Foods from the Forest: A Nutritional Analysis of Wild Plant Foods Used by the Baka Forager-Horticulturalists in Southeastern Cameroon

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2324-HS Research Master Thesis Archaeology Year 2, 1086THRSY

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Leiden, 05-12-2023

Final Version

Acknowledgements

I would like to thank dr. A. G. Henry for her continued support during the course of writing this thesis. The discussions that we have had over the last 1.5 year have undoubtedly been fruitful for the development of the research goals of this project, and for steering the project into the right directions. I would also like to thank dr. A. G. Henry for allowing me access to the Baka wild edible plant samples collected by Sandrine Gallois, during fieldwork in Cameroon in 2018, and for putting her trust in me for conducting the chemical analysis without having any prior experience in the lab. Furthermore, I would like to thank prof. V. Fogliano for allowing me to work in his Food Science lab at Wageningen University & Research. I greatly enjoyed my time in the lab and grew passionate for conducting chemical nutritional analyses. Furthermore, I would like to thank all the lab technicians from the Food Quality and Design department. Without their guidance and support, the chemical nutritional analysis would have never had become a reality. Lastly, I would like to thank my friends and family for their continued support.

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1. Introduction

1.1 Foods From the Forest

‘’*Life was better in the past* is a foundational and reoccurring idea in Western thought. Origin stories, from the lost Garden of Eden to the Golden Age of ancient Greece, describe a utopian past where humans lived in harmony with nature and were healthy and well nourished’’ (Figure 1.1) (Pontzer et al., 2018, p. 24). Today, a growing awareness of affluence together with a rise in mismatch diseases such as adult-onset diabetes, atherosclerosis, and gout, has garnered renewed public interest into the foraging lifestyle. In this sense, did *Homo sapiens* fabricate a clash between our evolutionary past and our modern behaviour and technology (Heying & Weinstein, 2021, p. 73)?

Figure 1.1: Wood engraving picturing the Garden of Eden, originally published in 1877. The image offers an artistic interpretation of humanity living in harmony with nature (from https://www.istockphoto.com/nl/vector/genesis-wood-engraving-published-in-1877-gm184971747-18870890).

The often romanticized past way of life is the hunter-gatherer lifestyle, which has allowed the genus *Homo* to thrive for 99.6% of its total time on Earth, until the advent of agriculture some 10,000 years ago (ka) (Cordain et al., 2000, p. 682; O'Keefe et al., 2010; Sahlins, 1998). Foraging refers exclusively to subsistence tactics reliant upon non-produced foodstuffs or other wild resources. These resources can be managed and preserved to a slight degree (Winterhalder, 1981, p. 13). Although the amount of hunter-gatherer societies is steadily decreasing through processes of globalisation (e.g., Gallois et al., 2020), many of the recent historical ones have been studied and documented into the ethnographic record. Since foraging societies provide the only data for human behaviour independent of agricultural influence they are crucial for broadening our understanding on the range of lifeways that humans have developed over the course of our existence. The diversity present in the subsistence behaviours of modern-day foragers allows us to develop more substantial models of human behavioural evolution (Marlowe, 2005). The complete spectrum of qualities maintained by foraging populations has had profound influence on the evolution of hominin behavioural capacity, morphologies, and social formations. Of particular interest is exploring those qualities or behaviours that are either shared or non-shared between individual hunter-gatherer societies (Winterhalder & Smith, 1981).

Interest in the relationships between culture and environment is longstanding. For decades, a focal point in archaeological and anthropological investigation has been the importance of interaction between culture and environment, frequently called subsistence strategy (Meggers, 1954). More specifically, a great area of interest is the dietary practice of our ancestors. Diet has been one of the main drivers in the origin, evolution, and behaviour of our species as it determines characteristics ranging from social behaviour, habitat choice, body size, and life history strategies (Teaford et al., 2023). Furthermore, changes in diet are commonly associated with key developments in our evolution such as brain expansion, cooperation, tool making, and family formation (Crittenden & Schnorr, 2017). However, a significant limitation of studying Pleistocene diet is the fragmentary nature of the archaeological record. Relying solely upon the archaeological record has been described as ''navigating in the vicinity of an iceberg: more than four-fifths of what is of interest is not visible'' (Teaford et al., 2023, p. 2). To overcome this problem, ethnographic and archaeological records have been combined to potentially elucidate our understanding of the influences of our species' dietary practice on our evolutionary trajectory (Winterhalder & Smith, 2000). It is broadly recognized that employing analogies of extant foragers to past humans can be problematic in nature. However, when studying evolutionary adaptations of humans we need to measure these traits in foraging societies instead of in agricultural populations (Marlowe, 2005).

As much as dietary composition and food choice were important during our past evolution, so are they still a core facet in all of the human societies today. Not only are ancient dietary practices relevant for archaeologists and anthropologists, even public health is frequently drawing upon our hunter-gatherer past to help explain and solve contemporary health issues. Nevertheless, extant hunter-gatherer behaviour differs from early hominin behaviour. The study of contemporary hunter-gatherers still gives us the best modern analogue available for potential models (Andrikopoulos, 2016; Chang & Nowell, 2016; Cordain et al., 2002; Cordain, 2012b; Österdahl et al., 2008; Pontzer et al., 2018; Tarantino et al., 2015). Hunter-gatherer diet can be recruited as a reference standard for the evolution of human nutrition (Crittenden & Schnorr, 2017) given that modern hunter-gatherers live a lifestyle most similar to that of our ancestors. Some authors have argued that taken in aggregate, the average diets of hunter-gatherers could represent the ideal human diet (Cordain, 2002, 2012; Cordain et al., 2000; Eaton & Konner, 1985; Wolf, 2010). Thus, nutritional and dietary studies on extant hunter-gatherers are of great relevance for understanding both past and present subsistence behaviours. Approaches vary from investigating fossil, archaeologic, or ethnographic records, alongside chemical nutrient analyses of plant and animal foods (Cordain et al., 2000). However, the variability among hunter-gatherer diets remains undervalued in these studies.

Most studies of foraging theory, ethnographic fieldwork, and dietary and nutritional analyses, have focused on foraging populations occupying the African plains and savannas (Henry et al., 2019). The focus on savanna-dwelling foraging societies has meant that other habitats have often been overlooked. In particular, the rainforest has been deemed as too hostile for human occupation, despite our closest living relatives, the chimpanzees, living almost exclusively in these regions (Collins & McGrew, 1988; Scerri et al., 2022; Van Leeuwen et al., 2020). Previous ethnographic research highlighted the inaccessible nature of the rainforest alongside a lack of abundant carbohydrate and protein resources (Bailey et al., 1989). In short, the requirements for human subsistence and significant technological developments were deemed absent (Roberts et al., 2015). However, the rainforest is anything but barren. Over half the world's existing animal and plant species reside within rainforests, making their homes in these oldest and most complex land-based ecosystems on Earth (Roberts & Petraglia, 2015).

Consequentially, significantly less nutritional studies concerned with populations occupying the African rainforests have been conducted. However, recent research argues for a key role of rainforest environments to the evolution of our genus (Mercader, 2002; Roberts et al., 2015; Roberts, 2022; Scerri et al., 2022). The African tropical rainforest is argued to have been integral to the out of Africa dispersal of *Homo erectus*, a species that had already dispersed to as far as Southeast Asia at around 1.2 million years ago (Ma). Moreover, forests in the Amazon and Congo Basin are argued to have served as refugia during extreme climatic conditions such as those present during the last glacial maximum (LGM) (Maley, 1991; Mercader, 2002; Roberts, 2022, p. 35; Thomas, 2000). The earliest hominin presence within African rainforests is documented by an isolated upper molar from Ishango, Democratic Republic of Congo, and is dated to c. 2 Ma (Orijemie, 2022). This region, the Congo Basin, offered a climatic refugia up to around 18 ka, during the terminal LGM (Maley, 1991, p. 87; Mercader, 2002; Roberts, 2022; Sato et al., 2012). Hence, rainforest-type environments and ecosystems have been shaped by human agency for millennia and must be included in the narratives and contexts of deep human history and human evolution (Scerri et al., 2022).

Currently, one of the societies occupying the Congo Basin rainforest is that of the Baka forager-horticulturalists, of which some 30,000 - 40,000 individuals live in Cameroon (Gallois et al., 2016; 2017; 2020; Sato et al., 2012; Yamauchi et al., 2000; Yasuoka, 2006). Though the Baka are slowly transitioning into a sedentary lifestyle similar to their Bantu neighbours due to pressure from government settlement processes (Gallois et al., 2016, 2017, 2020; Yamauchi et al., 2000; Yasuoka, 2006), they still partake in long-term foraging trips known as *molongos* (Sato et al., 2012; Yasuoka, 2006). They also rely heavily on a variety of foraged foods for their daily subsistence. Therefore, the Baka provide an excellent opportunity for investigating subsistence behaviour within groups that forage in an African rainforest-type environment. During a fieldwork trip in 2018, a range of wild edible plant foods were voluntarily gifted by the Baka living in the villages of Bizam, Bosquet, Elonda, and Kungu (Figure 1.2). The plants were gifted for botanical research and nutritional analyses.

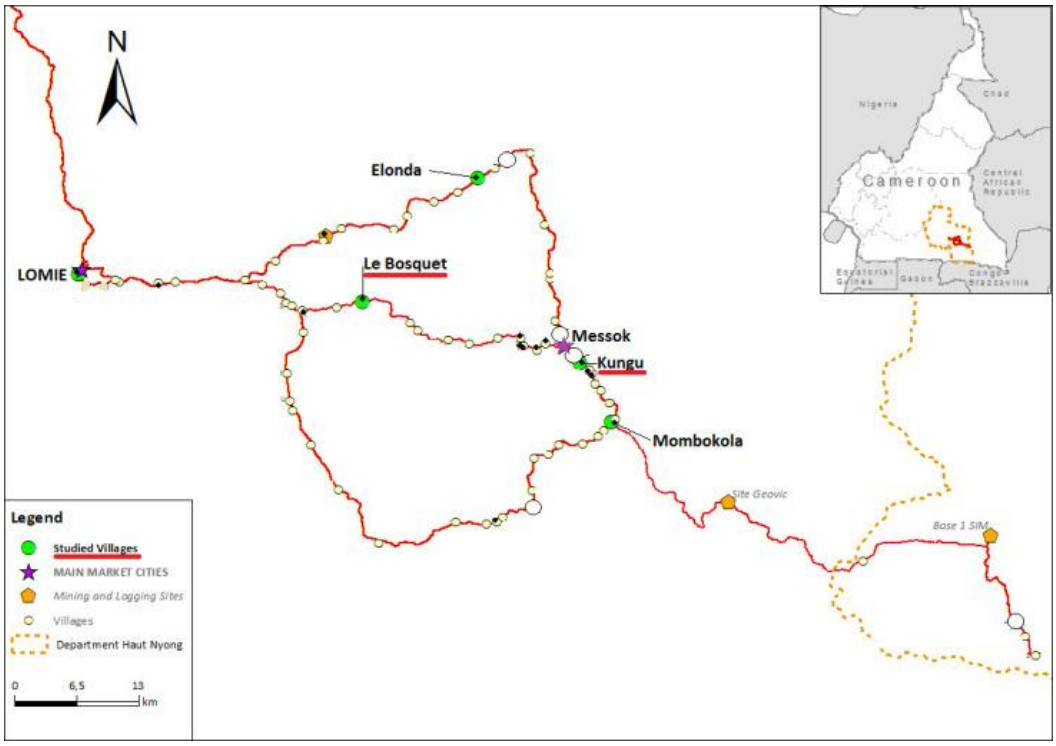

Figure 1.2: Map of the study area in Southeastern Cameroon, the Congo Basin. Note that Elonda is part of the studied villages for this project, and that Bizam is not presented on this map (from Gallois et al., 2021, p. 4, Figure 1).

1.2. Research Goals

This thesis presents a nutritional analysis conducted on 30 wild edible plant food samples collected during fieldwork in 2018. Chemical analysis of the samples was conducted at the Food and Quality Design department of Wageningen University & Research. Here, macronutrient profiles were established for each individual sample. All samples were homogenized, and all macronutrient data was expressed in amount of macronutrient/100 gr of dry matter for easy comparison to data published in the literature. Dry matter values were converted into fresh weight macronutrient ratios to better reflect macronutrient intake during consumption of the wild edible plants. The main goals of this research project are to present novel data on the macronutrient composition of wild, edible plant taxa used by the Baka in Southeastern Cameroon, and to test to what extent it is feasible to establish a universal macronutrient profile for human nutrition.

The main research question to be examined is: *Is there an ideal ancestral diet, or ratio of macronutrients?*

Additional sub-questions to be answered are:

- How does the species-level nutritional data from the analysis compare to data published in the literature and what are the implications for variability in hunter-gatherer nutrition?
- What is currently known about the consumption of rainforest foods by the Baka?
- How does Baka dietary- and macronutrient composition compare to that of other foraging societies?
- To what extent does the Baka macronutrient profile compare to the so-called "*Paleodiet*", a modern dietary trend that is supposedly based on a universal hunter-gatherer diet.
- How can studying hunter-gatherer diets help us understand Western health issues?

1.3. Outline

Chapter 2 introduces the general research question and background to understanding the Baka diet. The first section presents the past and current status quo in studies on hunter-gatherer nutrition. Next, the relevance of studying modern-day foraging diet will be justified. Of key importance are the relevance and possible contributions that ethnography and anthropology have for the broader field of archaeology. Next, key issues and developments within the field of hunter-gatherer studies are elaborated upon, to adequately nestle this thesis project in its niche. Chapter 2 goes on to explain the *Paleodiet* and its prescribed universal, ancestral dietary- and macronutrient guidelines. Since the Baka occupy the Congo Basin rainforest in Southeastern Cameroon, this environment will be elaborated upon as well. Next, each macronutrient is explained both chemically and biologically. Subsequently, the Baka forager-horticulturalists of Cameroon are introduced, as they play the main role in the case study presented in this thesis. A brief overview of previous research on the Baka is given, after which Baka dietary composition and long-term foraging trips are explored in detail.

Following Chapter 2, Chapter 3 explains and justifies the materials and methods used for this thesis project. Here, the study community, methodological considerations, sample information, and information on the laboratory processing are presented. Chapter 4 presents the results obtained during the chemical nutritional analysis in the food science lab of Wageningen University & Research. The macronutrient composition as well as the energy content measured for 30 wild edible plants used by the Baka are presented. The distribution of macronutrients and total energy content are explored per food type and within food types. Chapter 5 proposes the discussion, starting with a synthesis of the comparisons made between Baka nutritional data and previously published nutritional data. Next, the Baka rainforest nutrition profile is reverse-engineered and presented for the first time. After establishing the Baka rainforest nutritional profile, the data is put in perspective by comparison with aggregate hunter-gatherer dietary profiles. Lastly, the results are discussed in terms of their implications for public health and the *Paleodiet* movement. Chapter 6 will present the reader with the overall conclusion. Here, the main results are briefly stated, alongside implications and future directions for the field of studying the evolution of human nutrition.

2. Background

2.1. Studies on Hunter-Gatherer Nutrition

2.1.1. The Relevance of Studying Hunter-Gatherer Diets

The hunter-gatherer modes of subsistence were the only lifestyles available to pre-agrarian *Homo* and its predecessors. Until the advent of agriculture some 10,000 years ago, hominins thrived and moved into all parts of the world by surviving through foraging and hunting (Cordain et al., 2000; O'Keefe et al., 2010; Sahlins, 1998). Unsurprisingly, the subsistence behaviours and diets maintained before agriculture have had profound effects on the evolution of our lineage. Not only is diet part of the core facets of social identity and the structure of culture, it played a key role during important moments of our evolutionary trajectory (Crittenden & Schnorr, 2017). Adaptations in social behaviour, body size, life-history, and geographic range have all been influenced by past dietary adaptations (Teaford et al., 2023). Moreover, changes in diet are commonly associated with key developments in our evolution such as brain expansion, increased cooperation, tool-making, and family formation (Crittenden & Schnorr, 2017). In particular, it is generally agreed upon that a dietary shift to higher quality and more nutrient dense food items resulted in the co-evolvement of extreme intelligence, long lifespans, extended juvenile dependence, male provisioning support, and support of reproduction by older post reproductive individuals (Kaplan et al., 2000). As such, subsistence strategies, or rather the relationships between culture and environment, have long been a focal point in both archaeological and anthropological research (Meggers, 1954). Experts in nutritional ecology now acknowledge that knowledge of past human diet is axiomatic for broadening our understanding on the evolution of our lineage and our past ecology (Teaford et al., 2023).

Traditionally, archaeological dietary enquiries have focused on the analysis of dental morphology and dental topography. Thanks to recent advances in technology, the repertoire of analytical tools has expanded to allow high-resolution investigation into fossil dental calculus, dental microwear, and biogeochemical signatures through stable isotopes and trace elements However, these often impactful research methods are all subject to the fragmentary nature of the palaeolithic archaeological record. Currently, the main crux in studying Pleistocene diet is the limiting scope and incompleteness of the archaeological record (Teaford et al., 2023). In the last decades, this Achilles' heel has received its due treatment by the hands of a potent combination of the archaeological and ethnographic records. The ultimate goal of this potent symbiosis is to help elucidate the influences of our species' dietary practices on our evolutionary trajectory (Winterhalder & Smith, 2000).

In order to fully grasp these issues, acknowledging the plurality of foraging lifestyles is imperative, as both extinct and extant hunter-gatherers have developed a large number of unique lifeways (Marlowe, 2005). However, the lifestyles assumed to have been maintained by extinct Pleistocene hunter-gatherers and earlier hominins are now suggested to be extinct in their true forms (Cordain et al., 2000). Almost all modern foraging societies practice diets consisting of both wild- and farmed foods, as well as subsidized foods provided by aid organizations and the government (Headland et al., 2002). It is broadly accepted that analogies of extant foragers to those of the past are potentially problematic in nature. Compared to Pleistocene hunter-gatherers, modern ones have access to complex technologies such as the bow-and-arrow, harpoons, nets, blowguns, and spears. This diversity in technology led to an increased range of cultural variation. Likewise, extinct hunter-gatherers, although having access to different toolkits, can be assumed to have as much variation in their lifeways as seen within foragers today. Comparing extant and extinct hunter-gatherer societies is much like comparing two different mosaics. Still, attempts to better understand evolutionary adaptations in diet greatly benefit from being complemented by measurements of particular traits in modern foragers instead of in agricultural societies (Marlowe, 2005). Scholars generally agree upon the idea that using observations of extant hunter-gatherer behaviours is the best modern analogue for establishing potential hypotheses and models concerned with past dietary practice (Andrikopoulos, 2016; Chang & Nowell, 2016; Cordain, 2012; Cordain et al., 2002; Österdahl et al., 2008; Pontzer et al., 2018; Tarantino et al., 2015).

For these studies it is of crucial importance to avoid the marginalization of hunter-gatherer societies. A large amount of previous anthropological and ethnographical studies were greatly influenced by problematic and outdated paradigms. Foraging societies were regarded as simple and primitive, and indigenous people were often seen as ‘’living fossils” or ‘’savages” (Porr & Matthews, 2017, p. 1063). African indigenous people were portrayed to exhibit some sort of ancestral pattern, implicitly arguing that they are not modern, and less evolved (Ackermann, 2019; Athreya & Ackermann, 2019; Porr & Matthews, 2017). The epistemological roots of anthropology and ethnography sprung from problematic, colonial ontologies. When maintained within the academic field concerned with human origins, these roots facilitate problematic power relationships between Western scientists and extant hunter-gatherers. All scholars focusing on foraging societies should refrain from maintaining these problematic assumptions and should be aware of the field’s epistemological roots. Academics investigating extant foragers should aim to empower these societies, and to create increasingly multivocal and inclusive narratives on foraging lifeways and human evolution (Athreya & Ackermann, 2019).

Lastly, an often controversial issue is the defining of what a 'hunter-gather' or 'forager' is. Commonly, foraging refers to subsistence tactics that rely exclusively upon non-produced foodstuffs or other wild resources. These resources can be preserved and managed to a slight degree (Winterhalder & Smith, 1981). A generally accepted rule of thumb is that foragers only obtain their food and extra requirements from wild natural sources (Woodburn, 1980, p. 95). Foraging behaviours are expected to not deliberately alter the gene pool of any of the exploited species (Panter-Brick et al., 2001).

2.1.2. Key Issues and Developments

Current research among hunter-gatherer societies is being conducted by a plethora of academic fields, ranging from anthropology's behavioural and quantitative dietary data to nutrition chemistry's, human biology's, and microbiology's analysis of human samples such as saliva, stool, urine, blood, serum, hair, and dental calculus. Together, the disciplines of anthropology, biochemistry, and biology draw from multiple lines of evidence. Where anthropologists prefer investigating human behaviours such as division of labour, food sharing, and social network formation, the chemistry- and biology-based disciplines focus their attention towards highly technical data. Scholars partaking in these fields have grown adept at combining their data to construct holistic narratives on foraging societies and their lifeways (Crittenden & Schnorr, 2017).

One caveat arising from these differential academic backgrounds is the potentially problematic integration of biological data into larger evolutionary and ethnographic contexts (Crittenden & Schnorr, 2017). This thesis presents an analysis that overlaps with nutrition science's dealings with food composition, and anthropology's attempts at the qualitative and evolutionary aspects of hunter-gatherer diet. In particular, data on the chemical composition of the food items in question is rare. No comprehensive study on the macronutrient contents of wild plant and animal taxa used in completely non-Westernized societies exists (Cordain et al., 2000). Westernized societies are those that are considered as highly educated, industrialized, rich and democratic (Henrich et al., 2010). Information on the nutritional quality of non-Westernized diet largely influences our capacity of solving issues in biology, anthropology and evolutionary medicine (Crittenden & Schnorr, 2017). Since the 2000's, academic interest in sociological, demographic, evolutionary, and human health science studies has seen a revival, due to the studied populations shifting into wage economies (Cordain et al., 2000; Crittenden & Schnorr, 2017; Headland et al., 2002). Figure 2.1 demonstrates the quantitative increase of ethnographic inquiries concerned with the reproduction, distribution, and production strategies maintained by foraging populations. The graph clearly illustrates a significant increase in research interest throughout the last decades.

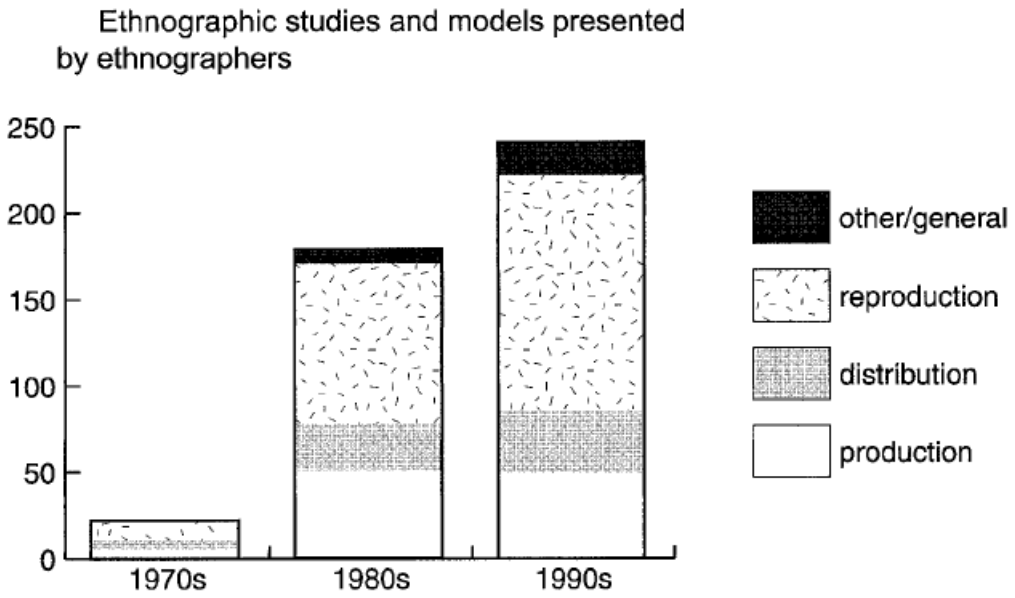

Figure 2.1: Ethnographic studies and models presented by ethnographers. Bar graphs showing the quantity of ethnographic studies and models published alongside their respective research focus, from the 1970s onwards (adapted from Winterhalder & Smith, 2000, p. 52, Figure 1).

Today, much of the sources referred to for investigating the diet composition of foragers consist of nutritional summary papers. These papers obtained their data from anecdotal ethnographic records. The anecdotal nature of these sources resulted in a lack of comparability and compatibility between their data, as they all use their own differential methodologies. One such source is the often cited *Ethnographic Atlas* (Murdock, 1967). This work is a cross-cultural index conveying the information of anthropologists that were originally researching issues other than dietary- and nutrient composition within hunter-gatherer societies. More importantly, the fact that no uniform unit of measurement was used for establishing subsistence reliance upon specific food items, caused criticism on the use of the index (Crittenden & Schnorr, 2017). Nonetheless, meta-analyses based on large-scale indexes have become the norm. For instance, Cordain et al. (2000) published a meta-analysis based on the *Ethnographic Atlas* (Murdock, 1967) including data from 229 populations. Their analysis suggests that circumpolar societies are most reliant upon fished and hunted animal food items. In these tundra-type environments, fish and meat provide 85% of the total diet (in weight). The contribution of animal products to foraging diets is lower in grassland ecosystems at 62 – 42 %, and lower in forests with a contribution of 80 – 52 %. The reliance upon wild plant food items is suggested to be underestimated due to the inclusion of circumpolar populations in the data sample as well as the way in which energy estimates were obtained (Crittenden & Schnorr, 2017).

The importance of plant-based food items towards the diets of Palaeolithic populations is proposed to be overshadowed to an even greater extent (Hardy et al., 2015, 2022). More recent indexes reevaluated the ratio of animal to plant (A:P ratio) food items in foraging diets (e.g., Marlowe, 2005). Although cross-cultural meta-data obtained from such indexes does not come without limitations, they do provide meaningful overviews of the potential contributions of plant- and animal-based food items to hunter-gatherer diets in varying environments (Crittenden & Schnorr, 2017). The importance of investigating different environments has been hinted at in earlier research and reviews, but still a low amount of studies has centred its attention on nutrient variation across habitats (Henry et al., 2019). In addition, remarkably few studies have combined all possible methodological avenues available to the study of diet composition. Close to no studies combine nutritional chemistry, estimated consumption patterns, estimated production patterns, and raw food weights transported back to camp (Crittenden & Schnorr, 2017). Hence, this thesis hopes to help address the methodological shortcoming constituted by the discipline's lack in comparability of data. To do this, data on estimated consumption patterns and raw food weights brought back to camp are combined with novel chemical nutritional data. Furthermore, the obtained macronutrient data has been converted into measurement units commonly used such as kcal, g/dry weight and g/fresh weight, so future research may benefit from, and easily compare with, the nutritional data obtained for the Baka.

2.2. The *Paleodiet*

Central to this thesis is the notion of hunter-gatherer diet being recruited as a potential reference standard for modern human nutrition (Cordain, 2002, 2012; Cordain et al., 2000; Crittenden & Schnorr, 2017; Eaton & Konner, 1985; Marlowe, 2005; Wolf, 2010). Some scholars have stressed the importance of ecological- and habitat variation within hunter-gatherer subsistence strategies and diet, postulating that there is no such thing as a universal ancient human diet (Konner & Eaton, 2010). However, nutritionists and anthropologists use aggregated data derived from modern-day foragers to help understand and solve issues in modern health (Cordain et al., 2000; Pontzer et al., 2018). The modern industrialized lifestyle (Henrich et al., 2010) has had many benefits, but also negative effects such as a rise in prevalence of non-communicable diseases (Milton, 2000; Pontzer et al., 2018). With the romanticized hunter-gatherer lifeway in its mind, public health increasingly exploited our hunter-gatherer past to explain the known increases in the prevalence of diabetes, cancer, obesity, and heart disease. A common assumption is that if we emulate hunter-gatherer diets we might prevent diseases of civilization such as diabetes type 2, coronary heart disease, and obesity, as none of the extant foraging societies suffer from these diseases so long as they refrain from maintaining a sedentary lifestyle and consuming Western foods (Milton, 2000). Many scholars postulate that industrialized environments are radically different from the environments in which humans evolved, and that these changes ultimately led to non-communicable diseases (Heying & Weinstein, 2021). For instance, the World Health Organization (WHO) noted that obesity is for a large part caused by an increase in physical inactivity as a consequence of increased urbanization and the sedentary nature of modern forms of work and transportation, alongside an increase in the consumption of calorie-dense foods (Pontzer et al., 2018).

Undeniably, an evolutionary perspective could provide essential understandings that may aid in mitigating such health issues. The elephant in the room is that this potential evolutionary understanding is only as good as our understanding of past lifeways. To attempt a diagnosis of the evolutionary causes of modern disease, a thorough understanding of past physical activity levels, diets, and disease profiles is required. Much of our current understandings are based on extant foraging societies. Subsequently, a large part of this work does not include any quantitative measurements of physical activity levels or diets in these living societies, let alone any such data for extinct populations (Pontzer et al., 2018). Studies that did attempt some sort of quantitative analysis on modern-day foraging diets have mostly drawn from the *Ethnographic Atlas* (Murdock, 1967) . These studies argued that most hunter-gatherer societies obtain 50% or more of their calories from meat. In comparison, the calorie-dense and starch-heavy modern diets are argued to contain

relatively less protein (Eaton & Konner, 1985). Subsequently, the now somewhat outdated index by Murdock (1967) laid the foundation for the *Paleodiet* movement (Cordain, 2002, 2012a; Wolf, 2010), in which high-protein and high-fat diets are advocated, and in which sugars and cereals are actively avoided (Cordain, 2002, 2012; Pontzer et al., 2018; Wolf, 2010). For the remaining sections of this thesis, any modern diet trend touching on what we ate in the past is referred to as ''*Paleodiet"* (e.g., (Cordain, 2002, 2012; Wolf, 2010). The data and arguments presented concerned with the *Paleodiet* are not reflective of the actual nutritional- or dietary compositions maintained by our ancestors or other early hominins.

The catalyst for the *Paleodiet* movement consists of a seminal paper from Eaton and Konner (1985). Here, it is proposed that foraging societies living in inland semitropical environments obtain 50 – 80% of their food (in weight) from plant sources, with the remaining 20 – 50 % consisting of animal food items. They propose an average daily macronutrient profile for the Palaeolithic, consisting of a daily 3000 kcal intake, of which 251.1 g is protein, 71.3 g is fat, 333.6 g is carbohydrate, and 45.7 g is fibre. The ratio of plant against animal food items for this diet is estimated to consist of 35% meat and 65% plants (Eaton & Konner, 1985, p. 286). Other authors such as Lee (1996) claim that there is increasing evidence indicative of tropical, savannah/grassland, and coastal foraging societies being mostly reliant upon animal food items. As mentioned before, Cordain et al. (2000) re-analysed all 229 hunter-gatherer societies recorded by Murdock (1967). The authors propose a revised plant to animal food ratio for daily dietary intake, consisting of 62% plant foods and 38% animal foods. Furthermore, they found that on average, dietary protein intake constitutes some 19 – 35% of the total daily energy intake. Carbohydrates provide between 22 – 40% of total daily energy intake, and fat between 28 – 47%.

The *Paleodiet* was further refined by Cordain and colleagues in the 2000s. Here they argued that, compared to modern-day western diets, the average hunter-gatherer diet is exceedingly high in protein content, but low in carbohydrate content (Cordain et al., 2000). Cordain (2002, p. 10) further claims a genetic blueprint has co-evolved along the subsistence behaviours that *Homo* maintained during its extended foraging phase. This blueprint entails the ideal macronutrient profile that we were originally designed to eat. In *The Paleodiet* (2002) Cordain proposes that the ideal dietary ratio for any hunter-gatherer includes 19 – 35% of the total energy intake from protein, 22 – 40% from carbohydrates, and 28 – 47% from fats. 55% Of the total energy intake is derived from meats and fish. Generally, *the Paleodiet* proposes that one should eat a relatively high amount of animal protein and a small amount of carbohydrates (Cordain, 2002, p. 22), and particularly to completely refrain from consuming cereals and legumes (Cordain, 2002, p. 20). The total amount of daily kcal intake is left out of the dietary equation (Cordain, 2002, p. 21).

In 2012, Cordain published a revised version of the *Paleodiet*, known as *the Paleodiet revised* (2012). Again, he states that the *Paleodiet*, but also early hominin diets, mainly consist of lean animal foods and that starchy food items such as tubers were not consumed, and should not be consumed at all, resulting in a high protein intake but a low carbohydrate intake. Typically, the *Paleodiet* is proposed to only contain seafood, fruits, lean meats and vegetables, whereas carbohydrates from starchy food items are to be avoided. In particular, explicitly listed food items to be avoided are starchy tubers, manioc, cassava roots, potatoes, and yams (Cordain, 2012, p. 143). In comparison the first edition of *the Paleodiet* (2002), the revised edition does present a universal daily caloric intake. According to Cordain (2012), the perfect foraging diet should consist of a 2200 kcal intake per day. The ideal macronutrient profile adhering to this is that of the following ratios: 190 g of protein (33% of total energy), 142 g of carbohydrates (25% of total energy), and 108 g of fat (42% of total energy) (Cordain, 2012, p. 48). These macronutrient ratios are prescribed for a female individual of 25 years old. The author states that chimpanzee diets, the fossil record, data on nutrients in plants and animals, as well as contemporary hunter-gatherer diets have been studied to attain this universal dietary composition, for which 55% of the total energy intake should derive from animal foods (Cordain, 2012, p. 64).

An attempt at solving public health issues through the recruitment of hunter-gatherer dietary practice together with a proposed ancestral dietary profile was published by Wolf (2010). Wolf (2010) proposes the '*Paleo solution: The original human diet'* to decrease the prevalence of obesity and inflammatory diseases. Furthermore, his *Paleodiet* promises to address reduced physical activity levels maintained by large parts of Westernized individuals. In this work, a 30-day dietary program is proposed and presented as the ancestral diet for *Homo sapiens*, though the largest part of the food items and ingredients used derive from domesticated animal and plant species. The list also includes ample examples of processed foods that were not available during the Pleistocene. Interestingly, Wolf (2010) exclusively proposes his ideal macronutrient plan for the cohort of 25 year old females, similarly to Cordain (2012). However, both authors fail to present any data on body composition and physical activity levels adhering to the chosen cohort. Wolf's (2010) recommended caloric intake aligns with that of Cordain (2012) and is set at 2200 kcal per day. However, Wolf (2010, p. 205) advices a daily intake of 217 g of protein (38% of total energy), 129 g of carbohydrates (23% of total energy), and 100.3 g of fat (39% of total energy). Whereas Cordain (2012) did not recommend any set amount of daily TDF intake, Wolf (2010, p. 205) states that an intake 42.5 g of fibres per day is ideal. In Wolf's (2010) recommended ancestral diet, the ratio between carbohydrate and protein intake is skewed towards protein even more than Cordain's (2012) proposed ratio (38% of protein and 23% of carbohydrates as opposed to 33% of protein and 25% of carbohydrates). Again, the proposed ideal foraging diet is dominated by meat, seafood and poultry (Wolf, 2010, p. 193). The consumption of fruit and starchy tubers is not recommended (Wolf, 2010, p. 189).

Evidently, the overrepresentation of animal food consumption in Palaeolithic and hunter-gatherer nutrition has a tight grip on the *Paleodiet* and modern health solutions. Contrasting evidence is offered by authors who found that out of eight foraging societies, only four obtain more than 50% of their calories from meat (Kaplan et al., 2000), in comparison to the 55% postulated by Cordain (2002; 2012) and Wolf (2010). The meta-analysis conducted on the *Ethnographic Atlas* (Murdock, 1967) by Kaplan et al. (2000) suggests that societies below 45° latitude rely upon diets equal in meat and plant proportions, representing the first mismatch between the *Paleodiet* movement and data from small-scale foraging societies (Pontzer et al., 2018). A second mismatch between the proposed *Paleodiet* (Cordain, 2002) and data from small-scale foraging societies used in public health, is that most of the foraging diets are carbohydrate laden, and consist of tubers, plantains, rice and manioc (Pontzer et al., 2018).

There appears to be little to no consensus on what a universal macronutrient composition should be. On the contrary, it is evident that extant hunter-gatherer societies maintain excellent cardiovascular and metabolic health despite a high reliance on starchy foods, and a great diversity of macronutrients. Given the great similarity in health profiles between the different groups constituting modern-day foraging societies, it appears it is the environment as opposed to the genetics, that maintains the successful health profiles noted for modern foraging societies (Pontzer et al., 2018). This suggests the important role of habitat variation and its effects on the nutritional qualities of food items, that ultimately effect health status. Moreover, it illustrates the pluralistic nature of nutritional adaptations and their outcomes, and the profound effects that dietary practices in our deep past may have had to our own evolution over space and time. In this framework of documenting the variation in dietary behaviours, and coupling that with detailed nutritional data, this thesis combines chemical nutritional data and estimated consumption patterns from the Baka to elucidate foraging subsistence behaviours within a rainforest-type environment. Since the rainforest-type environment present in Southeastern Cameroon constitutes the main geographical context of this analysis and is the ecological context from which the samples were collected, the following section will introduce the required background information for this ecological setting.

2.3. The Rainforest-Type Environment

The ecological framework in which this nutritional analysis is situated is that of the tropical rainforest in the Congo Basin (Figure 2.2). As such, it important to establish a concise overview of the potential role the rainforest has played in the evolution of our lineage, and how studying a foraging society in this type of environment can help address questions in human evolution. Traditionally, tropical forest environments have been portrayed as too hostile for hominin occupation throughout prehistory (Bailey et al., 1989). The consensus has been that tropical forests are pristine environments, bereft of any human influences. In general, rainforests were considered too tough to navigate whilst lacking sufficient sources of carbohydrates and protein (Roberts et al., 2015). On the contrary, the savanna and grassland environments have been regarded as the cradle of humankind. Subsequently, most studies focused only on a narrow set of habitats. Studies looking into the variability of subsistence behaviours within different habitats remain scarce (Henry et al., 2019). However, we now know that the African tropical rainforest has been of key importance to the hominin lineage, even though the rainforest has previously been described as too resource-poor (Scerri et al., 2022). Though most apes, including *Gorilla gorilla* and *Pan troglodytes*, occupy rainforest-type environments in which they are fully capable of meeting their nutritional demands (Head et al., 2011; Mercader, 2002)

Figure 2.2: Photograph of the Congo Basin rainforest (from https://unearthed.greenpeace.org/2016/11/17/congo-basin-rainforest-an-ecosystem-on-the-edge/).

For central Africa, the first evidence for hominin occupation derives from a tooth, suggesting that at least some early hominin populations subsisted in mixed environments located next to the edges of the central African rainforest at c. 2.5 Ma (Crevecoeur et al., 2014). This report suggests that the notion of hominin presence in rainforests only occurring after the advent of agriculture is not true. Palaeoclimatic reconstructions based on marine and terrestrial records show that the hominin presence in Gabon, in the Congo Basin between 850 – 650 ka coincided with a humid period and forest expansion (Braucher et al., 2022; Gosling et al., 2022). Moreover, profound shifts in the hydro-climate of Africa starting at c. 1 Ma potentially have resulted into an even more wetter environment. These shifts co-occur with the first appearances of *Homo sapiens* outside of Africa. The archaeological record corroborates this profound period of climatic fluctuations with the Lupemban lithic technocomplex, which has been related to mixed resource acquisition in the African rainforests between 270 – 170 ka (Taylor, 2022). During times of climatic hardship, the African rainforests have been argued to have served as climatic refugia, even when conservative parameters are applied to the climate models (Blinkhorn et al., 2022). Together, these lines of evidence suggest that rainforests can no longer be regarded as peripheral to the early human past (Scerri et al., 2022).

Additionally, it is now believed that watershed moments in our evolution have taken place within a rainforest-type environment (Mercader, 2002). For instance, the development of bipedalism was already present in rainforest dwelling *Orrorin* at c. 5.8 Ma, before the expansion of grassland and savanna-type environments (deMenocal, 2004). Additional fossil evidence is in favour of early hominin rainforest occupations, as *Ardipithecus ramidus* has already been ascribed to woodland and rainforest-type environments at c. 4.4 Ma (White et al., 2009) (Figure 2.3).

