen, L.; Lotti, L., Food neophobia among the finns and related responses to familiar and unfamiliar foods. *Food quality and preference* **2001,** 12, (1), 29-37.

54. Barcelos, M. D. d.; Aguiar, L. K.; Ferreira, G. C.; Vieira, L. M., Willingness to try innovative food products: a comparison between british and brazilian consumers. *Brazilian administration review* **2009,** 6, (1), 50-61.

55. Butler, R. A.; Laurance, W. F., New strategies for conserving tropical forests. *Trends in ecology & evolution* **2008,** 23, (9), 469-72.

56. Blasa, M.; Gennari, L.; Angelino, D.; Ninfali, P., Fruit and vegetable antioxidants in health. In *Bioactive foods in promoting health - fruits and vegetables*, 1st ed.; Watson, R. R.; Preedy, V. R., Eds. Academic press: Burlington, 2010; p 737.

57. Cheeseman, K. H.; Slater, T. F., An introduction to free radical biochemistry. *British medical bulletin* **1993,** 49, (3), 481-493.

58. Halliwell, B.; Gutteridge, J. M. C., *Free Radicals in Biology and Medicine*. Oxford University Press: USA, 1999.

59. Duthie, G.; Crozier, A., Plant-derived phenolic antioxidants. *Current opinion in clinical nutrition and metabolic care* **2000**, (3), 447-451.

60. Ames, B. N.; Gold, L. S., Endogenous mutagens and the causes of aging and cancer. *Mutation research* **1991**, (250), 3-16.

61. Sun, J.; Chu, Y.-F.; Wu, X.; Liu, R. H., Antioxidant and antiproliferative activities of common fruits. *Journal of agriculture and food chemistry* **2002**, (50), 7449-7454.

62. Liu, R. H., Potential synergy of phytochemicals in cancer prevention. Mechanism of action. *Journal of nutrition* 2004, pp 3479S-3485S.

63. Liu, R. H., Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *American journal of clinical nutrition* 2003, pp 517S-520S.

64. Chu, Y.-F.; Sun, J.; Wu, X.; Liu, R. H., Antioxidant and antiproliferative activities of common vegetables. *Journal of agriculture and food chemistry* 2002, pp 6910-6916.

65. Liu, M.; Li, X. Q.; Weber, C.; Lee, C. Y.; Brown, J.; Liu, R. H., Antioxidant and Antiproliferative Activities of Raspberries. *J of agriculture and food chemistry* 2002, pp 2926-2930.

66. Proteggente, A. R.; Pannala, A. S.; Paganga, G.; Van Buren, L.; Wagner, E.; Wiseman, S.; Van De Put, F.; Dacombe, C.; Rice-Evans, C. A., The antioxidant activity of regularly consumed fruit and vegetable reflects their phenolic and vitamin C composition. *Free radical research* 2002, pp 217-233.

67. Dong, M.; He, X., Liu, R.H., Phytochemicals of black bean seed coats: Isolation, structure elucidation, and their antiproliferative and antioxidative activities. *Journal of agriculture and food chemisty* 2007, pp 6044-6051.

68. Eberhardt, M. V.; Lee, C. Y.; Liu, R. H., Antioxidant activity of fresh apples. *Nature* 2000, pp 903-904.

69. Boudet, A.-M., Evolution and current status of research in phenolic compounds. *Phytochemistry* 2007, pp 2722-2735.

70. Dragsted, L. O.; Strube, M.; Larsen, J. C., Cancer-protective factors in fruit and vegetables: biochemical and biological background. *Pharmacology & toxicology* 1993, pp 116-35.

71. Rice-Evans, C.-A.; Miller, N. J.; Bolwell, P. G.; Bramley, P. M.; Pridham, J. B., The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free radical research* 1995, pp 375-383.

72. Rice-Evans, C.; Miller, N.; Paganga, G., Antioxidant properties of phenolic compounds. *Trends in plant science* **1997,** 2, (4), 152-159.

73. Boivin, D.; Blanchette, M.; Barrette, S.; Moghrabi, A.; Baliveau, R., Inhibition of cancer cell proliferation and suppression of TNF-induced activation of NFκB by edible berry juice. *Anticancer research* **2007,** 27, (2), 937-948.

74. Ray Sahelian, M. D., Index of herbs, supplements, and natural therapies. In RAYSAHELIAN.COM: 2005

75. Ghasemzadeh, A.; Jaafar, H. Z. E.; Rahmat, A., Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young Ginger (*Zingiber officinale Roscoe*). *Molecules* **2010,** 15, (6), 4324-4333.

76. Society, A. C., *Cancer Facts & Figures 2007*. American Social Society , Inc: Atlanta, 2007.

77. Surh, Y.-J., Cancer chemoprevention with dietary phytochemicals. *Nature reviews.Cancer* **2003,** 3, (10), 768-780.

78. Seeram, N. P.; Adams, L. S.; Zhang, Y.; Lee, R.; Sand, D.; Scheuller, H. S.; Heber, D., Blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit growth and stimulate apoptosis of human cancer cells in vitro. *Journal of agricultural and food chemistry* **2006,** 54, (25), 9329-39.

79. He, X.; Liu, R. H., Cranberry phytochemicals: Isolation, structure elucidation, and their antiproliferative and antioxidant activities. *Journal of agricultural and food chemistry* **2006,** 54, (19), 7069-74.

80. Liu, M.; Li, X. Q.; Weber, C.; Lee, C. Y.; Brown, J.; Liu, R. H., Antioxidant and antiproliferative activities of raspberries. *Journal of agricultural and food chemistry* **2002,** 50, (10), 2926-30.

81. Meyers, K. J.; Watkins, C. B.; Pritts, M. P.; Liu, R. H., Antioxidant and antiproliferative activities of strawberries. *Journal of agricultural and food chemistry* **2003,** 51, (23), 6887-92.

82. McCusker, J., *How to measure and evaluate community health : a self-teaching manual for rural health workers*. Macmillan: London, 1990.

83. Segall-Corrêa, A. M.; Marin-Leon, L., Food Security in Brazil: the proposal and application of the Brazilian Food Insecurity Scale from 2003 to 2009. *Segurança alimentar e nutricional* **2009,** 16, (2).

84. Pitanga, F. J.; Lessa, I., Anthropometric indexes of obesity as an instrument of screening for high coronary risk in adults in the city of Salvador--Bahia. *Arquivos brasileiro de cardiologia* **2005,** 85, (1), 26-31.

85. Liu, C.; Xie, B.; Chou, C.-P.; Koprowski, C.; Zhou, D.; Palmer, P.; Sun, P.; Guo, Q.; Duan, L.; Sun, X.; Anderson Johnson, C., Perceived stress, depression and food consumption frequency in the college students of China seven cities. *Physiology & behavior* **2007,** 92, (4), 748-754.

86. Abadio Finco, F. D. B.; Finco, M. V. A., An Assessment of the Brazilian Popular Restaurant Program as an Integrated Action for Food Security and Health Promotion. In *Food Security: quality management, issues and economic implications*, Jones, M. A.; Hernandez, F. E., Eds. Nova Science Pub Incorporated: Hauppauge, 2012.

87. Schofield, W. N., Predicting basal metabolic rate, new standards and review of previous work. *Human nutrition. Clinical nutrition* **1985,** 39 Suppl 1, 5-41.

88. FAO *Human Energy Requirements. Report of a joint FAO/WHO/UNU. Expert Consultation*; Rome, 2004.

89. Nelson, M.; Black, A. E.; Morris, J. A.; Cole, T. J., Between- and within-subject variation in nutrient intake from infancy to old age: estimating the number of days required to rank dietary intakes with desired precision. *American journal of clinical nutrition* **1989,** 50, (1), 155-67.