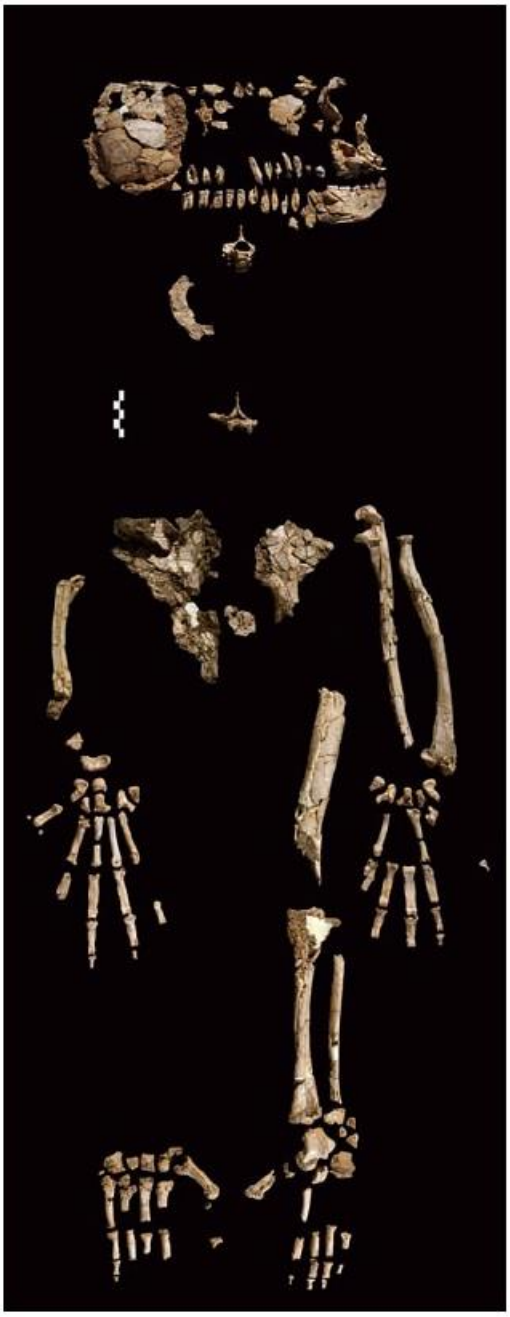

Figure 2.3: The ARA-VP-6/500 Ardipithecus ramidus skeleton recovered within the Sagantole Formation close to the Awash River in Ethiopia. The fossil was recovered during excavations in 1994-1995. This transitional hominin species preferred forested environments and lived c. 4.4 Ma (from White et al., 2009, p. 77, Figure 3).

Together, Late Miocene fossils ascribed to the genera of *Ardipithecus*, *Orrorin* and *Sahelanthropus* all problematize the idea of human evolution occurring only in savannas (deMenocal, 2004; White et al., 2009). Even later stages of human evolution are also likely to have occurred in rainforest habitats. Flashing forward to the dispersal of *Homo erectus* towards Southeast Asia at c. 1.2 Ma, it is known that tropical rainforests were already widespread (Figure 2.4). These environments undoubtedly influenced local hominin adaptations that can be evidenced in for instance later *Homo floresiensis* and *Homo luzonensis* (Scerri et al., 2022). On the contrary, the vast savanna and grasslands known today were only established after c. 1.2 Ma and c. 0.6 Ma (deMenocal, 2004). The species to have exploited the African rainforests most intensely is *Homo sapiens*. Due to most scholarly interest having been received by savanna and grassland environments, it is still unknown when *Homo sapiens* first exploited tropical forests. But, tropical environments evidently represent an important hominin habitat (Scerri et al., 2022).

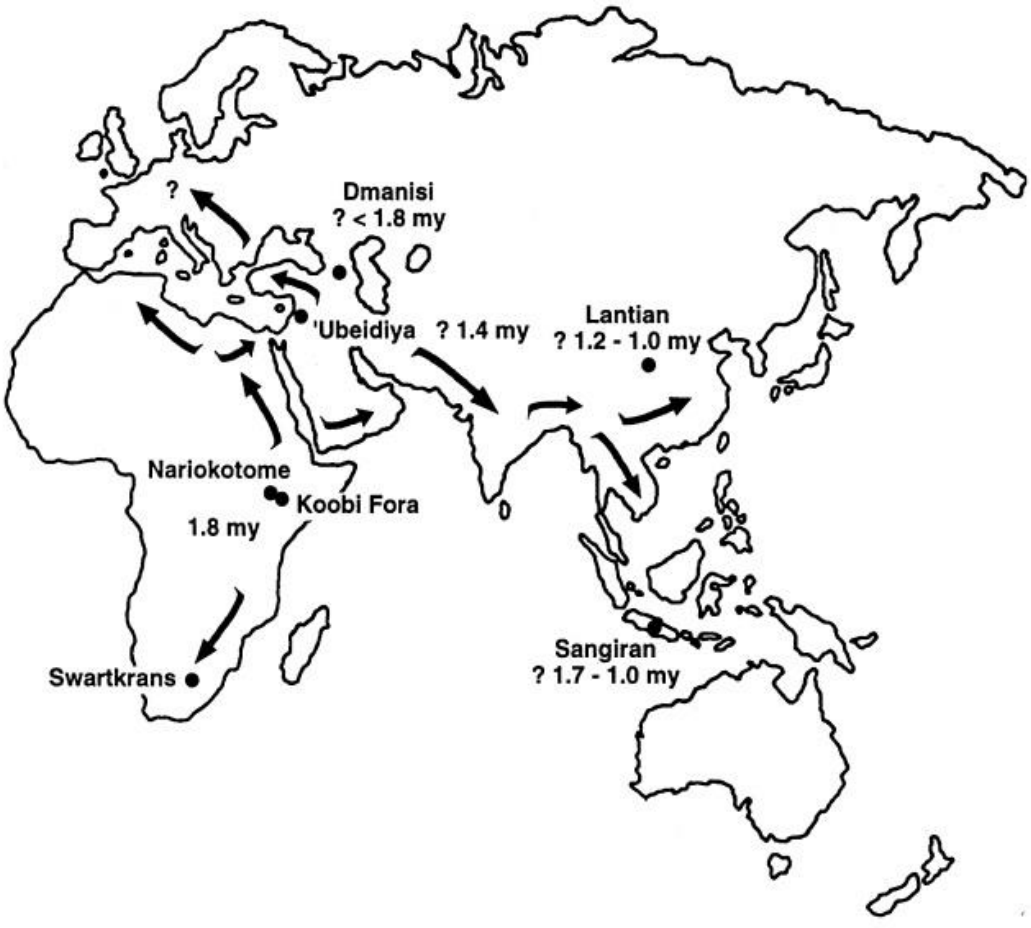

Figure 2.4: Map showing the possible dispersal routes for Homo erectus*. At Lantian, China, Homo erectus was present as early as 1.2 Ma, in an environment that is reconstructed as tropical rainforest (from Rightmire, 2001, p. 78, Figure 1).*

2.3.1. The Congo Basin

The archaeological records alongside the phytolith records have indicated that African foragers already lived in the Congo Basin during the late Pleistocene and early Holocene, at some 19 ka (Mercader et al., 2000). Interestingly, palaeoclimatic reconstructions indicate that the Congo Basin served as a climatic refuge during the Last Glacial Maximum up until around 18 ka (Maley, 1991, p. 87; Mercader, 2002; Roberts, 2022; Sato et al., 2012; Thomas, 2000). In a similar vein, prolonged late Pleistocene occupations in the rainforests of South Asia have been attested to at c. 20 ka (Roberts et al., 2015). The extensive amount of rainforest plants offer sufficient quantities of oils, carbohydrates and starches for hunter-gatherer societies to have sustained themselves. Recently, a new date for the earliest hominin presence in the Congo Basin has been reported by Braucher et al. (2022). The authors dated a small lithic assemblage found on a terrace above the Ogooué River in Gabon to a minimum age of 850 – 650 ka. All-together, the data on early rainforest occupations remain largely fragmentary in nature, possibly due to research and preservation biases (Scerri et al., 2022). Though it is feasible to maintain that the African tropics and the Congo Basin share a long history of hominin occupation.

Today, the Congo Basin suffering heavily from deforestation. The rainforest in Cameroon still covers some 175,000 km^3, which is close to 37% of the Cameroonian national territory. Estimates from 1997 indicate that some 3000 km^3 of forest is destroyed on an annual basis, with another 60.000 km^3 already under concession to timber companies. In response, the Cameroonian government started reforms aimed at the sustainable management of its natural resources from the 1990s onwards. Culminating in the formation of a Ministry of Environment and Forestry (MINEF) and a National Forestry Action Program (NEMP). Overall, the Cameroonian rainforest is characterized by two dry seasons and two wet seasons. Annual rainfall decreases with an increasing distance from the coast and may differ from 2950 mm in coastal localities to 1670 mm land inwards. The temperature varies little, and on average is 25 °C year-round (Tchouto, 2004). The evergreen forests of Cameroon still offer a rich environment to a wide variety of peoples. As such, the Congo Basin has been an important ecological setting for both past and modern foragers. The subsistence behaviours observed in foraging societies occupying the Congo Basin today are potentially more deeply rooted than previously thought. Exploring these subsistence behaviours may help elucidate the potential lifeways that are suggested to have been available to earlier hominins occupying similar environments.

Though the rainforest was deemed too hostile for human occupation without the aid of agriculture (Bailey et al., 1989), this type of environment offers ample nutrients if one knows where to look and possesses the required knowledge of the landscape. A central component of this analysis consists of macronutrient data on wild edible plants from the Congo Basin. The nature of these macronutrients will be explained both chemically and biologically in the following section.

2.4. Macronutrients and their Roles

Since macronutrient profiles will be presented and novel macronutrient data have been obtained, the role of these macronutrients requires further explanation. Every individual food item is composed of a unique suite of chemicals. On a molecular level, chemical constituents form compounds known as macronutrients and micronutrients. The former includes fats, proteins, and carbohydrates, whereas the latter consists of minerals, vitamins, colourants, flavours, toxicants, and additives. Macronutrients are those compounds that form the largest part of an individual's dietary intake. In terms of energy per gram of macronutrient, fat provides 9 kcal, protein 4 kcal, carbohydrates 4 kcal, and total dietary fibre (TDF) 2 kcal (Fellows, 2017). The following section will describe the molecular structures and the biological functions of each individual macronutrient. This thesis measured TDF ratios separately from carbohydrate fractions. However, TDF consists of complex carbohydrates. Thus, TDF is sometimes included into the carbohydrate fraction presented in nutritional studies. This analysis treats TDF as a separate macronutrient, but the role and structure of fibres are explained in the carbohydrate section.

2.4.1. Carbohydrates

Carbohydrates are known to form the largest part of dry matter in plants. They encompass multiple forms of naturally occurring sugars. In general, all the occurring sugar units are referred to as -saccharides. Simple sugars are known as monosaccharides. Carbohydrates can form into more complex forms of sugars known as disaccharides, trisaccharides, oligosaccharides and polysaccharides as shown in Figure 2.5 (Fellows, 2017; LibreTexts Biology, 2023). Carbohydrates play two major roles in an organism's physiology, namely, providing energy and structure. Simple forms of sugar such as monosaccharides are readily available for cells and can be recruited immediately after their consumption. Monosaccharides mostly occur as crystalline solids and are water-soluble. These carbohydrates are usually referred to as glucose, fructose and galactose. The most essential monosaccharide is glucose ($C_6H_{12}O_6$). Through the metabolization of carbohydrates, the chemical compound is conversed into energy via a process called hydrolysis (Fellows, 2017). Chemically, the following reaction occurs (LibreTexts Biology, 2023):

$$C_6H_{12}O_6\ (s) + 6\ O_2\ (g) \rightarrow 6\ CO_2 + 6\ H_2O\ (l) + \text{energy}$$

Here, the (s) represents the solid phase of a chemical, whereas (g) represents the gaseous phase, and (l) the liquid phase.

Monosaccharides can bind to form larger chains of carbohydrates known as polymers. Polymers such as di-, tri-, oligo-, and polysaccharides need to be digested in order for them to be recruited as energy source. Often these polymers are used for the storing of energy instead of providing readily available energy. In plants, the energy storage molecules are amylase or amylopectin, also known as starch, and in animals energy is stored in a compound known as glycogen (LibreTexts Biology, 2023).

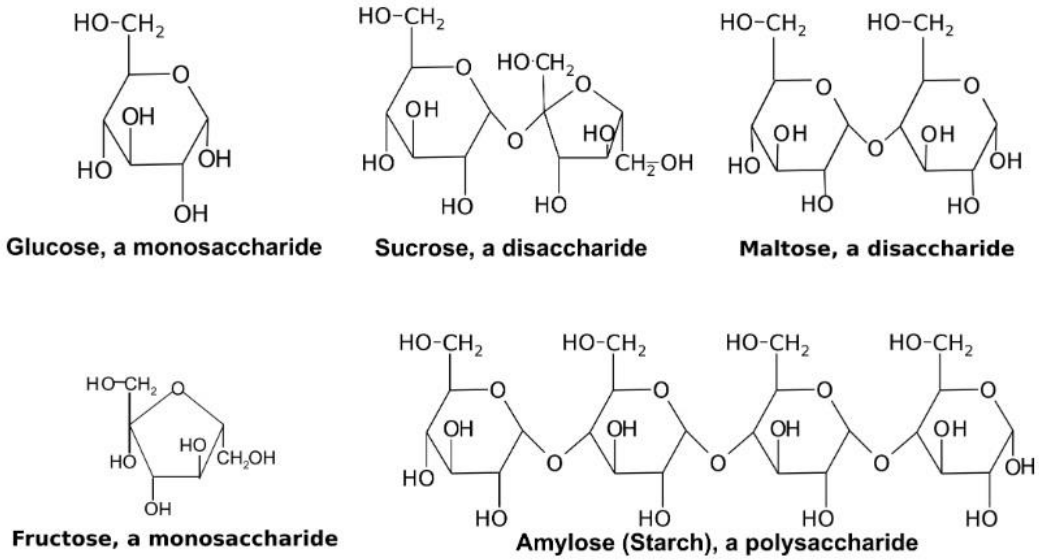

Figure 2.5: Schematic representation of different forms of carbohydrates. Depicted are glucose, sucrose, maltose, fructose, and amylose structures (adapted from LibreTexts Biology, 2023, https://bio.libretexts.org/Bookshelves/Biotechnology/Bio-OER_(CUNY)/02%3A_Chemistry/2.07%3A_Carbohydrates).

Oligosaccharides are comprised of 3 – 10 units of monosaccharides and commonly found in vegetables such as cabbage, broccoli, beans and in cereals, oats, wheat, and most fruits. Oligosaccharides are part of the total dietary fraction (TDF) of a specific food item. They are indigestible and mostly pass the digestive tract unaltered. Fermentation of oligosaccharides by commensal bacteria may occur in the large intestine, which is why TDF is considered to provide about 2kcal/g on average. Oligosaccharides have pre-biotic qualities, meaning that they are beneficial for the gut microbiome. TDF is selectively used by probiotic bacteria, resulting in the suppressing of pathogens as well as an increased uptake of nutrients. TDF can provide increased gut mobility, reduced risk of chronic disease, enrichment of colonic microbiota, and the reabsorption of bile acids (Crittenden & Schnorr, 2017). Polysaccharides occur most frequently in natural settings. Within plants these compounds are referred to as starch, cellulose, gum and pectin. In animals the glycogen is stored in muscle tissues and the liver. The indigestible carbohydrate compounds present in animals are known as chitin. Polysaccharides play an important role in controlling the viscosity and the physical and functional properties of foods, as their

structure enables them to form hydrogen bonds with water. The polysaccharides known as starches are mainly found in tubers and seeds and occur as granules containing millions of starch molecules. Commonly, 70 – 80% of the starches occur as amylopectin, and 20 – 30% as α-amylose. Starches can be either slowly or rapidly digestible.

2.4.2. Lipids

Lipids, or fats, belong to a class of macromolecules serving as long-term energy storage. Once digested and stored fats play roles in insulation, cell structure, cell signalling, and water sealing. The basic structure of a fat molecule consists of three fatty acid chains connected to a glycerol molecule, a structure commonly referred to as a triglyceride shown in Figure 8. Moreover, lipids can occur as either monoacylglycerols, or di- and triacylglycerols depending on the number of fatty acid chains present (Fellows, 2017; LibreTexts Biology, 2023).

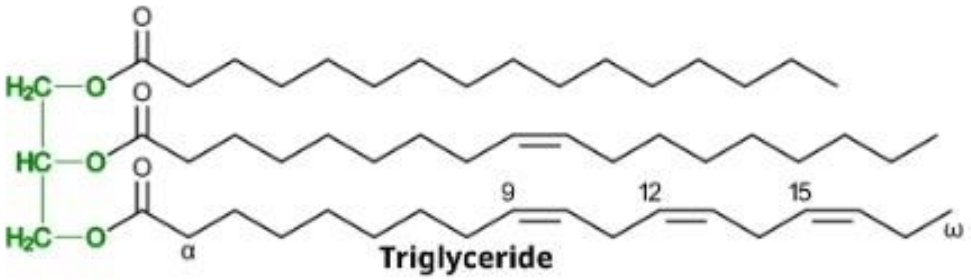

Figure 2.6: Schematic representation of a triglyceride molecule. The fatty acid chains are depicted in black, with the glycerol molecule in green (adapted from LibreTexts Biology, 2023, https://bio.libretexts.org/Bookshelves/Biotechnology/Bio-OER_(CUNY)/02%3A_Chemistry/2.07%3A_Lipids).

Fats can occur in three separate forms. The first is known as a saturated fatty acid. They are usually sourced from animals and are solid at room temperature. The polyunsaturated fatty acids mostly derive from plants and are liquid at room temperature. Polyunsaturated fats have double hydrogen bonds on specific points in their fatty acid chains. The last commonly known type of lipid is that of trans-fatty acid. These lipids are predominantly man-made and although they may contain double bonds, are solid at room temperature (LibreTexts Biology, 2023). Usually, fats are consumed directly as cooking oils, butter, or salad and cooking oils deriving from marine oils, animal fats, and plant oils. Furthermore, the oils of nuts are very distinct in taste. Oils from pecan, walnut, pistachio, cashew, almond, and hazelnut are commonly used in salads or as culinary oils. Since fats are considerably high in energy content, they remain a crucial focal point of studies investigating obesity, diabetes, and cardiovascular disease. Diacylglycerols usually contain a phosphate group alongside their glycerol group, forming a phospholipid. These type of lipids are important for structuring cell membranes. Phospholipids are commonly extracted

to serve as emulsifying agents. Another important type of lipid is that of sterols, or cholesterol. They occur in vegetables, vegetable oils, fruits, seeds, and legumes as phytosterols and serve to lower blood cholesterol levels (Fellows, 2017).

2.4.3. Proteins

Proteins occur in a large variety of structures. Fundamentally, proteins are polymers of amino acids. Proteins are commonly referred to as the building blocks of the body, as they provide much of the functional and structural capacity of cells. However, amino acids can also serve as energy sources. In turn, amino acids are monomers acting as the building blocks of proteins. Amino acids always occur with group of NH_2, or an amino group, with the addition of a COOH group known as a carboxyl group. The last component is known as the 'R' group, which consists of a specific hydrocarbon chain. The variation along this chain in each amino acid results in the diversity of proteins known. Taken together, there are 20 different amino acids that may form a protein structure. There are a total of 9 essential amino acids. These are the structures an organism cannot produce itself. They must be obtained from food. In contrast, non-essential amino acids can be produced within an organism. Lastly, conditional amino acids are mostly recruited during times of somatic stress and illness (Fellows, 2017; LibreTexts Biology, 2023).

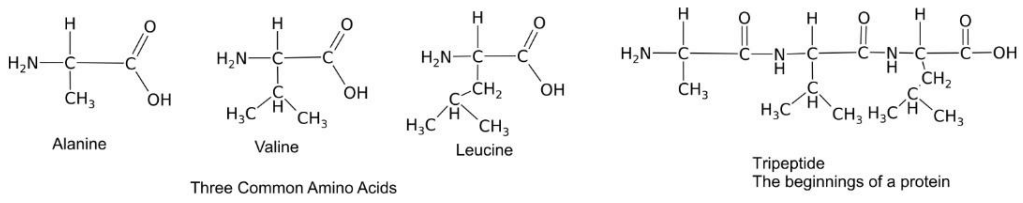

Figure 2.7: Schematic representation of amino acids. A tripeptide formed by peptide bonds is shown on the right (adapted from LibreTexts Biology, 2023, https://bio.libretexts.org/Bookshelves/Biotechnology/Bio-OER_(CUNY)/02%3A_Chemistry/2.07%3A_Proteins).

Single amino acids make up the building blocks of proteins, and are usually joined together into long chains through the formation of peptide bonds (Fellows, 2017; LibreTexts Biology, 2023), as shown in Figure 2.7. Distinctions can be made between small peptides (containing <25 amino acids) known as oligopeptides, and longer peptide chains (containing >25 amino acids) known as polypeptides. The sequence of the amino acids within a peptide are crucial for its function. However, there are more levels to the functioning of a protein. The ways in which amino acids interact with each other causes the unique three-dimensional shape, which ultimately produces the very wide variety of proteins and enzymes (Fellows, 2017). Proteins may form structural components known as elastin or collagen. They may constitute enzymes that serve to regulate and control growth, repair of somatic tissue, and metabolic activity. In addition, the proteins known as actin and

myosin are the building blocks of muscle tissue. Other proteins serve as hormones such as insulin, or as transfer proteins such as haemoglobin, as storage proteins, antibodies, or protective proteins. The function of each protein is determined by its primary, secondary, tertiary, and quaternary structure. That is, the sequence of the amino acids, the local three-dimensional shapes formed by hydrogen bonding, the general three-dimensional structure of the peptide chain, and the final protein structure as the result of multiple peptide subunits bonding together (LibreTexts Biology, 2023) as shown in Figure 2.8. While humans have the ability to perceive protein, as a newly accepted flavour known as umami, we cannot generally discriminate among protein types (Zhang et al., 2019). Proteins mostly determine food structure thanks to their hydrophobic and hydrophilic qualities. These traits influence the perceived wateriness, crumbliness, or spreadability during consumption (Fellows, 2017).

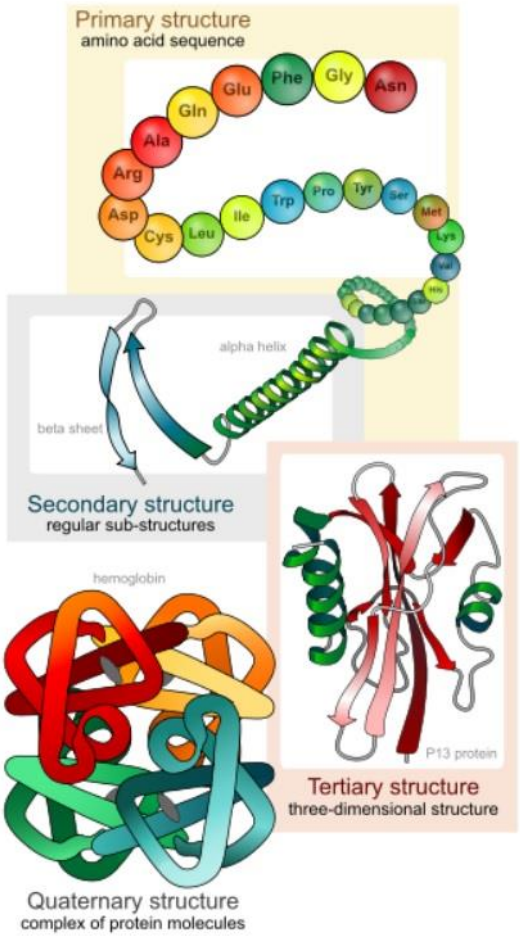

Figure 2.8: Schematic representation of the primary, secondary, tertiary, and quaternary structures. Together these structures form a protein (adapted from LibreTexts Biology, 2023, https://bio.libretexts.org/Bookshelves/Biotechnology/Bio-OER_(CUNY)/02%3A_Chemistry/2.07%3A_Proteins).

2.5. A Case Study: The Baka Forager-Horticulturalists of Cameroon

This thesis presents novel data on the nutritional qualities of 30 samples coming from wild edible plant taxa used by the Baka, to test the validity of aggregated hunter-gatherer dietary profiles. As such, it is paramount to introduce the Baka with their lifeways and ecology.

2.5.1. The Baka

The Baka are a group of forager-horticulturalists living in Cameroon, northwestern Congo, the Central African Republic and northeastern Gabon (Figure 2.9).

Figure 2.9: Political map of Western and Central Africa. The map indicates the position of Cameroon in relation to the Republic of the Congo, Gabon, and the Central African Republic (from https://www.istockphoto.com/nl/vector/west-central-africa-political-map-gm509252731-46165290).

Most Baka occupy the eastern province of Cameroon. An estimated 30,000 people belonging to the Baka societies currently occupy these regions (Gallois, 2016; Gallois et al., 2020). The Baka are also known as the Bayaka, Babinga, Bibayaka, Bebayaga, Bangombe, and Bibaya. The total size of Baka territory within the Congo Basin is estimated at some 100,000 km^2 (Gallois, 2016). Under the French Mandate's government in the 1950s, the Baka transitioned to living in semisedentary locales close to settlements of the Bantu cultivators (Yasuoka, 2006).

During the last 50 years, the Baka have depended on both forest and agricultural products. In addition, the Baka have faced a multitude of challenges in regards to deforestation and defaunation of the forest, alongside the Cameroonian government's settlement program in their area. where they live. Most Baka now occupy villages located close to logging roads, where they engage in wage labour and participate in agricultural activities (Gallois et al. 2020). The main crops cultivated by the Baka are cassava and plantain. The Baka also trade food items with the Bantu, in exchange for their hunted animal meat or labour (Yasuoka, 2006). Baka mobility has been reduced significantly and their foraging excursions are commonly organized around the agricultural seasons (Gallois et al., 2020). During the rainy seasons (major rainy season Sep-Nov, minor rainy season Apr-May(Sato et al., 2012)), the Baka are known to orchestrate small hunting camps constituted by a range of one to five households during which the main hunting method is cable snare hunting. In general, these camps are located some 10 – 20 km away from the Baka settlements. Moreover, the Baka have been recorded to hunt using guns obtained from the Bantu, although Baka men prefer the use of spears whenever the opportunity presents itself. Interestingly, during the dry season (Dec-Mar (Sato et al., 2012)) the Baka embark on a foraging expedition known as a *molongo*. A *molongo* is regarded as a two or three month period of migration into the forest, consisting of 10 or more households. *Molongo* camps are located considerably deeper into the forest than the rainy season hunting camps, being some 20 – 50 km away from the nearest settlements. Furthermore, in contrast to the hunting expeditions, the Baka are solely reliant upon wild food items during a *molongo*. In particular, wild yams are of crucial importance to Baka subsistence during these long-term foraging expeditions (Sato et al., 2012; Yamauchi et al., 2000; Yasuoka, 2006).

2.5.2. Earlier Research on the Baka

The first records including mention of the Baka date back to 1888, in which French explorer P. Crampel (Figure 2.10) documented his observations and interactions with the Baka peoples in Gabon (Crampel, 1890).

Figure 2.10: Portrait photograph of Paul Crampel. Crampel was a French explorer, and the first to document the Baka in the literature (from https://en.wikipedia.org/wiki/Paul_Crampel).

Subsequent reports in which the Baka appeared were established during French expeditions attempting to delimit the border between French Gabon and German Cameroon. These earlier reports lacked ethnographic accuracy. Although interesting, literature on the Baka was scarce and of low scientific quality (Gallois, 2016). As stated by Winterhalder and Smith (2000, p. 52), the quantity of ethnographic studies into foraging societies increased during the 1980s. The resurgence of interest is paralleled by the increase of ethnographic research conducted on the Baka, a trend that commenced somewhat earlier in the 1970s. The growing corpus of literature on the Baka investigated their language and oral tradition, religion, and music. Many of these studies focused on different regions within Baka territory. As such, they provide insights into the potential diversity present within Baka societies (Gallois, 2016). Subsequent studies shifted focus towards Baka subsistence behaviours, such as their use of wild plants and methods of paracultivation of wild yams. Japanese research has largely contributed to elucidating Baka lifeways during the 2000s.

These studies mainly adopted a human ecology approach (Gallois, 2016) and will be frequently drawn upon in this analysis (e.g. Sato et al., 2012; Yasuoka, 2006).

Although all Baka live in the Congo Basin rainforest, previous research has corroborated the diversity that is present within this one cultural group. Depending on the country in which they live, the governmental policies may differ. In addition, localization of settlements within the same country has resulted in significant diversity (Gallois et al., 2016). Consequentially, the results presented in this thesis are highly contextualized and offer high-resolution insights into Baka foraging behaviours and dietary compositions for those individuals living in Bizam, Bosquet, Elonda, and Kungu. As opposed to the dietary profiles established in large-scale indexes and the *Paleodiet* movement. The results presented in this thesis convey an accurate representation of the actual dietary- and macronutrient compositions of the Baka, a foraging society within an African rainforest-type environment.

2.5.3. Baka Dietary Composition

The samples used to reconstruct Baka dietary composition in this thesis were collected during an ethnobotanical survey conducted by Gallois (Gallois et al., 2020). The main goal of the survey was to assess current food behaviours and representations among the Baka during the nutritional transition the Baka are currently undertaking. In the survey, data from 536 participants is explored to shine extra light on Baka representations of food as well as their food behaviours. Informants were asked to list all food items consumed during the previous 24 hour period. The key meal for the Baka is their evening meal, which is often the only meal of the day. These meals mainly consist of a starchy base combined with a saucy dish. The most commonly recalled food items are listed in Table 2.1, alongside the source of each food item. In 93% of dietary recalls, the consumption of starchy food items was highlighted (Gallois et al., 2020, p. 4). Next, leafy vegetables were present in 71% of the recalls, with condiments, spices and beverages being listed in 72% of the recalls. Mammal muscle meat was recalled in 36.4% of cases, organ meat in 9.7% and fish in 11.4%. Most starchy foods consumed within Baka settlements are cassava and plantain.

Only 3% of the recalled starchy plant food items were wild plant taxa. The market is an important food source for the Baka, providing sweets, rice, spices, condiments, and vegetables, as shown in Table 1. In comparison to the small amount of starchy plant foods sources from the forest, 89% of the consumed fish and meat is obtained from the forest. Furthermore, 61% of leafy green vegetables are sources from the forest. In total, the Baka have been recorded to consume 14 different wild plant taxa. The reported wild plant taxa consist of starchy white tubers (*Dioscorea spp.*), dark green leaves (*Gnetum africanum*), fruits (*Aframomum spp.* and *Trichoscypha acuminata*), legumes and nuts (*Irvingia excelsa*, *Panda oleosa*, and *Irvingia gabonensis*), and spices (*Baillonella toxisperma*, *Afrostyrax lepidophyllus*, and *Ricinidendron heudelotii*). The most consumed wild plant taxa is *Gnetum africanum*, The species was included in 40.7% of dietary recalls, with the highest consumption rate recorded during the major dry season. Other species were reported in considerably less recalls, with the second highest recalled food item being bush mango (*Irvingia spp.*, a type of nut) at 8.4% of recalls.

Table 2.1: Overview of the most commonly listed food items and their consumption rates, with indication of their sources (n=2377 recalls from 536 individuals (from Gallois et al., 2020, p.2).

Food type	**Overall**		**Source (%)**		
	Number	%	Cultivated	Wild	Market
Cereals	243	10.2	1.7	0.0	98.4
White tubers	2214	93.1	45.2	2.7	56.5
Green leafy vegetables	1686	70.9	40.5	61.3	9.4
Vitamin-A fruits	392	16.5	73.5	0.5	26.0
Other fruits	227	9.6	95.6	1.3	2.6
Mammal muscle meat	865	36.4	0.0	89.3	10.6
Organ meat	231	9.7	0.0	99.6	2.1
Fish	271	11.4	0.0	97.1	3.0
Legumes, nuts and mushrooms	617	26.0	17.5	51.2	36.5
Oils and fats	1190	50.1	65.6	35.1	5.4
Sweets	46	1.9	0.0	23.9	76.1
Spices	1722	72.4	76.5	5.2	96.6

In addition to their general consumption patterns, the Baka were also asked to identify their preferred food items. Of the 10 most preferred foods, eight were starchy foods. The three most preferred starchy foods are cassava, plantain and cocoyam. The third to sixth most preferred starchy food items were wild yams (*Dioscorea spp.*). Interestingly, the Baka regard *Gnetum africanum*, a leafy green, as the only wild prestige food. Moreover, the Baka report *Dioscorea* yams as their preferred food item when they could hypothetically obtain any food item they wanted without costs. All in all, the Baka are heavily reliant upon cultivated starchy food items such as plantain and cassava. Carbohydrates are mainly provided by starchy tubers, whereas fibres, proteins and minerals are mostly derived from cassava and cocoyam leaves. Nonetheless, important nutrients are still provided by wild edible plant taxa. Furthermore, wild plants remain important for Baka cosmology and cultural identity. In particular, wild yams play an important role during the *molongo* (Gallois et al., 2020, pp. 6–9).

2.5.4. Baka Long-Term Foraging Expeditions

The above data represents a typical diet while living in villages near the agricultural plots, and indeed agricultural foods represent the majority of calories. However, the Baka also undertake longer foraging trips, the *molongo*, during which they subsist almost entirely on wild foods (Sato et al., 2012; Yasuoka, 2006). The dietary intake, dietary composition, and the macronutrient qualities of the consumed food items during these foraging trips offer an excellent opportunity for establishing a dietary overview of a foraging group within an African rainforest-type environment. By exploring how this dietary profile compares to those of the *Paleodiet*, we can test the assumptions and guidelines established by Cordain (2002; 2012) and Wolf (2010).

2.5.5.1. The Wild Yam Question

In the 1980s, scholars generally agreed that human subsistence without the aid of agriculture in rainforest-type environments was impossible. The forest's resources for human subsistence were deemed too scarce, too spatially dispersed, and to seasonally variable (Bailey et al., 1989). At the time, the 'wild yam question' was a central topic of debate. Wild yams present a rich and major source of energy for humans in tropical environments, but it was assumed that these yams were of insufficient nutritional quality to support human occupations (Yasuoka, 2006). To assess the wild yam question, Yasuoka (2006) followed the Baka during long-term foraging expeditions. During two periods, from August 2001 to September 2002 and from January to August 2003, all subsistence behaviours expressed by the Baka from Zoulabot Ancien were recorded. For most of the *molongo*, all animal and plant foods brought back to camp were recorded. Furthermore, a comparison between diet in the village and in the forest was conducted by recording and

weighing all food items consumed during meals at eight different households, during a period of 13 days. The energy intake per adult was calculated through daily fresh weight consumed per adult-consumption day (Yasuoka, 2006).

A total of 19 households partook in a *molongo*, comprised of 17 married men, 22 married women, 14 unmarried girls and boys over 12 years old, 27 children from 2-12 years old, and nine infants below the age of 2. The foraging trip lasted for 74 days, of which 44 were spent in a single camp, Mongungu. On the remaining days, the camp moved location each day. On moving days, first a new location was decided upon, then the men went looking for honey and animals to hunt, whereas the females were preoccupied with the construction of huts. While the group was occupying the Momgungu camp, the foraging activities would start at around 08:00. The men set out hunting snares, while the women went out to forage wild yams, predominantly harvesting *Dioscorea spp.* yams. All of the gathering and hunting activities took place within a range of 3 km from the camp, resulting in intensive usage of an area comprising some 30 km^2. During the hunts, men would kill their prey with either guns, spears, machetes and their bare hands. This hunting method was responsible for 17% of the total capture, totalling up to 1002.5 kg of meat. The largest component of the total capture was obtained with snare traps, accounting for 83% of the total capture. In total, snare hunting yielded 1930.4 kg of meat (Yasuoka, 2006). Based on data published by Wu-Leung (1968), the energy intake of meat was calculated to be 150 kcal per 100 g consumed. Overall, meat obtained through hunting supplied 446 daily kcal in the first *molongo* period, and 604 daily kcal in the second. On average, hunting supplied 0.36 kg of available edible meat per day, averaging 536 kcal per day (Yasuoka, 2006). Table 2.2 gives an overview of the energy intake available through meat procurement.

Table 2.2: Overview of Baka energy intake by hunting game in kcal, per consumption-day (from Yasuoka, 2006, p. 286).

Period of hunting phase	**Feb. 13-Mar.10[1]**	**Mar. 9-April. 27[2]**	
Main hunting method	**Spear and gun**	**Cable snare**	**Total**
Total fresh weight[3] (kg)	791	2142	2933
Days of consumption	24	50	74
Adult consumption-days	1596	3325	4921
Total weight (edible weight[4]) per consumption day (kg)	0.50 (0.30)	0.64 (0.39)	0.60 (0.36)
Energy intake[5] per consumption-day (kcal)	446	605	536

[1]Before arriving at Mongungu

[2]After the arrival at Mongungu

[3]Including edible parts

[4]The edible ratio was estimated at 0.6

[5]Energy intake = Fresh weight X Edible ratio X Energy value

During the *molongo*, the plant component of the diet consisted exclusively of wild plant taxa. In total, 5446.6 kg of wild yams were gathered, providing each individual 1.64 kg per consumption-day. In total, five different yams different species of yam were gathererd and identified to species level: *Dioscorea praehensilis*, *D. burkiliana, D. minutiflora*, *D. semperflorens*, and *D. mangenotiana*. Here, the edible ratio of wild yams is proposed to be 0.8, and the energy value assigned to the yam species is 120 kcal per 100 g of fresh weight based on data published by Wu-Leung (1968). Moreover, the total amount of energy per day available through gathering is estimated to be 1786 kcal, totalling up to 77% of the total daily energy intake (2322 kcal). Of the total daily energy intake, wild yams provided 68%, with *D. praehensilis* providing 56% alone. Aside from yams, *Irvingia spp.* and *Panda oleosa* seeds were also gathered, alongside mushrooms and honey. In total, 17 species of *Dioscorea* grow in the Cameroonian rainforest (Hladik & Dounias, 1993). Having annual stems and annual tubers, the dry season is the best season for harvesting wild yams. This period of the year coincides with the period in which the Baka embark upon long-term foraging trips. Taken together, the Baka were able to survive for 74 days without relying upon agricultural foodstuffs. Their main source of energy were wild yams, with meat providing just 23% of the daily energy intake (Yasuoka, 2006), as shown in Table 2.3. The dietary A:P ratio during a Baka foraging trip is estimated to be 23:77.

Table 2.3: Energy intake (kcal/consumption-day, percentage given within brackets) for all food items consumed during either a molongo *camp or in the village (from Yasuoka, 2006, p. 288).*

	***Molongo* camp**	**Village**
Wild yams and yam-like plants	1573 [68]	226 [10]
Other wild plants and mushrooms	30 [1]	46 [2]
Honey	183 [8]	13 [1]
Meat	536 [23]	40 [2]
Fish	-	101 [4]
Plantain	-	1018 [47]
Other cultivated plants	-	110 [5]
Oil palm fruit	-	693 [29]
Palm wine	-	40 [2]
Total	2322 [100]	2374 [100]

The total daily energy intake is relatively similar for both a consumption-day during a *molongo* and within the village. The data form Yasuoka (2006) has shown that the Baka are capable of surviving within the rainforest for prolonged periods, without the aid of agriculture. As such, the original hypothesis by Bailey et al. (1989) stating that the rainforest-type environment is too hostile for human occupation without relying upon agriculture, appears significantly less likely. Further field trips observing Baka foraging behaviours were conducted by Sato et al. (2012), as such reports remained scarce. The main goal for re-examining the 'wild yam question' was to further elucidate how the Baka live off the land (Sato et al., 2012, p. 130).

2.5.5.2. The Wild Yam Question Re-Examined

To further verify the answer to the wild yam question, Sato et al. (2012) joined the Baka on two 20-day *molongo* trips. Again, no agricultural foods were consumed, aside from salt and pepper. During the first foraging trip in the dry season of August 2003, 12 adults and 4 infants participated in the *molongo*. In the second trip that took place during the rainy season in October 2005, 16 adults and 7 infants participated. All of the participants lived in the village of Ndongo, in Southeastern Cameroon. For both foraging trips, the Baka participants selected the same locale as their main campsite, close to the base of mount Bek. This region is particularly known for its richness in wild tubers. Participants heading in or out of camp to forage were timed every day and questioned about their activities in the forest. All foodstuffs brought back to camp were identified and weighed, and estimated ratios of edible portions were determined for tubers, nuts, leaves, meat, termites, and snails. Next, the total energy intake and daily energy intake per participant were estimated (Sato et al., 2012).