90. Bhargava, A.; Guthrie, J. F., Unhealthy eating habits, physical exercise and macronutrient intakes are predictors of anthropometric indicators in the women's health trial: feasibility study in ninority Populations. *The british journal of nutrition* **2002,** 88, (6), 719-28.

91. A.O.A.C., *Official methods of analysis* 16th ed.; A.O.A.C. International: Gaitherburg, 2000; Vol. 2.

92. Kammerer, D.; Carle, R.; Schieber, A., Characterization of phenolic acids in black carrots (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef.) by high-performance liquid chromatography/electrospray ionization mass spectrometry. *Rapid communications in mass spectrometry : RCM* **2004,** 18, (12), 1331-40.

93. Singleton, V. L.; Rossi, J. A. J., Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of enology and viticulture* **1965,** 16, 144-158.

94. Sariburun, E.; Şahin, S.; Demir, C.; Türkben, C.; Uylaşer, V., Phenolic content and antioxidant activity of raspberry and blackberry cultivars. *Journal of food science* **2010,** 75, (4), C328-C335.

95. Sellappan, S.; Akoh, C. C.; Krewer, G., Phenolic compounds and antioxidant capacity of Georgia-Grown blueberries and blackberries. *Journal of agricultural and food chemistry* **2002,** 50, (8), 2432-2438.

96. Cao, G.; Prior, R. L., Measurement of oxygen radical absorbance capacity in biological samples. *Methods of enzymology* **1999,** 299, 50-62.

97. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C., Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology & medicine* **1999,** 26, (9-10), 1231-7.

98. Benzie, I. F.; Strain, J. J., The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry* **1996**, (239), 70-76.

99. Benzie, I. F.; Strain, J. J., Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. In *Methods of enzymology*, Academic Press: 1999; Vol. Volume 299, pp 15-27.

100. Al-Duais, M.; Müller, L.; Böhm, V.; Jetschke, G., Antioxidant capacity and total phenoilcs of Cyphostemma digitatum before and after processing; use of different assays. *European food research and technology* **2009,** 228, (5), 813-821.

101. Al-Duais, M.; Mueller, L.; Boehm, V.; Jetschke, G., Antioxidant capacity and total phenoilcs of *Cyphostemma digitatum* before and after processing; use of different assays. *Eur Food Res technol* **2008,** 228, 813-821.

102. Wolfe, K. L.; Liu, R. H., Cellular antioxidant activity (CAA) assay for assessing antioxidants, foods, and dietary supplements. *Journal of agricultural and food chemistry* **2007,** 55, (22), 8896-8907.

103. Schütz, K.; Kammerer, D.; Carle, R.; Schieber, A., Characterization of phenolic acids and flavonoids in dandelion (*Taraxacum officinale* WEB. ex WIGG.) root and herb by high-performance liquid chromatography/electrospray ionization mass spectrometry *Rapid communications in mass spectrometry : RCM* **2005,** 19, 179-186.

104. Yoon, H.; Liu, R. H., Effect of selected phytochemicals and apple extracts on NF-kappaB activation in human breast cancer MCF-7 cells. *Journal of agricultural and food chemistry* **2007,** 55, (8), 3167-73.

105. Gong, J. P.; Traganos, F.; Darzynkiewicz, Z., A Selective procedure for DNA extraction from apoptotic cells applicable for gel electrophoresis and flow cytometry. *Analytical biochemistry* **1994,** 218, (2), 314-319.

106. Doppler, W., *Contribution of the Farming Systems Approach to Regional Food Security and Rural Infrastructure*. LIT Verlag Münster-Hamburg: 1993; Vol. I.

107. DIEESE, Estatísticas do Meio Rural. In DIEESE (Statistics and socioeconomics research department): São Paulo, 2006.

108. Tai, D. A., *Family resources and their impact on living standard and food security of farmers in the mountainous farming systems in northwest Vietnam*. Margraf Verlag: Weikersheim, 2004.

109. Marini, M.; Pieroni, O., Relación entre la familia y el entorno social. Tipología de las familias agrícolas en una zona marginal (Calabria). In *Cambio rural en Europa. Colóquio de Montpellier.*, Research, A., Ed. Ministerio de Agricultura, Pesca y Alimentacion.: Madrid, 1987; pp 205-247.

110. Berdegue, J.; Reardon, T.; Escobar, G., La cresciente importancia del empleo y el ingresso rurales no agricolas. In *Desarrollo de las economias rurales*, ECHEVERRIA, R. G., Ed. Banco Interamericano de Desarrollo – BID: Washington, DC, 2001.

111. Reardon, T.; Vosti, S., Links between rural poverty and the environment in developing countries: asset categories and investment poverty. . *World development* **1995,** 23, (9), 1495-1506.

112. Kitchaicharoen, J., *Farming & Rural System economics*. Margraf Publishers: Weikersheim, 2003; Vol. 47, p 172.

113. Kitchairaroen, J., *Socio-economic assessement of the farm eesources and living standards of different ethnic groups. A case from northern Thailand.* Margraf Verlag: Weikersheim, 2003; Vol. 47.

114. Abu Shaban, A., *Socio-Economic Assessment of Using Treated Wastewater in Irrigated Agriculture.* Margraf Verlag: Weikersheim, 2007.

115. Greene, W. H., *Econometric analysis*. 6th ed.; 2008.

116. Hill, R. C.; Griffiths, W. E.; Lim, G. C., *Principles of econometrics*. 3rd ed.; 2008.

117. Lockey, A. S.; Handley, R.; Willett, K., 'Clearance' of cervical spine injury in the obtunded patient. *Injury* **1998,** 29, (7), 493-7.

118. Liu, R. H.; Finley, J., Potential cell culture models for antioxidant research. *Journal of agricultural and food chemistry* **2005,** 53, (10), 4311-4.

119. Wolfe, K. L.; Kang, X.; He, X.; Dong, M.; Zhang, Q.; Liu, R. H., Cellular antioxidant activity of common fruits. *Journal of agriculture and food chemistry* 2008, pp 8418-8426.

120. Fabre, N.; Rustan, I.; de Hoffmann, E.; Quetin-Leclercq, J., Determination of flavone, flavonol, and flavanone aglycones by negative ion liquid chromatography electrospray ion trap mass spectrometry. *Journal of the american society for mass spectrometry* **2001,** 12, (6), 707-715.

121. Markham, K. R.; Mabry, T. J., A procedure for the ultraviolet spectral detection of ortho-dihydroxyl groups in flavonoids. *Phytochemistry* **1968,** 7, (7), 1197-1200.

122. de Rijke, E.; Out, P.; Niessen, W. M. A.; Ariese, F.; Gooijer, C.; Brinkman, U. A. T., Analytical separation and detection methods for flavonoids. *Journal of chromatography A* **2006,** 1112, (1-2), 31-63.

123. Moraes, S. L. d.; Tomaz, J. C.; Lopes, N. P., Liquid chromatography–tandem mass spectrometric method for determination of the anti-inflammatory compound vicenin-2 in the leaves of *L. ericoides* Mart. *Biomedical chromatography: BMC* **2007,** 21, (9), 925-930.

124. Hong, Y. J.; Tomas-Barberan, F. A.; Kader, A. A.; Mitchell, A. E., The flavonoid glycosides and procyanidin composition of deglet noor dates (*Phoenix dactylifera*). *Journal of agricultural and food chemistry* **2006,** 54, (6), 2405-2411.

125. Gil-Izquierdo, A.; Riquelme, M. T.; Porras, I.; Ferreres, F., Effect of the rootstock and interstock grafted in Lemon Tree (*Citrus limon* (L.) Burm.) on the flavonoid content of lemon Juice. *Journal of agricultural and food chemistry* **2003,** 52, (2), 324-331.