During both *molongo* trips the Baka engaged in digging for yam tubers, collecting honey, nuts, edible fungi, and termites. These activities were done by both men and women. For snare hunting, exclusively men set and checked the hunting snares. The food items brought back to camp consisted of multiple food types. For wild yams 5 different species were collected, 12 mammal species were captured, 18 freshwater fish species were caught, 2 species of nuts were collected, 16 species of fungi were gathered, alongside honey and termites. In both the rainy- and dry seasons, the gathered yams and hunted meat amounted to more than 90% of the total weight of food items brought back into camp. In the rainy season, the amount of yams was higher. On the contrary, the amount of fish and meat was larger during the dry season. As was observed by Yasuoka (2006), Sato et al. (2012) also record *D. praehensilis* to be the most frequently collected wild yam species (more than 90% of the collected tubers). During the first trip 636.6 kg of *D. praehensilis* was collected,

with an even larger amount of 1030.6 kg having been collected during the second trip. Furthermore, close to all of the collected nuts belonged to *Panda oleosa*.

In the course of the first trip 22.9 kg of *P. oleosa* nuts were collected, and 44.3 kg in the second trip. The higher yield recorded for the second trip may in part be caused by a higher number of participants. A small amount of *Irvingia spp.* nuts was only collected during the first trip, adding up to 0.1 kg. Yams were commonly collected by couples. These couples each collected 9-12 kg of yams during the dry season, and 10-16 kg per day during the rainy season. The differences in mean weight collected are not indicative of resource shortage during either of the two seasons. As such, it is suggested that seasonal variation in resource availability did not elevate the gathering pressure for yams. The same was found to hold true for the collection of nuts and the hunting of mammals. The consumption of wild tubers was estimated at 1.4 kg per consumption-day in fresh weight during the dry season, with 1.5 per consumption-day for the rainy season. The estimated amount of meat consumed per consumption-day was 0.4 kg during the dry season, and 0.3 during the rainy season (Sato et al., 2012). Table 2.4 presents the fresh weight per food item brought back into camp for the two foraging trips. In total, the total energy intake during the foraging trip was estimated at 2528-2865 kcal in the dry season and 2479-2777 kcal in the rainy season, averaging to 2662.25 kcal per consumption-day. In total, wild tubers supplied 60% of the total energy intake (Sato et al., 2012), a somewhat lower amount than the 68% reported by Yasuoka (2006). The total amount of procured meat supplied 15-20% of the daily energy intake, with nuts supplying around 10%. The total daily protein intake per consumption-day was estimated at 114.5 – 146.1 g in the dry season and 93.0 – 125.4 g in the rainy season, averaging to 124.25 g of protein per consumption-day. The A:P ratio (animal food to plant food ratio and their contributions to the total daily energy intake) recorded is estimated at around 20:80 in terms of energy. However, animal foods supplied 60% of the total daily protein intake (Sato et al., 2012). Lastly, Sato et al. (2012) found the total energy intake could be considered as the daily energy expenditure, which allowed an estimation for the basal metabolic rate (BMR) of Baka foragers. During the dry season the BMR for males is estimated at 1413 kcal/day, and the BMR of females at 1141 kcal/day. Throughout the rainy season the average male BMR is estimated at 1425 kcal/day, and at 1190 kcal/day for females (Sato et al., 2012).

Again, wild tubers were the main suppliers of dietary energy. Within the evergreen rainforest in Southeastern Cameroon, *D. praehensilis* and *D. semperflorens* are argued to support a foraging lifestyle in a similar type of environment. No other food items in the area are able to replace the dietary contribution of these tubers. As such, it is argued that *D. praehensilis* tubers are essential for maintaining a foraging lifestyle within the African tropical rainforest. In addition, nuts, honey, fish, game, and insects are crucial sources for both protein and energy. These food items would have had played important roles in supporting a foraging lifeway. Moreover, a key implication deduced from the survey is that subsistence systems solely based on foraging within the African rainforest require a relatively large energy expenditure of some 2662.25 kcal per day.

Table 2.4: Overview of all foodstuffs brought back to camp during the course of two long-term foraging trips, expressed in total fresh weight (kg) and fresh weight brought back per consumption-day (from Sato et al., 2012, p. 135).

	Aug. 2003			Oct. 2005		
Food type	**Total fresh weight (kg)**	**(%)**	**Weight/CD[1] (kg)**	**Total fresh weight (kg)**	**(%)**	**Weight/CD (kg)**
Wild yams	660.7	59.4	2.75	1092.9	74.7	3.29
Seeds	23.0	2.1	0.10	44.3	3.0	0.13
Leaves	-	-	-	0.0	0.0	0.00
Fruit	13.6	1.2	0.06	-	-	-
Fungi	3.0	0.3	0.01	8.7	0.6	0.03
Mammal	343.1	30.9	1.43	290.6	19.9	0.88
Bird	-	-	-	0.0	0.0	0.00
Bird's egg	0.3	0.0	0.00	-	-	-
Snake	3.6	0.3	0.02	-	-	-
Fish	49.5	4.5	0.21	12.9	0.9	0.04
Crustaceae	0.3	0.0	0.00	0.1	0.0	0.00
Snail	0.8	0.1	0.00	1.7	0.1	0.01
Termite	5.9	0.5	0.02	9.3	0.6	0.03
Honey	8.0	0.7	0.03	3.1	0.2	0.01

[1]Weight/CD = Weight brought to camp per participant per day

3. Materials and Methods

The materials and methods chapter explains how and why the data was collected during this research project. Furthermore, the chapter elaborates on where the samples of edible foods come from and how they are used by the Baka. Table 3.1 provides an overview of all samples analysed and presents the sample area (Bizam; Bosquet; Elonda; Kungu), sample code, plant part, species name, Baka name, the fresh weight of the sample after collection, the dry weight of the sample after milling, and the date of collection in the field. In addition, this chapter highlights the ethical approval obtained for the analysis to be accepted by both the Baka and the Cameroonian government's ethical requirements. This thesis does not take advantage of the Baka people, nor of any official Cameroonian property. No samples have been obtained and analysed without prior consent by the aforementioned parties.

In the next section a concise summary is offered on the habitat and known uses of the particular sample in question. If any specific uses of the plant are recorded for the Baka specifically, this information is presented as well. The subsequent section describes the laboratory processing, including the protocols (see Appendix A) applied in the Wageningen Food Science lab, the calculations used to obtain the macronutrient data presented, and the justification for obtaining these data. All raw data is included and accessible in Appendix B.

3.1. Study Communities and Sample Collection

This thesis presents novel data on the macronutrient composition of wild edible plant foods. All samples were collected from four communities of the Baka forager-horticulturalists within the Congo Basin of south-eastern Cameroon: Bizam (n=3), Bosquet (n=16), Elonda (n=8), and Kungu (n=3). Firstly, Free Prior and Informed Consent was obtained in all four villages. Participants were asked to provide plant samples, and to explain how they were used. In exchange, they were provided with a small compensation: either a bar of soap or a small portion of rice. In addition, this study adheres to the Code of Ethics of the International Society of Ethnobiology and received agreement from the Ethical Committee from the Ministery of Health of Cameroon (n°2018/06/1049/CE/CNERSH/SP). The samples were collected by Sandrine Gallois, then a post-doctoral researcher at the Faculty of Archaeology in the group led by Amanda Henry. Most samples were collected in February and March 2018 during the major dry season, with a handful being collected in October 2018, during the major rainy season (Gallois et al., 2020). The samples acquired for analysis are reflective of the wild plant foods collected by the Baka during foraging trips. The samples do not include food items belonging to the categories of meat, honey, and insects. Several plant parts are represented, as reflected in Table 3.1.

The predominant part of the samples was processed by the Baka to prepare them for consumption. All food items were sundried and then stored within sample coded zip lock bags containing packets of silica gel to reduce moisture and spoilage. The sample code reflect the sampling location. Where 'BOS' stands for the village of Bosquet, 'ELO' for Elonda, 'KUN' for Kungu, and 'BIZ' for Bizam.

3.1.1. Methodological Considerations

To convey the potential contribution of a specific food item to the total diet of foraging societies, values are presented in both dry matter for comparison to previously published data, and fresh weight values in cases where actual consumption ratios play a key role. Through calculating carbohydrate content by subtraction, this analysis may present an overestimation of the contribution of carbohydrates to the diet. In addition, to present the energy content of a specific food item, the most appropriate factor of 'kcal' has been used (Crittenden & Schnorr, 2017). It should be noted that other researchers have expressed their concerns for the use of nutrient values in food composition tables. As the inherent qualities of plant nutrition and the bioavailability of these nutrients widely differ (Wollstonecroft et al., 2008). The main factors contributing to this potential variability are those of habitat, plant age, soil type, and seasonality (Henry et al., 2019; Paine et al., 2019). In particular, carbohydrate content is known to be altered by plant age (Hamlen et al., 1972).

Table 3.1: Overview of the entire subsample analysed in the chemical nutritional analysis, with species name, vernacular name, sample code, part of the plant, weight of the sample when collected, date of sampling, and sample weight after milling.

Species	Baka name	Sample code	Part of sample	Weight of sample when collected (gr)	Date of sampling	Weight after milling
Musanga cecropioides	Kpe Bombo	BOS 001	Fruit	135	13-02-2018	21.7
Aframomum subsericum	Tondo na Njiyi	BOS 002	Fruit	35	14-02-2018	6.3
Undetermined	Akpopo	BOS 003	Mushroom	100	14-02-2018	12.2
Hilleria latifolia	Sumba	BOS 004	Leaves	360	14-02-2018	32.1
Aframomum danielli	Tondo na Mongamba	BOS 005	Fruit	45	14-02-2018	9.4
Oryza sativa	Lee (domesticate)	BOS 006	Seed	175	14-02-2018	72.7
Arachis hypogaea	Peanuts	BOS 007	Seed	95	14-02-2018	72.9
Gaertnera cf. longivaginalis	Mpong mpong	BOS 010	Leaves	120	14-02-2018	12.1
Dioscorea sp	Epange	BOS 011	Tuber	220	15-02-2018	18.7
Klainedoxa gabonensis	Bokoko	BOS 012	Leaves	200	15-02-2018	30.8
Panda oleosa	Kana	BOS 013	Nuts	110	15-02-2018	42.7

Aframomum danielli	Tondo na Mongamba	BOS 015	Fruit	390	16-02-2018	83.1
Dioscorea minutifora	Kuku na Bele	BOS 016	Tuber	30	15-02-2018	21.9
Trichoscypha acuminata	Mbel	BOS 017	Bark	7	15-02-2018	8.1
Irvingia excelsa	Payo	KUN 001	Nuts	195	28-02-2018	23.2
Dioscorea burkiliana	Ba na Bele	KUN 002	Tuber	145	28-02-2018	32
Telfairia cf. occidentalis	Motumbelumbe	KUN 003	Seeds		10-10-2018	26.8
Dioscorea sp.	Keke na Bele	ELO 001	Tuber	105	19-03-2018	22.2
Dioscorea praehensilis	Sapa na Bele	ELO 002	Tuber	120	19-03-2018	22.1
Dioscorea sp.	Ba na Bele	ELO 003	Tuber	100	19-03-2018	25.1
Dioscorea praehensilis	Sapa na Gba (cultivated)	ELO 004	Tuber	125	20-03-2018	27
Dioscorea sp.	Ba na Bele	ELO 005	Tuber	105	20-03-2018	34
Dioscoreophyllum cumminsii	Mbi	ELO 006	Tuber	55	21-03-2018	9.2
Gnetum africanum	Koko	ELO 007	Leaves	240	21-03-2018	26.4
Dioscorea burkiliana	Keke na Bele	ELO 008	Tuber	80	21-03-2018	17.1
Ricinodendron heudelotii	Gobo	BIZ 001	Seeds			35.6
Baillonella toxisperma	Moabi	BIZ 002	Nuts			28.5
Undetermined	Kutu	BOS 018	Mushroom	80	27-09-2018	
N/A (Processed food)	Nutcake	BIZ 003	N/A			42.7

3.2.Sample Information

The plants collected for this project represent several different taxa. To identify the plants, Sandrine asked the provider to give us the name, which was then double-checked against herbarium specimens. The survey was conducted during a botanical survey in April and May 2019. During forest walks, seven women and nine men were asked to identify wild edible plants. Standard botanical methods were applied to create voucher specimens, of which one duplicate was stored in Naturalis Biodiversity Centre in Leiden, a duplicate voucher was stored in the National Herbarium of Cameroon. The third duplicate voucher was present in the field during identification of the plants. When identification failed, names were based on published nomenclature referred to in the electronic supplementary material published with the paper by Gallois et al. (2020) (Gallois et al., 2020). The following section described each individual sample on its habitat and potential uses.

Musanga cecropioides

The *Musanga cecropioides* canopy tree from the family of Urticaceae (sample BOS 001) is a native African, fast-growing pioneer plant frequently present in neotropical forests. It is the most abundant pioneer tree species found in large disturbed patches of these forests, and is commonly known as a parasol tree (Astaras et al., 2008; McKey, 1988). In Cameroon, the tree is known to be used as traditional folk medicine. The bark of *M. cecropioides* is primarily exploited for the treatment of gastrointestinal disorders and other type of infectious disorders in humans (Kouitcheu Mabeku et al., 2011). In addition, research on *M. cecropioides* leaf extract has indicated anti-inflammatory activities in animal models (Sowemimo et al., 2015) The Old World monkey species of *Mandrillus sphinx* (mandrills) occupying the forests of southwestern Cameroon is known to consume the seed-bearing fruit of *M. cecropioides*. Other Central African primates such as chimpanzees rely on the fruit as well (Serckx et al., 2015). The fruits (Figure 3.1) are most available during the major dry season in February and March (Astaras et al., 2008). The Baka exploit this parasol tree for its edible fruits or for the medicinal qualities found within its bark.

Figure 3.1: Photograph of Musanga cecropioides *fruits. The fruits are put on top of the characteristic parasol-like leaves (from https://www.quintadosouriques.com/nl/store/zaden/bomen/kurkhout-boom-paraplu-boom/).*

Aframomum subsericum

The African *Aframomum subsericum* (sample BOS 002) is a herb from the family of Zingiberaceae. It is part of a taxonomically understudied but frequently collected group of plants. Climate change and man-made habitat destruction are decimating *Aframomum spp.* populations throughout Africa. The genus is currently in rapid decline. The *Aframomum spp.* herbs are commonly found in light gaps and forest margins, or in old fields and along roads. Most of the genus' species are used for the treatment of toothache, diarrhoea, fever, stomach aches, and other inflammatory conditions. Use of the herb as sexual stimulant and laxative is also recorded. Furthermore, the herbs have antiulcer, anticancer, and antimicrobial qualities (Amadi et al., 2016). The Baka are known to consume the fruits and seeds of multiple species of *Aframomum*. Seeds are either dried, or roasted and crushed, so they can be added to a dish later. Fruits are readily eaten or sold at nearby markets. The seeds can also be processed into seed oil for sale (Gallois et al., 2021).

Aframomum danielli

Similar to *Aframomum subsericum*, *A. danielli* (Figure 3.2) (sample BOS 005, BOS 015) is an herb of the Zingiberaceae family. Its genus is under severe pressure due to the effects of environmental destruction. The species of *A. danielli* is regarded as possessing antimicrobial effects (Amadi et al., 2016). Its seeds are known to have been used in the treatment of erectile dysfunction in folkloric medicine. The seeds are also used in soups, and are commonly used as a spice in other dishes. *A. danielli* seeds contain important essential oils known to have anti-inflammatory, anti-browning, and antioxidant.

Furthermore, the seeds are rich in phenolic compounds and amino acids (Adefegha et al., 2017). Traditionally in Cameroon, the herb is used for treating infections, parasitic diseases, and for controlling arthropod pests. In Cameroon, essential oils are commonly exploited from the herb's leaves (Kamte et al., 2017). As they do *A. subsericum*, the Baka are known to sell *A. danielli* as a spice on local markets. The Baka refer to four species of *Aframomum* as '*Tondo*', including *A. subsericum, A. danielli, A. sceptrum*, and *A. cf. longipetiolatum* (Gallois et al., 2021).

Figure 3.2: Photograph of the leaves of Aframomum danielli *(from https://en.wikipedia.org/wiki/Aframomum_daniellii).*

Hilleria latifolia

The perennial herb known as *Hilleria latifolia* grows in the tropical forests of Africa and South America and is part of the Phytolaccaceae family (sample BOS 004). The plant is well-known for its uses as medicine in Ghanaian traditional medicine. Its leaves (Figure 3.3) are found to effective for the treatment of rheumatism and otalgia. In Congo the leaves are used in treating skin disease and the flowers for asthma. In Ivory Coast *H. latifolia* leaves are used in the treatment of headache and feverish pain. The plant has seen extensive use as an anti-inflammatory, and anti-infective agent (Abotsi et al., 2011; Amponsah et al., 2014). The Baka collect *H. latifolia* leaves for sustenance. They pound *H. latifolia* leaves in a mortar or cut them into thin slices. They then add the processed leaves to stews for consumption (Gallois et al., 2021).

Figure 3.3: Photograph of the leaves of Hilleria latifolia *(from https://www.mozambiqueflora.com/speciesdata/image-display.php?species_id=171310&image_id=3).*

Oryza sativa

Oryza sativa, commonly known as rice (Figure 3.4) (sample BOS 006), belongs to the family of grasses known as Poaceae. It is one of the most important cereal crops in the world. *O. sativa* is a domesticated species of which some 600 million tonnes is harvested on an annual basis. Although only 3% of this annual yield comes from Africa, rice consumption in Cameroon has increased significantly (Nguefack et al., 2005). The production of rice in Cameroon has seen an increase, with an annual yield of 313084 tonnes of rice produced in 2019. Rice is most commonly milled into flour or consumed in the form of fufu. *O. sativa* is well-suited for brewing, and is used in the treatment of hair and skin.

The rice husks are commonly used as fuel (Ambindei et al., 2022). The Baka are known to buy rice from merchants (Gallois et al., 2020). Rice has been recorded to be packed by Baka when departing on foraging trips (Yasuoka, 2006). Furthermore, the Baka have stated that rice is an important starchy food item in their meals (Gallois et al., 2020).

Figure 3.4: Photograph of Oryza sativa *and its fruits (from https://www.mozambiqueflora.com/speciesdata/image-display.php?species_id=171310&image_id=3).*

Arachis hypogaea

Arachis hypogaea is an annual oil seed known as peanut or groundnut (sample BOS 007) belonging to the Leguminosae family (Figure 3.5). The groundnut is native to South America, but is now cultivated in a variety of environments between 40°N and 40°S (Sharma & Bhatnagar-Mathur, 2006). In Africa some 5.3 million ha of peanuts are grown (Stalker, 1997). *A. hypogaea* is one of the most crucial legume crops cultivated in developing countries of the semiarid tropics, as they are a rich source of fats and dietary protein (Sharma & Bhatnagar-Mathur, 2006). Aside from human consumption of groundnut seeds, *A. hypogaea* leaves are used as animal feed alongside the leftover meal after oil extraction of the seeds (Stalker, 1997). In the Congo Basin within Cameroon, the peanut is among the most important legumes cultivated (Ngo Nkot et al., 2008).

Figure 3.5: Photograph of Arachis hypogaea*. Note that the groundnuts are not visible, but located underneath the soil (from https://www.inaturalist.org/taxa/63205-Arachis-hypogaea).*

Gaertnera cf. longivaginalis

Gaertnera cf. longivaginalis (BOS 010) is part of the genus *Gaertnera* which consists of endemic small tropical trees and shrubs (Figure 3.6). The genus belongs to the Rubiaceae family, and encompasses some 88 identified species (Jongkind, 2018; Malcomber & Taylor, 2009). Other information on the species is scarce. The plant exclusively occurs in West Africa (Jongkind, 2018)

Figure 3.6: Photograph of Gaertnera cf. longivaginalis *leaves and its budding flowers (from https://en.wikipedia.org/wiki/Gaertnera).*

Dioscorea spp.

Yam, or *Dioscorea spp.* is a tuber-producing, annual vine belonging to the family of Dioscoreaceae (Samples BOS 011 *Dioscorea sp.*; BOS 016 *D. minutiflora*; KUN 002 *D. burkiliana*; ELO 001 *Dioscorea sp.*; ELO 002 *D. praehensilis*; ELO 003 *Dioscorea sp.*; ELO 004 *D. praehensilis* (cultivated); ELO 005 *Dioscorea sp.*; ELO 008 *D. burkiliana*). The *Dioscorea* genus encapsulates some 600 species. Taxonomic delimitation is vague for *Dioscorea* species. Some tubers can only be identified up to genus level (Gallois et al., 2021). The white tuber (Figure 3.7) is a crucial staple for people in subtropical and tropical regions of Asia, Africa, South America, the Pacific Islands, and the Caribbean. In Africa, *Dioscorea* is most key in the 'yam zone', ranging from Ghana, Ivory Coast, Togo, Nigeria, Benin, and Cameroon. An estimated 92.2% (67.3 million metric tonnes) of Earth's global

yield of *Dioscorea* (73 million metric tonnes) is produced in the yam zone. Of this annual yield, 0.9% is produced in Cameroon. There are 10 major *Dioscorea* species cultivated globally, of which the included samples are excluded. The yam's underground tubers are an important source of proteins, carbohydrates, and vitamins. *Dioscorea* tubers have a low glycaemic index giving good protection against obesity and diabetes. The Baka 'paracultivate' yam species such as *D. praehensilis*, *D. minutiflora*, and *D. burkiliana*. By 'paracultivating' the Baka procure their tubers in such a way that the vine can be repeatedly exploited (Azeteh et al., 2019). When asked what food they prefer if any food item would be available, the Baka listed the wild yam (*D. cf. praehensilis*) (Gallois et al., 2020). *D. praehensilis* provides the bulk of the available food during Baka foraging trips (Sato et al., 2012). It is the most frequently collected tuber of the genus *Dioscorea* (Yasuoka, 2006).

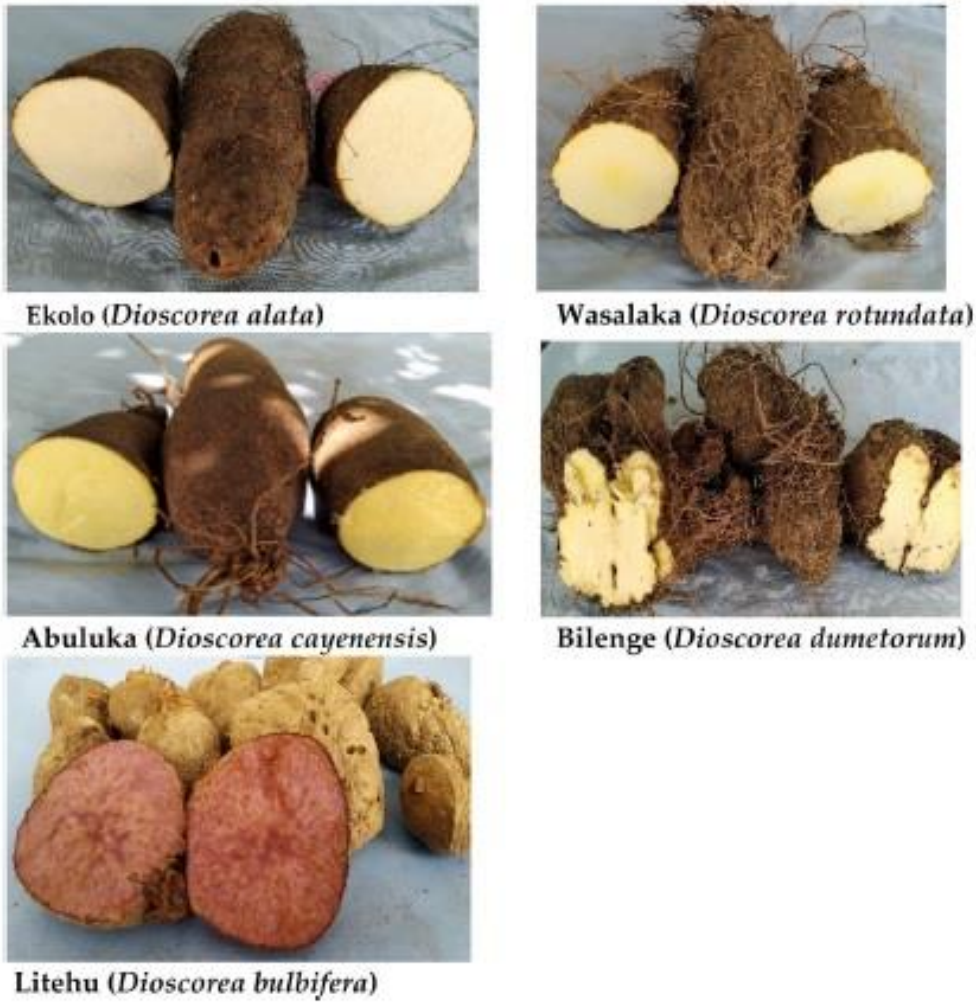

Figure 3.7: Photograph of multiple species of Dioscorea *yam. The yams depicted include* D. alata, D. rotundata, D. cayenensis, D. dumetorum, *and* D. bulbifera *(from Adejumobi et al., 2022, p. 7. Figure 4). Note that the top four depicted yams are white yams, similar to the Dioscorea yams included in this nutritional analysis. The native names come from Kisangani peoples living in the Democratic Republic of Congo.*

Klainedoxa gabonensis

Klainedoxa represents one of three genera belonging to the Irvingiaceae family (sample BOS 012). Of this genus, two species are present in Cameroon: *Klainedoxa gabonensis* and *Klainedoxa grandifolia*. *K. gabonensis* is a large tree that grows in the evergreen forests of Cameroon, Congo, Guinea, Sudan, Gabon, and Uganda. In Cameroon, the bark and leaves (Figure 3.8) of the tree are used for its medicinal properties in the treatment of swelling, inflammation, rheumatism, diarrhoea, and bacterial infections (Wansi et al., 2010). Recently, the mechanical properties of *K. gabonensis* timber have been shown to be suitable for construction (Boadu et al., 2017). The Baka process *K.* gabonensis leaves for consumption in stews. They also consume the seeds and fruits of the tree, and process and sell the seeds' oil (Gallois et al., 2021).

Figure 3.8: Photograph of Klainedoxa gabonensis *leaves (from https://www.zambiaflora.com/speciesdata/image-display.php?species_id=185420&image_id=1).*

Panda oleosa

The *Panda oleosa* evergreen forest tree's (sample BOS 013) habitat ranges from Congo to Liberia (Harris, 2002). It is part of the Pandaceae family, and is a native species to Central and Western Africa. In Congo, *P. oleosa* is exploited for traditional medicine in treating HIV/AIDs and diabetes. The tree is collected from the wild for use as source of timber and food as well (Muhoya et al., 2017). The Baka collect seed nuts from the tree (Figure 3.9). They collect the fallen seeds from the forest floor, break them with machetes and remove the kernel. During a field survey, nearly all of the nuts collected were *P. oleosa* nuts as they are large and easy to find (Sato et al., 2012; Yasuoka, 2006). When dietary recalls were proposed to the Baka, *Panda oleosa* was among the most frequently listed species. The species is imperative for the Baka to meet the daily requirement of fat intake (Gallois et al., 2021). Commonly the nuts are sold on local markets but are preferred by the Baka for their own consumption (Gallois et al., 2020).

Figure 3.9: Photograph depicting a mid-section of a Panda oleosa *nut with the edible kernel encapsulated in the left half of the nut (from http://www.westafricanplants.senckenberg.de/root/index.php?page_id=14&id=2583).*

Trichoscypha acuminata

Trichoscypha acuminata is a small tree (sample BOS 017) is part of the Anacardiaceae family (Figure 3.10). Its habitat ranges from Gabon, Congo, to Cameroon. The tree is cultivated and undergoing the process of domestication in Africa. *T. acuminata* is commonly found in the central and southern regions of Cameroon. Here, the tree is mainly exploited for its fruit and it has supported Indigenous peoples for millennia. Chimpanzees and gorillas are known to consume the fruits as well (Hu et al., 2007). The fruits are consumed raw. The species is exploited exponentially due its high commercial and dietary benefits. To prevent extinction by unsustainable fruit harvesting there is an urgent need for domestication (Tsobeng et al., 2020). The Baka are known to collect and consume the fruits of *T. acuminata* (Gallois et al., 2020). The bark is known to be used for the treatment of constipation in children. A decoction of the bark is used for treating dysmenorrhoea, haemorrhages during pregnancy, rheumatics, headaches, or it is used as aphrodisiac (Burkill, 1994).

Figure 3.10: Photograph of Trichoscypha acuminata *tree and its bark (from https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:71836-1).*

Irvingia excelsa

Irvingia excelsa (sample KUN 001) is an economic farm tree found in West Africa. The trees can be found in the wild evergreen forests, but also in compound farms and outlaying fields. The seed's kernel is used as thickening agent in soups. The kernels are also high in fat. The nuts are better known as either bush mango or African mango (Figure 3.11). *Irvingia* shells are often used as fuel, and the bark serves as preservative in palm wine. *I. excelsa* also contributes economically as the kernels can be sold on markets for high prices (Ejiofor et al., 1987; Ugwumba et al., 2013). In Cameroon the plant is used for a variety of medical treatments. *I. excelsa* is used to relieve male infertility, sexual weakness, and male urogenital tract complaints. It has been shown *in vivo* that *I. excelsa* extract significantly reduces rat prostate size (Njamen et al., 2020). The tree is one of the most economically viable species in Western Africa, as it is a source of income, food, raw material and pharmaceutical material (Ugwumba et al., 2013). The Baka listed *Irvingia spp.* nut kernels as second most frequently consumed wild plant food on an annual basis. The mango kernels are easily stored and can be prepared as fatty paste or roasted for consumption (Gallois et al., 2020).

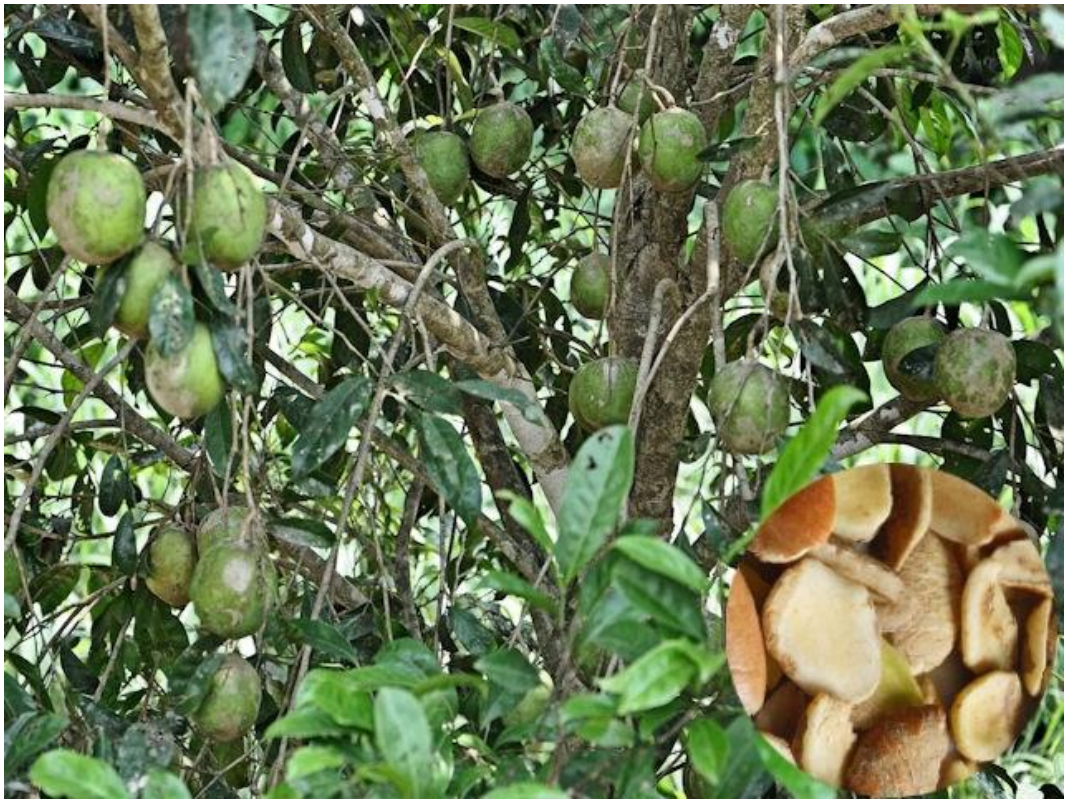

Figure 3.11: Photograph of Irvingia gabonensis. *The species is relatively similar* to Irvingia excelsa. *The photograph shows the nuts attached to the tree, with the nut kernels in the bottom right (from https://www.lafermedeleo.eu/nos-futurs-fruitiers-irvingia-gabonensis-en.html).*

Telfairia cf. occidentalis

The fluted gourd/pumpkin (sample KUN 003), or *Telfairia occidentalis*, is a perennial species native to Western Africa and is part of the Cucurbitaceae family. It mostly occurs in Nigeria in its cultivated form. The intensive cultivation stems from the vine's nutritious and palatable leaves, and its oil-rich seeds for oil manufacture and cooking. The seeds are recorded to be high in protein. Its fruits are among the largest known (Figure 3.12) (Idris, 2012; Okoli & Mgbeogu, 1983). The seeds are readily edible and usually are processed into powder for soups, or fermented into porridge. *Telfairia occidentalis* leaves are regularly consumed as vegetable (Idris, 2012). The leaves are rich in minerals such as potassium, calcium and iron, as well as vitamins. The plant has also been proven to have antidiabetic, antioxidant, haematological, antiplasmodial, anticancer, antimicrobial, anti-inflammatory and sedative properties (Eseyin et al., 2014).

Figure 3.12: Photograph of Telfairia cf. occidentalis*. The fluted gourd can be seen hanging from the vines, inside are its seeds that are used in cooking, among other parts of the plant (from https://www.virboga.de/Telfairia_occidentalis.htm).*

Dioscoreophyllum cumminsii

Dioscoreophyllum cumminsii belongs to the family of Menispermaceae and is also known as the 'serendipity berry'. This tropical rainforest vine produces fruits that are renowned for its sweet protein monellin that is commonly used as a natural sweetener (Oloyede et al., 2015). Currently, the plant is studied for its potential benefactors in insulin resistance, increasing of blood glucose, and the treatment of inflammation and oxidative stress (Ajiboye et al., 2016).

Gnetum africanum

Gnetum africanum, known as 'wild spinach' in English (sample ELO 007), is a shade-loving evergreen climbing liana part of the Gnetaceae family. It is found in preforest fallow, abandoned farmlands, dense forest, secondary forest and equatorial forest, and grows abundantly in tropical and subtropical Asia, South America, and Central Africa. Within Africa, there are two species of *Gnetum*: *Gnetum africanum* and *Gnetum bucholzianum*. The plant is of great importance to a plethora of forest-dwelling peoples (Ali et al., 2011). The leafy vegetable is eaten in 4-5 countries, by some 15-20 million people in total (Isong et al., 1999). The vegetable is very palatable and nutritious. Its leaves (Figure 25) are also used in the treatment of sore throast, enlarged spleens, pains of child-labour, and as antidote for snake bites (Ekop, 2007). The Baka consume 'koko'(*G. africanum*) more frequent than they do any other wild edible plant food. In addition, it is the only wild plant food considered as a prestige food item. The leafy vegetable is usually processed into thin slices. The consumption of 'koko' is relatively new in the area of Elonga as the Baka mentioned there was no prior consumption before 40 years ago. *G. africanum* leaves are rich in protein and likely used by the Baka as a substitution for meat during times of little hunting success. The leaves are also the most sold wild plant food on the international market (Gallois et al., 2020; Gallois et al., 2021).

Figure 3: Photograph of Gnetum africanum *leaves (from https://globalfoodbook.com/interesting-facts-about-gnetum-africanum-okazi-leaf).*

Ricinodendron heudelotii

Ricinidendron heudelotii (BIZ 001) is a West African deciduous tree commonly found in secondary forests. The tree is part of the Euphorbiaceae. The use of the tree varies from applications in construction to medicinal treatments for leprosy and elephantiasis (Shiembo et al., 1997). Furthermore, the plant has been applied as antidote and as treatment for cough, yellow fever, malaria, rheumatism, and stomach pain (Tamuno-Boma, 2016). Specifically, in Cameroon the fruits of the tree (Figure 3.13) are a vital source of cash income, as farmers sell the fruits on local markets. The seeds of the tree are valued as soup ingredient, but they are also used as an addition to a plethora of dishes. The seed oil is known to be processed into varnish and soap (Shiembo et al., 1997).

Figure 3.13: Photograph of Ricinodendron heudelotii *branch. The fruits containing its seeds are ready for harvesting (from https://www.inaturalist.org/taxa/338839-Ricinodendron-heudelotii).*

Baillonella toxisperma

The tree species of *Baillonella* toxisperma (sample BIZ 002) is among the most characteristic African trees. The tree is highly valued for its timber. Indigenous communities use its bark for the treatment of child birth shocks and rheumatism (Fungo et al., 2015). Its nut is used for consumption, and its oil for cosmetic and cooking purposes. *B. baillonella* is exploited in alarming rates, with some 100,000 m^3 harvested on an annual basis. Some 3.2 tonnes of bark were estimated to have been traded in Cameroon in 2000. The tree is put on the International Union for the Conservation of Nature's (ILCUN) list of 'vulnerable' species (Doucet et al., 2009). In the study area, this tree is the most exploited tree species of the region (Gallois et al., 2020). *B. toxiserpma* nuts (Figure 3.14) are exclusively available for only a few weeks per year for the Baka. The Baka's consumption of the nut peaks during the major rainy season. Although the nut is only available for a short window of time the Baka list the nut amongst the most salient wild edible food items. The Baka consume the *B. toxisperma* nut, and gather them from the forest floor. The nut is also among the most sold species due to its highly valued oil (Gallois et al., 2020; Gallois et al., 2021).

Figure 3.14: Photograph of Baillonella toxisperma *nuts (from https://www.pinterest.se/pin/722124121495545464/).*

Nutcake

The nutcake tested in the analysis is the sole processed food item included in the subsample. Baka are known to store seeds by pressing them into an oily cake (Gallois et al., 2021). *Irvingia* kernels are the preferred constituents in creating such a nutcake. The only species reported to be used in the making of a nutcake are *Irvingia gabonensis* and *Irvingia excelsa*. To prepare the cake, the Baka dry the bush mango kernels for two to three weeks, after which they rack them above a fire. Then, after roasting the kernels, they are pounded in a mortar separating an oily dough from the oil itself. A pot is then lined with banana leaves, upon which the dough and oil are poured. Subsequently, the oil and dough are mixed together. The cake is then left to dry in the sun for an additional two days, providing time for the oil to be absorbed back in to the dough. These cakes can be stored for periods up to two years. When required, the Baka scrape of portions of the cake resulting in a nutritious powder that is then added to their dishes (Gallois et al., 2020).

Undetermined mushrooms

The Baka regularly use a wide variety of fungi, but it was not possible to identify the species that were collected for this study. Nevertheless, we provide their nutritional qualities to give a general picture of the potential values of these mushrooms.

3.3. Laboratory Processing

This work contributes to a growing body of nutritional data for Indigenous plant use and broadens the scope of potential nutrient reference data for evolutionary anthropologists currently studying hunter-gatherer diets and lifeways.

The macronutrient analyses of the plant items were conducted in Laboratory of Food Chemistry of the department of Food Quality & Design (FQD) at Wageningen University & Research, the Netherlands. We measured dry weight, crude fat, protein, total phenol, and TDF contents. The fraction containing carbohydrates was calculated through difference. Total energy content (kcal) was calculated with the formula:

$$kcal = (carbohydrate \times 4) + (protein \times 4) + (fat \times 9) + (TDF \times 2)$$

Where protein is the average value of the duplicate protein values of the sample, fat is the average value of the duplicate fat values of the sample, TDF is the measured amount of TDF of the sample, and carbohydrate was obtained through difference of all other fractions of the sample. All macronutrients used in the equation are given in grams. The energy (kcal) values used for each macronutrient are commonly used in the food science industry (Charrondiere et al., 2004). Only the edible portions of the plants are equated into the macronutrient profiles of each sample. All data are given as g/100 g dry matter (or % dry matter) and all analyses were performed in duplicate.

3.3.1. Sample Preparation

Every sample was homogenized by a cryogenic laboratory mill. For robust analytical results, homogenization of organic samples is crucial. The mill (6875D Freezer/Mill® Dual-Chamber Cryogenic Grinder) (Figure 3.15) freezes the samples in liquid nitrogen and then pulverizes them with a magnetically driven impactor. Due to low temperatures, complex molecules found within nutritional items are not degraded by heat (Spex SamplePrep, n.d.) can be detrimental for the nutritional analysis (Marlett, 1990).

Figure 3.15: Photograph of the 6875D Freezer/Mill® Dual-Chamber Cryogenic Grinder. On the left side of the picture the cold-proof safety gloves and safety mask can be seen. On the right side, the device used to open the cannisters can be seen. Photograph by Author, 2022).

Before milling, sample dry weight was measured. The optimum volume, weight, grinding time, and impact frequency are determined by sample properties. All samples were pulverized for two cycles at a rate of 15 counts per second with a pre-cool time of 1 minute.

Figure 3.16: Photograph documenting the ongoing process of categorizing the wild edible plant samples. Photography by Author, 2022.

Depending on the hardness and fattiness of the sample the cycle duration ranges from 2.5 minutes to 5 minutes, with harder and fatter samples skewing towards longer cycle duration. Food items were categorized into the groups of: tubers, leaves, fruits, nuts, seeds, entire (e.g., bark or mushroom) (Figure 3.16). Bark was mechanically sawn by hand for it to fit the grinding vial. Leaves were pulverized by hand before milling. The Moabi fruit, or *Baillonella toxisperma* nuts, were encased in tough shells that were cracked open mechanically with a hammer. Tubers, seeds, and fruits were put into the grinding vials without any particular precautions. The remaining inedible parts of these samples such as roots or fragmented parts of encasings were removed by hand prior to milling. Afterwards, the homogenized sample weight was measured again on a Kern 440-49A precision weighing scale. The powdered samples were stored in 100ml plastic sample containers, in a freezer at -20 °C. The powdered samples make up the total dry matter for all the analyses conducted. Subsamples were taken from the powders for all analyses except for the determination of total dietary fiber, for which the defatted samples obtained by the Soxhlet procedure were used, as shown in Figure 3.17.

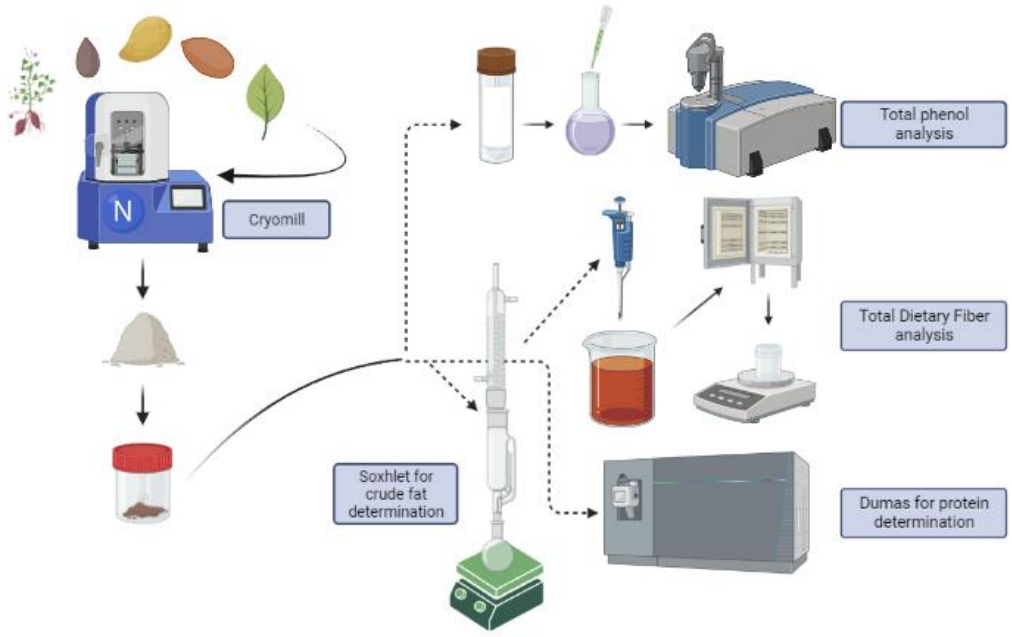

Figure 3.17: Schematic representation of the lab workflow. After the milling process, powdered samples were used for Soxhlet extraction, total phenol analysis, TDF analysis, and protein analysis. Figure made in Biorender.com, by Author, 2022.

3.3.2. Fat Determination

To determine the crude fat content for each sample, lipids were extracted with petroleum ether (P. E. 40-60°C; Merck 1.01774.2500) using a conventional Soxhlet-system (Gerhardt Laboratory Systems, Königswinter, Germany), following 'Manual nr. 38' of the FQD (25-03-2019) (see Appendix A) (Figure 3.18). The Soxhlet extraction is a frequently used form of leaching, a method to transfer crude fat molecules from their solid state into a liquid phase, or a solid-liquid extraction (Jensen, 2007). Leaching separates compounds of interest from insoluble high-molecular-weight fractions (fat) and removes any compounds that could interfere in the later steps of the analytical process (Luque De Castro & Priego-Capote, 2010).

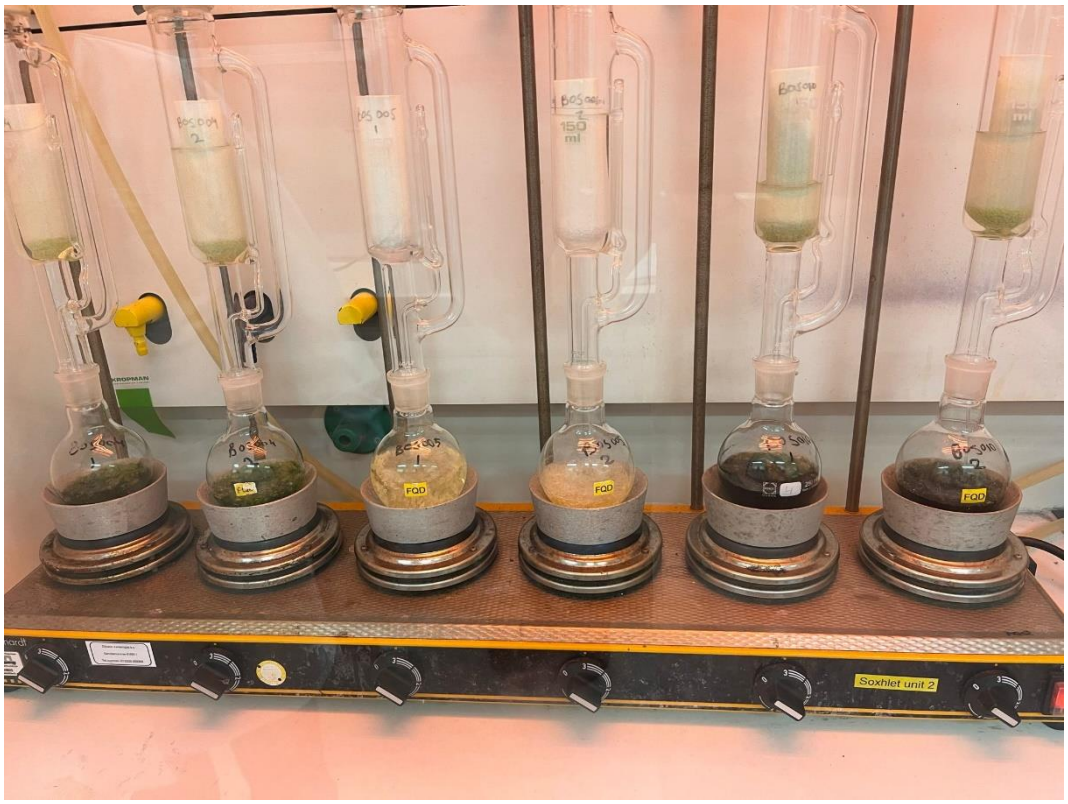

Figure 3.18: Photograph of a Soxhlet extraction. The white thimbles holding the powdered samples can be seen in the Soxhlet chambers. The heating elements are all put on Gerhardt position 3. The petroleum ether is boiling and continuously cycling the extractant through the thimble. Photography by Author, 2022.

The dry sample matter is placed in a thimble that is put into the Soxhlet chamber. The thimbles should be covered with cotton cloths. Empty 250 ml flat bottom flasks were weighed on a Mettler Toledo analytical scale after adding boiling stones to enhance heat transfer. Around 200 ml of petroleum ether was added to the flat bottom flask as solvent. Flasks are placed on the heating element (Gerhardt position 3). The cooking petroleum ether evaporates from the distillation flask into the Soxhlet chamber leaching the sample into the extractant. The extractant condenses under the cooling effect of the condenser and fills the thimble chamber until the liquid reaches the over-flow-point.

This carries the extracted compounds back into the flask, constituting one cycle. All extractant returns to the flasks after the extraction is done, with the extractant containing all lipids that were present in solid state sample before the process. The petroleum ether is then evaporated using Rotation Evaporators of Büchi (Figure 3.19) under vacuum following 'Manual nr. 37' of the FQD (25-03-2019). Compounds of high-molecular-weight are fractionated without any decomposition through rotary vacuum evaporation, leaving exclusively the undamaged fatty compounds within the flasks (Burch, 1929). The flasks were put in the fume hood for overnight evaporation of any remaining petroleum ether. The dry flasks were weighed after the overnight evaporation. The empty flask weight was subtracted from the extracted flask weight, resulting in the measured amount of crude fat (Figure 3.20). To establish the percentage of fat of the sample, the weight of extracted fat was divided by the total amount of sample weight put into the thimble, multiplied by 100.

Figure 3.19: Photograph of a Buchi rotary evaporator. The 250 ml flask is currently attached under vacuum whilst being heated and rotated. Photograph by Author, 2022).

Dependent on apparent crude fat value of the sample and the amount of sample available, 1.5-3.0 g was used for the extraction. More fatty (e.g., nuts or seeds) were extracted for a duration of 6 hours, less fatty samples were extracted for 3. During each run, samples of a similar food category were extracted within the same amount of extraction cycles. The defatted sample s remaining in the thimbles were stored in a fume hood for TDF determination.

Figure 3.20: Photograph of the extracted lipids from sample BIZ 003, duplicate 2. Here, the 250 ml flasks are shown after complete evaporation of petroleum ether. Photograph by Author, 2022.

3.3.3. Protein Determination

The determination of protein was done by measuring nitrogen content using the Dumas combustion method following 'Manual nr: 39' of the Chemistry Group at FQD (see Appendix A). Dumas offers a relatively quick and durable method for protein analysis. It requires a small amount of sample, and is well suited for solids. Moreover, Dumas is able to measure Nitrate-N (NO_3-N), a compound that commonly forms a large part of nitrogen present in plant samples (Sader et al., 2004). Standard series were established by combusting D-Methionine 99+%. The blanks consisted of 8 μm cellulose powder. Both the blanks and samples were put in tin cups, then weighed on a Mettler Toledo XPR6UD5 precision scale (0.0000 mg precision). During Dumas complete combustion, samples are oxidised in a metal column filled with oxidation chemicals under high temperature using pure oxygen. Released gases are transported through reduction, with helium as carrier gas. The gases are filtered by water filters and carbon dioxide filters, and transferred onto a separation column. Here, the nitrogen is separated from the other gases and measured by mass spectrometry with the Flash EA 1112 Protein analyser. The %N (total nitrogen measured relative to total sample weight) measurements were subtracted by the %N of the adhering blank. The corrected %N was then multiplied by a conversion factor of 6.25 - the nitrogen-to-protein conversion factor - to obtain the corrected % of protein in the dry matter of the sample. The protein data presented here is novel. As such, the conventional conversion factor of 6.25 was applied and protein values may appear slightly higher than expected (Charrondiere et al., 2004; Krul, 2019).

3.3.4. Total Dietary Fiber Analysis

The TDF analysis was done following the AACC method 32.05.01 and AOAC Method 985.29 (see (McCleary et al., 2013) as stated in the Megazyme TDF Assay Procedure K-TDFR-100A/K-TDFR-200A 04/17 manual (see Appendix A). Both methods are based on the analysis of Prosky et al. (1985) that uses a combination of gravimetric and enzymatic procedures. The enzymatic gravimetric treatment mimics digestion and leaves behind fibre residue (Phillips et al., 2019). TDF is constituted by hemicellulose, cellulose, oligosaccharides, lignin, pectins, and gums and waxes (Dhingra et al., 2012). Relative to other gravimetric methods, the 32-05.01 procedure results in high TDF results due to the usage of α-amylase (McCleary et al., 2013). Gravimetric analyses following Prosky et al. (1985) yield values within 10% of chemically determined values (Marlett, 1990, p. 35). The AACC 32.05.01 and AOAC Method 985.29 measure polysaccharides as fibre. The analysis was conducted on dried and defatted (fat content > 10% of sample) duplicate samples of 1 g. Samples were dried overnight at 105°C, alongside the celite added to the

crucibles. For obtaining ash weight the crucibles were put into a Heratherm oven and incinerated for 5 hours at 525°C.

A phosphate buffer was added to 1 g of dried sample, together with 50 µl α-amylase. Samples were then incubated at 98 - 100°C for 30 minutes. The pH was adjusted to 7.5 and 100 µl of protease was added, followed by an incubation period of 30 minutes at 60°C. Next, the pH was adjusted to 4.5 and 200 µl of amyloglucosidase was added (Figure 3.21).

Figure 3.21: Photograph of all enzymes used for the TDF analysis, according to the TDF Assay Procedure K-TDFR-100A/K-TDFR-200A 04/17 manual. Photograph by Author, 2022.

The sample was then incubated at 60°C for another 30 minutes. Next, the samples were precipitated with ethanol heated to 60°C (Figure 3.22).

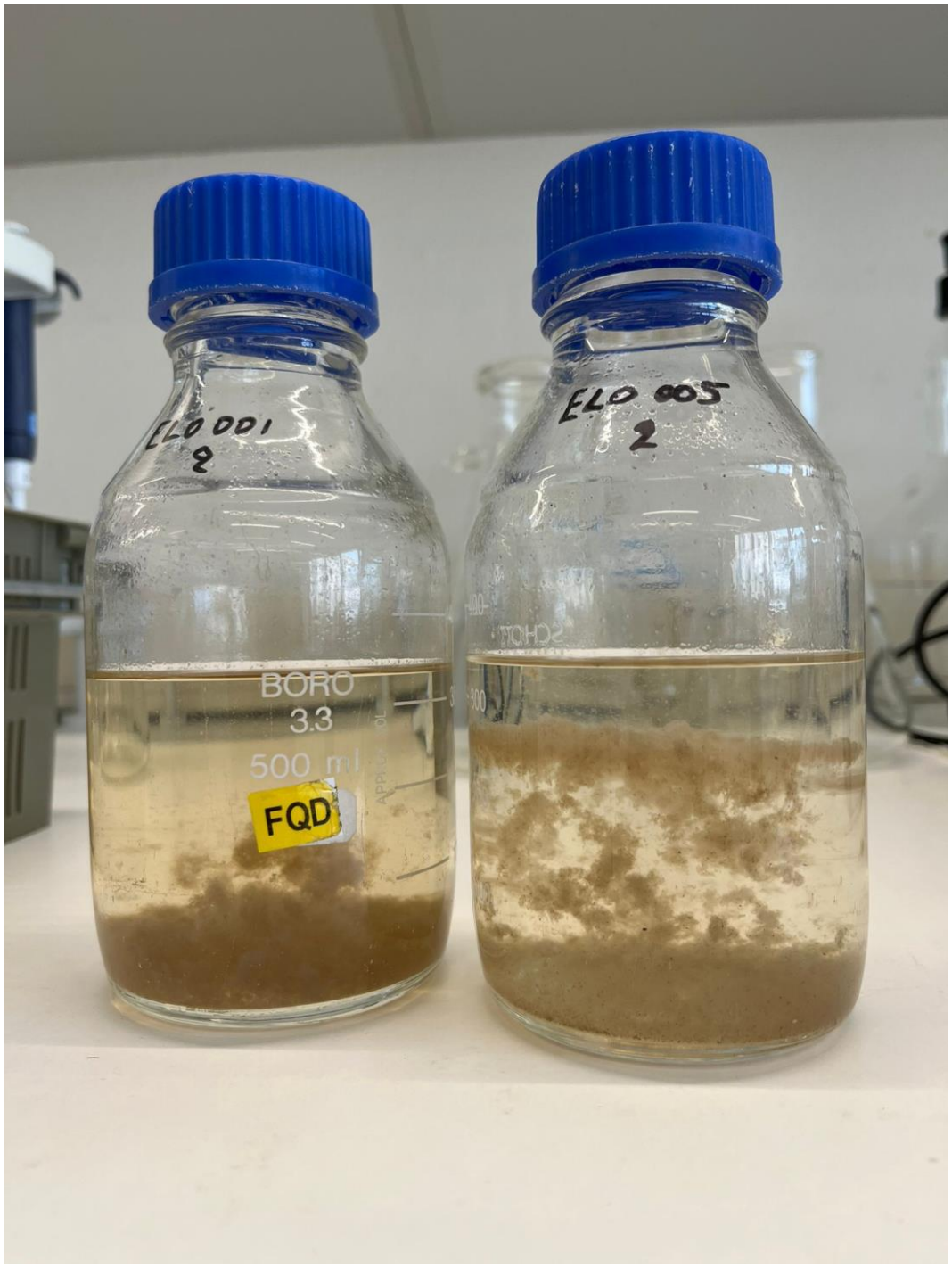

Figure 3.22: Photograph of sample ELO 001, duplicate 2, and ELO 005, duplicate 2, after precipitation with heated ethanol. Photograph by Author, 2022.

Vacuum pumps were used for the filtration. Samples were poured into the celite crucibles. Sample containers were washed in the following sequence: 3* 20 ml of 78% ethanol → 2* 10 ml of 95% ethanol → 2* 10 ml acetone. Then the residue inside the crucible was left to dry overnight at 105°C and weighed after drying. Lastly, samples were incinerated. The sample duplicates were assigned for either protein or ash determination. The following formula was used to calculate TDF:

$$Total\ Dietary\ Fiber\ (\%) = \frac{\frac{R_1 + R_2}{2} - p - A - B}{\frac{m_1 + m_2}{2}} \times 100$$

Where R_1 = residue weight of duplicate 1 from m_1; R_2 = residue weight of duplicate 2 from m_2. Here, m_1 is the sample weight of duplicate 1 and m_2 is the sample weight of duplicate 2. A= ash weight from R_1; p = protein weight from R_2.

To calculate B (blank) for referencing:

$$B = \frac{BR_1 + BR_2}{2} - BP - BA$$

Where BR = the blank residue, BA = blank ash from BR_2 and BP = blank protein from BR_1. To simplify the calculations the Megazyme *Mega-Calc*™ was used (www.megazyme.com). The final %TDF obtained by the above calculations were corrected for fat as the samples were defatted in preparations for the analysis. Lastly, to convert macronutrient values from either dry weight concentration (DW) to wet weight/fresh weight concentration (WW) the following formulas were used:

To convert values given in fresh weight to values in dry weight:

$$DW = WW \times \frac{100}{(100\% - moisture\ percentage)}$$

To convert values given in dry weight to values in fresh weight:

$$WW = DW \times \frac{(100\% - moisture\ percentage)}{100}$$

Where '100' is the total value given for the macronutrient up for conversion, the moisture percentage is obtained by calculating the water weight through subtracting the dry weight from the fresh weight, then subsequently dividing the water content by the fresh weight and dividing by a 100. In some instances the moisture percentage may already be available in the literature.

3.3.5. Total Phenol Analysis

The determination of the total phenolic content of the plant samples was done according to the Folin & Ciocalteu (FC) method in 'Protocol nr: 4' of the FQD, based on Swain and Hillis (1959) (see Appendix A). The FC method's main advantage is that it responds fairly equal to different phenols. However, its disadvantage is that the method responds to sugars and sulphur dioxide as well (Waterhouse, 2001). Despite this, FC analysis is relatively simple as it requires common analytical equipment. A large body of phenol data has been established through the FC method and its results are readily comparable to a large corpus of similar data (Singleton et al., 1999). The FC method is known as a antioxidant capacity (AOX) method involving the transfer of a single electron (SET). In most plants antioxidants constitute the predominant part of phenolics present (Dudonné et al., 2009). Hence, the FT method is applicable for estimating total phenolic content in plants (Everette et al., 2010). The phenolic compounds are oxidised by the FC reagent, originally proposed by Folin and Ciocalteu (1927) for measuring tyrosine. The products of this metal oxide reduction have a blue colour (Figure 3.23) and maintain a broad light absorption with a wavelength of a maximum of 765 nm.

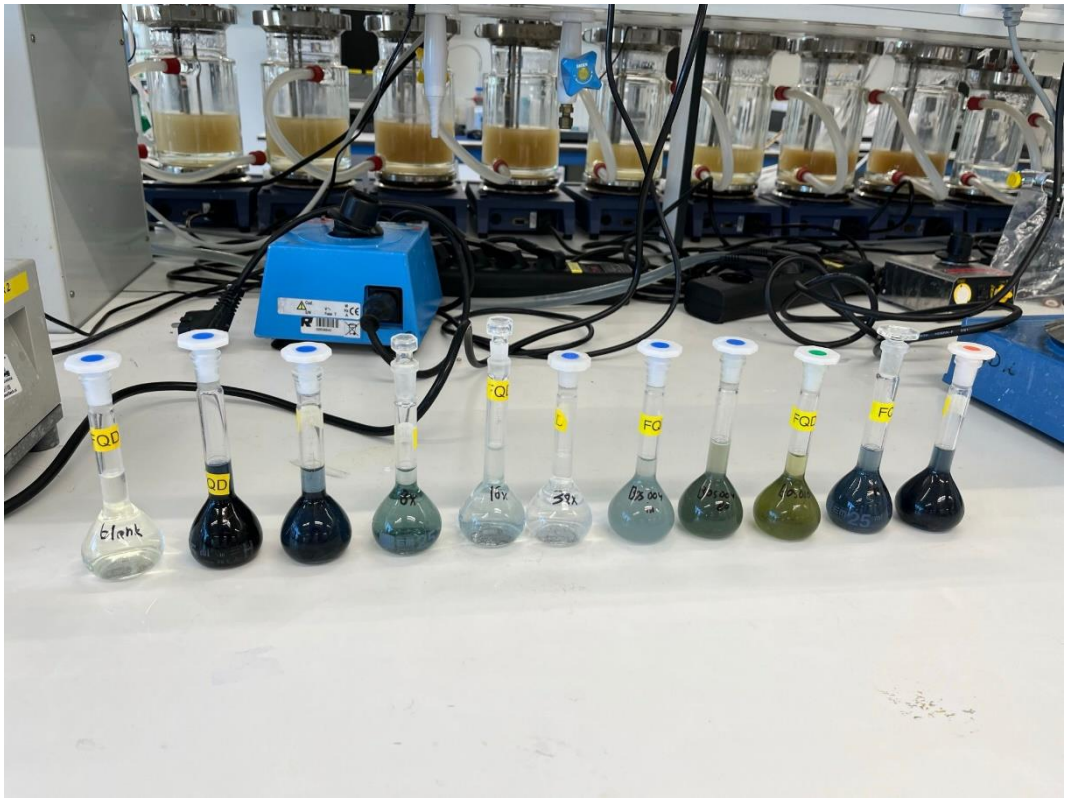

Figure 3.23: Photograph of a range of samples after the phenolic compounds had oxidized under the effect of the Folin and Ciocalteu reagent. Note that samples with a high total phenolic content appear dark-blueish (the two 25 ml flasks on the far right), whereas low phenolic contents are reflected by increased transparency as visible in the lefthand-side blank, or the 32x diluted stock solution. Photograph by Author, 2022).

The concentration of total phenols is proportional to the intensity of light absorption (Waterhouse, 2001). The intensity of light absorption was measured with the Agilent Technologies Cary 60 UV-Vis spectrophotometer. To convert the measured light absorption to mg total phenol/g of dry matter the following formula was used:

$$C = C_1 \times V/m$$

Here, C is the total phenolic content in mg/g in Gallic Acid Equivalent (GAE), C_1 is the concentration of gallic acid established from the calibration curve in mg/ml, V is the volume of sample extract in ml, and m is the weight of the sample used in g (see Siddiqui et al., 2017).

To establish the calibration curve, a stock solution of gallic acid was made by adding 40 ml of gallic acid (Merck 149-91-7) to 40 ml of demi water (i.e.: an aqueous solution of gallic acid of 1mg/ml). This stock solution was diluted with the factors of 2*, 4*, 8*, 16* and 32* to create a series of 5 standards for the calibration curve. The sodium carbonate (Na_2CO_3) solution was prepared by adding 70 g of Na_2CO_3 to 100 ml of demi water. Before usage, the Na_2CO_3 solution was stirred at room temperature for 1 hour.

All samples were weighed in solid state to 0.500 g on a Mettler Toledo analytical scale. Then, 5 ml of 100% methanol was added to create a suspension for the extraction. The suspension was then mixed for 15 minutes in a Heidolph Shaker, speed setting '8'. Samples were then placed in a centrifuge for 10 minutes at 4700 rpm. The supernatant of the aliquots was then transferred through 0.20 µl PTFE filters using 5 ml syringes. Subsequently, 5 ml of 100% methanol was added to the extracted supernatant and shaken again for 15 minutes. The aliquots were then centrifuged for 10 minutes at 4700 rpm again, The supernatant was transferred with 0.20 µl PTFE filters to complete the second extraction. Lastly, the extractions were subjected to one last round of centrifuging at 4700 rpm, for 10 minutes. Each time, 10 ml transparent aliquots were extracted. Volumetric flasks of 25 ml were filled with 5 ml of demi water, or 6 ml of demi water if the flask was assigned to a calibration blank. Then, 1.0 ml of the sample's extracted supernatant was added, followed by 1.0 ml of FC reagent (Merck 109001.0500), then 1.0 ml of saturated Na_2CO_3 solution was added. The volume was adjusted to add up to a total of 25 ml. The flasks were shaken by hand and then shielded from any light sources by wrapping aluminium foil around the flasks for 15 minutes. Spectrophotometer measurements were done at 750 nm for 0.8000 seconds with two replicate measurements.

3.4. Limitations

Due to limited time and resources the analysis was conducted in duplicates instead of the preferred triplicates. In addition, due to the amount of sample available and the amount of measurements conducted, no meaningful statistical testing could be done on the presented data. However, when too large of a discrepancy was noted between duplicate measurements of the same sample, the measurement was repeated. No anomalous measurements or outliers are included in the reported data. Furthermore, the sample preparation and chemical analyses conducted were destructive in nature. Leftover homogenized samples are currently being stored at the Faculty of Archaeology, Leiden University. Lastly, as the chemical analyses were conducted on homogenised dried samples, water content was not present in any of the samples. The dry weights used do not equate to fresh edible weights. Since the reported food items are almost always consumed when fresh, data presented in the first section of the results chapter are not representative of any of the food items' actual macronutrient ratios during consumption. In light of this, the percentage of moisture content per sample was calculated, whenever the sample weight after sample collection in the field and the weight after sun drying were recorded. This estimated water percentage was used to convert the macronutrient ratios into fresh weight ratios to better portray the macronutrient ratios present in the fresh wild plants.

4. Results

This chapter presents the results obtained by the nutritional analyses conducted in the Food Science lab of Wageningen University & Research. The data are elaborated upon through descriptive statistics. The first section showcases the macronutrient composition, alongside the energy content and phenolic content of each individual sample. Here, the freshly obtained data is compared to previously published data from the literature, whenever possible. Subsequently, an overview is given that shows the mean macronutrient amounts per food type. The samples have been divided into six individual food type categories: leaves (n=5), fruits (n=5), nuts (n=3), tubers (n=10), seeds (n=3), and mushrooms (n=2). The subsample of rice that was analysed is not included in the seed category type as it constitutes a domestic species. In the next section, the taxa within each food type category are compared side by side with globally consumed domestic species of a similar food type. Mushrooms will not be discussed on a within food type level, as the two sampled mushroom species could not be identified to genus level.

4.1. Macronutrient Composition, Energy Content, and Total Phenolic Content

Musanga cecropioides

The fruits of sample BOS 001, or 'Kpe Bombo' in Baka, have relatively large amounts of TDF and carbohydrates. In comparison, the protein and crude fat contents are low. *Musanga cecropioides* contains 331.5 kcal/100 g as shown in the macronutrient profile given in Table 4.1.

No other data on the macronutritional composition of *M. cecropioides* fruits has been published. For this taxa only leaf nutritional properties have been published (Shemishere et al., 2018). However, these data are generally not comparable to those of fruits. As such, nutritional data for the fruit of *Musanga loe-errerae* published by Kagoro-Rugunda (2020) allows for a better nutritional comparison.

Table 4.1: Macronutrient profile established for Musanga cecropioides *fruit including energy content, in g per 100 g dry weight. Previously published macronutrient data is added for comparison.*

Baka name, *Genus species*	Crude fat	Protein	TDF	Carbohydrate	Energy (kcal/100 g dry weight)
	(g / 100 g dry weight)				
Kpe Kombo *Musanga cecropioides*	5.8	6.4	48.6	39.2	331.5
N/a *Musanga loe-errerae* (Kagoro-Rugunda, 2020)	3.7	10.1	22.5	63.7[1]	309.8[2]

[1] The carbohydrate content was obtained by difference.

[2] The energy content was calculated with the formula listed in the Methods chapter for calculating energy content.

Comparing the relative proportions of the nutrient composition for *M. cecropioides* and *M. loe-errerae* appears to show general overlap in total energy, protein, and crude fat contents. Broader differences are present between the TDF and carbohydrate contents, possibly resulting from different analytical methods.

The total phenolic content of the fruit is 5.8 mg gallic acid equivalent (GAE) per gram of sample. This is significantly lower than the total phenolic content established for its leaves (385.5 mg GAE/g) (Sowemimo et al., 2015).

Aframomum subsericum* and *Aframomum danielli

The fruit of BOS 002, *Aframomum subsericum* was found to be relatively high in carbohydrate content and TDF content. The fat content is relatively low. The protein content is relatively low as well. In total, the fruit of *A. subsericum* contains 326.3 kcal/100 g of dry matter. No other data on the macronutritional composition of *A. subsericum* is available. The analysis for *A. danielli* (BOS 005 and BOS 015) yielded relatively low protein and fat values. The TDF content measured is also relatively low, whereas the carbohydrate content is considerably high (BOS 005). The total energy content for *A. danielli* (BOS 005) was found to be 410.2 kcal/100 g of dry matter. For BOS 015 the crude fat content measured was relatively low. Likewise, the protein content measured was proportionately low as well. The carbohydrate content was relatively high, and the TDF content was measured to be average. The total energy content measured for BOS 015 was 396.6 as shown in Table 4.2. No previously published data on the nutritional composition of *A. subsericum*, nor *A. danielli* is available. *Aframomum alboviolaceum* fruit and *Aframomum spp.* fruit have undergone chemical analyses (see Herzog et al., 1994; Wu-Leung, 1968). Additional values for the protein and energy contents of *A. danielli* were available, although only the seeds have been analysed (see Adegoke & Skura, 1994).

The proportional nutrient composition of *A. danielli* (BOS 005 and BOS 015) suggests relatively little intraspecies variation. Both their crude fat and protein contents show no noteworthy discrepancies. BOS 005 is shown to be higher in carbohydrate content when compared to BOS 015, whereas BOS 015 is suggested to have a higher TDF content in comparison to BOS 005. The relative proportion of the nutrient profile obtained for *A. subsericum* shows no large differences with that of *A. alboviolaceum*. Compared to the values of *Aframomum spp., A. subsericum* is suggested to contain less fat and protein than other *Aframomum* species. The proportional nutrient composition obtained for *A. danielli* compared to that of *A. alboviolaceum* suggests that *A. danielli* fruit is higher in crude fat content than the aforementioned fruit. Compared to *Aframomum spp.*, the results for *A. danielli* show a relatively higher fat content for the latter, and a relatively higher protein content for the former. Altogether, *Aframomum spp.* fruits are relatively high in carbohydrate content as well as in TDF content. Crude fat and protein values are relatively low overall. Data from Popovich et al. (1997) largely deviates in the TDF and carbohydrates contents published.

The total phenolic analysis for *A. subsericum* yielded a total phenolic content of 60.8 mg GAE/g of dry matter. No previous data on the total phenolic content of *A. subsericum* fruit, nor *A. danielli* fruit has been published. The determination of total phenolic content for *A. danielli* resulted in values of 18.7 mg GAE/g of dry matter for BOS 005, and 11.3 mg GAE/g of dry matter for BOS 015. *A. subsericum* total phenolic content for its fruit appears proportionately higher than the values measured for *A. danielli.* Between both *A. danielli* samples BOS 005 yielded arelatively higher total phenolic content in comparison to BOS 015. Data on the total phenolic content of *Aframomum danielli* seeds and *Aframomum melegueta* seeds are available. *A. danielli* seeds are reported to have a total phenolic content of 3.8 mg GAE/g. The seeds of *A. melegueta* are suggested to contain 3.9 mg GAE/g of total phenols (Adefegha & Oboh, 2012). These data suggest the phenolic content of *Aframomum* seeds to be lower than values measured for *Aframomum* fruits.

Table 4.2: Macronutrient profile established for Aframomum subsericum *and* Aframomum danielli *fruits including energy content, in g per 100 g dry weight. Previously published macronutrient data is added for comparison.*

Baka name, *Genus species*	Crude fat (g / 100 g dry weight)	Protein (g / 100 g dry weight)	TDF (g / 100 g dry weight)	Carbohydrates (g / 100 g dry weight)	Energy (kcal/100 g dry weight)
Tondo na Niivi *Aframomum subsericum*	1.5	3.7	40.6	54.2	326.3
Tondo na Mongamba *Aframomum danielli* (BOS 005)	8.2	5.5	15.3	71	410.2
Tondo na Mongamba *A. danielli* (BOS 015)	8.7	4.3	23.4	63.6	396.6
N/a *Aframomum*	2.4	5.5	35.8	56.32	215.8

alboviolaceum (Herzog et al., 1994)					
N/a *Aframomum spp.* (Wu-Leung, 1968)	4.5	8.1	-	81.93	360.7
N/a *Aframomum sp.* (Popovich et al., 1997)	0.2	4.0	83.7	5.7	2084

[1] Nutritional values published in g/100 g of edible matter were converted to g/100 g of dry matter by subtracting the mean moisture content from 100%. The 'edible portion' was divided by the calculated dry matter percentage and multiplied by 100.

[2] Carbohydrate content given for Herzog et al. (1994) was calculated through difference of the dry matter nutritional values.

[3] The carbohydrate content given by Wu-Leung (1968) includes fibre content.

[4] The energy content was calculated with the formula listed in the Methods chapter for calculating energy content.

Hilleria latifolia

The chemical analysis of the leaves of *Hilleria latifolia* (sample BOS 004) suggested a relatively high amount of carbohydrate content, as well as a relatively high amount of TDF. The 'Sumba' leaves contain a relatively average amount of protein and a relatively low amount of fat. In total, the energy content of *H. latifolia* is suggested to be 350.8 kcal/100 g of dry matter. The inedible part of the plant (the leaf stems) were also analysed. In contract, the stems are suggested to contain less energy. The values for protein, fat and carbohydrates are all lower than those of the analysed edible portion of the sample. The TDF content of the stems was found to be higher. The total phenolic content is estimated at 6.7 mg GAE/g of dry matter for the edible portion and 6.2 mg GAE/g of dry matter for the stems.

Table 4.3: Macronutrient profile established for Hilleria latifolia *leaves including energy content, in g per 100 g dry weight. Previously published macronutrient data is added for comparison. Data on the inedible stems of the leaves is included.*

Baka name, *Genus species*	Crude fat	Protein	TDF	Carbohydrates	Energy (kcal/100 g dry weight)
	(g / 100 g dry weight)				
Sumba *Hilleria latifolia* (edible)	2.3	19.7	30.4	47.5	350.8
Sumba *Hilleria latifolia* (inedible)	1.2	13.7	44.3	40.8	317.3
N/a *Hilleria latifolia* (Wu-Leung, 1968)[1]	5.1	26.1	19.1	49.7	396.6

[1] Nutritional values published in g/100 g of edible matter were converted to g/100 g of dry matter by subtracting the mean moisture content from 100%. The 'edible portion' was divided by the calculated dry matter percentage and multiplied by 100.

Additional nutritional data on *H. latifolia* leaves has only been published by Wu-Leung (1968) and has been included in Table 4.3. Wu-Leung (1968) found a protein value relatively higher in comparison to the one reported in this analysis. The author reported a relatively higher amount of fat and a relatively similar amount of. Wu-Leung (1968) furthermore reported a relatively lower amount of TDF. In total, an energy value of 280.3 kcal/100 g of dry matter is reported (Wu-Leung, 1968).

Oryza sativa

Rice (sample BOS 006) yielded relatively low values for protein and fat contents, a somewhat higher TDF content, and a relatively high carbohydrate content. The energy content is estimated at a total of 387.2 kcal/100 g of dry matter. Rice is shown to contain a relatively high amount of carbohydrates, and relatively low amounts of protein, fat, and TDF. Fat content is the lowest of all macronutrients measured. The total phenolic content was determined to be 3.4 mg GAE/g of dry matter.

Table 4.4: Macronutrient profile established for Oryza sativa *fruit including energy content, in g per 100 g dry weight. Previously published macronutrient data is added for comparison.*

Baka name, *Genus species*	Crude fat (g / 100 g dry weight)	Protein (g / 100 g dry weight)	TDF (g / 100 g dry weight)	Carbohydrates (g / 100 g dry weight)	Energy (kcal/100 g dry weight)
Lee *Oryza sativa*	0.5	6.7	12.1	80.8	378.2
N/a *Oryza sativa* (Frei & Becker, 2005)	2.0	9.5	-	-	-
N/a *Oryza sativa* (NARO, 2011)	3.3	7.7	-	86.6	-
N/a *Oryza sativa* (USDA, 2014)[1]	3.2	7.7	3.9	91.1	431.8
N/a *Oryza sativa* (USDA, 2023)[1]	1.1	7.9	3.2	90.4	404.3

[1] Nutritional values published in g/100 g of edible matter were converted to g/100 g of dry matter by subtracting the mean moisture content from 100%. The 'edible portion' was divided by the calculated dry matter percentage and multiplied by 100.

The sampled rice is similar in its relative proportion of macronutrients to the relative proportions reported in other databases (Frei & Becker, 2005; NARO, 2011; USDA, 2014, 2023) as shown in the overview given in Table 4.4. The rice acquired by the Baka is suggested to be relatively higher in TDF content and somewhat lower in crude fat- and protein content when compared to earlier data. Published data on the total phenolic contents of Brazilian non-pigmented *O. sativa* suggests a value of 0.94 mg GAE/g of dry matter (Adom & Liu, 2002). Total phenolic contents for pigmented rice genotypes such as brown-reddish and black rice have a mean level of 4.3 FAE (ferulic acid equivalent)/g/ (De Mira et al., 2009). Relatively, the total phenolic content of Cameroonian rice averages between the studied non-pigmented and pigmented rice phenotypes.

Arachis hypogaea

The seeds of *Arachis hypogaea* (peanuts, sample BOS 007) have a relatively high crude fat content, more than half of *A. hypogaea*'s dry matter consists of lipids. The peanuts also contain a relatively large amount of protein. The carbohydrate and TDF content are relatively low. Related to the high fat content measured, the energy content of the peanut is relatively high at 635.1 kcal/100 g of dry matter as shown in Table 4.5. The total phenolic content measured is 8.5 mg GAE/g of dry matter. Other known total phenol values are given by Sebei et al. (2013). Here four values are given for peanut seeds coming from four different cultivators respectively: 1.35 mg GAE/g, 1.35 mg GAE/g, 2.1 mg GAE/g, and 1.0 mg GAE/g (Sebei et al., 2013). In contrast, the measurements for peanuts proposed in this analysis are relatively higher for total phenolic content.

Table 4.5: Macronutrient profile established for Arachis hypogaea *seeds including energy content, in g per 100 g dry weight. Previously published macronutrient data is added for comparison.*

Baka name, *Genus species*	Crude fat	Protein	TDF	Carbohydrates	Energy (kcal/100 g dry weight)
	(g / 100 g dry weight)				
Peanuts *Arachis hypogaea*	51.4	28.4	11.1	9.1	635.1

N/a *Arachis hypogaea* (USDA, 2023)[1]	45.4	24.4	8.4	27.8	617.8
N/a *Arachis hypogaea* (PROTA4U, n.d.)[1]	52.6	27.6	9.1	17.2	606.4[3]
N/a *Arachis hypogaea* (Campos-Mondragón et al., 2009)[2]	51.6	24.9	3.7	21.0	655.4

[1] Nutritional values published in g/100 g of edible matter were converted to g/100 g of dry matter by subtracting the mean moisture content from 100%. The 'edible portion' was divided by the calculated dry matter percentage and multiplied by 100.