126. Caristi, C.; Bellocco, E.; Panzera, V.; Toscano, G.; Vadalà, R.; Leuzzi, U., Flavonoids detection by HPLC-DAD-MS-MS in lemon juices from sicilian cultivars. *Journal of agricultural and food chemistry* **2003,** 51, (12), 3528-3534.

127. Siciliano, T.; De Tommasi, N.; Morelli, I.; Braca, A., Study of flavonoids of *Sechium edule* (Jacq) Swartz (*Cucurbitaceae*) different edible organs by liquid chromatography photodiode array mass spectrometry. *Journal of agricultural and food chemistry* **2004,** 52, (21), 6510-6515.

128. Sánchez-Rabaneda, F.; Jáuregui, O.; Casals, I.; Andrés-Lacueva, C.; Izquierdo-Pulido, M.; Lamuela-Raventós, R. M., Liquid chromatographic/electrospray ionization tandem mass spectrometric study of the phenolic composition of cocoa (*Theobroma cacao*). *Journal of mass spectrometry* **2003,** 38, (1), 35-42.

129. Pacheco-Palencia, L. A.; Duncan, C. E.; Talcott, S. T., Phytochemical composition and thermal stability of two commercial açai species, *Euterpe oleracea* and *Euterpe precatoria*. *Food chemistry* **2009,** 115, (4), 1199-1205.

130. Ferreres, F.; Ribeiro, V.; Izquierdo, A. G.; Rodrigues, M. Â.; Seabra, R. M.; Andrade, P. B.; Valentão, P., *Rumex induratus* leaves: interesting dietary source of potential bioactive compounds. *Journal of agricultural and food chemistry* **2006,** 54, (16), 5782-5789.

131. Han, J.; Ye, M.; Qiao, X.; Xu, M.; Wang, B.-r.; Guo, D.-A., Characterization of phenolic compounds in the Chinese herbal drug Artemisia annua by liquid chromatography coupled to electrospray ionization mass spectrometry. *Journal of pharmaceutical and biomedical analysis* **2008,** 47, (3), 516-525.

132. Qiao, X.; He, W.-n.; Xiang, C.; Han, J.; Wu, L.-j.; Guo, D.-a.; Ye, M., Qualitative and quantitative analyses of flavonoids in *Spirodela polyrrhiza* by High-performance liquid chromatography coupled with mass spectrometry. *Phytochemical analysis* **2011**, n/a-n/a.

133. Ferreres, F.; Silva, B. M.; Andrade, P. B.; Seabra, R. M.; Ferreira, M. A., Approach to the study of C-glycosyl flavones by ion trap HPLC-PAD-ESI/MS/MS: application to seeds of quince (Cydonia oblonga). *Phytochemical analysis* **2003,** 14, (6), 352-359.

134. Park, S. H.; Kim, H.; Rhyu, D. Y., Flavonoids from the stems of eastern picklypear *Opuntia humfusa*, *Cactaceae*. *Jounal of applied biological chemistry* **2007,** 50, (4), 254-258.

135. Seeram, N. P.; Lee, R.; Scheuller, H. S.; Heber, D., Identification of phenolic compounds in strawberries by liquid chromatography electrospray ionization mass spectroscopy. *Food chemistry* **2006,** 97, (1), 1-11.

136. Kim, H.; Park, S. H., Metabolic profiling and fiscrimination of two cacti cultivated in Korea using HPLC-ESI-MS and multivariate statistical analysis. *Journal of korean society of applied biological chemistry* **2009,** 52, (4), 346-352.

137. Lee, J.; Mitchell, A. E., Quercetin and isorhamnetin glycosides in onion (*Allium cepa* L.): varietal comparison, physical distribution, coproduct evaluation, and long-term storage stability. *Journal of agricultural and food chemistry* **2011,** 59, (3), 857-863.

138. Gariboldi, E.; Mascetti, D.; Galli, G.; Caballion, P.; Bosisio, E., LC-UV-Electrospray-MS-MS mass spectrometry analysis of plant constituents inhibiting xanthine oxidase. *Pharmaceutical research* **1998,** 15, (6), 936-943.

139. Gutzeit, D.; Wray, V.; Winterhalter, P.; Jerz, G., Preparative isolation and purification of flavonoids and protocatechuic acid from Sea Buckthorn juice concentrate (*Hippophaë rhamnoides* L. ssp. *rhamnoides*) by high-speed counter-current chromatography. *Chromatographia* **2007,** 65, (1), 1-7.

140. Bastos, D. H. M.; Saldanha, L. A.; Catharino, R. R.; Sawaya, A. C. H. F.; Cunha, I. B. S.; Carvalho, P. O.; Eberlin, M. N., Phenolic antioxidants identified by ESI-MS from yerba maté (*Ilex paraguariensis*) and green tea (*Camelia sinensis*) extracts. *Molecules* **2007,** 12, 423-432.

141. Pawlowska, A. M.; Oleszek, W.; Braca, A., Quali-quantitative analyses of flavonoids of *Morus nigra* L. and *Morus alba* L. (*Moraceae*) fruits. *Journal of agricultural and food chemistry* **2008,** 56, (9), 3377-3380.

142. Marín, A.; Ferreres, F.; Tomás-Barberán, F. A.; Gil, M. I., Characterization and quantitation of antioxidant constituents of sweet pepper (*Capsicum annuum* L.). *Journal of agricultural and food chemistry* **2004,** 52, (12), 3861-3869.

143. Piao, X. L.; Mi, X. Y.; Tian, Y. Z.; Wu, Q.; Piao, H. S.; Zeng, Z.; Wang, D.; Piao, X., Rapid identification and characterization of antioxidants from *Ligularia fischeri*. *Archives of pharmacal research* **2009,** 32, (12), 1689-94.

144. Shi, P.; He, Q.; Song, Y.; Qu, H.; Cheng, Y., Characterization and identification of isomeric flavonoid O-diglycosides from genus Citrus in negative electrospray ionization by ion trap mass spectrometry and time-of-flight mass spectrometry. *Analytica chimica acta* **2007,** 598, (1), 110-118.

145. Wang, H.; Feng, F., Identification of components in Zhi-Zi-Da-Huang decoction by HPLC coupled with electrospray ionization tandem mass spectrometry, photodiode array and fluorescence detectors. *Journal of pharmaceutical and biomedical analysis* **2009,** 49, (5), 1157-1165.

146. Amico, V.; Napoli, E. M.; Renda, A.; Ruberto, G.; Spatafora, C.; Tringali, C., Constituents of grape pomace from the Sicilian cultivar `*Nerello Mascalese*'. *Food chemistry* **2004,** 88, (4), 599-607.

147. Jerz, G.; Gutzeit, D.; Winterhalter, P., Characterization of acylated flavonoid glycosides from Sea Buckthorn (*Hippophaë rhamnoides*) juice concentrate by preparative HSCCC/ESI-MS-MS. In *Flavor and health benefits of small fruits*, American Chemical Society: 2010; Vol. 1035, pp 253-265.

148. Hessler, J. A.; Budor, A.; Putchakayala, K.; Mecke, A.; Rieger, D.; Banaszak Holl, M. M.; Orr, B. G.; Bielinska, A.; Beals, J.; Baker, J., Atomic force microscopy study of early morphological changes during apoptosis. *Langmuir* **2005,** 21, (20), 9280-9286.

149. Häcker, G., The morphology of apoptosis. *Cell and tissue research* **2000,** 301, (1), 5-17.

150. Patel, T.; Gores, G. J.; Kaufmann, S. H., The role of proteases during apoptosis. *The FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **1996,** 10, (5), 587-97.

151. Martin-Renedo, J.; Mauriz, J. L.; Jorquera, F.; Ruiz-Andres, O.; Gonzalez, P.; Gonzalez-Gallego, J., Melatonin induces cell cycle arrest and apoptosis in hepatocarcinoma HepG2 cell line. *Journal of pineal research* **2008,** 45, (4), 532-40.

152. Hengartner, M. O., The biochemistry of apoptosis. *Nature* **2000,** 407, (6805), 770-6.

153. Jänicke, R. U.; Sprengart, M. L.; Wati, M. R.; Porter, A. G., Caspase-3 Is Required for DNA Fragmentation and Morphological Changes Associated with Apoptosis. *Journal of biological chemistry* **1998,** 273, (16), 9357-9360.

154. Blair, R. A., *An Assessment of Agricultural Risk and Diversification on Farming Familie's Living Standard under Fuzzy Conditions: A case from Guyana*. Margraf Verlag: Weikersheim, 2007; Vol. 90.

155. Finco, M. V. A.; Finco, F. D. B. A., Biodiesel and the regional sustainable development: impact on farm income and food security in northern Brazil. *Segurança alimentar e nutricional* **2011,** 18, 21-30.

156. Finco, M. V. A.; Doppler, W., *Jatropha curcas* and *Ricinus communis* in Brazilian Cerrado: a farming and rural systems economics approach. *Informe GEPEC (Online)* **2009,** 13, 56-65.

157. IBGE *Pesquisa nacional de amostra de domicílios (PNAD)/ National Household Survey*; Rio de Janeiro, 2004.

158. Doppler, W., Contribution of the farming systems approach to regional food security and rural infrastructure. In *Regional food security and rural infrastructure*, Thimm, H. U.; Hahn, H.; Reinhard, I.; Schulz, D., Eds. LIT Verlag: Münster-Hamburg, 1993; Vol. I.

159. Fine, E. J.; Feinman, R. D., Thermodynamics of weight loss diets. *Nutrition & metabolism* **2004,** 1, (1), 15.

160. Dos Anjos, L. A.; Ferreira, B. C.; de Vasconcellos, M. T.; Wahrlich, V., Energy expenditure of adults in the city of Niterói, state of Rio de Janeiro: nutrition, physical activity and health survey--PNAFS. *Ciência e saúde coletiva* **2008,** 13, (6), 1775-84.

161. Velasquez-Melendez, G.; Gazzinelli, A.; Correa-Oliveira, R.; Pimenta, A. M.; Kac, G., Prevalence of metabolic syndrome in a rural area of Brazil. *São Paulo medical journal* **2007,** 125, (3), 155-62.

162. Marinho, S. P.; Martins, I. S.; Perestrelo, J. P. P.; Oliveira, D. C. d., Obesidade em adultos de segmentos pauperizados da sociedade. *Revista de nutrição* **2003,** 16, 195-201.

163. Velasquez-Melendez, G.; Martins, I. S.; Cervato, A. M.; Fornes, N. S.; Marucci, M. F.; Coelho, L. T., Relationship between stature, overweight and central obesity in the adult population in Sao Paulo, Brazil. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity* **1999,** 23, (6), 639-44.

164. Han, T. S.; Feskens, E. J.; Lean, M. E.; Seidell, J. C., Associations of body composition with type 2 diabetes mellitus. *Diabetic medicine : a journal of the british diabetic association* **1998,** 15, (2), 129-35.

165. Carey, V. J.; Walters, E. E.; Colditz, G. A.; Solomon, C. G.; Willett, W. C.; Rosner, B. A.; Speizer, F. E.; Manson, J. E., Body fat distribution and risk of non-insulin-dependent diabetes mellitus in women. The Nurses' Health Study. *American journal of epidemiology* **1997,** 145, (7), 614-9.

166. McKeigue, P. M.; Shah, B.; Marmot, M. G., Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. *Lancet* **1991,** 337, (8738), 382-6.

167. Hassapidou, M.; Fotiadou, E.; Maglara, E.; Papadopoulou, S. K., Energy intake, diet composition, energy expenditure, and body fatness of adolescents in northern Greece. *Obesity* **2006,** 14, (5), 855-62.

168. Subar, A. F.; Kipnis, V.; Troiano, R. P.; Midthune, D.; Schoeller, D. A.; Bingham, S.; Sharbaugh, C. O.; Trabulsi, J.; Runswick, S.; Ballard-Barbash, R.; Sunshine, J.; Schatzkin, A., Using intake biomarkers to evaluate the extent of dietary misreporting in a large sample of adults: the OPEN study. *American journal of epidemiology* **2003,** 158, (1), 1-13.

169. WHO *Energy and protein requirements: Report of a joint FAO/WHO/UNU expert consultation.* Geneva, 1985.

170. Sanchez-Castillo, C. P.; Lara, J. J.; Villa, A. R.; Aguirre, J.; Escobar, M.; Gutierrez, H.; Chavez, A.; James, W. P., Unusually high prevalence rates of obesity in four Mexican rural communities. *European journal of clinical nutrition* **2001,** 55, (10), 833-40.

171. Hill, J. O., Understanding and addressing the epidemic of obesity: an energy balance perspective. *Endocrine reviews* **2006,** 27, (7), 750-61.

172. Yang, E. J.; Kerver, J. M.; Park, Y. K.; Kayitsinga, J.; Allison, D. B.; Song, W. O., Carbohydrate intake and biomarkers of glycemic control among US adults: the third National Health and Nutrition Examination Survey (NHANES III). *American journal of clinical nutrition* **2003,** 77, (6), 1426-33.

173. Wu, T.; Giovannucci, E.; Pischon, T.; Hankinson, S. E.; Ma, J.; Rifai, N.; Rimm, E. B., Fructose, glycemic load, and quantity and quality of carbohydrate in relation to plasma C-peptide concentrations in US women. *American journal of clinical nutrition* **2004,** 80, (4), 1043-9.

174. Flood, A.; Peters, U.; Jenkins, D. J.; Chatterjee, N.; Subar, A. F.; Church, T. R.; Bresalier, R.; Weissfeld, J. L.; Hayes, R. B.; Schatzkin, A., Carbohydrate, glycemic index, and glycemic load and colorectal adenomas in the Prostate, Lung,

Colorectal, and Ovarian Screening Study. *American journal of clinical nutrition* **2006,** 84, (5) 1184-92.

175. Florencio, T. T.; Ferreira, H. S.; Cavalcante, J. C.; Luciano, S. M.; Sawaya, A. L., Food consumed does not account for the higher prevalence of obesity among stunted adults in a very-low-income population in the Northeast of Brazil (Maceio, Alagoas). *European journal of clinical nutrition* **2003,** 57, (11), 1437-1446.

176. Gazzaniga, J. M.; Burns, T. L., Relationship between diet composition and body fatness, with adjustment for resting energy expenditure and physical activity, in preadolescent children. *American journal of clinical nutrition* **1993,** 58, (1), 21-8.

177. Miller, W. C.; Lindeman, A. K.; Wallace, J.; Niederpruem, M., Diet composition, energy intake, and exercise in relation to body fat in men and women. *American journal of clinical nutrition* **1990,** 52, (3), 426-30.

178. Matos, A. C.; Ladeia, A. M., Assessment of cardiovascular risk factors in a rural community in the Brazilian state of Bahia. *Arquivos brasileiro de cardiologia* **2003,** 81, 297-302.

179. Willett, W. C., Dietary fat and obesity: an unconvincing relation. *American journal of clinical nutrition* **1998,** 68, (6), 1149-50.

180. Roberts, S. B.; Pi-Sunyer, F. X.; Dreher, M.; Hahn, R.; Hill, J. O.; Kleinman, R. E.; Peters, J. C.; Ravussin, E.; Rolls, B. J.; Yetley, E.; Booth, S. L., Physiology of fat replacement and fat reduction: effects of dietary fat and fat substitutes on energy regulation. *Nutrition reviews* **1998,** 56, (5 Pt 2), S29-41; discussion S41-9.