[2] Macronutrient values listed from Campos- Mondragón et al. (2009) are the mean values obtained from all chemical compositions reported by the authors.

[3]The energy content was calculated with the formula listed in the Methods section of Chapter 3 for calculating energy content.

In comparison, for raw peanut the USDA reported a somewhat lower fat and protein content, but a higher carbohydrate content. The reported TDF content is relatively average, and the total energy content of 617.8 kcal/100 g of dry matter is somewhat lower in comparison (USDA, 2023). Additional data corroborates that the sampled peanut is relatively high in TDF and low in carbohydrate content in comparison (Campos-Mondragón et al., 2009; PROTA4U, n.d. ; USDA, 2023).

Gaertnera cf. longivaginalis

The sundried leaves of *Gaertnera cf. longivaginalis* **(**BOS 010) yielded a high amount of measured protein content. The leaves are also relatively high in TDF content. The crude fat content is very low relative to the other macronutrient values obtained. Carbohydrate content is low relative to the other nutritional constituents as well. In total the *G. cf. longivaginalis* leaves contain 323.7 kcal/100 g of dry matter as shown in Table 4.6. The total phenolic content measured is 3.9 mg GAE/g. No additional data on *G. cf. longivaginalis* was available during the time of writing.

Table 4.6: Macronutrient profile established for Gaertnera cf. longivaginalis *leaves including energy content, in g per 100 g dry weight.*

Baka name, *Genus species*	Crude fat	Protein	TDF	Carbohydrates	Energy (kcal/100 g dry weight)
	(g / 100 g dry weight)				
Mpong mpong *Gaertnera cf. longivaginalis*	2.6	39.0	44.7	13.7	323.7

Dioscorea spp.

Yams belonging to the genus of *Dioscorea* all yielded relatively high values for carbohydrate content, except for the *Dioscorea sp.* from Bosquet and *Dioscorea minutiflora* from Bosquet. On the contrary, compared to all other yams, *Dioscorea sp.* and *D. minutiflora* have relatively high TDF contents. Overall, *Dioscorea* yams are relatively low in crude fat content. Interspecies variation does occur. *Dioscorea praehensilis* from Elonda stands out with its relatively high crude fat content. Next to *D. praehensilis*, *Dioscorea burkiliana* from Kungu yielded a high crude fat value, as well as a relatively higher amount of protein in comparison to the other tubers in its genus. A relatively higher amount of protein was also measured in the aforementioned *D. praehensilis* from Elonda. The average amount of energy content for the yams is 387.4 kcal/100 g of dry matter, with those tubers with a higher TDF content lowering the mean value, and those with higher protein/fat values increasing the mean value, respectively. The nutritional profile of the cultivated 'Sapa na Gba' (Elonda, *D. praehensilis*) overlaps with the nutrient profiles of other proportionately average yams listed as shown in Table 4.7.

The total phenolic content for *D. minutiflora* is relatively very high (32.9 mg GAE/g) in comparison to the measured content of the other *Dioscorea* yams. The second highest total phenolic content was measured for *Dioscorea sp.* (7.8 mg GAE/g). The remainder of total phenolic contents for the yams ranges between 4.2 – 1.7 mg GAE/g, with a mean value of 2.9 mg GAE/g. In comparison Sakthidevi and Mohan (2013) reported values of 6.8 mg GAE/ g and 12.1 g GAE mg/ g for *Dioscorea alata*. Additional published values for *D. alata* are 4.2 mg GAE/g, 0.7 mg GAE/g, 1.8 mg GAE/g, 0.7 mg GAE/g, and 2.3 mg GAE/g Values for another species, that of *Dioscorea esculenta* are reported at 1.6 mg GAE/g and 1.1 mg GAE/g (Cornago et al., 2011).

Table 4.7: Macronutrient profile established for Dioscorea spp. *tubers including energy content, in g per 100 g dry weight. Previously published macronutrient data is added for comparison.*

Baka name, *Genus species*	Crude fat (g / 100 g dry weight)	Protein (g / 100 g dry weight)	TDF (g / 100 g dry weight)	Carbohydrates (g / 100 g dry weight)	Energy (kcal/100 g dry weight)
Epange (Bosquet) *Dioscorea sp.*	1.5	4.4	74.3	19.7	259

Kuku na Bele (Bosquet) *Dioscorea minutiflora*	0.8	4.0	72.4	22.8	259
Ba na Bele (Kungu) *Dioscorea burkiliana*	16.3	5.2	11.5	67.0	458.6
Keke na Bele (Elonda) *Dioscorea sp.*	0.2	5.8	6.7	87.3	387.6
Ba na Bele (Elonda) *Dioscorea sp.*	0.3	5.1	2.1	92.5	397.5
Keke na Bele (Elonda) *Dioscorea burkiliana*	0.4	7.2	12.5	79.8	377.1
Sapa na Bele (Elonda) *Dioscorea praehensilis*	14.2	9.6	2.4	73.9	466.3
Sapa na Gba (Cultivated, Elonda) *Dioscorea praehensilis*	0.3	6.7	6.1	86.9	389.5
Ba na Bele (Elonda)	0.3	4,7	5.1	89.9	391.5

Dioscorea sp.					
N/a *Dioscorea spp.* (Wu-Leung, 1968)[1]	0.7	6.1	2.6	89.7	383.9
Yam (USDA, 2019)[1]	0.6	5.0	13.5	91.7	419.2[2]

[1] Nutritional values published in g/100 g of edible matter were converted to g/100 g of dry matter by subtracting the mean moisture content from 100%. The 'edible portion' was divided by the calculated dry matter percentage and multiplied by 100.
[2]The energy content was calculated with the formula listed in the Methods chapter for calculating energy content.

Additional data on *Dioscorea spp.* nutritional composition is scarce. Wu-Leung (1968) has published average numbers (n=13) for *Dioscorea spp.* Wu-Leung's (1968) proposed nutritional profile is proportionately similar to the average amounts of macronutrients recorded for *Dioscorea* yams in this analysis. The proposed TDF content is relatively on the lower end of the spectrum, similar to the listed crude fat content. Again, carbohydrate is relatively very high. The energy content of 383.9 kcal/100 g of dry matter falls close to the mean value of 387.4 kcal/100 g of dry matter. Data published by the USDA (2019) for 'yam' has a relatively high TDF and carbohydrate content, resulting in a relatively high caloric content in comparison to the mean values listed as shown in Table 4.7.

Intraspecies variation in *D. praehensisilis* is most evident for crude fat content, TDF content, and carbohydrate content. In *D. burkiliana* intraspecies variation occurs in al the recorded macronutrients. The largest variation is evidenced in crude fat and protein contents, as well as TDF content.

Klainedoxa gabonensis

Results for the leaves of *Klainedoxa gabonensis* (BOS 012) suggest a relatively very high crude fat content. The leaves also contain a relatively large amount of TDF. In contrast, the carbohydrate content is relatively low. The protein content is suggested to be relatively low as well. The suggested total energy content of *K. gabonensis* leaves is 575.5 kcal/100 g of dry matter as shown in Table 4.8. The total phenolic content of the leaves is suggested to be 8.9 mg GAE/g.

K. gabonensis and other *Klainedoxa* species are understudied, no data on the nutritional composition of *K. gabonensis* leaves is available for comparison at the moment of writing.

Table 4.8: Macronutrient profile established for Klainedoxa gabonensis *leaves including energy content, in g per 100 g dry weight.*

Baka name, *Genus species*	Crude fat	Protein	TDF	Carbohydrates	Energy (kcal/100 g dry weight)
	(g / 100 g dry weight)				
Bokoko *Klainedoxa gabonensis*	48.7	10.6	33.9	6.9	575.5

Panda oleosa

The nuts of *Panda oleosa* almost consist of 50% fat, with a suggest crude fat content. Relative to the crude fat content, the protein content and the TDF content are sizeable. The carbohydrate content is relatively low compared to those of the other available macronutrient profiles. The high caloric content of the *P. oleosa* nut (578.6 kcal/100 g of dry matter) is primarily dictated by the suggested high crude fat content. The total phenolic content of the nut is measured to be 8.9 mg GAE/g. Compared to the total phenolic content of 49.6 mg GAE/g (fresh weight) proposed by Fungo et al. (2019) is relatively higher than the total phenolic content of dry matter measured in this analysis.

Table 4.9: Macronutrient profile established for Panda oleosa *nuts including energy content, in g per 100 g dry weight. Previously published macronutrient data is added for comparison.*

Baka name, *Genus species*	Crude fat	Protein	TDF	Carbohydrates	Energy (kcal/100 g dry weight)
	(g / 100 g dry weight)				
Kana *Panda oleosa*	44.0	24.2	20.8	11.0	578.6
N/a *Panda oleosa* (Fungo et al., 2019)[1]	61.3	4.8	6.5	41.5	749.9[2]
N/a *Panda oleosa* (PROTA4U, n.d.)[1]	70.4	20.9	-	4.5	680.3

[1] Nutritional values published in g/100 g of edible matter were converted to g/100 g of dry matter by subtracting the mean moisture content from 100%. The 'edible portion' was divided by the calculated dry matter percentage and multiplied by 100.

[2]The energy content was calculated with the formula listed in the Methods chapter for calculating energy content.

The proportion of the *P. oleosa* macronutrient profile reported here overlaps most with the one reported on PROTA4U (n.d.). Although relatively large discrepancies are evident in the reported crude fat, carbohydrate, and energy contents listed in Table 4.9. The crude fat content presented in this analysis is relatively much lower, whereas the protein, and carbohydrate contents are considerably higher. Compared to the nutrient profile reported by Fungo et al. (2019) the reported crude fat content is again considerably lower. On the contrary, the protein content measured is considerable higher relatively. The TDF content is also relatively higher than the one mentioned by Fungo et al. (2019). Whereas the carbohydrate content is considerably lower, an opposite pattern compared to the one observed by comparing values presented here and the ones given by PROTA4U (n.d.). The energy contents proposed by Fungo et al. (2019) and PROTA4U (n.d.) are relatively much higher than the one reported here. Overall, the intraspecies variation in nutrient composition suggested is of notable size.

Trichoscypha acuminata

No previous nutritional data on *Trichoscypha acuminata* bark (BOS 017) is available. This analysis found a relatively low crude fat content, as well as a relatively low protein content as shown in Table 4.10. On the other hand, the TDF content is relatively very high, and the measured carbohydrate content is higher relative to the protein and fat values. A total energy content of 254.9 kcal/100 g of dry matter was obtained for the bark. The total phenol analysis yielded a value of 75.5 mg GAE/g.

Table 4.10: Macronutrient profile established for Trichoscypha acuminata *bark including energy content, in g per 100 g dry weight.*

Baka name, *Genus species*	Crude fat (g / 100 g dry weight)	Protein (g / 100 g dry weight)	TDF (g / 100 g dry weight)	Carbohydrates (g / 100 g dry weight)	Energy (kcal/100 g dry weight)
Mbel *Trichoscypha acuminata*	0.8	3.7	74.6	20.8	254.9

Overall, the TDF content is relatively high for the bark of *T. acuminata*. In comparison, the crude fat and protein content are relatively very small. The carbohydrate content is somewhat average, as almost 21% of the bark is suggested to consist of carbohydrates.

Irvingia excelsa

The nutritional- and phenolic data for *Irvingia excelsa* nuts are novel. Relatively, *I. excelsa*, or bush mango, is very high in crude fat content. The protein and carbohydrate contents are somewhat low in comparison to the amount of crude fat. TDF is relatively low. Due to the high amount of fat, the energy content of the nut is high as shown in Table 4.11. The total phenolic content is measured to be 5.9 mg GAE/g. Previously published data indicated a total phenolic content of 3.8 mg GAE/g for fresh *Irvingia gabonensis* nuts (Olayiwola et al., 2013).

Table 4.11: Macronutrient profile established for Irvingia excelsa *nuts including energy content, in g per 100 g dry weight. Previously published macronutrient data is added for comparison.*

Baka name, *Genus species*	Crude fat (g / 100 g dry weight)	Protein (g / 100 g dry weight)	TDF (g / 100 g dry weight)	Carbohydrates (g / 100 g dry weight)	Energy (kcal/100 g dry weight)
Payo *Irvingia excelsa*	76.2	6.9	11.0	5.9	758.9
N/a *Irvingia gabonensis* (Wu-Leung, 1968)[1]	72.6	7.9	-	17.5[3]	706.0
N/a Irvingia gabonensis (Platt, 1962)1	69.8	8.9	-	15.6	726.2
N/a Irvingia excelsa (Popovich et al., 1997)	0.6	4.5	85.7	4.2	206.22

[1] Nutritional values published in g/100 g of edible matter were converted to g/100 g of dry matter by subtracting the mean moisture content from 100%. The 'edible portion' was divided by the calculated dry matter percentage and multiplied by 100.

[2] The energy content was calculated with the formula listed in the Methods chapter for calculating energy content.

[3]The carbohydrate content given by Wu-Leung (1968) includes fibre content.

Macronutrient profiles available for the kernels of *Irvingia gabonensis* proportionately overlap with the macronutrient profile obtained for *I. excelsa*. *Irvingia* nuts appear to all consist of some 70% crude fat and circa 7 – 9% protein. The energy content of the nuts are relatively similar to one another. The analyses published by Platt (1962) and Wu-Leung (1968) did not account for TDF. As such, a discrepancy is notable between the carbohydrate values of earlier data and data given in this analysis. Conspicuously, Popovich et al.'s (1997) are suggestive of a relatively very high TDF content and a very low crude fat content, whereas the reported protein content aligns with the one presented here and the carbohydrate content aligns with that of Wu-Leung (1968). The total energy content proposed by Popovich et al. (1997) indicates a large discrepancy with the other published data, due to the high amount of TDF measured.

Telfairia occidentalis

Relatively, the seeds of *Telfairia occidentalis* are highest in crude fat content. The seeds are also suggested to contain a considerate amount of protein and TDF. The carbohydrate content could not be estimated as there was no difference remaining between the fractions of macronutrients, the total sum of macronutrients recorded added to above 100 g/100 g of dry matter.. The calculated total energy content of the bottle gourd seeds is 574.0 kcal/100 g of dry matter. The measured total phenolic content for the *T. occidentalis* seeds is 5.4 mg GAE/g. A total phenolic content of 46.7 mg GAE/g has been noted for *T. occidentalis* leaves by Louis et al. (2017). No other total phenolic data for *T. occidentalis* seeds was available at the time of writing. The total phenolic content of bottle gourd seed's oil suggested by Abdel-Razek et al. (2021) is 15.5 mg GAE/g, a relatively higher measurement in comparison to the one proposed in this analysis.

The macronutrient for bottle gourd seeds reported in this analysis is proportionally similar in crude fat content to the profiles reported by Wu-Leung (1968) and Esuoso et al. (1998). Although relatively, the protein content obtained in this analysis is higher than those suggest by the additional data. In addition, notable carbohydrate contents are noted by both Wu-Leung (1968) and Esuoso et al. (1998) suggestive of methodological issues possibly having occurred during the TDF analysis of *T. occidentalis*. The energy content noted by Esuoso et al. (1998) is notably higher than the one noted in this analysis and by Wu-Leung (1968) as shown in Table 4.12.

Table 4.12: Macronutrient profile established for Telfairia occidentalis *seeds including energy content, in g per 100 g dry weight. Previously published macronutrient data is added for comparison.*

Baka name, *Genus species*	Crude fat	Protein	TDF	Carbohydrates	Energy (kcal/100 g dry weight)
	(g / 100 g dry weight)				
Mortumbelumbe *Telfairia occidentalis*	46.0	28.9	28.1	-	574.0
N/a *Telfairia occidentalis* (Wu-Leung, 1968)[1]	48.0	21.6	-	25.1[2]	578.9
N/a *Telfairia occidentalis* (Esuoso et al., 1998)[1]	51.8	17.1	9.9	17.6	726.2

[1] Nutritional values published in g/100 g of edible matter were converted to g/100 g of dry matter by subtracting the mean moisture content from 100%. The 'edible portion' was divided by the calculated dry matter percentage and multiplied by 100.

[3]The carbohydrate content given by Wu-Leung (1968) includes fibre content.

Dioscoreophyllum cumminsii

The results for the tuber of *Dioscoreophyllum cumminsii* (ELO 006) propose a relatively high carbohydrate content. In comparison, the other macronutrient contents were found to be relatively low. In total, an energy content of 391.9 kcal/100 g of dry matter was obtained for the tuber as shown in Table 4.13. The total phenolic content measured is 3.7 mg GAE/g.

Table 4.13: Macronutrient profile established for Dioscoreophyllum cumminsii *tubers including energy content, in g per 100 g dry weight.*

Baka name, *Genus species*	Crude fat	Protein	TDF	Carbohydrates	Energy (kcal/100 g dry weight)
	(g / 100 g dry weight)				
Mbi *Dioscoreophyllum cumminsii*	0.9	7.2	6.2	85.7	391.9

Gnetum africanum

The leaves of *Gnetum africanum* contained a relatively small amount of crude fat, a relatively large amount of protein, a relatively large amount of carbohydrates, and a relatively very high TDF content. The determined total energy content is 307.9 kcal/100 g of dry matter. The total phenolic content measured for the leaves of *G. africanum* is 8.2 mg GAE/g. Other data on total phenolic content of *G. africanum* leaves has been published by Odukoya et al. (2006) suggesting values of 5.5 mg TAE (tannic acid equivalent)/g and 5.4 mg TAE/g.

Comparing the nutritional profiles of Wu-Leung (1968) and Isong et al. (1999) indicates that *G. africanum* leaf is high in protein content overall. The data on total energy content of the leaves shows some variation dictated by the ratio of measured TDF content against the measured carbohydrate content as shown in Table 4.14.

Table 4.14: Macronutrient profile established for Gnetum africanum *leaves including energy content, in g per 100 g dry weight. Previously published macronutrient data is added for comparison.*

Baka name, *Genus species*	Crude fat	Protein	TDF	Carbohydrates	Energy (kcal/100 g dry weight)
	(g / 100 g dry weight)				
Koko *Gnetum africanum*	3.0	21.8	53.6	21.6	391.9
N./a *Gnetum africanum* (Isong et al., 1999)	-	17.9	28.6	43.8	307.1
N/a *Gnetum buchholzianum* (Wu-Leung, 1968)[1]	4.3	19.7	-	71.5[2]	341.1[3]

[1] Nutritional values published in g/100 g of edible matter were converted to g/100 g of dry matter by subtracting the mean moisture content from 100%. The 'edible portion' was divided by the calculated dry matter percentage and multiplied by 100.

[2]The carbohydrate content given by Wu-Leung (1968) includes fibre content.

[3]The energy content was calculated with the formula listed in the Methods chapter for calculating energy content.

Ricinodendron heudelotii

The seeds of *Ricinodendron heudelotii* predominantly consist of crude fat. They are also high in protein content as well as in carbohydrate content. The TDF content of *R. heudelotii* seeds is relatively low. In total, 100 g of dry *R. heudelotii* seed matter contains some 601.6 kcal. The total phenolic content was measured to be 6.9 mg GAE/g. Data from Ene-Obong et al. (2018) corroborates a relatively low amount of total phenolic content for *R. heudelotii* (3.6 mg GAE/g).

Table 4.15: Macronutrient profile established for Ricinodendron heudelotii *seeds including energy content, in g per 100 g dry weight. Previously published macronutrient data is added for comparison.*

Baka name, *Genus species*	Crude fat	Protein	TDF	Carbohydrates	Energy (kcal/100 g dry weight)
	(g / 100 g dry weight)				
Gobo *Ricinodendron heudelotii*	43.2	22.7	7.2	26.9	601.6
N/a *Ricinodendron heudelotii* (Adome et al., 2022)	51.8	25.8	-	3.3	-
N/a *Ricinodendron heudelotii* (Wu-Leung, 1968)[1]	45.6	22.4	24.8[2]	-	560.8

[1] Nutritional values published in g/100 g of edible matter were converted to g/100 g of dry matter by subtracting the mean moisture content from 100%. The 'edible portion' was divided by the calculated dry matter percentage and multiplied by 100.

[2]The carbohydrate content given by Wu-Leung (1968) includes fibre content.

In comparison to previously published nutritional profiles for *R. heudelotti*, the nutritional composition of its seeds presented here proportionately overlap, accounting for the fact that Wu-Leung (1968) has included carbohydrates and fibres together. All of the published data suggest a protein content of over 40% and a protein content ranging between 22 – 26% as shown in Table 4.15. Overall, the results published here present a relatively higher carbohydrate content as TDF analysis was not conducted by neither Adome et al. (2022) and Wu-Leung (1968).

Baillonella toxisperma

The analysis of the nuts of *Baillonella toxisperma* indicates a particularly high crude fat content. Relative to the remaining macronutrients tested, the carbohydrate content is also relatively high. In comparison, the protein content and TDF content are relatively low. The energy content of the 'moabi' nut is suggested to be considerately high at some 718.3 kcal/100 g of dry matter. The total phenolic analysis suggested a value of 7.7 mg GAE/g. For the fruit of *B. toxisperma*, a total phenolic content of 6.9 mg GAE/g is suggested by Fungo et al. (2015).

Table 4.16: Macronutrient profile established for Baillonella toxisperma *nuts including energy content, in g per 100 g dry weight. Previously published macronutrient data is added for comparison.*

Baka name, *Genus species*	Crude fat	Protein	TDF	Carbohydrates	Energy (kcal/100 g dry weight)
	(g / 100 g dry weight)				
Moabi *Baillonella toxisperma*	66.2	7.5	6.4	19.8	718.3
N/a *Baillonella toxisperma* (Fungo et al., 2015)[1]	12.5	1.5	7.3	120.7[2]	-

[1] Nutritional values published in g/100 g of edible matter were converted to g/100 g of dry matter by subtracting the mean moisture content from 100%. The 'edible portion' was divided by the calculated dry matter percentage and multiplied by 100.

[2]Note that Fungo et al.'s (2015) value exceeds a 100%

No other nutritional data for the nuts of *B. toxisperma* is available, but data for its fruits has been published (Fungo et al., 2015).

When comparing the relative proportions of the nutritional profile to the profile published by Fungo et al. (2015) there is one large discrepancy. The results from Fungo et al. (2015) suggest a relatively high carbohydrate content together with a relatively low crude fat content as shown in Table 4.16. On the contrary, the results presented here suggest a relatively very high crude fat content, but a relatively low carbohydrate content. This discrepancy is to be expected in comparisons between fruits and seeds/nuts of a specific plant.

Nutcake

The nutcake (BIZ 003) analysed during the analysis was relatively very high in crude fat content. On the other hand, the carbohydrate content was relatively very low. The protein content was found to be slightly higher than the carbohydrate content. The TDF content was slightly higher relative to the protein and carbohydrate contents. The energy content of the nutcake is remarkably high with an estimated 751.3 kcal/100 g of dry matter as shown in Table 4.17. The total phenolic content measured to 7.3 mg GAE/g.

Table 4.17: Macronutrient profile established for the processed nutcake including energy content, in g per 100 g dry weight.

Baka name, *Genus species*	Crude fat	Protein	TDF	Carbohydrates	Energy (kcal/100 g dry weight)
	(g / 100 g dry weight)				
Nutcake (processed) *N/a*	75.7	7.9	13.7	2.8	751.3

Undetermined mushroom (BOS 003 and BOS 018)

The undetermined mushroom labelled BOS 003 is relatively high in TDF and in protein content. In comparison, the crude fat content and carbohydrate content appear to be relatively low. In total, the mushroom (BOS 003) contains a relatively average amount of kcal (306.3 kcal/100 g of dry matter). 'Kutu' is relatively low in crude fat, relatively low in protein content, relatively low in carbohydrate content, but relatively high in TDF content as shown in Table 4.18. The total energy content for BOS 018 is estimated at 225.4 kcal/100 g of dry matter. The total phenol content determined for BOS 003 is relatively low, at 6.4 mg GAE/g of dry matter. Likewise, the total phenolic content for BOS 018 is relatively low, at 1.1 mg GAE/g of dry matter.

Table 4.18: Macronutrient profile established for two unidentified mushroom specie known as 'Akpopo' and 'Kutu' including energy content, in g per 100 g dry weight. Previously published macronutrient data is added for comparison.

Baka name, *Genus species*	Crude fat	Protein	TDF	Carbohydrates	Energy (kcal/100 g dry weight)
	(g / 100 g dry weight)				
Akpopo *Undetermined*	2.6	28.1	53.5	15.7	306.1
Kutu *Undetermined*	0.6	6.6	88.8	4	225.4
N/a *Agaricus spp.* (Wu-Leung, 1986)[1]	1.9	11.5	66.1[2]	-	290.5

[1] Nutritional values published in g/100 g of edible matter were converted to g/100 g of dry matter by subtracting the mean moisture content from 100%. The 'edible portion' was divided by the calculated dry matter percentage and multiplied by 100.

[2]The carbohydrate content given by Wu-Leung (1968) includes fibre content.

'Akpopo' is relatively higher in crude fat, considerably higher in protein content, and relatively higher in carbohydrate content in comparison to 'Kutu'. The latter having a higher TDF content. The nutritional composition published by Wu-Leung (1968) for *Agaricus spp.* mushrooms aligns more with the profile obtained for 'Akpopo'. Although, the TDF content for 'Kutu' is in closer range to that of *Agaricus spp.*

4.2. Macronutrient Distributions

4.2.1. Macronutrient Distributions and Energy Contents per Food Type

A comprehensive overview of the average amounts of macronutrients per food type is given in Figure 4.1, allowing for a comparison of macronutrient contents between each food type. All macronutrient percentages have been converted to their fresh weight ratios. This allows the data to represent the actual ratios of macronutrients in the samples during consumption, as opposed to dry weight which disregards moisture content. As such, the data better represents the potential contribution of a specific wild edible plant taxa for Baka nutrition.

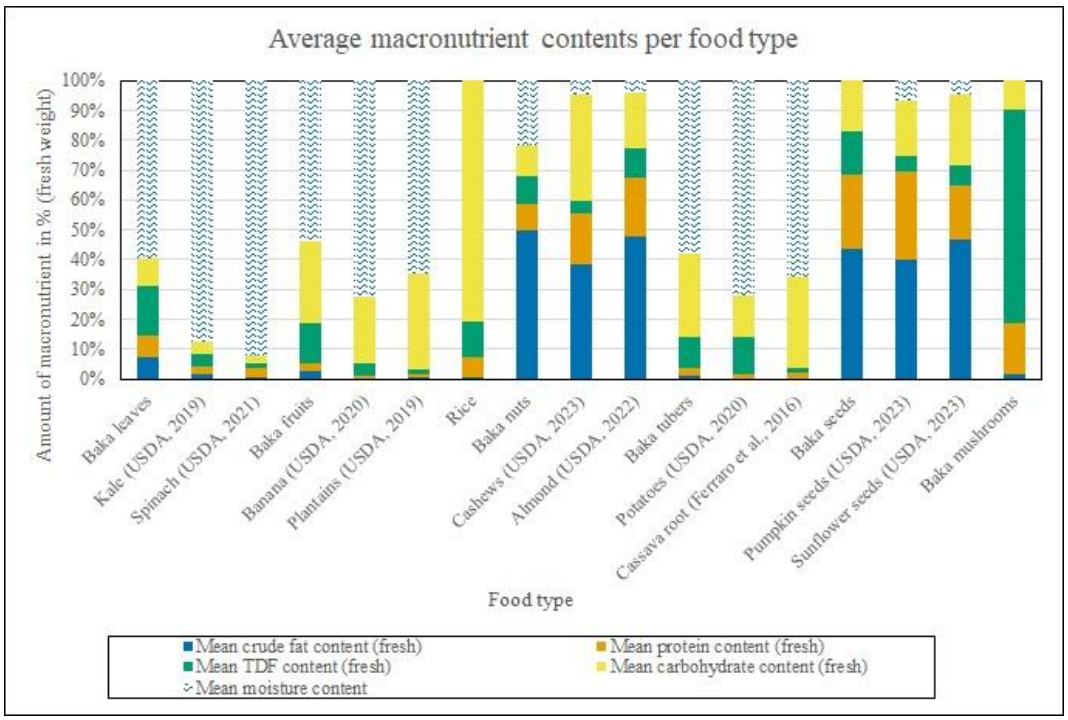

Figure 4.1: Graph showing the average macronutrient contents per food type, in % of fresh weight. Domesticate species have been plotted for comparison. Kale and spinach are compared with Baka leaves, banana and plantains with Baka fruits, cashews and almonds with Baka nuts, potatoes and cassava roots with Baka tubers, and pumpkin and sunflower seeds with Baka seeds.

Baka leaves, tubers, and fruits have a relatively high average percentage of moisture content in comparison to Baka nuts, seeds, and mushrooms. Both Baka tubers and Baka fruits maintain the highest mean percentages of carbohydrates. Baka seeds are also suggested to be a viable option for carbohydrate intake. Baka mushrooms are the most nutrient dense food items available, alongside rice. Baka nuts, alongside Baka seeds are suggested to be the most viable sources of crude fat. All other food types are relatively low in fat. The average protein content is highest in Baka seeds. Relatively, Baka leaves, Baka mushrooms, and Baka nuts provide an average amount of protein and are presented as adequate sources of protein, whereas the Baka tubers and Baka fruits are less viable options for dietary protein intake. The average fat percentage of Baka leaves appears somewhat

high considering their food type category. However, this is caused by *Klainedoxa gabonensis* leaves, which contains relatively large amounts of crude fat. Baka tubers are mainly sources of carbohydrates, but also provide a considerable amount of TDF. Baka leaves, Baka fruits, and Baka tubers are more macronutrient-dense than their domesticated alternatives. The Baka wild edible plant foods offer a balanced macronutrient profile for Baka individuals partaking in foraging trips. In the case of protein, rich protein sources such as mammal meat and insects are complementary to the available plant-based macronutrients.

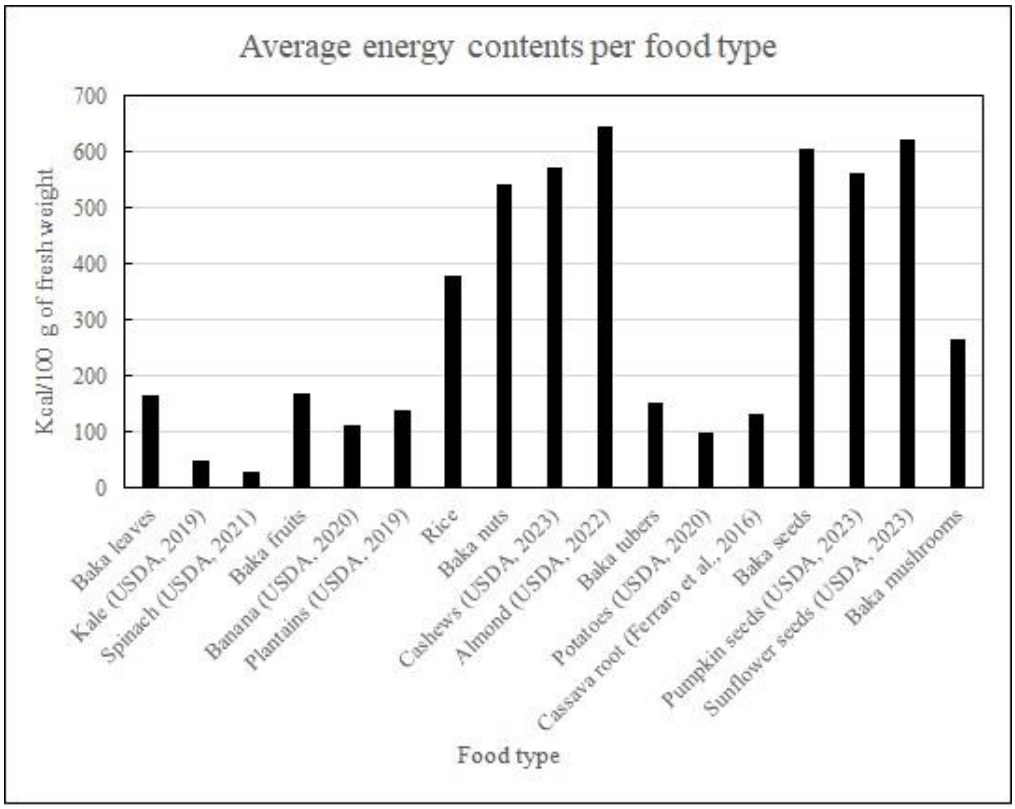

Figure 4.2: Graph showcasing the mean energy content per food type, in kcal/100 g of fresh weight. Domesticate species have been plotted for comparison.

As shown in Figure 4.2, Baka seeds contain the highest average amount of energy. Baka nuts contain a relatively high average energy content as well. These high energy contents are largely governed by the relatively high mean crude fat contents of both nuts and seeds. The relatively high average energy content of mushrooms is largely caused by the lack of moisture content of the samples. Baka fruits, leaves, and tubers all have relatively similar average energy contents, although their macronutrient compositions differ. Baka fruits and tubers offer a solid amount of metabolizable energy, since they mainly consist of carbohydrates. Domesticate nuts are suggested to contain more energy than the wild Baka alternatives. For the other food categories, the Baka wild plant taxa provide more energy.

4.2.2. Macronutrient Distributions and Energy Contents Within Food Types

This section will give an overview showcasing all individual taxa with their average macronutrient- and energy contents. In these overviews, macronutrient contents and energy contents of domesticated crops are plotted alongside the sampled taxa, to compare wild edible plant foods to domesticated plant foods consumed globally.

4.2.2.1. Leaves

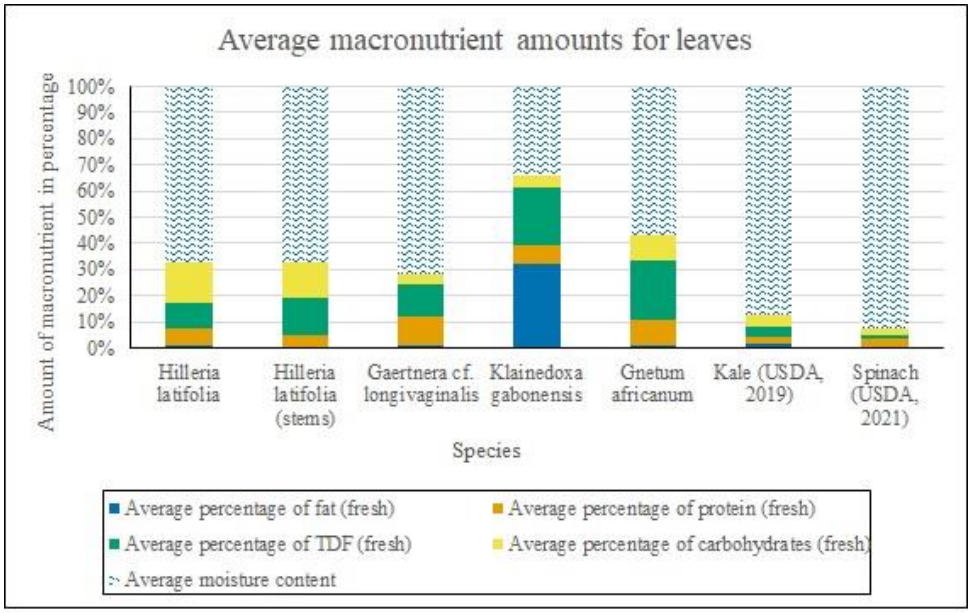

Figure 4.3: Graph showcasing the average macronutrient profiles for all sampled Baka leaves, alongside the domesticate crops of kale and spinach. For Hilleria latifolia*, both the edible and inedible plant parts were analysed.*

Figure 4.3 illustrates that both the domestic crops of kale and spinach are particularly high in average moisture content, in comparison to the Baka leaf species. *Klainedoxa gabonensis* leaves are suggested to be considerably high in their average crude fat content. All of the other reported leaf taxa have relatively low average crude fat contents. The Baka leaves contain higher TDF contents in comparison to their domesticated alternatives. The average protein percentage is highest in *G. africanum* and *Gaertnera cf. longivaginalis* leaves, with *K. gabonensis* and *H. latifolia* leaves having intermediate average protein contents. The remaining leaves are relatively low protein. All in all, the two domestic species of kale and spinach contain the least macronutrients (USDA, 2019, 2021). For the wild plants in particular, *G. africanum* and *K. gabonensis* are dense in macronutrients. Overall, the Baka leaf species are more nutrient-dense than their domesticate crop alternatives, and offer good amounts of TDF, and protein and carbohydrates to a lesser extent.

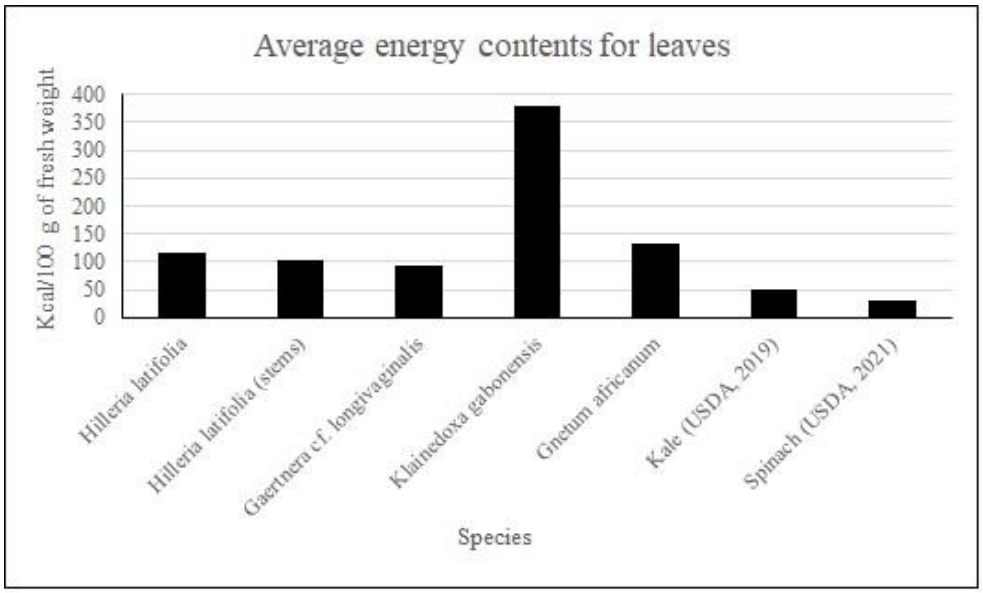

Figure 4.4: Graph showcasing the average energy contents of all sampled Baka leaves, alongside the domesticate crops of kale and spinach. For Hilleria latifolia*, both the edible and inedible plant parts were analysed.*

The average energy contents given in Figure 4.4 indicate that *K. gabonensis* is highest in caloric content, more than doubling the average energy contents of all other leaf species. The high fat fraction present within the leaf results in a higher energy content. All other Baka leaves sampled contain relatively similar amounts of energy. Both of the domestic species are relatively low in comparison to the energy contents of the wild plant taxa, as the domestics have been suggested to contain higher amounts of moisture. The energy content of the inedible stems of *Hilleria latifolia* maintain a similar energy content in comparison to its edible counterpart. The less palatable stems have been shown to contain higher amounts of TDF, and thus provide less metabolizable energy than the leafy part.

4.2.2.2. Fruits

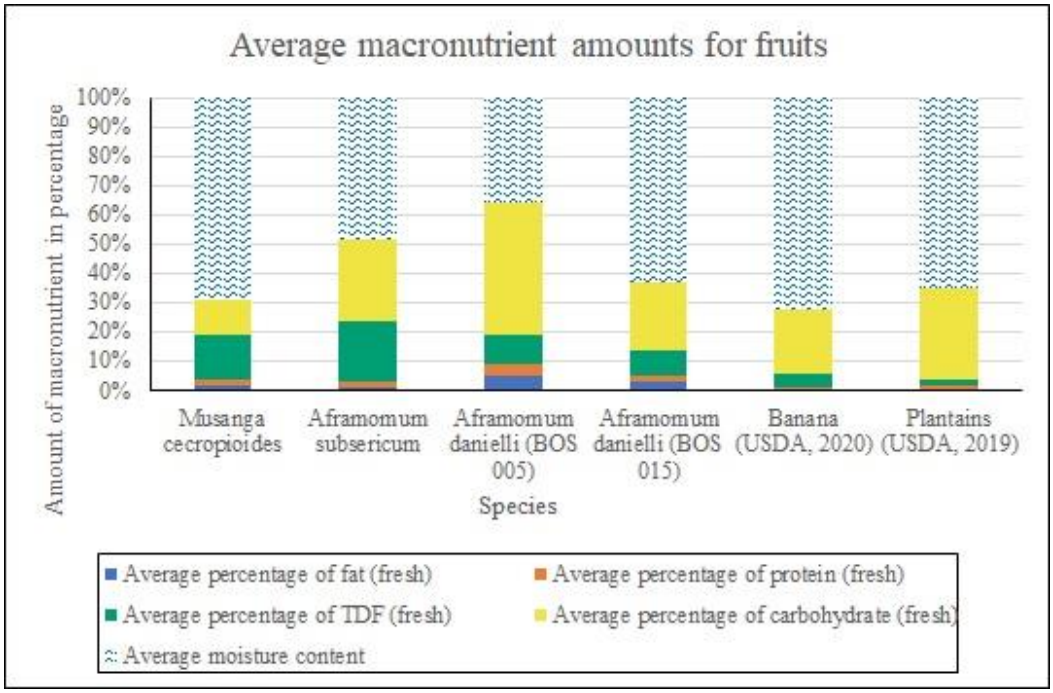

Figure 4.5: Graph showcasing the average macronutrient profiles for all sampled Baka fruits, alongside the domesticate crops of banana and plantain.

Closer investigation into the macronutrient profiles of fruits reveals that fruits are most dense in carbohydrates, as shown in Figure 4.5. Two separate subsamples of *A. danielli* are presented (BOS 005 and BOS 015). These two samples were harvested on separate days and show some within species variation, largely in their carbohydrate and TDF contents. Plantains (USDA, 2019) contain a potent amount of carbohydrates, as do bananas (USDA, 2020). They consist of a relatively similar macronutrient profile in comparison to the wild Baka counterparts. Though the Baka wild edible plants contain larger amounts of TDF, protein, and fat to a slight degree. Baka fruits are suggested to be excellent sources for carbohydrates.

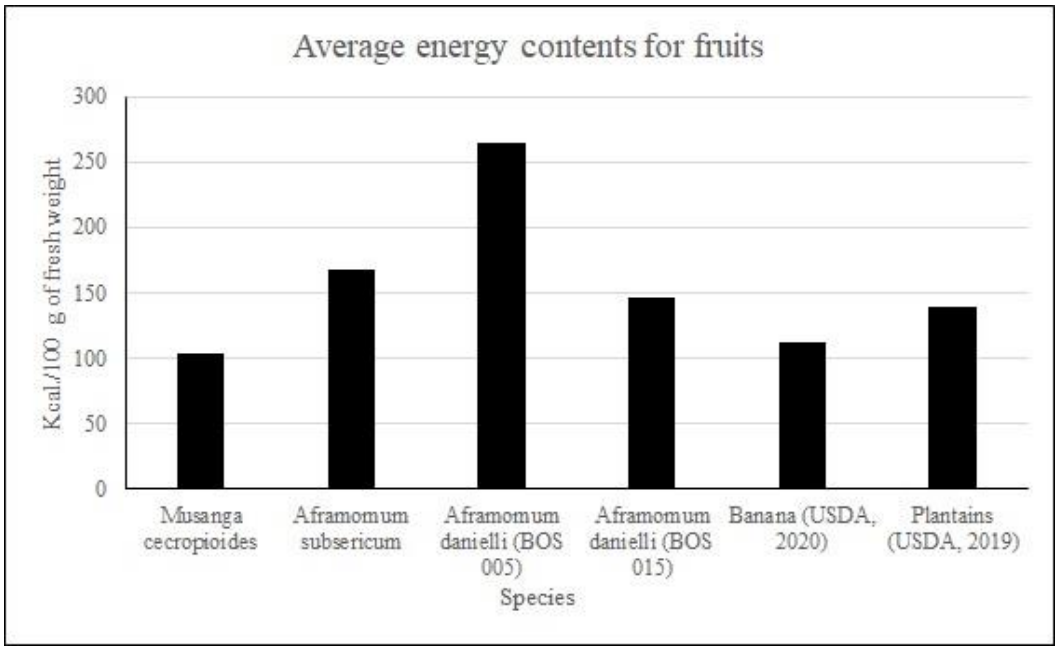

Figure 4.6: Graph showcasing the average energy contents of all sampled Baka fruit, alongside the domesticate crops of banana and plantain.

The energy content measured in *Aframomum danielli* (BOS 005) is considerably higher compared to all other energy contents reported, as reflected in Figure 4.6. The high energy content reported for BOS 005 is proposed to be influenced by plant age. BOS 015, also an *A. danielli* fruit, contains a similar amount of energy to the other fruit species. The remainder of the Baka fruits and domesticated fruits maintain relatively similar energy contents. The domesticated fruits and the wild Baka fruits mainly differ in their macronutrient compositions.

4.2.2.3. Tubers

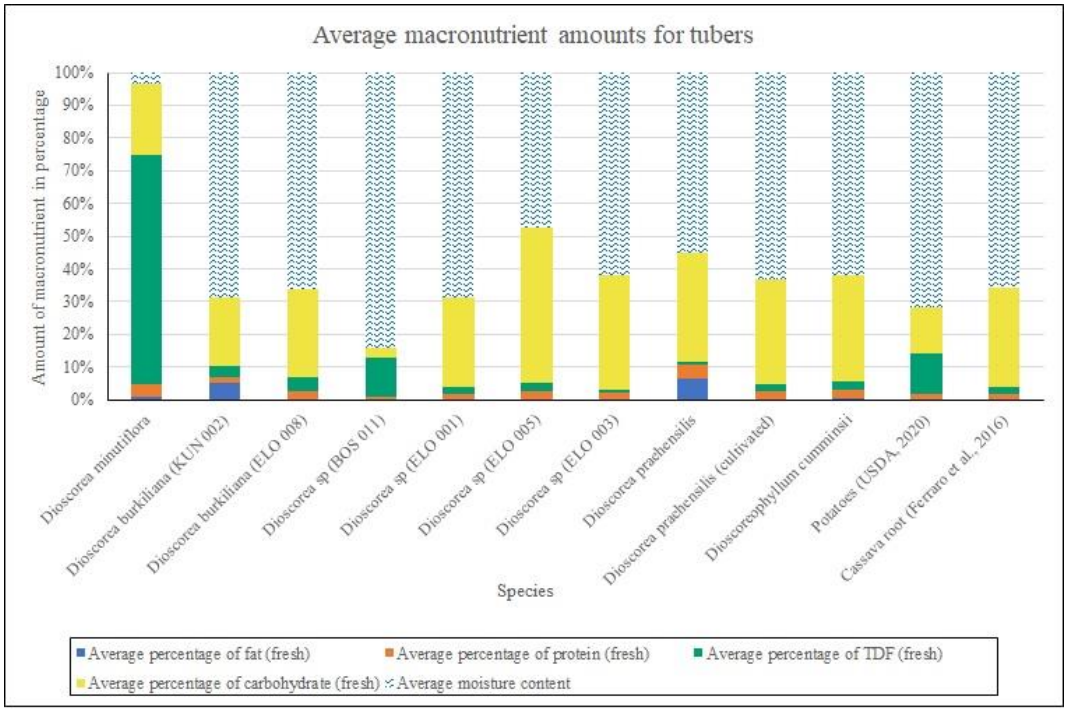

Figure 4.7: Graph showcasing the average macronutrient profiles for all sampled Baka tubers, alongside the domesticated crops of potato and cassava.

The most predominant macronutrient present in Baka tubers is that of carbohydrates, as shown in Figure 4.7. The only exception are the tubers of *Dioscorea minutiflora*, which are suggested to maintain relatively high TDF contents. The average carbohydrate percentages of all reported tubers are mostly similar. *Dioscorea sp*. (ELO 005) contains the largest fraction of carbohydrates. None of the reported tubers contain considerable amounts of crude fat. The average protein content is relatively similar for all tubers. All tubers but *D. praehensilis* and *D. minutiflora* contain more than 50% moisture. Overall, tubers are suggested to be reliable sources of carbohydrates. For BOS 011, the carbohydrate to TDF ratio is suggested to converse of what is reported for all other tuber species. The average macronutrient profile reported for wild edible tubers overlaps with that of the domesticated cassava roots (Ferraro et al., 2016). In contrast, potatoes have a relatively high TDF content in comparison to all the other tubers, bar BOS 011. Intraspecies variation in *Dioscorea* occurs mostly in moisture, TDF, and carbohydrate contents as a result of differential plant age and/or habitat.

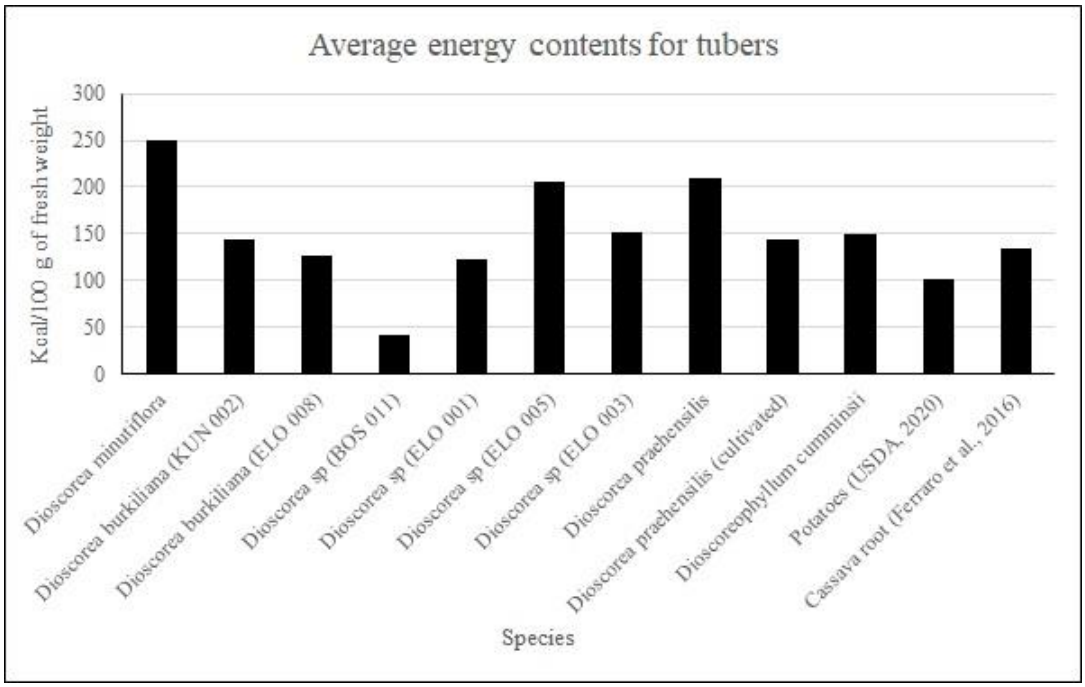

Figure 4.8: Graph showcasing the average energy contents of all sampled Baka tubers, alongside the domesticated crops of potato and cassava.

The average energy content is highest in *D. minutiflora*, as shown in Figure 4.8. This is largely under the effect of the high TDF- and low moisture content reported for this species. *Discorea sp.* (BOS 011) is lowest in nutrient density and in energy content. *Dioscorea sp.* (ELO 005) and *D. praehensilis* are somewhat high in energy content, whereas the remaining tubers contain relatively similar amounts of energy. *Dioscorea* yams are suggested to maintain some degree of nutrient variation, on a within- and between species level. Though, the variation within macronutrient profiles of the wild white yams is stronger in comparison to the variation noted for the energy contents. This variability is likely caused by plant age. The domesticated alternatives of potato and cassava offer similar amounts of energy as the Baka tubers, although potato contains somewhat less energy on average. Overall, *Dioscorea* tubers are readily available in the environment, and are a crucial source of energy for the Baka. As reported by Yasuoka (2006), wild yams provided 68% of Baka total daily energy intake during a long-term foraging trip, with *D. praehensilis* providing 56% on its own.

4.2.2.4. Nuts

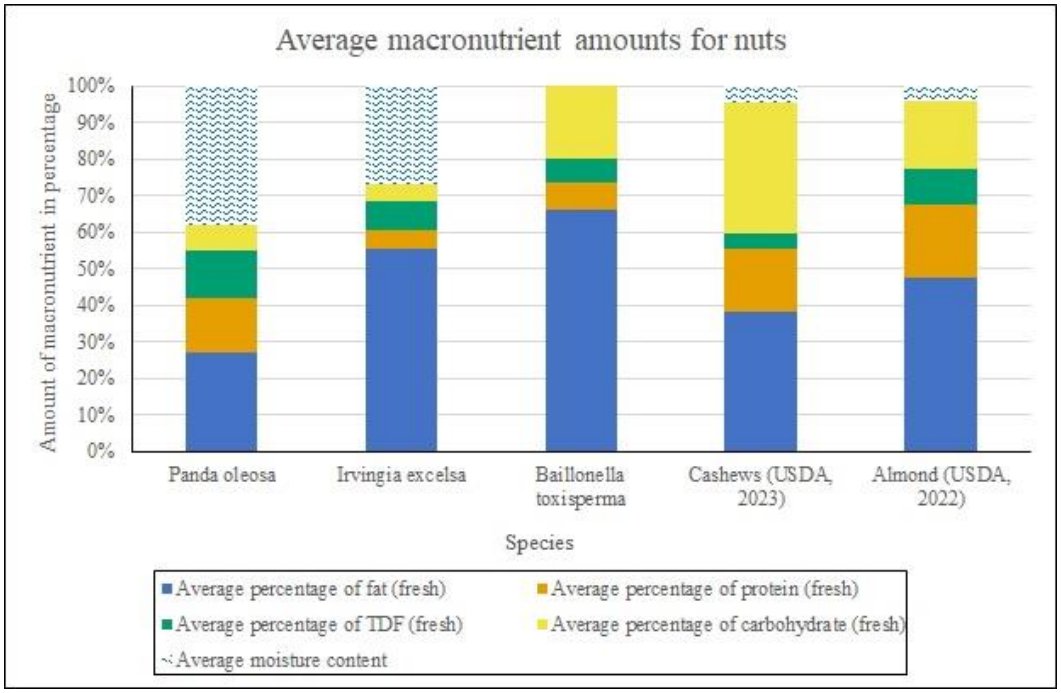

Figure 4.9: Graph showcasing the average macronutrient profiles for all sampled Baka nuts, alongside the domesticated cashews and almonds.

Overall, the most largest fraction in any nut consists of fat, as shown in Figure 4.9. The ratios in *P. oleosa* are somewhat skewed towards the average percentage of TDF content. The domesticated almonds and cashews appear more nutrient dense, but *B. toxisperma* contains the highest average percentage of crude fat by far, and the lowest moisture content of all the reported nuts. *I. excelsa* also provides more crude fat than the reported domesticated nuts. Overall, the Baka wild edible nuts are potent sources for crude fat, with *P. oleosa* providing good amounts of protein and TDF, and *B. toxisperma* providing a relatively good amount of carbohydrates. *P. oleosa* is of particular importance for Baka individuals partaking in a *molongo* (Sato et al., 2012).

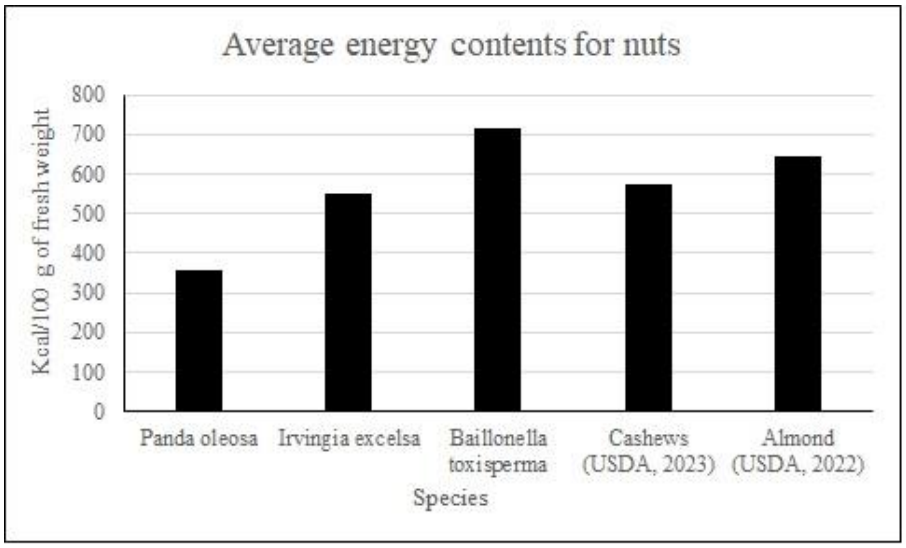

Figure 4.10: Graph showcasing the average energy contents of all sampled Baka nuts, alongside the domesticated cashews and almonds.

The average energy content is highest *B. toxisperma*, as the nut is highest in crude fat content. Next are almonds, which are relatively high in crude fat, carbohydrates and protein. *P. oleosa* nuts are suggested to be lowest in average caloric contents, mainly due to its relatively low fat and carbohydrate contents. Overall, nuts are proposed to be high in caloric content, and thus good sources of metabolizable energy, as shown in Figure 4.10. The only wild plant used by the Baka containing considerably less energy in comparison to cashews and almonds is that of *P. oleosa*.

4.2.2.5. Seeds

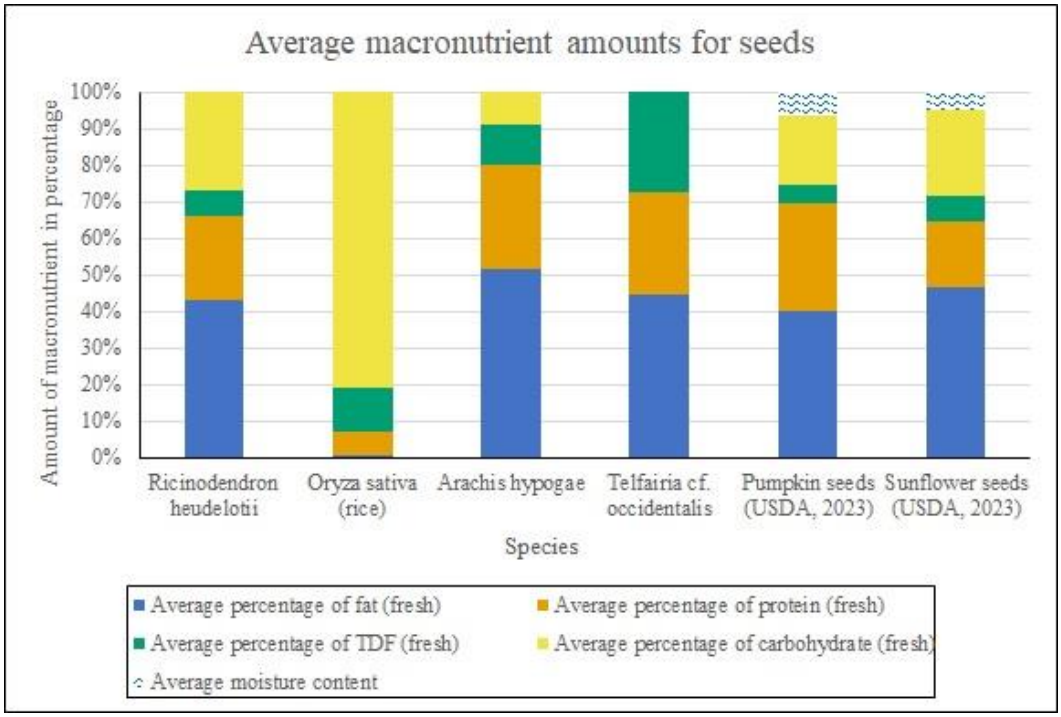

Figure 4.11: Graph showcasing the average macronutrient profiles for all sampled Baka seeds, alongside the domesticated pumpkin seeds and sunflower seeds.

For the food type category of seeds, the only reported moisture contents in this analysis belong to those of the two domesticated species of pumpkin- and sunflower seeds. All of the listed seeds contain relatively high average percentages of crude fat, as shown in Figure 4.11. Seeds contain a relatively high percentage of protein, except for the domesticated sunflower seeds. The one seed that differs most from all others is rice, which contains a considerably large amount of carbohydrates and close to no fat. Overall, seeds appear low in their average TDF percentages, with the only exception being the seeds of *Telfairia cf. occidentalis*. They are also the only seeds that do not contain any carbohydrates. Overall, seeds offer balanced macronutrient ratios. Apart from rice, the Baka seeds are especially good sources of protein and fat. The macronutrient profiles reported for the wild edible seeds relatively overlap with that of the domesticated species listed. Though there is a tendency for somewhat higher TDF fractiosn to occur in the wild Baka seeds.

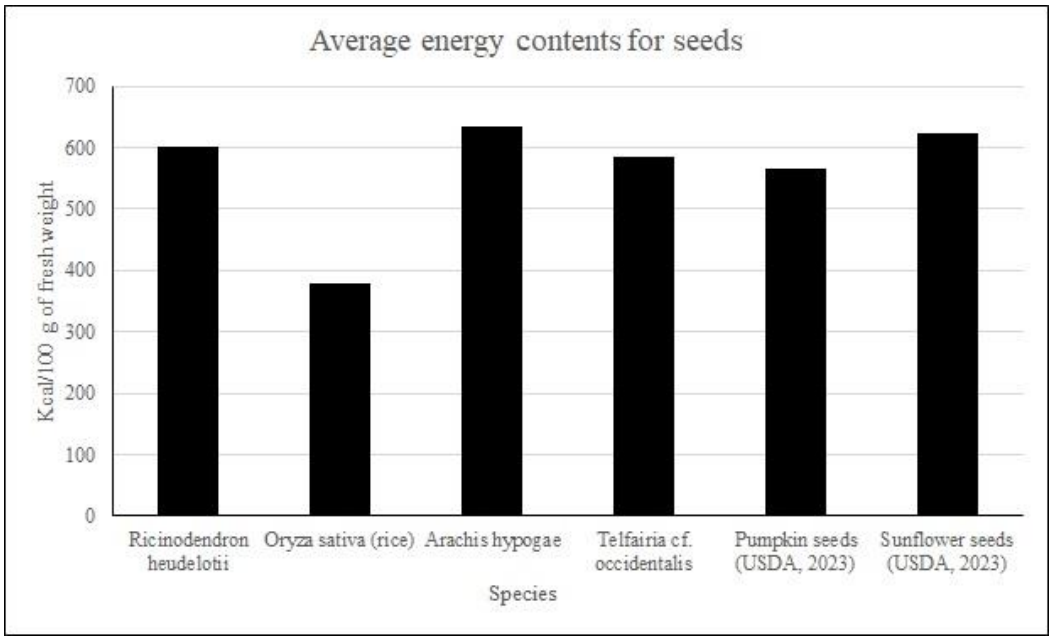

Figure 4.12: Graph showcasing the average energy contents of all sampled Baka seeds, alongside the domesticates of pumpkin seeds and sunflower seeds.

The average energy content is suggested to be highest in *Arachis hypogaea* seeds, as shown in Figure 4.12. In general the energy content of the Baka seeds are relatively similar. Only rice maintains a lower energy content, due to the fact that its seeds contain a larger fraction of carbohydrates as opposed to fats. The domesticated species of sunflower seeds and pumpkin seeds offer a similar amount of energy as the Baka seeds.

5. Discussion

The following section of this thesis explores and discusses the research aims and questions stated in Chapter 1. This thesis aims to elucidate the plausibility of an ideal hunter-gatherer diet, much like the dietary profiles postulated by the *Paleodiet* movement imply. To explore this question, I defined several sub-questions. The first additional research question explores how Baka macronutrient data compares to data published in the literature, and what the nature of the Baka nutrient data implies for other nutritional studies. Such a comparison helps in highlighting potential uniformity or variability within hunter-gatherer nutrition, in this case of nutrition derived from plant foods. The next supportive research question explores what we know about Baka rainforest consumption. Through combining ethnographic observations on Baka food intake per consumption-day and macronutrient data, the average daily macronutrient intake for the Baka are established and discussed. In turn, these data can be used in comparisons to generalised dietary composition data derived from large-scale indexes. Here, the goal is to answer how Baka dietary- and macronutrient composition compare to that of the *Paleodiet*. Lastly, the potential role of studying early hominin diet in solving or explaining contemporary health issues is discussed.

5.1. Comparing the Baka Macronutrient Data to Previously Published Data

The macronutrient data obtained for the plant taxa used by the Baka have been compared in great detail to previously published data in Chapter 4. Since a large body of ethnographic nutritional studies undervalue the nutritional and dietary variability within and between foraging societies (Henry et al., 2019), the first section of the discussion examines the variability present within the obtained Baka macronutrient data. By taking a bird's eye view on the data presented in Chapter 4, the nature of the variability present in the Baka macronutrient data can be assessed, and any meaningful patterns present in the data can be examined. This section examines the potential contributions of plant age and habitat towards variability within the nutritional qualities of wild plants per foot type category. to shine a light on whether or not Baka wild plants nutritional qualities are in favour of generalised macronutrient profiles for foraging societies. If a given plant taxa, or plant food type category, tends to significantly vary in its nutritional qualities, the notion of uniform hunter-gatherer nutrition becomes increasingly less likely.

5.1.1. Baka leaves

As shown in Figure 4.3, the macronutrient profiles obtained for analysed Baka leafy greens are relatively uniform in nature. Exclusively *Klainedoxa gabonensis* deviates most markedly from all other leafy green species in its macronutrient profile. In terms of energy content, *K. gabonensis* is the only species to largely differ from all other species (Figure 4.4). The between species nutritional variability for the tested Baka leaves is relatively uniform in nature. For *Hilleria latifolia* (Table 4.3), Wu-Leung's (1968) results suggest double the amount of fat in comparison to the data from this project. The protein content is suggest to be higher, whereas the TDF content is suggested to be lower. *H. latifolia* thus reflects some variability in its nutritional qualities across different habitats. *Gnetum africanum* was the other leafy green species for which previous macronutrient was readily available (Table 4.14). Comparing the data from this study to data from Isong et al. (1999), considerable variability is evident in the carbohydrate and TDF fractions in the plant's nutritional composition. For leaves, different species growing in the same habitat exhibit relatively uniform nutritional qualities. However, a within species comparison across habitats suggests considerable variability within the nutritional qualities of *H. latifolia* and *G. africanum* leaves.

5.1.2. Baka fruits

The tested Baka wild edible fruits show relatively similar macronutrient profiles to one another, as shown in Figure 4.5. However, within the taxa of *Aframomum danielli*, marked variability in the TDF and carbohydrate fractions is present within a similar habitat. The energy provided by wild edible fruits used by the Baka sketches a similar pattern (Figure 4.6). The macronutrient data for the Baka wild fruits suggests that nutritional variation as a result of habitat is less likely, and that the nutritional qualities between species are relatively uniform. Nutritional data on *Aframomum spp.* given in Table 4.2, suggests that the carbohydrate and TDF fractions present the largest part of the variability, likely caused by the effects of plant age. As opposed to leaves, the nutritional qualities of fruits are suggested to be more susceptible to variation under the influence of plant age, instead of habitat.

5.1.3. Baka tubers

The wild tubers used by the Baka showcase significant nutritional variability between species (Figure 4.7), as well as within species under the effect of plant age. The macronutrient mostly subject to variability are those of TDF and carbohydrates. The same pattern can be discerned for their energy contents (Figure 4.8). Comparing data from this study to data on *Dioscorea spp.* from Wu-Leung (1968) (Table 4.7), considerate variability within TDF and carbohydrate contents is evident. As such, the nutritional qualities of tubers are mainly affected by plant age and habitat, both on a within- and between species level. Their energy contents are suggested to be relatively uniform. For *D. praehensilis*, the cultivated alternative is suggested to contain somewhat less protein and more TDF.

5.1.4. Baka nuts

The wild nuts used by the Baka are relatively similar in their nutritional qualities on a between species level (Figure 4.9). Although their average moisture content tends to fluctuate, ultimately leading to differential nutritional densities and energy contents (Figure 4.10). Comparing the Baka data from *Panda oleosa* to data from Fungo et al. (2019) and PROTA4U (n.d.) for *P. oleosa,* highlights significant variability within the fractions of fat, protein, TDF and carbohydrates within species level, under effect of habitat (Table 4.9) . Data from Wu-Leung (1968) generally overlaps with the data obtained for the Baka *P. oleosa* nuts. However, data from Popovich et al. (1997) showcases significant variability in the fat, TDF, and carbohydrate fractions across habitat, on a within species level. The same variability applies to the nutritional qualities of Baka *Baillonella toxisperma* nuts and those analysed by Fungo et al. (2015) (Table 4.16). Nuts are suggested to maintain variable nutritional qualities on a within- and between species level, across and within habitat.

5.1.5. Baka seeds

Apart from the included domesticate rice seeds, the wild Baka seed species reflect relatively similar macronutrient profiles between species (Figure 4.11). The carbohydrate and TDF contents are mainly subject to variation, likely under the effect of plant age. The same pattern arises when comparing the Baka data on *Ricinodendron heudelottii* to data from Adome et al. (2022) and Wu-Leung (1968) (Table 4.15). For *Telfairia occidentalis* nuts the TDF and carbohydrate factions are suggested to be variable under the effect of habitat, as shown by comparing the Baka data with data from Wu-Leung (1968) and Esuoso et al. (1998) (Table 4.12). The nutritional qualities of Baka seeds mainly vary in their moisture, TDF, and carbohydrate content. On a within species level, the same pattern of variability is evident. The nutritional qualities of Baka seeds are suggested to be subject to the effects of both plant age and habitat on a within- and between species level.

For the fruits of *Musanga cecropioides* (BOS 001), the leaves of *Gaertnera cf. longivaginalis* (BOS 010), *Klainedoxa gabonensis* (BOS 012), the bark of *Trichoscypha acuminata* (BOS 017), the tubers of *Dioscoreophyllum cumminsii* (ELO 006) and the processed nutcake (BIZ 003), no previously published macronutrient data was readily available in the literature. Hence, future chemical nutrition analyses on these taxa may further broaden our understanding on their macronutrient profiles and the nature of their nutritional variability. Table 5.1 offers a quantitative overview on the nature of the nutritional variability discussed for the Baka wild plant taxa.

5.1.6. The implications for nutritional studies

Comparison with the Baka macronutrient data and previously published nutritional data shows that the nutritional qualities of Baka leaves, tubers, nuts, and seeds, are all influenced by the effect of habitat on a within species level, as seen in Table 5.1. The nutritional qualities of Baka fruits are largely affected by plant age on within species level, as are those of seeds. On a between species level, Baka tubers, nuts, and seeds are influenced by the effects of habitat on a between species level. Baka tubers and seeds also show a tendency to be affected by plant age on a between species level. As stated in Chapter 3, the inherent qualities of plant nutrition widely differ (Wollstonecroft et al., 2008). By comparing Baka data with the literature, it is evident that considerable nutritional variability is present within the plant species consumed by the Baka. The data indicate that the nutritional qualities of wild plant taxa used by the Baka are not supportive of a holistic, uniform plant-based dietary profile for foraging societies as is often suggested in cross-cultural comparisons. The effect of plant age is suggested to cause significant variability within the TDF and carbohydrate components of wild plants. In addition, habitat greatly effects the nutritional qualities of wild edible plants. Considering that the bioavailability of specific plant taxa also differs across habitats, it appears unlikely that any given foraging society can maintain a relatively similar plant-based diet in comparison to any other given foraging society, unless they occupy regions geographically near to one another. Even then, the consumption of similar plant taxa does not result in similar macronutrient intakes. Lastly, as Henry et al. (2019) stated, future research would greatly benefit from focussing more on the effects of habitat on the nutritional qualities of plant foods.

Table 5.1: Overview of the variability present within the nutritional qualities of wild plants used by the Baka. Within brackets the leading cause of nutritional variability is indicated.

Relatively uniform nutritional qualities (within species)	**Relatively uniform nutritional qualities (between species)**	**Relatively differential nutritional qualities (within species)**	**Relatively differential nutritional qualities (between species)**
		Baka leaves [habitat]	Baka tubers [plant age and habitat]
		Baka fruits [plant age]	Baka nuts [habitat]
	Baka leaves	Baka tubers [plant age and habitat]	Baka seeds [plant age and habitat]
	Baka fruits	Baka nuts [habitat]	
		Baka seeds [plant age and habitat]	

The insights gained from high-resolution nutritional analyses like this are imperative for a better understanding on the nature of foraging nutrition, and in turn the dietary practices of hunter-gatherers and our ancestors. Overarching narratives that dominate the discourse on early *Homo*- and hunter-gatherer subsistence behaviours and diets are mostly based upon large-scale syntheses of cross-cultural, low-resolution indexes such as the *Ethnographic Atlas* by Murdock (1967) (Crittenden & Schnorr, 2017). Meta-analyses such as the ones published by Cordain (2002) and Eaton and Konner (1985) are inevitably guilty of overgeneralisation, undervaluing the variability that is present within extant- and extinct hunter-gatherer dietary practices. However, indexes such as the *Ethnographic Atlas* (Murdock, 1967) and subsequent analyses based thereon can be of heuristic value. Still, their large scale should not overshadow the contribution of smaller scale, high-resolution analyses. The variability within nutritional qualities presented here, would better serve as being heuristically projected onto the dietary compositions of extant- and extinct hunter-gatherers, alongside that of early hominins. This variability highlights the importance of nutritional chemical analyses like the one conducted here, towards constructing narratives on foraging nutrition.

Comparing and using modern foraging dietary compositions to reconstruct Pleistocene ones is indeed like working with two different mosaics and their different parts (*sensu* Marlowe, 2005). Large-scale analyses and indexes may serve as a broad frame for the mosaic, but the smaller scale analyses serve as the individual pieces. These smaller pieces may consist of different lines of evidence, such as estimated consumption patterns, nutritional chemistry, and raw food weights brought back to camp (Crittenden & Schnorr, 2017). Even then, the mosaic that constitutes the subsistence behaviour of modern-day foragers does not reflect the mosaic that constitutes early hominin subsistence behaviours, it merely serves as the best option scholars have for comparison. The often overshadowed variability within extant hunter-gatherer diets may result in overgeneralized and thus incorrect assumptions on the evolution of human nutrition and early hominin dietary compositions. The implication of the nutritional patterns observed in Baka forest nutrition shows that the variability in extant hunter-gatherer nutrition should be accounted for in future studies on foraging nutrition and Pleistocene nutrition, for more accurate portrayals of the complexities inherent to dietary practices.

5.2. Baka Rainforest Nutrition

Currently, no study has yet combined nutritional chemistry, estimated consumption patterns, and raw food weights brought back to camp for the Baka forager-horticulturalists. With the macronutrient data presented in this thesis and the ethnographic observations from Sato et al. (2012) and Yasuoka (2006), Baka rainforest nutrition can be quantified per consumption-day. In order to establish a macronutrient profile for the Baka, the ethnographic data on energy intake per consumption-day and total edible weight of food items brought back to camp per consumption-day (Sato et al., 2012; Yasuoka, 2006) will be used to reverse engineer the Baka macronutrient profile per consumption-day. All data presented by these authors solely concern wild edible plant taxa collected by the Baka during long-term foraging trips. As such, the nutritional profile presented here reflects the average daily macronutrient intake of a foraging society within a rainforest-type environment, completely independent of agricultural produce. The estimated energy intakes per food category per consumption-day given by both Sato et al. (2012) and Yasuoka (2006) are presented in Table 5.2. The mean macronutrient percentages presented in fresh weight in Chapter 4.2. are used in the calculations. The dietary contributions reported by both Sato et al. (2012) and Yasuoaka (2006) were recorded at different Baka groups at different periods of time.

Tubers, of which the bulk consists of *Dioscorea*, supply the highest amount of energy per consumption-day. Wild yams are available year-round and play a crucial role for the Baka because of their starch rich nature. Though meat only provides 19% of the daily energy intake, game is estimated to supply 60% of the daily protein intake (Sato et al., 2012). Sato et al. (2012) have reported five species of wild yam being gathered by the Baka, all species belonging to the genus of *Dioscorea*. Similarly, Yasuoka (2006) reported the consumption of five species of wild yam exclusively belonging to the genus *Dioscorea*. The tuber sample in this thesis is well-suited to represent the adhering macronutrient intake for Baka tuber consumption. Furthermore, Sato et al. (2012) report that almost all nuts collected during the *molongo* were *Panda oleosa* nuts. Thus, the newly obtained macronutrient values for *P. oleosa* are used to calculate the daily macronutrient intake through the consumption of nuts. The reported fungi consumed could not be identified to species level. Vernacular names were presented for mushrooms recorded by Yasuoka (2006), but none of these correspond to the vernacular names belonging to the samples of this project, that being 'akpopo' and 'kutu'. The average values of the unidentified mushroom samples will be used to calculate the macronutrient intake through the consumption of fungi. To calculate the protein intake by the consumption of meat, the estimate of 17.9 g of protein per 100 g of meat is maintained as given by Sato et al. (2012), who derived their nutritional data for mammal meat from Wu-Leung (1968)

Table 5.2: Total energy intake (in kcal) per food type category per consumption-day [percentages given in brackets] during a molongo.

Food categories	**Total energy intake (kcal) m*olongo* camp/CD** (Yasuoka, 2006)	**Total energy intake (kcal) *molongo* camp/CD** (Sato et al., 2012)	**Average energy intake (kcal) *molongo* camp/CD**
Wild yams and yam-like plants	1573 [69]	1755 [69]	1664 [65]
Nuts	-	295 [12]	295 [12]
Fungi	-	4 [0.2]	4 [0.2]
Honey	183 [8]	40 [2]	112 [4]
Meat	536 [23]	443 [17]	490 [19]
Total	2292 [100]	2537 [100]	2565 [100]

5.2.1. Reverse Engineering the Baka Macronutrient Profile per Consumption-Day

Having established the nutritional qualities of 30 wild edible plant foods used by the Baka, alongside the average Baka energy intake per consumption-day during a long-term foraging trip, the average Baka macronutrient profile per consumption-day can be estimated for the first time. In Table 5.3 below, the estimated amount of fresh weight per food category required to obtain the average energy intake per consumption-day is given for the plant-based food categories. Around 1 kg of *Dioscorea spp*. needs to be consumed to attain some 1664 kcal on a daily basis. For nuts, some 80 g is required to obtain the 295 kcal listed. Lastly, a small amount of 1.5 g is needed to obtain the 4 kcal through the consumption of fungi.

Table 5.3: Estimated amounts of fresh weight (in g) needed per food type category to obtain the average energy intake per consumption-day given for all plant-based food type categories (after Sato et al., 2012; Yasuoka 2006)..

Food categories	**Average energy intake (kcal) *molongo* camp/CD**	**Amount of fresh weight required (g) [average kcal/100 g of fresh weight]**
Wild yams and yam-like plants	1664 [65]	1078.1 [154.35]
Nuts	295 [12]	82.5 [357.62]
Fungi	4 [0.2]	1.5 [265.68]

As recorded by Sato et al. (2012) individual Baka members brought back 2.75 kg of *Dioscorea spp.* wild yams per consumption-day during the first observed *molongo*. During the second tracked *molongo*, the amount was even higher at 3.29 kg per cooperator. These numbers show that the bioavailability of *Dioscorea spp*. in the study area is sufficient for a group of at least 16 individuals. A rough estimate suggests that the collected amount of white yams provides enough energy for 32 individuals. For *Panda oleosa*, a total of 22.9 kg was gathered and brought back to camp during the first long-term foraging trip, whereas 44.3 kg was collected during the second trip. In total 16 Baka individuals were present during the first trip, meaning that each cooperator brought back 71.6 g of *P. oleosa* nuts per consumption-day. During the second trip a total of 23 participants were present. Here, each cooperator brought back an average amount of 96.3 g of *P. oleosa* nuts per consumption-day. Across both foraging trips, the average amount of *P. oleosa* nuts brought back to camp is estimated at 83.95 g per consumption-day. The data suggest a 100% consumption rate for *P. oleosa* nuts during a *molongo*. Sato et al. (2012) report that each cooperator brought back 10 g of fungi to camp per consumption-day during the first *molongo*, with triple the

amount during the second foraging trip. It appears the Baka are able to amount surpluses of both wild yams and fungi. Table 5.4 showcases the macronutrients obtained from plant foods per consumption-day during a *molongo*, according to the nutritional data obtained in this project and the ethnographic data reported by Sato et al. (2012) and Yasuoka (2006). Note that the values provided in Table 5.4 are aggregate values calculated by combining both the data from Sato et al. (2012) and Yasuoka (2006).

Table 5.4: The average amounts of macronutrients obtained (in g) per consumption-day during a long-term foraging trip. Values depicted are the aggregate values calculated from data published by both Sato et al. (2012) and Yasuoka (2006)..