181. Sawaya, A. L.; Grillo, L. P.; Verreschi, I.; da Silva, A. C.; Roberts, S. B., Mild stunting is associated with higher susceptibility to the effects of high fat diets: studies in a shantytown population in São Paulo, Brazil. *The journal of nutrition* **1998,** 128, (2 Suppl), 415S-420S.

182. Leite de Vasconcellos, M. T.; Portela, M. C., Body mass index and its relationship to nutritional and socioeconomic variables: a linear regression approach to a Brazilian adult sub-population. *Cadernos de saúde pública* **2001,** 17, (6), 1425-36.

183. Ludwig, D. S., The glycemic index: Physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. *JAMA: The Journal of the american medical association* **2002,** 287, (18), 2414-2423.

184. Newby, P. K.; Muller, D.; Hallfrisch, J.; Qiao, N.; Andres, R.; Tucker, K. L., Dietary patterns and changes in body mass index and waist circumference in adults. *American journal of clinical nutrition* **2003,** 77, (6), 1417-25.

185. Toeller, M.; Buyken, A. E.; Heitkamp, G.; Cathelineau, G.; Ferriss, B.; Michel, G., Nutrient intakes as predictors of body weight in european people with type 1 diabetes. *International journal of obesity and related metabolic disorders : journal of the international association for the study of obesity* **2001,** 25, (12), 1815-22.

186. Bouche, C.; Rizkalla, S. W.; Luo, J.; Vidal, H.; Veronese, A.; Pacher, N.; Fouquet, C.; Lang, V.; Slama, G., Five-week, low-glycemic index diet decreases total fat mass and improves plasma lipid profile in moderately overweight nondiabetic men. *Diabetes care* **2002,** 25, (5), 822-8.

187. Heitmann, B. L.; Lissner, L.; Sorensen, T. I.; Bengtsson, C., Dietary fat intake and weight gain in women genetically predisposed for obesity. *American journal of clinical nutrition* **1995,** 61, (6), 1213-7.

188. Gisela P.M. Barreto, M. T. B. a. A. Z. M., Bioactive compounds from several tropical fruits and correlation by multivariate analysis to rree radical scavenger activity. *Journal of the brazilian chemical society* **2009,** 20, 1856-1861.

189. Abadio Finco, F. D. B.; Kammerer, D. R.; Carle, R.; Tseng, W.-H.; Böser, S.; Graeve, L., Antioxidant Activity and Characterization of Phenolic Compounds from

Bacaba (Oenocarpus bacaba Mart.) Fruit by HPLC-DAD-MSn. *Journal of agricultural and food chemistry* **2012**.

190. Thaipong, K.; Boonprakob, U.; Crosby, K.; Cisneros-Zevallos, L.; Hawkins Byrne, D., Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of food composition and analysis* **2008,** 19, (6-jul), 669-675.

191. Wu, X.; Beecher, G. R.; Holden, J. M.; Haytowitz, D. B.; Gebhardt, S. E.; Prior, R. L., Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of agricultural and food chemistry* **2004,** 52, (12), 4026-4037.

192. Sariburun, E.; Sahin, S.; Demir, C.; Cihat, T.; Uylaser, V., Phenolic Content and Antioxidant Activity of Raspberry and Blackberry Cultivar. *Journal of Food Science* **2010,** 74, 328-335.

193. Cevallos-Casals, B. A.; Cisneros-Zevallos, L., Stoichiometric and kinetic studies of phenolic antioxidants from andean purple corn and red-fleshed sweetpotato. *Journal of agricultural and food chemistry* **2003,** 51, 3313−3319

194. Prior, R. L.; Wu, X.; Schaich, K., Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of agricultural and food chemistry* **2005,** 53, (10), 4290-302.

195. Benzie, I. F.; Strain, J. J., The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* 1996, pp 70-76.

196. Ou, B.; Hampsch-Woodill, M.; Flanagan, J.; Deemer, E. K.; Prior, R. L.; Huang, D., Novel fluorometric assay for hydroxyl radical prevention capacity using fluorescein as the probe. *Journal of agricultural and food chemistry* **2002,** 50, (10), 2772-2777.

197. Huang, D.; Ou, B.; Prior, R. L., The chemistry behind antioxidant capacity assays. *Journal of agricultural and food chemistry* **2005,** 53, (6), 1841-56.

198. Seeram, N. P.; Aviram, M.; Zhang, Y.; Henning, S. M.; Feng, L.; Dreher, M.; Heber, D., Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. *Journal of agricultural and food chemistry* **2008,** 56, (4), 1415-22.

199. Blando, F.; Gerardi, C.; Nicoletti, I., Sour Cherry (*Prunus cerasus* L.) anthocyanins as ingredients for functional foods. *Journal of biomedicine and biotechnology* **2004,** 5, 253-258.

200. Schauss, A. G., Açaí (*Euterpe oleracea* Mart.): A macro and nutrient rich palm fruit from the Amazon Rain Forest with demonstrated bioactivities *In Vitro* and *In Vivo*. In *Bioactive foods in promoting health*, Ronald Ross, W.; Victor, R. P., Eds. Academic Press: San Diego, 2010; pp 479-490.

201. Khanavi, M.; Saghari, Z.; Mohammadirad, A.; Khademi, R.; Hadjiakhoondi, A.; Abdollahi, M., Comparison of antioxidant activity and total phenols of some date varieties. *DARU - Journal of faculty of pharmacy, Tehran university of medical sciences* **2009,** 17, (2), 104-108.

202. C. Vijaya Kumar Reddy, D. S., M. Raghunath, Antioxidant activity of fresh and dry fruits commonly consumed in India. *Food research international* **2010,** 43 285–288.

203. Khanavi M, S. Z., Mohammadirad A., Khademi R., Hadjiakhoondi A., Abdollahi M. , Comparison of antioxidant activity and total phenols of some date varieties *DARU* **2009,** Vol. 17, 104-108.

204. Percival, S. S.; Talcott, S. T.; Chin, S. T.; Mallak, A. C.; Lounds-Singleton, A.; Pettit-Moore, J., Neoplastic transformation of BALB/3T3 cells and cell cycle of HL-60 Cells are inhibited by Mango (*Magnifera indica* L.) juice and mango juice extracts. *Journal of nutrition* 2006, pp 1300-1304.

205. Song, W.; Derito, C. M.; Liu, M. K.; He, X.; Dong, M.; Liu, R. H., Cellular antioxidant acitivity of common vegetables. *Journal of agriculture and food chemistry* 2010, pp 6621-6629.

206. Mosmann, T., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of immunological methods* 1983, pp 55-63.

207. Bruggisser, R.; Von Daeniken, K.; Jundt, G.; Schaffner, W.; Tullberg-Reinert, H., Interference of plant extracts, phytoestrogens and antioxidants with the MTT tetrazolium assay. *Planta medica* 2002, pp 445-448.

208. Liu, Y.; Peterson, D. A.; Kimura, H.; Schubert, D., Mechanism of Cellular 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) Reduction. *J. Neurochem.* 1997, pp 581-593.

209. Bernhard, D.; Schwaiger, W.; Crazzolara, R.; Tinhofer, I.; Kofler, R.; Csordas, A., Enhanced MTT-reducing activity under growth inhibition by resveratrol in CEM-C7H2 lymphocytic leukemia cells. *Cancer letters* **2003,** 195, (2), 193-199.

210. Berridge, M. V.; Tan, A. S., Characterization of the Cellular Reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT): Subcellular Localization, Substrate Dependence, and Involvement of Mitochondrial Electron Transport in MTT Reduction. *Archives of biochemistry and biophysics* **1993,** 303, (2), 474-482.

211. Liu, Y.; Peterson, D. A.; Kimura, H.; Schubert, D., Mechanism of cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction. *Journal of neurochemistry* **1997,** 69, (2), 581-593.