Amounts in fresh weight (g)	Wild yams and yam-like plants	Nuts	Fungi	Total
Amount of fresh weight required (g)	1078.1	82.5	1.5	
Fat (g)	14.6	22.5	0.0	37.1
Protein (g)	26.3	12.3	0.3	38.9
Carbohydrates (g)	302.6	5.6	0.2	308.4
TDF (g)	108.8	10.6	1.1	120.5
Macronutrients (g)				504.9

Regarding macronutrients, out of the total amount of wild edible plants consumed 61% is made up of carbohydrates. The second most occurring macronutrient is that of TDF, constituting some 24%. The protein and fate intake per consumption-day are relatively similar to one another, with protein accounting for 7.7% of the total macronutrient profile, and fat for 7.3% as shown in Figure 5.1. On average, the Baka consume some 119.8 g of protein per consumption-day (Sato et al., 2012), of which 38.9 g is derived from plants, meaning that meat provides some 67.5% of the total protein intake per consumption-day. For comparison, data on daily protein intake from developed countries indicates that the animal to plant protein intake ratio is 2.1:1 on aggregate (Messina et al., 2023, p. 329), suggesting that Western protein intake consists of 67.8% animal protein. In the past years health organizations have advised Western individuals to up the intake of plant protein, as following these recommendations is said to reduce the risks of diabetes, cardiovascular disease, and cancer. The Baka have a similar animal to plant protein intake ratio, but a troublesome prevalence of non-communicable diseases has never been noted. The possible

health benefits previously related to a higher plant protein intake, are more likely the result from the non-protein components of whole food sources of plant protein (Messina et al., 2023).

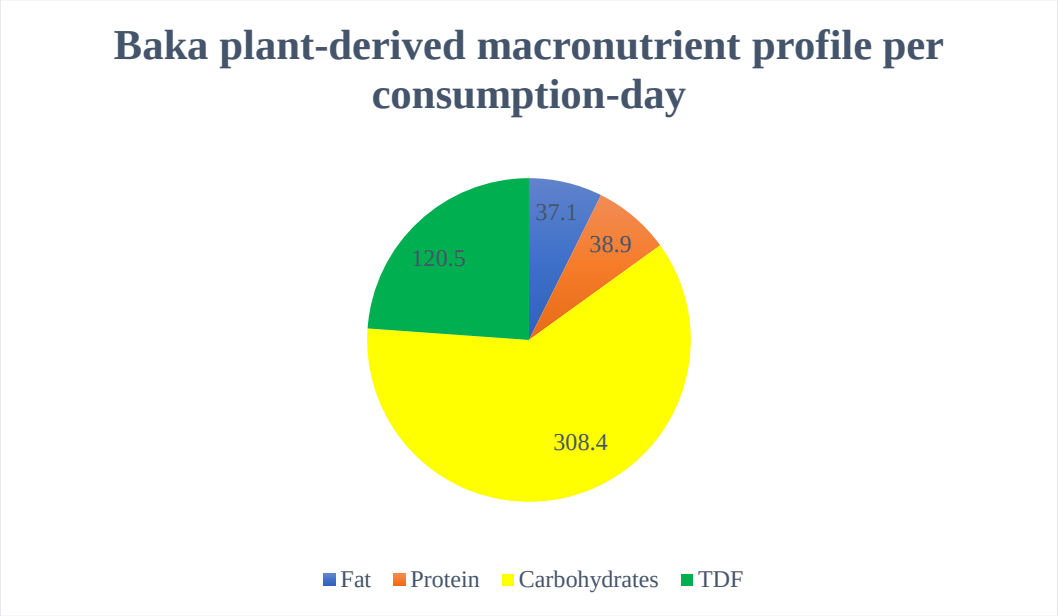

Figure 5.1: Pie chart showcasing the plant-derived macronutrient ratios (in g) per consumption-day for the Baka.

Ryan-Harshman and Aldoori (2006) have published new dietary reference intakes for industrialized societies. Their data is presented in the context of addressing obesity and diabetes in Western society. The recommended amount of carbohydrates per consumption-day sits at 130 g. The estimated amount of protein needed for adult men and women is 0.8 g per day, per kg of bodyweight. For the Baka, Sato et al.'s (2012) data suggests an average bodyweight of 50 kg. As such, the recommended amount of protein per day for Baka males and females is estimated at 40 g, according to the new dietary references pubished by Ryan-Harshman and Aldoori (2006). Additionally, females require an additional 25 g of protein during gestation. The recommended TDF intake per day for adult males is 38 g, whereas for women it is 25 g (Ryan-Harshman & Aldoori, 2006). Lastly, fats should provide 20 – 35% of the total daily energy intake. In the case of the Baka the recommended daily intake of fat ranges between 57 - 99.8 g of fat. Compared to Westernized diets (Ryan-Harshman & Aldoori, 2006), the Baka easily consume more than the recommended amount of fibre per day with some 120 g of TDF intake per day. Similarly, the Baka manage to consume more than double the daily amount of recommended carbohydrate intake. The Baka also manage to consume close to 40 g of protein through the consumption of wild edible plants alone, the addition of meat totalling a daily protein intake of some 120 g. Lastly, the Baka consume some 37 g of fat per day through the consumption of wild edible plants, which is 35.1% short of the minimal recommended amount of daily fat intake.

The macronutrient dietary guidelines published by Harshman and Aldoori (2006) state that 45-65% of the total daily energy intake should come from carbohydrates, 20-35% from fat and 10-35% from protein. The Baka obtain 13.2% of their total energy intake per day from plant-based fat, 48% from carbohydrates, and only 6% from protein, with a remaining 9.4% from TDF. The TDF fraction can potentially be attributed to that of the carbohydrate fraction. Through this lens, the Baka meet the recommended daily energy intake from carbohydrates, while almost meeting the recommended daily energy intake from protein. However, they fall short in meeting the recommended daily energy intake from lipids. The shortcomings in macronutrient intakes are likely complemented by animal foods.

Although modern-day Baka society relies heavily upon agricultural produce (Gallois et al., 2020), these data nonetheless offer novel insights into the dietary composition of a foraging group within a rainforest-type environment. Overall, Baka foraging nutrition is sufficient enough for maintaining fitness. As such, a foraging society within a rainforest-type environment can survive aided by the required knowledge of the landscape, but not agriculture. The rainforest occurring in the Congo Basin of Southeastern Cameroon, is suggested to be a rich environment for foraging groups, especially regarding its bioavailability of white tubers. This type of environment not only sustains the foraging lifestyle, it is capable of providing close to the recommended, Westernized dietary reference intakes. The rainforest-type environment type thus facilitates hominin occupations and is argued to have been an important ecological setting for early hominins (Mercader, 2002; Roberts, 2022; Roberts & Petraglia, 2015; Scerri et al., 2022).

5.3. Baka Dietary- and Macronutrient Composition Put in Perspective

As touched upon in Chapter 2, Murdock's *Ethnographic Atlas* (1967) is one of the first and most important cross-cultural indexes that aimed to investigate and categorize the dietary composition of a plethora of foraging societies. Subsequently, many authors proposed large-scale nutritional analyses such as the ones published by Cordain et al. (2000), Eaton and Konner (1985), and Marlowe (2005). Moreover, the *Atlas* was foundational for other large databases to be developed, such as Binford's *Frames of Reference* (2001) or Kelly's *The Foraging Spectrum* (1995), through which generalising analyses on hunter-gatherer nutrition and dietary composition were revised (e.g., Marlowe, 2005). To assess how the Baka's dietary- and macronutrient composition compare to those of other foraging societies, the large-scale dietary compositions reported by Cordain et al. (2000), Eaton and Konner (1985), and Marlowe (2005) will be compared to the data reported in this thesis. Using the results from Chapter 5.2., this section highlights the potential variability within hunter-gatherer dietary compositions that is commonly overshadowed by large-scale indexes, parallel to the often overlooked variability present within the nutritional qualities of plants used by foraging societies, as discussed in Chapter 5.1. The reference data will be described in chronological order according to the year of publishing, to elucidate any trends present within the discourse of hunter-gatherer nutrition at the time.

5.3.1. Baka Nutrition Compared to Eaton and Konner's Palaeolithic Nutrition

Based on ethnographic observations made in over 50 hunter-gatherer societies, Eaton and Konner (1985) maintain that foraging groups occupying semitropical habitats obtain some 50 – 80% of their food (in weight) from plants, whereas animal food items provide close to 20 – 50%. For groups living in grassland/savanna-type environments such as the Hadza from Tanzania, the San bushmen of the Kalahari, and the !Kung from the Kalahari, dietary animal to plant ratios align with the generalized A:P ratio of 35:65 (Eaton & Konner, 1985). Based on the nutritional data of foraging societies, Eaton and Konner (1985) propose an average daily macronutrient intake for Late Palaeolithic Human beings of 3000 kcal, of which 251.1 g is protein, 71.3 g is fat, 333.6 g is carbohydrate, and 45.7 is TDF. As postulated in Chapter 2.5.5.1., the Baka A:P ratio is roughly 23:77. In comparison to the grassland/savanna-type foraging groups, the Baka's A:P ratio is markedly skewed towards the contribution of plant foods. For a foraging group with similar A:P ratios as the Baka, Eaton and Konner (1985) propose that of the plant derived daily energy intake 19.6% should derive from protein, 44% from carbohydrates, and 16.4% from fats. However, as proposed in Chapter 5.2.1., the Baka obtain 6% from protein, 48% from carbohydrates and 13.2% from fat, and 9.4% from TDF. These data suggest that the Baka consume less protein per consumption-day than proposed by Eaton and Konner (1985), though they consume

somewhat higher amounts of carbohydrates, including the fraction of TDF. The portion of plant-based fat is relatively similar to the amount proposed by Eaton and Konner (1985). Evidently, the Baka are more reliant upon plant food items in comparison to a large quantity of hunter-gatherer societies. A potential reason for this differentiation is the fact that tropical forest plants provide more TDF and protein than do grassland/savanna plants (Paine et al., 2019). Hence, the dietary needs of the Baka are better provided for by plant-based food items than are those of a large component of foraging societies, in particular those occupying grassland/savanna-type landscapes.

5.3.2. Baka Nutrition Compared to Cordain et al.'s Worldwide Hunter-Gatherer Diets.

Based on Murdock's *Atlas* (1967) Cordain et al. (2000) gathered dietary and nutritional data on 229 foraging societies worldwide. This more recent analysis suggests an aggregate A:P ratio of 38:62 for all foraging societies. Furthermore, the suggested contribution of protein to the total daily energy intake ranges between 19 – 35%, with carbohydrates providing some 22 – 40%, and fat between 28 – 58%. In comparison to data published by Eaton and Konner (1985), Cordain et al. (2000) emphasize the contribution of animal based food. The highest possible contribution of plant foods is given in the A:P ratio of 35:65, in which 40% of the total daily energy intake should derive from carbohydrates. The Baka A:P ratio of 23:77 deviates considerably with what Cordain et al. (2000) deem plausible for the average foraging dietary composition. Large discrepancies are evident between the daily energy obtained from protein, carbohydrates, and fats between Cordain et al.'s (2000) generalised dietary composition and that of the Baka, as shown in Figure 5.2. The differences between the A:P- and macronutrient ratios given by Eaton and Konner (1985) and the presented Baka data appears to be even larger between Cordain et al.'s (2000) data and the Baka data. The Baka dietary- and macronutrient profile shows a higher reliance upon plant-based food items. In particular, the difference between the amount of plant-based carbohydrate intake recommended by Cordain et al. (2000) and the actual daily plant-based carbohydrate intake by the Baka is very large. Thus, the African rainforest-type environments of the Congo Basin in Southeastern Cameroon lend themselves for a higher reliance upon plant food items than do different habitats in the case of carbohydrates. The data suggest that compared to more balanced A:P ratios, the Baka would be more likely to complement their macronutrient requirements with animal foods. The African rainforest is suggested especially rich is plant-based carbohydrates. Furthermore, a diachronic trend towards emphasizing the importance of animal food in foraging diets can be noted for at least part of the discourse.

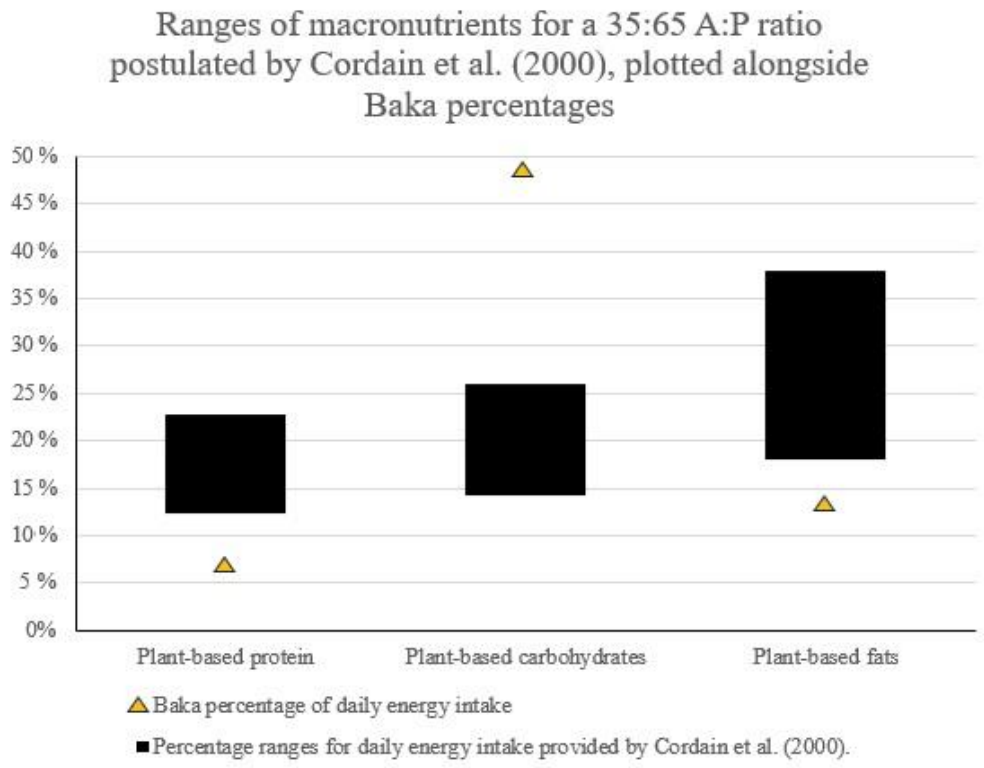

Figure 5.2: Plot showing the ranges of daily energetic contribution per macronutrient postulated by Cordain et al. (2000), alongside the daily energetic contributions per macronutrient established for the Baka. Data from Cordain et al. (2000) is calculated for an A:P ratio of 35:65.

5.3.3. Baka Nutrition Compared to Marlowe's Synthesis of Hunter-Gatherer Nutrition

Marlowe (2005) based his analysis on 478 foraging societies in total. Though Marlowe (2005) did not present any detailed macronutrient data, he does present the respective contribution of both hunting, gathering and fishing for foraging groups categorized into Old World foragers, New World foragers. According to Marlowe (2005), Old World foragers, such as the Baka, obtain 50.64% of their total dietary energy intake through gathering, 25% through hunting, and 23% through fishing. This would equate to an A:P ratio of around 33.7:66.3. New World foragers are argued to rely more heavily upon hunting and fishing, and less on gathering. Together, Old World and New World foragers obtain 36% from their total dietary energy from gathering, 33% from hunting, and 31% from fishing (Marlowe, 2005). As reported by Sato et al. (2012) and Yasuoka (2006), the Baka do not rely upon fishing during a *molongo*. The Baka however, derive 69% of their daily energy intake from plant foods, 23% from meat and 0% from fish according to the ethnographic observations of Yasuoka (2006). The interpretation presented by Marlowe (2005) emphasizes the contribution of animal foods to a higher degree than does the postulated Baka dietary A:P ratio. However, Marlowe's (2005) emphasis on animal foods is not as predominant as it is in the analyses of Cordain et al. (2000) and Eaton and Konner (1985).

5.3.4. An Overview of Comparisons

To more conveniently express the three individual comparisons made in the above three paragraphs, this section provides an overview given in Table 5.5.

Table 5.5: Synthesis for the three comparisons of dietary- and macronutrient profiles, with data from large-scale ethnographic indexes and the Baka.

	Eaton and Konner (1985)	Cordain et al. (2002)	Marlowe (2005)	Baka
Suggested A:P	35:65	38:62	33.7:66.3	23:77
Macronutrients (contribution to total plant-based daily energy intake in %)				
Fat	16.4%	28%	-	13.2%
Protein	19.6%	17.6%	-	6%
Carbohydrates [including TDF]	44%	20.2%	-	48% [57.9%]

The Baka are suggested to rely more heavily upon plant-based food items in comparison to foraging societies included in generalised syntheses of hunter-gatherer nutrition. The discrepancies noted between Baka dietary composition and the large-scale dietary compositions published by Cordain et al. (2002), Eaton and Konner (1985) and Marlowe (2005), may largely be governed by environmental constraints that govern food bioavailability and food choice across habitats. Both Eaton and Konner's (1985) and Cordain et al.'s (2000) analyses are skewed towards the contribution of animal foods in foraging diets. Whereas the analysis presented by Marlowe (2005) replicates this ratio less strongly. The A:P ratio noted for the Baka is markedly different from the A:P ratios noted in any of the explored analyses. The Baka are significantly less reliant upon plant-based protein and somewhat less reliant upon fat. The suggested amount of carbohydrates contributing to hunter-gatherer daily energy intake according to Cordain et al. (2000) is very low in comparison to those reported for the Baka and the subsample used by Eaton and Konner (1985).

In this project, the Baka are presented as being representative of foraging societies occupying the rainforest-type environment located in the Old World, close to the equator. It is argued that extant and extinct groups foraging the rainforests of Africa appear to rely more on plant-based food items than foraging societies in different types of ecological settings. In contrast to their savanna/grassland counterparts, the bioavailability of tubers is suggested to be crucial for foraging groups occupying the African rainforests. These data indicate the potential blind spots in studies of hunter-gatherer nutrition when exclusively large-scale nutritional indexes are used to establish dietary syntheses. The importance of high-resolution chemical nutritional studies is elucidated, as such studies greatly enhance our capacity to grasp the variability within forager dietary composition and subsistence behaviour, across habitats. The next section will describe the potential issues that may arise by maintaining such generalised ideas on foraging nutrition in more detail, as many studies still undervalue the suggested variability within hunter-gatherer diets

5.4. Baka Foraging Nutrition, the *Paleodiet* and Public Health

The *Paleodie*t trend is a populist movement aimed at restoring public health through replicating the dietary practice that our ancestors presumedly maintained. The works of Cordain (2002; 2012) and Wolf (2010) attempt to present a universal, ideal dietary profile for modern-day humans living in Westernized society. One main goal of the *Paleodiet* movement is to guide people in losing weight, as modern Westernized societies maintain a high prevalence of obesity, but also to reduce diabetes, increase sports performance, and reduce risk of hypertension. The presented universal dietary profiles mainly originated from analyses concerned with the large-scale indexes such as Murdock's *Ethnographic Atlas* (1967), with the addition of data on chimpanzee diets, the early hominin fossil record, and nutrients in plants and animals (Cordain, 2012). Cordain's (2002) *Paleodiet* suggests that 55% of the total daily energy intake should be derived from fish and meat. In addition, Cordain (2002) advises to avoid any consumption of starchy, carbohydrate rich plant foods. Instead, one should eat high amounts of animal protein whereas cereals and legumes should be completely refrained from. An overview of the recommended daily macronutrient intakes is given in Table 5.6. Interestingly, the advised total kcal intake per day is not given in the first edition of Cordain's (2002) *Paleodiet*, although daily kcal intake is one of the main factors for either weight loss or weight gain. Nevertheless, the percentages given in Table 5.6 are presented as a dietary blueprint for which our bodies have evolved over the course of our existence (Cordain, 2002). The revised edition of Cordain's *Paleodiet* (2012) aligns with the previous editions, stating that one should mainly consume lean animal food items instead of starchy food items such as tubers, ultimately leading to a dietary profile high in protein and low in carbohydrates. The revised edition states that a dietary intake of 2200kcal is ideal.

Another renowned advocate of the *Paleodiet* is Wolf, who published '*The Paleo solution: The original human diet*' in 2010. Again, one main aim is to help solve issues in public health, such as the high prevalence of obesity, diabetes, and inflammatory diseases. Similar to the revised edition of Cordain's *Paleodiet* (2012), the ideal daily energy intake is put at 2200 kcal. The daily amounts of macronutrients constituting the ideal ancestral diet according to Wolf (2010) are given in Table 5.6. To investigate the extent to which Baka nutrition represents the ideal human diet according to the *Paleodiet* movement, the daily dietary macronutrient intakes established for the Baka are also given in Table 5.6. The macronutrient ratios established by Cordain et al. (2000) were based on a large body of data derived from a broad range of foraging societies as presented in Table 5.5. Cordain's (2002; 2012) and Wolf's (2010) *Paleodiets* are the presumedly idealized ratios based upon these previous data.

Table 5.6: The ideal ancestral dietary profiles according to Cordain (2002; 2012) and Wolf (2010), compared with nutritional data from the Baka. The relative contribution to the total plant-based daily energy intake derived from plant foods is given between brackets.

Ideal daily macronutrient amount (g) [contribution to total plant-based daily energy intake in %]	Cordain (2002)	Cordain (2012)	Wolf (2010)	Baka
Fat	- [28 - 47%]	108 [42%]	100 [39%]	37.1 [15%]
Protein	- [19 - 35%]	190 [33%]	217 [38%]	119.8 [21%]
Carbohydrates	- [22 – 40%]	142 [25%]	129 [23%]	308 [54%]
TDF	-	-	42.5	121 [10%]
Total kcal	-	2200	2200	2287

In comparison to the assumed universal, ideal ancestral diets, the Baka consume significantly less fat on a daily basis. Furthermore, the Baka consume between roughly 33 – 50% less protein than the advised amount. On the contrary, the Baka consume double the amount of carbohydrates advised, corroborating the argument by Hardy et al. (2015; 2022) that the importance of carbohydrates towards our evolution has been crucial, but instead is generally undervalued. As much as plant-based food items are overshadowed in palaeoarchaeology and palaeobiology, studies in modern ethnography, anthropology, and nutrition appear to overemphasize the energetic contribution through the consumption of meat as well. The Baka almost completely derive this energy from starchy wild yams. Though, Cordain (2002; 2012) and Wolf (2010) claim that one should avoid these food items. The Baka consume around triple the advised amount for TDF. The total daily kcal intake for the Baka is relatively similar to the amounts advised by Cordan (2002; 2012) and Wolf (2010). However, the macronutrient profile that constitutes this energy intake differs considerably. As such, Baka nutrition does not represent the ideal *Paleodiet*. Subsequently, as implied by Cordain (2002; 2012) and Wolf (2010), Baka nutrition does not represent the ideal ancestral diet. On the contrary, the Baka rely upon starchy food items that are not

included in the *Paleodiet*. Many extant foraging populations do not express cases of diabetes, obesity or other inflammatory diseases, as long as they refrain from consuming Western foods and a sedentary lifestyle (Milton, 2000). Such a health profile is applicable to the Baka as well. Though, according to the scholars claiming to have the ideal human diet, starchy foods are one of the main contributors of obesity, diabetes, and inflammatory diseases. As such, these data are indicating that environment and lifestyle are leading factors in public health. Ratios in macronutrients, or ratios in overall dietary profiles are of less importance in solving these health issues. As noted by Milton (2000) and Pontzer (2018), there is great similarity in the health profiles of foraging societies despite quite different ethnicities. However, these similar health profiles are supported by a very wide array of dietary- and macronutrient profiles, as well as by broad spectrums of habitats and food choices. Hence, the ultimate goal of losing weight as postulated by Cordain (2002; 2012) and Wolf (2010) and reducing the prevalence of non-communicable diseases, has less to do with a proposed universal ancestral diet, but more with limiting the overall daily kcal intake. However, many Western individuals exceed their required daily energy intake. In addition, data on Baka foraging nutrition greatly challenges the perpetuated notion that Palaeolithic populations maintained high protein and low carbohydrate diets. The high amount of fibre consumed by the Baka, instead suggests the consumption of foods with lower glycaemic indexes than those of Western, processed food items (Pontzer, 2018).

The analysis into Baka nutrition highlights the importance of investigating other plausible solutions for public health, instead of attempting to establish generalised, universal dietary profiles. Factors such as overconsumption, physical activity, chronic stress or time spent outdoor impact health profiles on a holistic level. Hence, diet is not the sole factor responsible for a high prevalence of non-communicable diseases in industrialized societies. Although strongly related, the high variability within foraging diets and the nutritional qualities of wild edible plants, elucidates the importance of exploring other avenues to address issues in public health.

5.5. The Ideal Ancestral Diet

The lifeways of modern-day hunter-gatherers provide the best modern analogues for potential models of early hominin behaviours (Andrikopoulos, 2016; Chang & Nowell, 2016; Marlowe, 2005; Pontzer et al., 2018; Tarantino et al., 2015). The nutritional analyses conducted for the Baka provide a unique opportunity for establishing such an analogue for those foraging groups that occupy, and have occupied, the African rainforests. Modern-day Baka dietary practices do not represent ancestral dietary practices as proposed by the *Paleodiet* movement (e.g., Cordain, 2002; 2012; Wolf, 2010). This notion has been an underlying concept for much of the discussion, and for the central research question. In public health, a universal ancestral diet is a diet regarded as one that mitigates the occurrence of obesity, diabetes and inflammatory diseases (Pontzer, 2018). Here, the plausibility of the idea of an ideal dietary- and macronutrient profile as implied by the *Paleodiet* movement can be assessed through comparison with Baka foraging nutrition.

The chemical nutritional data obtained from 30 Baka wild edible plant samples suggests the presence of within- and between-species variability within the nutritional qualities of these plants. These data express the variability in exclusively one foraging society, across four different communities. Large-scale indexes base their nutritional analyses on over hundreds of foraging societies. Seeing that much nutritional variability is present within just one foraging society, the generalisation of dietary- and nutritional variability of over hundreds of hunter-gatherer societies appears to be problematic in nature. Consequently, a large amount of studies investigating hunter-gatherer nutrition undervalue the variability that is present within foraging diets. In actuality, studies dealing with hunter-gatherer nutrition are engaging in cross-cultural comparisons consisting of mosaics of large- and small-scale data (Crittenden & Schnorr, 2017). These studies often more strongly emphasize the generalised data as opposed to the contextualized, society specific data. The variability witnessed within the Baka wild edible plant subsample may well serve to be projected onto the nutritional qualities of plants used by other extant foraging societies, as inferences made through this lens better represent the reality of foraging diet and nutrition. In addition, this variability may be projected onto the nutritional qualities of Pleistocene wild edible plants and ancestral dietary compositions. Future chemical nutritional studies may greatly benefit by regarding plant age, plant part, soil type, habitat type, and seasonality during sample collection.

Moreover, the Baka are capable of assuring their survival and maintaining their fitness solely by foraging the rainforest of the Congo Basin (Sato et al., 2012; Yamauchi et al., 2000; Yasuoka, 2006). The rainforest provides the Baka with close to the recommended Westernized dietary intake references. The Baka are heavily reliant upon plant-based food items, in particular starch rich wild yams. The Baka dietary- and macronutrient profile shows little overlap with the perpetuated universal diets postulated by the *Paleodiet* movement (Cordain, 2002, 2012; Wolf, 2010). In addition, Baka nutrition considerably differs from the aggregate hunter-gatherer nutrition presented in the analysed large-scale indexes (Cordain et al., 2000; Eaton & Konner, 1985; Marlowe, 2005). Animal to plant ratios in foraging diets are suggested to significantly differ between different foraging societies across habitats. Whereas the total daily energy intake may be similar in foraging societies, the macronutrients constituting the total amount of energy differ greatly. Taken together, evidence for significant variability within the nutritional qualities of wild edible plants (Paine et al., 2019; Wollstonecroft et al., 2008), alongside considerable variability within dietary profiles of different foraging societies, suggests that there is no such thing as a universal foraging diet. The successful health profiles witnessed in almost all extant hunter-gatherer societies are suggested to be more closely related to differences in environment, habitats and lifestyle, rather than exclusively diet (Pontzer, 2018). Regarding Western issues in public health, it appears more worthwhile to explore different avenues in relationship to diet, that have to do with lifestyle, physical activity or environment. Previous dietary recommendations that vouch for an increase in plant-based protein intake to reduce the risk of diabetes, cancer, and obesity in Western society, seem to be beneficial due to the effects of the non-protein components of whole-plant foods (Messina et al., 2023). Though such food items are actively avoided by proponents of the *Paleodiet*. As such, there is much ambiguity as to what the best iteration and implementation of such a *Paleodiet*, or universal diet, should be. The overconsumption of processed food items with a high glycaemic index remains a central contributor to diseases of civilization, the resulting health issues are not rooted in specific macronutrient compositions of any given food item.

6. Conclusion

The often romanticized hunter-gatherer lifestyle is the only remaining avenue for exploring subsistence behaviours that are independent of agriculture to some extent (Marlowe, 2005; O'Keefe et al., 2010; Sahlins, 1998). One crucial component of our evolutionary past has been diet. Diet constitutes one of the main drivers in the origin, evolution, and behaviours of our species and has influenced changes in habitat, social behaviour, body size, and life history strategies. Watershed moments in human evolution commonly related to diet include developments in brain expansion, cooperation, tool making, and family formation (Crittenden, 2016; Teaford et al., 2023). To fully understand the evolution of our lineage, a broad understanding of what our ancestors ate is of key importance. The archaeological record of the Pleistocene is highly fragmentary in nature, therefore ethnographic and archaeological data have been combined for over decades to elucidate the influences of our species' dietary practice on our evolutionary trajectory (Marlowe, 2005; Winterhalder & Smith, 2000). Since the modern hunter-gatherer diets are probably more similar to the diets of our than are horticultural or agricultural diets, many modern-day foraging societies have research scholarly attention by researchers attempting to investigate the evolution of human nutrition. Even public health studies enquire upon modern-day hunter-gatherer dietary practice to potentially help solve modern health issues. These health complications, often referred to as diseases of civilization, include diabetes, obesity, and non-inflammatory diseases (Andrikopoulos, 2016; Cordain et al., 2002; Pontzer et al., 2018; Teaford et al., 2023). Much of this research has used large-scale indexes on foraging nutrition such as Murdock's *Ethnographic Atlas*, to establish average hunter-gatherer nutritional profiles (e.g., Cordain, 2002, 2012; Eaton & Konner, 1985; Marlowe, 2005; Wolf, 2010). Some of these generalised nutritional profiles gave rise to a mainstream nutrition trend, encapsulated in the *Paleodiet* movement. As such, this project's main aim was to investigate whether a universal macronutrient- and dietary profile actually exists as is implied by the *Paleodiet* movement.

To do this, a nutritional analysis on 30 wild edible plant samples used by the Baka forager-horticulturalists of Southeastern Cameroon was conducted. A bird's eye view on the Baka macronutrient data have indicated significant variability within the nutritional qualities of wild edible plants used by the Baka, on both a within- and between species level. Habitat and plant age are suggested to be key contributors to a lack of uniformity in the macronutrient compositions of wild plants and in turn, in hunter-gatherer dietary compositions. The Baka macronutrient data is not supportive of a universal, ancestral dietary composition as proposed by the *Paleodiet* movement. Though cultural factors in food choice were not included in this analysis, this study refrains from environmental

determinism as the obtained dietary- and macronutrient profiles derive from actual Baka subsistence behaviours. Future studies may further elucidate what drives the Baka to make any given foraging decision whilst they partake in long-term foraging trips. Forthcoming nutritional studies should instead focus more on the effects of plant age and habitat on food choice and the nutritional qualities of wild plants. High-resolution nutritional analyses hold the potential to highlight the uniqueness and variability present within foraging diets, traits that are usually undervalued by large-scale indexes on hunter-gatherer nutrition. However, the heuristic value of large-scale indexes on hunter-gatherer nutrition should not be undervalued (Crittenden & Schnorr, 2017). The variability noted for the nutritional qualities of wild plants, ultimately influencing hunter-gatherer dietary- and nutritional compositions, should be accounted for by future studies on foraging nutrition. In a similar vein, potential inferences on Pleistocene diets that refer to extant hunter-gatherer subsistence behaviours analogously, should consider the presence of significant variability within early hominin nutrition.

The African rainforest within the Congo Basin in Southeastern Cameroon presents sufficient nutrients for the Baka for long-term survival without the aid of agriculture (Sato et al., 2012; Yamauchi et al., 2000; Yasuoka, 2006). Moreover, the nutrients provided by the Congo Basin rainforest per consumption-day almost conform with Western daily dietary guidelines. As the Congo Basin is argued to have served as a climatic refuge during the LGM, subsistence behaviours observed within this ecological setting may have deeper roots in the past than previously thought. As such, the African rainforest-type environment can no longer be denied its crucial role in the evolution of hominin nutrition, nor in the evolution of the human lineage (Mercader, 2002; Roberts, 2022; Roberts et al., 2015; Roberts & Petraglia, 2015; Scerri et al., 2022). The Baka as representatives of foraging groups occupying the African rainforest, suggest that starch-rich plant food items are crucial for survival in said environment. Their A:P ratio of 23:77 is skewed towards the dietary contribution of plant foods more so than in any aggregate A:P ratio given by large-scale indexes and analyses thereof. The *Paleodiet* movement draws much from these aggregate nutritional profiles. Actual Baka rainforest nutrition does not align with any of the guidelines postulated by the *Paleodiet* movement. The nutritional- and dietary profile established for the Baka differs significantly from aggregate nutritional profiles that have been suggested previously for semi-tropical and grassland foraging societies. The Baka are highly reliant upon wild yams, supplying them with ample carbohydrates. However, the nutritional guidelines presented by the *Paleodiet* movement advise to abstain from starchy foods altogether. This discrepancy is another indication of how generalising analyses may naively undervalue the variability present within hunter-

gatherer diets. Interestingly, while the variability in nutritional profiles and dietary composition between foraging societies is high, they all maintain relatively successful health profiles when abstaining from Western processed food items and sedentary lifestyles

Public health studies using extant foraging diets to help solve and understand diseases of civilization would find more fruitful results by looking into other constituents of good health, such as physical activity, overconsumption, environment, stress, or time spent outdoor (Pontzer et al., 2018). Though the effects of non-protein components derived from whole plant foods are suggested as a promising contributors for reducing the risks diabetes, obesity and other non-communicable diseases in Western individuals (Messina et al., 2023). Allas, no specific macronutrient composition based on extant or extinct foraging diet offers a magical solution to Western health issues. It is argued that the main driver for successful health is unlikely to exclusively be diet. Hence, studying modern-day hunter-gatherer diet highlights the complexity of human nutrition and modern-day health issues, alongside the complexity of extant and extinct subsistence behaviours.

Overall, it is highly unlikely that a universal dietary- and macronutrients profile exists. The variation within both environmentally and culturally determined food choice, and the nutritional qualities of food items within and across habitat indicates that maintaining a relatively uniform macronutrient- and dietary profile across different habitats is unfeasible. The diseases of civilization are driven in large part by diet, but other avenues should be explored in combination with diet to propose holistic future solutions. Upcoming studies on the evolution of human nutrition and hunter-gatherer nutrition should attempt to combine different data such as amounts of food brought back to camp, chemical nutritional values, consumption rates, as well as salience of the given food items (Crittenden & Schnorr, 2017) in order to present high-resolution, contextualized narratives on foraging nutrition, and in turn Pleistocene nutrition.

The time is now to elucidate our understanding on the variability within hunter-gatherer dietary practice, as most if not all foraging societies are transitioning into agricultural and sedentary lifeways. Ultimately, the broad spectrum present within human nutrition, food choice, food availability, the nutritional qualities of foods, can be heuristically applied to the Pleistocene. To fully understand the complexity of Pleistocene diet, high-resolution studies into modern-day foraging nutrition may well serve as an important tool for filling the gaps presented by the fragmentary nature of the Pleistocene archaeological record. Instead of aiming to find the universal ancestral diet, scholars should aim to acknowledge the plurality of hunter-gatherer lifeways, and apply this diversity onto narratives on human

evolution and hominin deep history. Key moments in the evolutionary trajectory of *Homo* under effect of diet were subject to a broad range of variables. The resultant changes in brain size, cooperation, family structure, tool making and life history strategies are situated in frameworks more complex than previously implied by nutritional studies based on large-scale indexes. Ultimately, the potent combination of ethnographic data and archaeological data allows for a broader understanding on key adaptations in human evolution, such as the development of behavioural capacities, hominin morphologies and social formations. The ultimate goal being to elucidate the influence of our ancestors' dietary practices on our evolutionary past. This project has shown that certain insights can only be gained from multi-disciplinary research.

The renewed interest in hunter-gatherer nutrition presented by public health studies, offers a promising future for both archaeologists and ethnographers, as multi-disciplinary research constitutes the tip of the spear in this endeavour. Whilst broadening our understanding on the evolution of hominin nutrition, this new academic frontier may simultaneously help to understand and solve modern health issues on a global scale. The large temporal gap present between extant- and Pleistocene foraging subsistence behaviours may be bridged by the combination of primate nutrition, the early hominin fossil record, and extant foraging nutrition. This allows for investigating what components of foraging behaviours are either derived or ancestral. Such a phylogenetic approach will illuminate the nutritional evolution of the hominin lineage, without claiming extant foragers to be remnants of the past. Instead, diachronic narratives can be constructed by referring to the uniqueness of modern foraging behaviours, Pleistocene foraging behaviour, and primate foraging behaviour. To substantiate the uniqueness present in hunter-gatherer lifeways that so often leads to their romanticization, a crucial component of future studies on extant hunter-gatherer dietary practice is that of mythology. A more interpretive, qualitative approach highlights hunter-gatherer food choice based upon their contextualized social belief systems, social identities, connections with nature, and adhering ontologies. The focal point here being the inclusion of the voices of modern-day foraging societies themselves, for more inclusive, diverse, and less functionalist interpretations of the lifeways of hunter-gatherers.

Abstract

Diet has been one of the main drivers in the origin, evolution, and behaviour of our ancestors. Key moments in our evolutionary trajectory have been linked to changes in diet. These evolutionary developments include an increase in brain size, changes in habitat choice, adaptations in body size, and changes in life history. However, the Pleistocene archaeological record is highly fragmentary. To complement this lack of data, archaeology and ethnography have been combined for over decades. The predominant part of studies investigating early *Homo* subsistence behaviours and nutrition have focused on extant foraging populations from the African savannas to develop substantial models of human behavioural evolution. Though, studies focusing on hunter-gatherer nutrition have significantly undervalued the variability that is present within foraging diets. Furthermore, the rainforest-type environment is largely rejected its crucial role in the evolution of our lineage. The predominant part of studies have based their analyses on large-scale dietary indexes. Some authors have assumed that taken on aggregate, the average hunter-gatherer dietary profile can be recruited as a universal, ancestral diet. This led to the formation of the so-called *Paleodiet* movement, that attempts to help solve and understand public health issues known as the diseases of civilization such as obesity, diabetes, and other non-communicable diseases. However, it remains unclear what a uniform, ancestral *Paleodiet* should look like, or whether such a concept is feasible. Here, we show that hunter-gatherer nutrition is highly variable in nature, and that no uniformly applicable *Paleodiet* exists. By conducting a high-resolution macronutrient analysis on 30 wild edible plant taxa used by the Baka forager-horticulturalists from Southeastern Cameroon, we have shown that the nutritional qualities of wild edible plants are greatly affected by the effects of habitat and plant age, on a within- and between species level. Furthermore, we found that previously established aggregate hunter-gatherer nutritional profiles greatly differ from the reconstructed dietary- and macronutrient profiles established for the Baka. As Baka rainforest nutrition could be reverse-engineered, we illustrate that the Congo Basin rainforest-type environment provides enough macronutrients for hominins to sustain themselves without the aid of agriculture, and that the rainforest-type environment has been a crucial environment for the evolution of our lineage. Carbohydrates from starchy tubers are proposed to play a key role in Baka nutrition. Such underground storage organs are argued to have been important to early hominin nutrition within African rainforests. Public health studies may benefit by shifting their focus towards other components of Western lifestyle as more important contributors to diseases of civilization such as physical activity, stress, time spent outdoor, and overconsumption. We anticipate that future studies on extant foraging diet may greatly benefit from supplementing their use of large-scale hunter-gatherer nutritional indexes with high-resolution chemical nutritional data, as well as data

on weight of food brought back to camp, and estimated consumption patterns, to broaden our understanding on Pleistocene subsistence behaviour. We stimulate other research to partake in multi-disciplinary discourse for more increasingly diverse and inclusive narratives on human nutrition, Pleistocene subsistence behaviours, and human evolution. Lastly, to fully understand the influences of hominin dietary practices on the trajectory of our own evolution, it is imperative to acknowledge the plurality of both extant- and extinct hunter-gatherer lifeways, and to project a similar range of variability onto Pleistocene behaviours across different habitats.