212. Pagliacci, M. C.; Smacchia, M.; Migliorati, G.; Grignani, F.; Riccardi, C.; Nicoletti, I., Growth-inhibitory effects of the natural phyto-oestrogen genistein in MCF-7 human breast cancer cells. *European journal of cancer* **1994,** 30, (11), 1675-1682.

213. Pagliacci, M. C.; Spinozzi, F.; Migliorati, G.; Fumi, G.; Smacchia, M.; Grignani, F.; Riccardi, C.; Nicoletti, I., Genistein inhibits tumour cell growth *in vitro* but enhances mitochondrial reduction of tetrazolium salts: A further pitfall in the use of the MTT assay for evaluating cell growth and survival. *European journal of cancer* **1993,** 29, (11), 1573-1577.

214. Liu, W.; Dalgleish, A., MTT assays can underestimate cell numbers. *Cancer chemotherapy and pharmacology* **2009,** 64, (4), 861-862.

215. Sims, J.; Plattner, R., MTT assays cannot be utilized to study the effects of STI571/Gleevec on the viability of solid tumor cell lines. *Cancer chemotherapy and pharmacology* **2009,** 64, (3), 629-633.

216. Hanauske, A. R., *In vitro* assays for antitumour activity: more pitfalls to come? *European journal of cancer* **1993,** 29A, (11), 1502-3.

217. Virág, L.; Kerékgyáró, C.; Fachet, J., A simple, rapid and sensitive fluorimetric assay for the measurement of cell-mediated cytotoxicity. *Journal of immunological methods* **1995**, (185), 199-208.

218. Fernandes, I.; Faria, A.; Azevedo, J.; Soares, S.; Calhau, C. a. o.; De Freitas, V.; Mateus, N., Influence of anthocyanins, derivative pigments and other catechol and pyrogallol-type phenolics on breast cancer cell proliferation. *Journal of agricultural and food chemistry* **2010,** 58, (6), 3785-3792.

219. Johnson, J. L.; Bomser, J. A.; Scheerens, J. C.; Giusti, M. M., Effect of black Raspberry (*Rubus occidentalis* L.) extract bariation conditioned by cultivar, production site, and fruit maturity stage on colon cancer cell proliferation. *Journal of agricultural and food chemistry* **2011,** 59, (5), 1638-1645.

220. Felice, D. L.; Sun, J.; Liu, R. H., A modified methylene blue assay for accurate cell counting. *Journal of functional foods* **2009**, (I), 109-118.

221. Manson, M. M.; Farmer, P. B.; Gescher, A.; Steward, W. P., Innovative Agents in Cancer PreventionTumor Prevention and Genetics III. In Senn, H.-J.; Morant, R., Eds. Springer Berlin Heidelberg: 2005; Vol. 166, pp 257-275.

222. Greenwald, P.; Clifford, C. K.; Milner, J. A., Diet and cancer prevention. *European journal of cancer* **2001,** 37, (8), 948-965.

223. Delhalle, S.; Duvoix, A.; Schnekenburger, M.; Morceau, F.; Dicato, M.; Diederich, M., An introduction to the molecular mechanisms of apoptosis. *Annals of the New York academy of sciences* **2003,** 1010, (1), 1-8.

224. Ziedan, N. I.; Kadri, H.; Westwell, A. D., The development of pro-apoptotic cancer therapeutics. *Mini reviews in medicinal chemistry* **2008,** 8, (7), 711-8.

225. Enari, M.; Sakahira, H.; Yokoyama, H.; Okawa, K.; Iwamatsu, A.; Nagata, S., A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature* **1998,** 391, (6662), 43-50.

226. Janicke, R. U.; Ng, P.; Sprengart, M. L.; Porter, A. G., Caspase-3 is required for alpha-fodrin cleavage but dispensable for cleavage of other death substrates in apoptosis. *The Journal of biological chemistry* **1998,** 273, (25), 15540-5.

227. Li, L. Y.; Luo, X.; Wang, X., Endonuclease G is an apoptotic DNase when released from mitochondria. *Nature* **2001,** 412, (6842), 95-99.

228. Salvioli, S.; Barbi, C.; Dobrucki, J.; Moretti, L.; Pinti, M.; Pedrazzi, J.; Pazienza, T. L.; Bobyleva, V.; Franceschi, C.; Cossarizza, A., Opposite role of changes in mitochondrial membrane potential in different apoptotic processes. *FEBS letters* **2000,** 469, (2-3), 186-90.

229. Chung, K.-S.; Choi, J.-H.; Back, N.-I.; Choi, M.-S.; Kang, E.-K.; Chung, H.-G.; Jeong, T.-S.; Lee, K.-T., Eupafolin, a flavonoid isolated from Artemisia princeps, induced apoptosis in human cervical adenocarcinoma HeLa cells. *Molecular nutrition & food research* **2010,** 54, (9), 1318-1328.

230. Chou, C.-C.; Yang, J.-S.; Lu, H.-F.; Ip, S.-W.; Lo, C.; Wu, C.-C.; Lin, J.-P.; Tang, N.-Y.; Chung, J.-G.; Chou, M.-J.; Teng, Y.-H.; Chen, D.-R., Quercetin-mediated cell cycle arrest and apoptosis involving activation of a caspase cascade through the mitochondrial pathway in human breast cancer MCF-7 cells. *Archives of pharmacal research* **2010,** 33, (8), 1181-1191.

231. Hengartner, M. O., *Nature* **2000,** 407, (null), 770.

232. Boyce, M.; Degterev, A.; Yuan, J., Caspases: an ancient cellular sword of Damocles. *Cell death and differentiation* **2004,** 11, (1), 29-37.

233. Sohn, D.; Schulze-Osthoff, K.; Janicke, R. U., Caspase-8 can be activated by interchain proteolysis without receptor-triggered dimerization during drug-induced apoptosis. *The journal of biological chemistry* **2005,** 280, (7), 5267-73.

234. Jänicke, R., MCF-7 breast carcinoma cells do not express caspase-3. *Breast cancer research and treatment* **2009,** 117, (1), 219-221.

235. Mc Gee, M. M.; Hyland, E.; Campiani, G.; Ramunno, A.; Nacci, V.; Zisterer, D. M., Caspase-3 is not essential for DNA fragmentation in MCF-7 cells during apoptosis induced by the pyrrolo-1,5-benzoxazepine, PBOX-6. *FEBS letters* **2002,** 515, (1-3), 66-70.

236. Loegering, D. A.; Ruchaud, S.; Earnshaw, W. C.; Kaufmann, S. H., Evaluation of the role of caspase-6 in anticancer drug-induced apoptosis. *Cell death and differentiation* **2005,** 13, (2), 346-347.

237. Bernhard, D.; Tinhofer, I.; Tonko, M.; Hubl, H.; Ausserlechner, M. J.; Greil, R.; Kofler, R.; Csordas, A., Resveratrol causes arrest in the S-phase prior to Fas-independent apoptosis in CEM-C7H2 acute leukemia cells. *Cell death and differentiation* **2000,** 7, (9), 834-42.

238. Ekert, P. G.; Silke, J.; Vaux, D. L., Caspase inhibitors. *Cell death and differentiation* **1999,** 6, (11), 1081-6.

239. Bharti, A. C.; Takada, Y.; Aggarwal, B. B., PARP cleavage and caspase activity to assess chemosensitivity. *Methods in molecular medicine* **2005,** 111, 69-78.

9. Abbreviations

%	Per cent
<	Is less than
=	Is equal to
>	Is higher than
∫	Integral
°C	Degree Celsius
$\Delta\Psi_m$	Mitochondrial membrane potential
$\chi 2$	Chi-square test
µ	Micro
µg	Microgramm
µL	Microliter
A	Absorbance
AAPH	2,2`-azobis(2-amidino-propane)dihydrochloride
ABAP	2,2‘-azobis (2-amidipropane)
ABTS	2,2’-azinobis (3-ethyl-benzothiazoline-6-sulfonate)
Ac-VEID-AFC	Acetyl-Val-glu-ile-asp-7-amino-4-trifluoromethylcoumarin
AIF	Apoptosis inducing factor
$AlCl_3$	Aluminum chloride
ANOVA	Analysis of variance
AO	Acridine orange
APA	Environmental preservation area (*Área de Preservação Ambiental*)
APAF-1	Apoptotic protease activating factor 1
APS	Ammoiumpersulfate solution
Aubl.	Jean Baptiste Christophore Fusée Aublet (botanist)
AUC	Area under curve
BD	Benton Dickson
BMI	Body Mass Index
BMR	Basic metabolic rate

BSA	Bovine serum albumine
CAA	Cellular antioxidant capacity
CAD	Caspase-activated deoxyribonuclease
CAPS	N-cyclohexyl-3-aminopropanesulfonic acid
CAQ	Cellular antioxidant quality
Caspase	Cysteine-aspartic proteases
CID	Collision induced dissociation
cm	centimeters
CNS	National Health Board (*Conselho Nacional de Saúde*)
CNSAN	National conference of food and nutrition security (*Conferência Nacional de Segurança Alimentar e Nutricional*)
CO_2	Carbon dioxide
CTE	Catechin equivalent
cv.	Cultivar
Cyn-3-glcEq	Cyanidin-3-glucoside equivalent
DCFH-DA	2'-7'-dichlorofluorescein di-acetate
dd H_2O	double distilled water
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethylsulfoxid
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
DTT	Dithiothreitol
DW	Dry weight
e.g.	*exempli gratia* (for example)
EB	Energy balance
EB	Ethidium bromide
EBIA	Brazilian scale of food and nutrition security (*Escala Brasileira de Insegurança Alimentar*)
EC_{50}	half maximal effective concentration
ECL	Enhanced Chemiluminescence
EDTA	Ethylene-Diamine-Tetraacetic Acid

Eq.	equation
ESI-MS	electrospray ionization mass spectrometry
et al.	*et alii*
FACS	Fluorescence-activated cell sorting
FC	Folin-Ciocalteau
FCS	Fetal calf serum
FeSO4.7H2O	ferrous sulfate heptahydrate
FFQ	Food frequency questionnaire
FITC	Fluorescein isothiocyanate
FITC-Z-LEHD-FMK	Z-Leu-Glu(OMe)-His-Asp(OMe)-fluoromethyketone conjugated do FITC
FNS	Food and nutrition security
FNS	Food and nutrition security
FOSHU	Foods for specified health use
FRAP	Ferric reducing ability of plasma assay
FSC	Forward side scatter
FUFOSE	European Commission Concerted Action on Functional Food Science in Europe
FW	Fresh weight
g	Gramm
g	Gravitational acceleration
GAE	Gallic acid equivalent
GDP	Gross domestic product
GIZ	German agency for International cooperation (Deutsche *Gesellschaft für Internationale Zusammenarbeit***)**
h	hour
HAT	Hydrogen atom transfer
HBSS	Hank’s Balanced Salted Solution
HCL	Hydrochloric acid
HeLa	Cervical cancer cell line
HepG2	Human liver cancer cells

HPLC	High-performance liquid chromatography
i.e.	*id est* (that is)
IC_{50}	half maximal inhibitory concentration
ICAD	Inhibitor of caspase-activated deoxyribonuclease
ILSI	International Life Science Institute
JC-1	5,5’ ,6,6’-tetrachloro-1,1’ , 3,3’ – tetraethylbenzimidazole carbocyanide iodide
Kcal	Kilocalorie
KDa	KiloDalton
Kg	Kilograms
Km	Kilometers
L.	Carl Linnaeus (botanist)
LC	Liquid chromatography
Ln	Natural logarithm
M	Molar
m^2	Square meter
mA	Milli-Ampère
Mart.	Carl Friedrich Philipp von Martius (botanist)
MB	Methylene blue
MCF	Michigan Cancer Foundation
mg	Milligramm
min	Minute
MJ	Megajoule
mL	Milliliter
mm	Millimeters
mM	Millimolar
MS^n	Mass Spectrometry to the N-th Power
MTT	3,-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-tetrazolium bromide
MUH	4-Methylumbelliferyl heptanoate
n	Sample size (statistics)
Na_2HPO_4	Disodium hydrogen phosphate

Na_3VO_4	Sodium ortho-vanadate
NaF	Sodium fluoride
NaN_3	Sodium azide
$NaNO_2$	Sodium nitrite
NCD	Non communicable Diseases
nm	Nanometer
nM	Nanomolar
ORAC	Oxygen radical absorbance capacity
p	Significant probability
P/S	Penicillin-/Streptomycin-Solution
PAL	Physical activity level
PARP	Poly (ADP-Ribose) polymerase
PBS	Phosphate-buffered Saline
PCB	Phosphate citrate buffer
Pers.	Christian Hendrik Persoon (botanist)
pH	potential hydrogen
PI	Propidium iodide
PMSF	Phenyl-methyl-sulfonyl fluoride
PNAD	Brazilian national household survey (*Pesquisa Nacional por Amostra de Domicílios*)
PNPSB	National plan for promotion of sociobiodiversity product chains (*Plano Nacional das Cadeias de produtos da Sociobiodiversidade*)
PRONAF	National Program for the Family Agriculture (*Programa Nacional de Agricultura Familiar*)
PVDF	Polyvinyliden-Difluorid
QE	Quercetin Equivalent
R^2	Coefficient of determination
Rich.	Louis Claude Richard (botanist)
RP-HPLC	Reversed phase high performance liquid chromatography
rpm	Rounds per minute

s	Second
SD	Standard deviation
SDS	Sodiumdodecylsulfate
SDS-PAGE	Sodiumdodecylsulfate-Polyacrylamide-Gelelectrophoresis
SE	Standard error
see	Standard error of estimate
SET	Single electron transfer
SIT 571	Imatinib (4-[(4-methylpiperazin-1-yl)methyl]-N-(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)benzamide)
SPSS	Statistical package for the social sciences
TACO	Brazilian Food Composition Table (*Tabela Brasileira de Composição de Alimentos*)
TB	Trypan blue
TBS	Tris-buffered Saline
TBST	Tris-buffered Saline Tween-20
TE	Trolox equivalent
TEAC	Trolox equivalent antioxidant capacity assay
TEE	Total energy expenditure
TNF-alpha	Tumor necrosis factor-alpha
TPTZ	*2*,*4*,*6*-tris(*2*-*pyridyl*)-s-triazine
Tris Base	Tris(hydroxy)aminomethane
Tris-HCl	Tris(hydroxy)aminomethane-Hydrochloride
Trolox	6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid
UV	Ultraviolet
v	Volume
WC	Waist circumference
WHO	World Health Organization
Z-IETD-AFC	Z-Ile-Glu-Thr-Asp7-amido-4-trifluoromethylcoumarin

10. Curriculum Vitae

Fernanda Abadio Finco[4]

Personal Data

Full Name: Fernanda Dias Bartolomeu Abadio Finco

Name in Bibliographic Citation: ABADIO, F.D.B;
ABADIO FINCO, F.D.B;
FINCO, F.D.B.A.

E-mail: fabadio@gmail.com;
Date of birth: 7th October, 1977
Birthplace: Rio de Janeiro, Brazil
Citizenship: Brazilian
Marital Status: Married; no children.

[4] The current *Curriculum vitae* is constantly updated and permanently available in the Brazilian database of scientists (Lattes Platform) in the following website: http://buscatextual.cnpq.br/buscatextual/visualizacv.do?id=N727032

Education

2007 - 2012 - PhD

Institute of Biological Chemistry and Nutrition (140c), University of Hohenheim, Germany.

2001 - 2003 - Master in Food Science and Technology

Federal Rural University of Rio de Janeiro, UFRRJ, Brazil.

1996 - 2000 - B.Sc. Nutrition

Federal University of Rio de Janeiro, UFRJ, Brasil.

Research Areas

Molecular nutrition, Functional foods, Traditional Foods, Food and nutrition security.

Professional Experience

Since 2003 - Federal University of Tocantins (UFT), Brazil.
Position: Lecturer (Professor Assistente IV)

Awards

2012 - Bunge Foundation, Category: Youth scientist in Food and Nutritional Security
2010 - Rosani Cunha Award, Brazilian Ministry of Social Development
2005 - I Scientific Initiation Meeting, Federal University of Tocantins (supervisor degree).
1999 - XXI Scientific Initiation Meeting, Federal University of Rio de Janeiro (bachelor student degree).

Periodical Reviewer

Food Security ISSN: 1876-4517
African Journal of Agricultural Research ISSN: 1991- 637X
Brazilian Journal of Food and Nutrition e- ISSN: 2179-4448 1
African Journal of Business Management ISSN: 1990-3839

Publications

Journal Articles

ABADIO FINCO, F.D.B.; KAMMERER, R. C.; TSENG, W.H.; BÖSER, S. GRAEVE, L. Antioxidant activity and Characterization of phenolic compounds from bacaba (*Oenocarpus bacaba* Mart.) fruit by HPLC-DAD-MS^n Jounal of Agricultural and Food Chemistry, *60* (31), pp 7665–7673, 2012.

ABADIO FINCO, F.D.B. ; BOSER, S. ; Graeve, L. Antiproliferative activity of Bacaba (*Oenocarpus bacaba*) and Jenipapo (*Genipa americana L*.) phenolic extracts: a comparison of assays. Nutrition & Food Science, v. 43 n.2, 2012.

ABADIO FINCO, F.D.B. ; SILVA, I. G. ; OLIVEIRA, Renata Botelho. Physicochemical characteristics and antioxidant activity of three native fruits from Brazilian Savannah (Cerrado). Alimentos e Nutrição (UNESP. Marília), v. 23, n. 2 p. 1-4, 2012.

FINCO, M. V. A.; ABADIO FINCO, F.D.B. Biodiesel and the regional sustainable development: impacts on farm income and food security in northern Brazil. Segurança Alimentar e Nutricional, v. 18, p. 21-30, 2011.

FINCO, M. V. A.; ABADIO FINCO, F.D.B. The consumer willingness to pay for food services: an analysis of the Popular Restaurant Program in northern Brazil. Teoria e Evidencia Economica (UPF), v. 16, p. 350, 2011.

ABADIO FINCO, F.D.B.; MOURA, L. L.; SILVA, I. G. Physical and chemical properties of Honey from *Apis Mellifera L*. produced in South Tocantins State. Ciência e Tecnologia de Alimentos (Impresso), v. 27, p. 49-52, 2010.

ABADIO FINCO, F.D.B; DELIZA, R.; ROSENTHAL, A.; SILVA, C. The Effect of Extrinsic Product Attributes of Pineapple Juice on Consumer Intention to Purchase. Journal of International Food & Agribusiness Marketing, v. 22, p. 125-142, 2010.

PAIXÃO, A. N.; ARAÚJO, A. F. V. ; ABADIO FINCO, F.D.B. ; RAMOS, F. Caracterização da Demanda por alimentos Artesanais: Uma aplicação do Método de Avaliação Contingente na valoração do selo de origem de Palmas - TO. Amazônia (Banco da Amazônia), v. 3, p. 7-25, 2007.

FINCO, M.V.A.; ABADIO FINCO, F.D.B. . A disposição a pagar (DAP) por serviços de alimentação: uma estimativa para os restaurantes populares de Palmas/TO. Cadernos de Economia (UNOESC), v. 11, 2007.

Submitted articles

Abadio Finco, F.D.B.; Tseng, W-H.; Böser, S.; Kammerer, D. R.; Carle, R.; Graeve, L.Determination of phenolic contents and antioxidant capacity of extracts from *Genipa americana* L. (“Jenipapo”) fruit.

Book Chapter

ABADIO FINCO, F.D.B.; FINCO, M. V. A.; COELHO, A. F. S. An Assessment of the Brazilian Popular Restaurant Program as an Integrated Action for Food Security and Health Promotion. *In:* Maddox A. Jones; Francisco E. Hernandez. (Org.). Food Security: Quality Management, Issues and Economic Implications. : Nova Publishers, 2012.

FINCO, M.; ABADIO FINCO, F.D.B. Em busca do modelo de Gestão Social: uma análise do Programa Popular de Restaurantes Solidários através do Método de Valoração Contingente. II Encontro Nacional de Pesquisadores em Gestão Social. 2008.

FINCO, M. V. A ; CANÇADO, A.C.; ABADIO, F.D.B. Pesquisa de avaliação da II Feira Estadual de Economia Solidária - PAFES/Tocantins - 2006. *In*: CANÇADO, Airton Cardoso; PEREIRA, José Roberto; SILVA JÚNIOR, Jeová Torres.. (Org.). Economia solidária, cooperativismo popular e autogestão: as experiências de Palmas/TO.. Palmas: NeSol, 2007, p. 279-310.

Research Projects

2012 - 2013 - NoPa Project: EcoNutrition, Bilateral Cooperation project between Universität Hohenheim and Federal University of Tocantins. Funding: DAAD/CAPES/GIZ. Position: Coordinator.

2007 - 2012 - Community Based Nutrition Strategies: Assessing Savannah fruits as a health promotion strategy in Tocantins, Brazil. University of Hohenheim and Federal University of Tocantins. Funding: Eiselen Foundation Ulm, Germany and ISFE, Switzerland.

2005 - 2007 - Evaluation of Solidary Restaurants Programme in Palmas city, Brazil as an Integrated Action of Health and Food Security.Federal University of Tocantins. Position: Coordinator Funding: CNPq (National Council of Scientific Research, Brazil);
2004 - 2006 - Evaluation of local foods as a tool to design a culture-based menu for the School Feeding Programme in Palmas – TO. Federal University of Tocantins, Position: Coordinator. Funding: CNPq (National Council of Scientific Research, Brazil).

2004 - 2006 - Evaluation of components from local diet as a prevention tool to Non Communicable diseases in Tocantins State. Federal University of Tocantins, Position: Coordinator. Funding: Brazilian Ministry of Health.
2004 - 2005 - Nutritional and Sensory Evaluation of Foods Offered at Schools in Palmas city, TO. Federal University of Tocantins, Position: Researcher.

2005 - 2006 - Physical and Chemical Properties of Honey from South Tocantins State. Federal University of Tocantins, Position: Coordinator.

2004 - 2005 - Evaluation of Native Fruits from Brazilian Savannah as Alternative to Novel Foods Development. Federal University of Tocantins, Position: Coordinator

2002 - 2003 – Sensory evaluation and consumer studies of food processed by emergent methods (high hidrostatic pressure). EMBRAPA Food Tecnhology (Brazilian Enterprise for Agricultural Research). Position: Master Student.

1998 - 2000 – Optimization of pineapple juice clarifying process (*Ananas comosus, L. Merr*) by microfiltration and ultrafiltration and identification of clarified juices. Federal Universtiy of Rio de Janeiro. Position: Bachelor Student.

Declaration of authorship

With this statement I declare, that I have independently completed the doctoral thesis entitled: "**Health enhancing traditional foods in Brazil: an interdisciplinary approach to food and nutritional security**". The resources are taken directly or indirectly from external sources which are properly marked.

08.08.2012, Stuttgart- Hohenheim,

Signature