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Appendix A: Laboratory Protocols

A. 1. Cryo-Mill Protocol

Voorschrift nr: 88 Freezer/Cryo Mill 6875D 30-6- 2020 FQD

Manual: Freezer/Cryo Mill

Equipment

SPEX SamplePrep Freezer/Cryo Mill 6875D location: XX215

Vails

Working:
The Freezer/Cryo Mill is a cryogenic laboratory mill that freezes samples in liquid nitrogen, then pulverizes them with a magnetic driven impactor. Each sample is placed in a separate grinding vail which is immersed in a liquid nitrogen bath inside the mill. Thus there is no cross-sample contamination and the low temperature of the sample is maintained during grinding.

1. Method

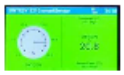
Reservations
Make your reservation using the digital outlook agenda.

Important

When working with the Freezer/Cryo Mill, it is mandatory to wear a face protector. As long as you work with the Freezer/Cryo Mill you keep this face protector on. When opening and slowly closing the Freezer/Cryo Mill, it is mandatory to wear cryo gloves.

If the nitrogen alarm goes off, you are obliged to leave the room and wait for the light to turn green again.
Without oxygen there is no life. Humans require 20,9% oxygen and are already quite unwell at rates below 16%. At 6% a human being will be dead within seconds. For this reason, the law sets a minimum of 19% and requires that 18% the space is (calmly) vacated.

Green: the space is safe to stay in (≥19% oxygen).
Orange: calmly leave/do not enter the space (<19% oxygen).
Red: leave the area immediately, and certainly do not enter it (less than 18% oxygen).



Vails.
Pressure can develop inside the vail after removing it from the Freezer/Cryo Mill. As the vail warms pressure can build causing the end plug to pop out with force and sample can be lost. Handle vails with care. Do not point toward face. Chemicals not compatible with polycarbonate vail include acetone, alcohols, organic solvents (Chloroform, ammonia), Diethylpyrocarbonate (DEPC), TriZol and phenols.

Nitrogene liquid
Filling liquid nitrogen tank is only performed by Erik Meulenbroeks

Sample Preparation:
- Fill (with small pieces) 4 vials (Less vails is also possible), maximum half full with your sample and add an impactor.
- Place 4 vials in the Freezer/Cryo Mill. The front 2 vials are grinding, the rear 2 vials are already pre-cooled.

SS Version June 2020 | Xandra Balder

Starting device:

- Switch on the extractor hood at full power (5) (big switch box) at the wall side on the right of the hood.
- Turn on the suction for the Freezer/Cryo Mill (small switch box)at 1 (Open)

- Close the Freezer/Cryo Mill
- Open the tap on the nitrogen tank, tap connected to the filling hose, counterclockwise.
- Switch on and set Freezer/Cryo Mill.

Click on the control panel:

- Click on "Dual" (or mono if you only have one sample) and "Auto fill".

- Settings for most plant tissue, insects, food products samples are:
Pre-cool 1 min, Run time 1 min, Cool time 0 min, Cycles 2, Rate 12 cps.
If this is not enough, you can adjust it.
- Set the settings with the "< >" arrows
- *Start grinding by pressing run.
- Device starts to fill up, this releases a lot of nitrogen vapor, that is normal.

Device will grind and indicate when it is ready.
Carefully open the device and remove one vial. Carefully close the device.
Open de vail immediately to prevent pressure build-up in the vail.
Carefully open the device and remove the second vail. Carefully close the device.
Also open the second vail immediately.
Place the 2 rear vials with sample to the front and the 2 new filled vials at the back.
Carefully close the Freezer/Cryo Mill.
Collect the samples from the open vails.
All of the Freezer/Cryo Mill® Grinding Vials may be superficially cleaned quickly and easily by placing them under running hand warm water with soap. Clean them immediately after use. Dry and refill.*

Repeat from * until * you have ground all the samples.

- Switch off the Freezer/Cryo Mill.

- Turn off the tap on the nitrogen tank, turn it clockwise. Turn off the Freezer/Cryo Mill and leave it open until the nitrogen evaporates. Set the extractor hood at power 2 (big switch box) and the suction for the Freezer/Cryo Mill (small switch box) at 1 (Open).

2.Cleaning

- Clean the Freezer/Cryo Mill at the end of the day or the following day when all the liquid nitrogen has evaporated.
- Turn off the extractor hood and set the suction for the Freezer/Cryo Mill on 2 (Closed).
- Clean the Freezer/Cryo Mill inside and outside with a cloth and soapy water. Also clean the table where the Freezer/Cryo Mill is located.

3.Remarks

The optimum volume, weight, grinding time and impact frequency for any sample ground in the Cryo Mill are determined by experimentation, the experience of the operator, and the requirements of the analyst. In most cases, the smaller the sample and the longer the grinding time, the finer the particle size will be. In cryogenic grinding the temperature also affect the outcome. As a rule, the colder the sample, the finer it can be ground.

A. 2. Soxhlet Protocol

The Soxhlet extraction.

1. Introduction

Soxhlet is used for total fat determination in food. In principle fat will be extracted out of a food product, using petroleum ether 40-60°C, special glassware, sample thimbles and a heating block, for at least 3 hours. It is used for solid food samples containing less than 10% water. If moisture content is higher pre-drying of samples is necessary. Samples should also be homogeneous.

2. Reagents

Petroleum ether 40-60°C; Merck 1.01774.2500 (P.R.) location X1210.FVC

Cleaning solutions:

Acetone for rinsing; VWR PROL20063.695 location X1210.FVC

Industrial soap:
Mix of 500 ml K500 soap, 250 ml 33% NaOH and 2000 ml of demi-water
or use "RBCA Teepol soap" ask Frans

Demineralised water

Chemicals and solutions:

Sea sand; Merck 1.07711.1000 location X1206.VCC

Hydrochloric acid, concentrated, Merck 1.00314.2500 location X1211.ACC

Hydrochloric acid, 4M;
Add 400 ml of demineralized water in a 1 Litre glass beaker and add slowly 200 ml concentrated hydrochloric acid while stirring.

3. Equipment and materials

Reservation: subscription in office agenda in fume hood in lab.2-06
(full name, date and number of samples)

Fume Hood location X1211

Heating blocks:
Labotech LT-6 heating blocks for 6 positions each (2 blocks available)= 12 positions and/or
Gerhardt EV16 heating blocks for 6 positions each (2 blocks available)=
12 positions. In total 24 positions.
Special glassware:
Coolers
Extractors (150 ml); Lenz Laborglasinstruments art.nr. 5395143
Location; storage room FQD " bordes".

Extraction thimbles (Whatman nr. 603; 33*118 mm) location X1211. 6-2

Flatbottomflasks, 250 ml Location X1208/9-13
Fat free cotton wool
Boiling chips
Dispenser, 50 ml with tubing, for adding petroleum ether
Rotation Evaporator(s) of Büchi; Reservation by Outlook digital agenda
Analytical balance (accuracy 0.1 mg)
Whatman 595 ½ filter discs, 150 mm (only for meat sample hydrolysis)
pH paper 0-14 (for meat sample hydrolysis)
Measuring cylinder, glass, 500 ml (for meat sample hydrolysis)
Watch glasses (for meat sample hydrolysis)
Glass beaker, 250 ml (for meat sample hydrolysis)
Glass beaker, 100 ml (for thimbles to weigh samples)
Glass beaker, 1 Litre (for making 4M HCl)
Glass beaker, 2 Litre (for evaporation of P.E. out of thimbles)
50 ml Measuring cylinder (for adding hydrolysis solution)
Glass funnels (only for meat sample hydrolysis)

4. Method

Sample preparation:

- Be sure samples are homogeneous and contain no more than 10% moisture. If so, pre-dry samples overnight in an oven at 102°C.
- For meat samples first hydrolysis of samples should be carried out described below ("Hydrolysis of meat samples")
- Weigh in extraction thimbles 1-5 grams of sample (amount depending on expected fat percentage: the higher fat % the lower the weight. Use a glass beaker of 100 ml ,to put thimbles in, and an analytical balance. If weight of sample is less than 5 grams, mix sample with Sea sand to total weight of 5 grams.
- Add a code to the thimbles with a pencil (not a penil) and close the extraction thimbles with a plaes of cotton wool and bring it in extractors.
- Add some boiling chips to the flat bottom flasks and weigh them on an analytical balance.
- Add, using the dispenser, at least 200 ml petroleum ether 40-60°C (but not more than 225 ml) in the flat bottom flasks with boiling chips.

Hydrolysis of meat samples:

- Weigh 3 to 5 grams of homogenised sample in a 300 ml Erlenmeyer flask. Add 50 ml 4M HCl and some boiling chips. Close the erlenmeijer flask with a watch glass and boil gently for 1 hour. Add 150 ml hot demineralized water and filter over a Whatman 595 1/2 , 150 mm, filter. Wash the erlenmeijer flask 3 times with hot water and filter. Wash the filter with hot water until the filtrate is neutral (check with pH paper). Spread the filter on a watch glass and dry it for at least 1 hour in an oven at 105°C. Place the filter in a Soxhlet thimble and proceed as described below.

Extraction procedure:

Before use: Use the Soxhlet device set-up and place it in a fume hood. Connect the coolers to each other within one heating assembly and turn the cold water tap open (slowly and not too far: check outlet of last cooler).

- **Connect the flat bottom flasks to the extractors and both to the coolers and place everything on a heating block.**
- **Turn on the heating block (Labotech blocks position "75%" and Gerhardt position "2").**
- **Wait till the petroleum ether boils and refluxes in the extractor**
- **Extraction should be done for at least 3 hours.**
- **After extraction time stop heating, but leave the cooling on. After boiling and refluxing of Petroleum ether stopped wait for half an hour to cool down. Close the water tap.**
- **Disconnect the flat bottom flasks from the extractors and the extractors from the coolers. Rinse all P.E into the flat bottom flasks (use closed side of extractor for pouring!). Take out the thimbles and bring them in a glass beaker. Keep this beaker in the fume hood one night for evaporation of P.E. inside the thimbles.**
- **Rinse the extractors with small amounts of P.E. (5ml) and collect the liquid in the flat bottom flasks.**

Evaporation of P.E.:

- **Evaporate the P.E in the flat bottom flasks using Rotation Evaporators of Büchi (lab.246) under vacuum (protocol 37).**
- **Leave the flat bottom flasks, after evaporating all P.E., overnight in a fume-hood to get rid of the last bit of P.E.**
- **Weigh the flat bottom flasks with boiling stones and fat on the same analytical balance as in the beginning of this experiment.**

Cleaning:

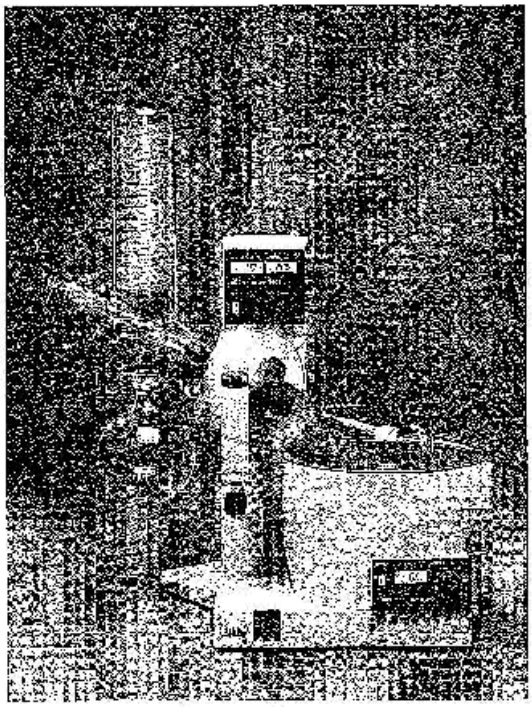
- **Clean the flat bottom flasks well with warm water and the industrial soap solution and leave them to dry on a drying rack. Throw the dried thimbles with sample waste in a waste basket or collect your dried samples for further analysis and throw the thimbles away.**
- **Clean the extractors 3 times with acetone and leave them to dry in the fume-hood. Acetone waste is collected in waste can 3 (halogen poor organic waste).**

A. 3. Buchi Rotary Evaporator Protocol

Using the Rotavapors

1. *Equipment*:

Rotavapor R-200 (Büchi, system 1) ; LAB. X1206
Rotavapor R-215 (Büchi, system 2) ; LAB. X1206
Rotavapor R-210 (Büchi, system 3) ; LAB. X1206
Heating Baths B-490 and 491 (Büchi); LAB. X1206
Vacuum Pumps V-500 and V-700 (Büchi); LAB. X1206
Vacuum controllers V-800 and V-850 (Büchi); LAB. X1206
Cooling Bath (Haake F3); LAB. X1206 (below the table with rotavapors)



Picture 8: R-200 Basic Glass assembly

2. *Method of use*:

Reservation: **Subscribe in digital agenda in Outlook in advance and ask a technician for first instruction on day of use**

Starting up: **Switch on cooling bath half an hour before start and check set temperature (10°C).**

- **At start of cooling, check if no water leakage occurs in the system(s)**
- **Check if flask(s) below condensers are empty. If not, throw waste in waste can nr.8 (toxic waste).**
- **Check if Water Bath(s) are filled with half tap water and half demi-water to work level (flask with content half in water)**
- **Switch on the equipment. Check if Rotavapor(s), Heating Bath(s), Vacuum Pump(s) and Controller(s) are on (lamps on). If not, then check electricity connection(s) and switch on manually again.**
- **Set temperature(s) of Heating Bath(s) and check after 10 minutes. Re-set if necessary. Rule: cooling water max.10°C, then bath temperature should be 60°C (vapour temp. in system is than 40°C)**
- **Be sure valve on Rotavapor cooler is closed**
- **Check in advance if flasks are not damaged, especially the edges**
- **Connect your flask to the evaporator using the "quick-action jack" for the right position and a plastic clamp for attachment of flask on evaporator (flask should not touch edge of Heating Bath(s)). Bring plastic safety screen in right position**
- **Turn on adjusting knob for rotation speed (not to slow and not to fast)**

Starting:

- **Place flask underneath waterlevel in heating baths using "quick-action jack".**
- **The Vacuum Controller(s) should show "Manual" on display. If not then press MENU→MODE→MANUAL**
- *Define set point controller V-800*→**press P set and then P arrow down. Be careful in the beginning, because sample liquid in flask can suddenly start to boil.**
- *Define set point controller V-850*→ **press P set and turn the knob on the controller slowly to approximately 800 mbar and then slowly to set point pressure. When liquid boils to quickly→ press Esc**
- **Standard performance: lower pressure first to approximately 800 mbar and then lower slowly to vacuum for boiling point at 40°C ", which is in relation to vapour temp. in system (see attached "Solvent Table) Press OK to confirm set pressure. Be sure that sample liquid in flask is not boiling to rapidly that losses occur. When that's the case→ press STOP**
- *Stopping*: **When no evaporation takes place anymore proceed as follows:**
  Lift the flask out of the Heating Bath(s) using the quick-action jack
  Stop the rotation speed.
  Aerobic conditions; **Press for Vacuum Controller V-800→ STOP and for V-850→Esc**
  Wait till pressure is atmospheric pressure (around 1000 mbar)
  Release the flask from the Rotavapor system(s)
  Anaerobic conditions; **use Nitrogen gas for aerating**
- **Open big yellow tap on the wall (turn left)**
- **Turn small valve below big yellow tap till a maximum of 0.5 bar is read on manometer**

- **Open a tap connected to (a) Rotavapor(s) (1, 2 or 3 for resp. system 1,2, or 3). Only perform this just before stopping evaporation!!**

End of work:

Empty all flasks, below the condensers and washing bottles, in the right chemical waste can (see chemical waste list on fume hood window!)
If necessary release the pressure of Nitrogen gas and close the valve(s) connected to the Rotavapor(s): close first valve B, then valve(s) 1,2 and/or 3, then big "Praxair" valve C by turning it completely to the left
Switch off electricity supply of the system(s). Check if all equipment is OFF
Switch off Cooling Bath MGW Lauda K4R electronic "Kühlen" on 0

3. Remarks

If samples contain water it's advised to use 3 times 1 ml of pure (100%) acetone and evaporate after each addition. Lower pressure to 70 mbar.
Conditions for Tomato puree : 70°C and 250 mbar pressure
Conditions for Carotenoids : 40°C and 270 mbar pressure

4. Literature

-Büchi instruction manuals

5. Contact persons:

- **Frans Lettink** **Tel : 0317-483231 E: frans.lettink@wur.nl**
- **Erik Meulenbroeks** **Tel: 0317 480 290 E: erik.meulenbroeks@wur.nl**

5.4 Solvent table

Table 5-6: Solvent table

Solvent	Formula	Molar mass in g/mol	Evaporation energy in J/g	Boiling point °C at 1013 mbar	Density in g/cm3	Vacuum in mbar for boiling point at 40 °C
Acetone	C_3H_6O	58.1	553	56	0.790	556
n-amylalcohol, n-pentanol	$C_5H_{12}O$	88.1	595	37	0.814	11
Benzene	C_6H_6	78.1	548	80	0.877	236
n-butanol tert. butanol	$C_4H_{10}O$	74.1	620	118	0.810	25
(2-methyl-2-propanol)	$C_4H_{10}O$	74.1	590	82	0.789	130
Chlorobenzene	C_6H_5Cl	112.6	377	132	1.106	36
Chloroform	$CHCl_3$	119.4	264	62	1.483	474
Cyclohexane	C_6H_{12}	84.0	389	81	0.779	235
Diethyl ether	$C_4H_{10}O$	74.0	389	35	0.714	atmospheric
1,2-dichloroethane	$C_2H_4Cl_2$	99.0	335	84	1.235	210
1,2-dichloroethylene (cis)	$C_2H_2Cl_2$	97.0	322	60	1.284	479
1,2-dichloroethylene (trans)	$C_2H_2Cl_2$	97.0	314	48	1.257	751
Diisopropyl ether	$C_6H_{14}O$	102.0	318	68	0.724	375
Dioxane	$C_4H_8O_2$	88.1	406	101	1.034	107
DMF (dimethyl-formamide)	C_3H_7NO	73.1		153	0.949	11
Acetic acid	$C_2H_4O_2$	60.0	695	118	1.049	44
Ethanol	C_2H_6O	46.0	879	79	0.789	175
Ethylacetate	$C_4H_8O_2$	88.1	394	77	0.900	240
Heptane	C_7H_{16}	100.2	373	98	0.684	120
Hexane	C_6H_{14}	86.2	368	69	0.660	335
Isopropylalcohol	C_3H_8O	60.1	699	82	0.786	137
Isoamylalcohol 3-methyl-1-butanol	$C_5H_{12}O$	88.1	595	129	0.809	14
Methylethylketone	C_4H_8O	72.1	473	80	0.805	243
Methanol	CH_4O	32.0	1227	65	0.791	337
Methylene chloride, dichloromethane	CH_2Cl_2	84.9	373	40	1.327	atmospheric
Pentane	C_5H_{12}	72.1	381	36	0.626	atmospheric
n-propylalcohol	C_3H_8O	60.1	787	97	0.804	67
Pentachloroethane	C_2HCl_5	202.3	201	162	1.680	13
1,1,2,2-tetrachloroethane	$C_2H_2Cl_4$	167.9	247	146	1.595	35
Tetrachlorocarbon	CCl_4	153.8	226	77	1.594	271
1,1,1-trichloroethane	$C_2H_3Cl_3$	133.4	251	74	1.339	300
Tetrachloroethylene	C_2Cl_4	165.8	234	121	1.623	53
THF (tetrahydrofurane)	C_4H_8O	72.1		67	0.889	357
Toluene	C_7H_8	92.2	427	111	0.867	77
Trichloroethylene	C_2HCl_3	131.3	264	87	1.464	183
Water	H_2O	18.0	2261	100	1.000	72
Xylene (mixture)	C_8H_{10}	106.2	379			25
o-xylene	C_8H_{10}	106.2		144	0.880	
m-xylene	C_8H_{10}	106.2		139	0.864	
p-xylene	C_8H_{10}	106.2		138	0.861	

A. 4. Dumas Flash Protein Analyser Protocol

Working with the Flash EA 1112 Protein analyser

1. Equipment, tools and chemicals

Flash EA 1112 Protein analyser ("DUMAS")		AFSG.BZ.118.FCH.X0230.
sample tray with 32 positions		
Eager 200 software		
Stove 60°C (for drying liquid samples)		AFSG.BZ.118.FCH.X0230.
Microbalance (Mettler-Toledo XA105		AFSG.BZ.118.FCH.X0230.
Tin Cups (Interscience; in box with transparent cover)		
Sample rack for tin cups		
Spatula (small)		
Pipet 200 microliter and tips (for liquid samples)		
Tweezer		
Tin Cup closing device with pestle (Interscience)		
for standard series		
D-Methionine 99+%	ACROS Organics227210250 CAS 348-67-4	AFSG.BZ.118.FCH.X0230.
for blank samples		
Cellulose	Aldrich 310697-50g powder 20 µm;	AFSG.BZ.118.FCH.X0230.

2. Method

Reservation;

Ask Frans or Erik for booking in digital agenda of device. (https://booking.labfacilities.wur.nl/public/portal/index/domain/39/catid/132)

Principle of DUMAS:

Dried or pre-dried homogeneous samples containing protein, methionine standards and blank (cellulose) samples are weight in tin cups and oxidised in the DUMAS in a metal column filled with oxidation chemicals at high temperature using pure oxygen and gases released are transported with helium as carrier gas through reduction, carbon dioxide and water filters onto a separation column on which nitrogen is separated and after that detected. A conversion factor is used to calculate nitrogen content into protein content. The results are good comparable to results obtained by the Kjeldahl method.

Sample preparation

Before every measurement it is necessary to clean the weighing tools thoroughly with ethanol. Samples for measurement should be homogeneous (dry samples milled and wet samples mixed well), because this is a microanalysis.

Dry samples: Tare tin cup, weigh maximum 15 mg in the tin cup, make note of the weight and close with closing device and tweezer. Then, wearing gloves, make small tin balls by rubbing the closed tin cups between your thumb and pointing finger. Next place tin cups in sample rack (add to this rack your name and FQD on a tape).

Wet samples: Tare tin cup, pipet 200 microliter in the tin cup, make note of the weight and place it in a sample rack. Add a tape with your name to this rack. Place the rack with tin cups in the 60°C stove nearby the DUMAS. Dry overnight. Next day close the tin cups carefully with a tweezer and use the closing device and, wearing gloves, make small tin balls by rubbing the closed tin cups between thumb and pointing finger. Put the samples back in the sample rack. Only one sample tray on the system should be used (position 0 to 31= total 32 positions) so refill the sample tray when you have more than 32 samples (blanks+ standards+ own samples).

Blank samples; weigh 10 to 15 mg of cellulose in tin cups, make note of the weight and close them as mentioned for dry and wet samples.
Standard samples: weigh approximately 2.5, 5.0, 7.5, 10.0,12.5 and 15.0 mg of D-Methionine in tin cups, make note of their weight and close them as mentioned for dry and wet samples.

Order of analysis: 1 Blank sample, 5 standard samples, 1 Blank sample, 10 samples of your own, 1 Blank sample, 10 samples of your own, 1 Blank sample etc. In the end of your series add a methionine sample (as Unknown) and the last sample should be a Blank sample.
Analyse your own samples at least in duplicate.

Instrument maintenance

Select the icon  on desktop and then  . The next window will appear:

Before starting, check if the system needs maintenance with the icon  . If maintenance is necessary ask one of the responsible persons of your department (see list below).

For maintenance ask:

FQD - Frans Lettink, Erik Meulenbroeks
(FCH - Emma Teuling, Peter de Gijsel)

Starting up system

Select 'Edit Elemental Analyser Parameters'  . Uncheck 'Set instrument to Standby', than select 'SEND' and 'OK'.
Perform a leak check (already possible without temperatures ready); Open the window 'View Elemental Analyser Status' with the  icon, and choos the tab 'special functions'. Choose 'leak check'. After 300 to 400 seconds carrier flow should be smaller than 5 ml/min and reference flow should be smaller than 10 ml/min.
Check temperatures in toolbars caption 'View, 'View Elemental Analyser Status' . The system is ready to use when 'ready led' on the system lights up. This takes about 30 minutes.

Preparation measurement

For every new sequence, a new folder 'yymmdd' should be made in your own folder. Copy the Method 'Template N measurement' in this folder from the 'Method' folder and adjust the sequence table of the method and save it in your folder with the new name.
Fill in the sample names , the file name, the weights and your conversion factor. Always fill in a file name otherwise after measurement you can't find your chromatograms! Opening your sample table can be done using Edit--> Sample Table.
When having less than 62 samples, always end with a standard methionine.

Actual (Act) should be checked in the row of your first sample in the Sample Table.

This should be a Blank sample.
When having less than 62 samples (maximum for a morning performance), always end with a blank sample (as Unknown) and then a methionine (as Unknown).

Saving your method--> File-> Save method

Starting measurement

Start the system with the icon  and click "force to standby" before starting the sequence.
Check if the first blank has a zero value and standard series have a R square of at least 0.99 (View--> Calibration curve) If so, continue with the analysis, otherwise ask the responsible technician of FQD Group (see maintenance list) to have a look at the instrument conditions.
Looking at results: Recalculation--> Summary results

Important remark: Don't leave the sample table and the Summary results screens open during measurement of your samples, because the DUMAS won't go on with sampling your samples!!!!!

Finalize measurement

Fill in the Excel file  **on the desktop on the PC**
It is important to have this data, so please fill in

Copy your own data to Excel (File-Export-) and give this file the same name as your DUMAS file.
Copy your Excel file to a USB stick for further data management.

3. *Remarks as a result of visit of Interscience company (Only for supervisors)*

a) **Re-introduce users with above-mentioned rules**
b) **Do not use double cups anymore**
c) **We tested the Oxygen supply and downgraded it. This results in less maintenance on Cupper column.**
d) **When bringing system to standby position for maintenance, wait 2 minutes before disconnecting anything**
e) **Be careful with replacing the catalyst tube. The inside coating of the oven is broken, so it is possible you hit the inside heating coil**
f) **Remove ash after each measuring series, a new metal ash catcher has been introduced**

A. 5. TDF Analysis Protocol

Note: For the Megazyme TDF Protocol, please follow this link:

https://www.megazyme.com/documents/Assay_Protocol/K-TDFR-200A_DATA.pdf, accessed on 29-10-2023.

A. 6. Total Phenol Determination Protocol

Determination of total phenol content according to Folin & Ciocalteu.

1. Introduction

The main tests for measuring polyphenolics are the Folin-Denis and Folin-Ciocalteu methods. Folin – Ciocalteu reagent consists of acidic compounds of molybdenum and tungsten ions. Folin reagent can be reduced by polyphenolic compounds. The product of this reaction is **heteropolymolybdenum blue**, which absorbs strongly at 750nm.

Gallic acid or Tannic acid is used as arbitrary reference standard. Gallic acid is a phenolic compound containing two hydroxyl (OH) groups that react with Folin reagent to produce the blue color. Tannic acid C76H52O46 is a mixture polygalloyl glucose and polygalloyl ester containing numerous phenol groups in the structure. By comparing the colorimetric index of a known quantity of the reference with the sample, the Gallic Acid Equivalence or Tannic acid Equivalence is calculated.

2. Reagents

Folin & Ciocalteau regents	Merck 109001.0500	AFSG.BZ.118.FQD.X1211.FVA
Na_2CO_3	Merck 6392	AFSG.BZ.118.FQD.X1206.VCC
Tannic acid	Sigma T 0125	AFSG.BZ.118.FQD.X1206.VCC
Gallic acid		AFSG.BZ.118.FQD.X1206.VCC

3. Equipment

Volumetric flask 25 ml
Spectrophotometer Cary 50
Braun multipress MP 50

4. Method

Calibration curve:
Prepare an aqueous solution of 1 mg/ml. This stock solution is diluted 2x, 4x, 8x, 16x, 32x.

Sample preparation: Prepare juices using the automatic juice extractor. Dilute the 10 times using demi water. Or extract samples according protocol no 8.

Satuarated Na_2CO_3 solution: Add 100 ml demi water to 70 gram Na_2CO_3. Stirr for 1 hour at room temperature

Test:

Pipette 5 ml of demi water, in a 25 ml volumetric flask. Add 1,0 ml of you're sample/ calibration sample or blank. Add 1,0 ml Folin Ciocalteu reagens. Subsequently add 1,0 ml of the saturated Na_2CO_3 solution. Adjust the volume till 25 ml. Swing the flasks several times. Measure the absorbance after 15 minutes at 750 nm.
Plot the polyphenol concentration against the absorbance. Calculate the phenol content in you're sample.

5. Remarks

The problem is, the polyphenolic constituents in extracts contain varying numbers of hydroxyl groups (i.e., one, two, three or more hydroxyl groups per molecule), which produce different results relative to gallic acid. In other words, two extracts with the same amount of polyphenols but different compositions of OH can produce two different results using the GAE method.

6. Literature.

Swain, T. and Hillis W.E. (1959). The phenolic constituents of Prunus domestica I.
The quantitative analysis of phenolic constituents. Journal of the Science of Food and Agriculture, 10, p. 63-68.

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Wrolstad, R.E., Wiley, 2001, Determination of Total Phenolics, in Current Protocols in Food Analytical Chemistry, I1.1.1-I1.1.8,

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Appendix B: Electronic Supplementary Material

B. 1. Baka Wild Edible Plant Foods Overview

Code of the sample	Part of the sample	Name	Average percentage of fat	Average percentage of protein	Average percentage of TDF	Total Phenol Average in mg / g dry weight	Average percentage of carbohydrates	Total kcal/100 gr dry mass
BOS 004 (edible)	leaves	*Hilleria latifolia (n=2)*	2.34	19.74	30.44	6.71	47.5	350.82
BOS 004 (inedible)	stems (leaves)	*Hilleria latifolia (stems) (n=2)*	1.17	13.70	44.29	6.15	40.8	317.27
BOS 010	leaves	*Gaertnera cf. longivaginalis (n=2)*	2.62	39.01	44.69	3.91	13.7	323.74
BOS 012	leaves	*Klainedoxa gabonensis (n=2)*	48.66	10.59	33.88	8.85	6.9	575.51
ELO 007	leaves	*Gnetum africanum (n=2)*	3.03	21.81	53.6	8.18	21.6	307.93
BOS 005	fruit	*Aframomum danielli (n=2)*	8.17	5.56	15.32	18.74	71	410.2
BOS 013	fruit (nuts)	*Panda oleosa (n=2)*	44.02	24.19	20.76	8.95	11	578.6
KUN 001	fruit (nuts)	*Irvingia excelsa (n=2)*	76.19	6.87	11.04	5.93	5.9	758.87
BIZ 002	fruit (nuts)	*Baillonella toxisperma (n=2)*	66.23	7.49	6.43	7.72	19.8	718.27

BOS 001	fruit	*Musanga cecropioides (n=2)*	5.75	6.44	48.62	5.75	39.2	331.51
BOS 002	fruit	*Aframomum subsericum (n=2)*	1.49	3.72	40.58	60.67	54.2	326.32
BOS 015	fruit	*Aframomum danielli (n=2)*	8.67	4.34	23.37	11.32	63.6	396.59
BOS 006	fruit	*Oryza sativa (rice) (n=2)*	0.46	6.69	12.08	3.41	80.8	378.16
BOS 011	tuber	*Dioscorea sp (n=2)*	1.52	4.41	74.32	7.76	19.7	258.95
BOS 016	tuber	*Dioscorea minutiflora (n=2)*	0.75	4.02	72.41	32.88	22.8	258.94
KUN 002	tuber	*Dioscorea burkiliana (n=2)*	16.32	5.23	11.47	4.21	67	458.64
ELO 001	tuber	*Dioscorea sp (n=2)*	0.21	5.80	6.72	3.29	87.3	387.62
ELO 002	tuber	*Dioscorea praehensilis (n=2)*	14.20	9.57	2.35	4.22	73.9	466.33
ELO 003	tuber	*Dioscorea sp (n=2)*	0.33	5.05	2.08	1.72	92.5	397.49
ELO 004	tuber	*Dioscorea praehensilis* (cultivated) (n=2)	0.32	6.67	6.06	1.74	86.9	389.47
ELO 005	tuber	*Dioscorea sp (n=2)*	0.33	4.77	5.11	2.27	89.8	391.45
ELO 006	tuber	*Dioscoreophyllum cumminsii (n=2)*	0.87	7.22	6.19	3.73	85.7	391.97

ELO 008	tuber	*Dioscorea burkiliana (n=2)*	0.43	7.21	12.54	1.72	79.8	377.06
BIZ 001	seeds	*Ricinodendron heudelotii (n=2)*	43.18	22.74	7.16	6.86	26.9	601.59
BOS 018	mushroom	*Undetermined (n=2)*	0.59	6.61	88.81	1.11	4	225.35
BOS 007	seeds	*Arachis hypogae (n=2)*	51.44	28.42	11.05	8.51	9.1	635.08
BOS 003	mushroom	*Undetermined (n=2)*	2.63	28.12	53.51	6.37	15.7	306.13
KUN 003	bottle gourd seeds	*Telfairia cf. occidentalis (n=2)*	46.05	28.87	28.14	5.4	NA	573.98
BIZ 003	nutcake	Processed foods (n=2)	75.72	7.88	13.65	7.25	2.8	751.31
BOS 017	bark	*Trichoscypha acuminata (n=2)*	0.83	3.73	74.62	75.51	20.8	254.89

Code of the sample	Part of the sample	Name	Average percentage of fat (fresh)	Average percentage of protein (fresh)	Average percentage of TDF (fresh)	Average percentage of carbohydrates (fresh)	Total kcal/100 gr (fresh)	Average moisture content
BOS 004 (edible)	leaves	*Hilleria latifolia (n=2)*	0.77	6.47	9.98	15.57	115.02	67.22
BOS 004 (inedible)	stems (leaves)	*Hilleria latifolia (stems) (n=2)*	0.38	4.49	14.52	13.37	103.95	67.23
BOS 010	leaves	*Gaertnera cf. longivaginalis (n=2)*	0.74	11.05	12.66	3.88	91.74	71.66
BOS 012	leaves	*Klainedoxa gabonensis (n=2)*	32.11	6.99	22.36	4.55	379.92	33.98
ELO 007	leaves	*Gnetum africanum (n=2)*	1.30	9.36	23.01	9.27	132.23	57.06
BOS 005	fruit	*Afromomum danielli (n=2)*	5.26	3.58	9.87	45.72	264.29	35.57
BOS 013	fruit (nuts)	*Panda oleosa (n=2)*	27.22	14.95	12.83	6.80	357.62	38.20
KUN 001	fruit (nuts)	*Irvingia excelsa (n=2)*	55.52	5.00	8.04	4.30	552.98	27.13
BIZ 002	fruit (nuts)	*Baillonella toxisperma (n=2)*	66.23	7.49	6.43	19.8	718.08	0.05
BOS 001	fruit	*Musanga cecropioides (n=2)*	1.79	2.00	15.13	12.20	103.15	68.89
BOS 002	fruit	*Aframomum subsericum (n=2)*	0.77	1.91	20.87	27.88	167.80	48.57

BOS 015	fruit	*Aframomum danielli (n=2)*	3.20	1.60	8.63	23.48	146.38	63.09
BOS 006	fruit	*Oryza sativa (rice) (n=2)*	0.46	6.69	12.08	80.8	378.30	0.00
BOS 011	tuber	*Dioscorea sp (n=2)*	0.24	0.70	11.82	3.13	41.17	84.10
BOS 016	tuber	*Dioscorea minutiflora (n=2)*	0.73	3.89	70.00	22.04	250.26	3.34
KUN 002	tuber	*Dioscorea burkiliana (n=2)*	5.13	1.64	3.61	21.06	144.17	68.57
ELO 001	tuber	*Dioscorea sp (n=2)*	0.07	1.82	2.11	27.44	121.87	68.56
ELO 002	tuber	*Dioscorea praehensilis (n=2)*	6.39	4.31	1.06	33.26	209.89	54.99
ELO 003	tuber	*Dioscorea sp (n=2)*	0.13	1.92	0.79	35.15	150.99	62.01
ELO 004	tuber	*Dioscorea praehensilis* (cultivated) (n=2)	0.12	2.46	2.23	31.98	143.26	63.22
ELO 005	tuber	*Dioscorea sp (n=2)*	0.17	2.50	2.68	47.04	205.06	47.61
ELO 006	tuber	*Dioscoreophyllum cumminsii (n=2)*	0.33	2.76	2.36	32.72	149.62	61.83
ELO 008	tuber	*Dioscorea burkiliana (n=2)*	0.15	2.43	4.23	26.93	127.23	66.26
BIZ 001	seeds	*Ricinodendron heudelotii (n=2)*	43.18	22.74	7.16	26.9	601.50	0.02
BOS 018	mushroom	*Undetermined (n=2)*	0.59	6.61	88.81	4	225.40	0.00

BOS 007	seeds	*Arachis hypogae (n=2)*	51.44	28.42	11.05	9.1	635.13	0.00
BOS 003	mushroom	*Undetermined (n=2)*	2.63	28.12	53.51	15.7	305.95	0.04
KUN 003	seeds	*Telfairia cf. occidentalis (n=2)*	46.05	28.87	28.14		586.22	0.00
BIZ 003	nutcake	Processed foods (n=2)	75.72	7.88	13.65	2.8	751.49	0.00
BOS 017	bark	*Trichoscypha acuminata (n=2)*	0.83	3.73	74.62	20.8	254.82	0.02

	Mean crude fat content (fresh)	Mean protein content (fresh)	Mean TDF content (fresh)
Baka leaves (n=5)	7.06	7.67	16.50
Kale, raw (n=5) (USDA, 2019)	1.49	2.92	4.1
Spinach, mature (n=8) (USDA, 2021)	0.6	2.91	1.6
Baka fruits (n=4)	2.75	2.27	13.62
Hadza fruits (n=6) (Murray et al., 2001)	0.8	0.46	7.57
Banana, ripe, raw (n=12) (USDA, 2020)	0.29	0.74	4.62
Plantains, raw (n=2) (USDA, 2019)	0.35	1.3	1.7
Rice (n=1)	0.46	6.69	12.08
Baka nuts (n=3)	49.65	9.15	9.10
Cashew (n=8) (USDA, 2023)	38.9	17.4	4.1
Almond (n=8) (USDA, 2022)	51.1	21.4	10.8
Baka tubers (n=10)	1.35	2.44	10.09
Hadza tubers (n=7) (Schoeninger et al., 2000)	0.6	3.03	9.24
Potatoes, raw (n=8) (USDA, 2020)	0.26	1.81	13.8
Cassava root (Ferraro et al., 2016)	0.03	1.9	1.9
Baka seeds (n=3)	46.89	26.68	15.45
Hadza baobab seeds (n=1) (Murray et al., 2001)	27.89	34.56	13.42
Pumpkin seeds (n=8) (USDA, 2023)	40	29.9	5.1
Sunflower seeds (n=8) (USDA, 2023)	48.4	18.9	7.2
Baka mushrooms (n=2)	1.61	17.37	71.16

Mean carbohydrate content (fresh)	Mean energy content (fresh)	Mean moisture content
9.33	164.57	59.43
4.42	48.57	89.60
2.64	30.8	92.40
27.32	170.41	54.03
26.9	131.78	64.27
23	112.38	75.3
31.9	139.35	65.2
80.8	378.30	0.00
10.30	542.89	21.79
36.3	573.1	4.81
20	647.1	4.26
28.07	154.35	58.05
12.35	85.4	74.78
16	101.18	81.1
30.5	133.67	65.5
18	607.62	0.00
0	416.09	24.13
18.7	564.6	6.62
24.5	623.5	4.87
9.85	265.68	0.00

Fat values avg, med + sum	
Leaves average	11.56
Leaves median	2.62
Fruits+nuts average fat value	26.37
Fruits+nuts median fat value	8.42
Tubers average	3.53
Tubers median	0.75
Seeds average	46.89
Seeds median	48.74
Nuts average	62.15
Nuts median	66.23
Fruits average	6.02
Fruits median	6.96
Leaves sum fat value	57.82
Tubers sum fat value	35.29
Nuts sum fat value	186.44
Fruits sum fat value	24.08
Seeds sum fat value	140.67
Protein avg med sum	
Leaves average	20.97
Leaves median	19.74
Tubers average	6.00
Tubers median	5.80
Seeds average	26.68
Seeds median	28.65
Nuts average	12.85
Nuts median	7.49
Fruits average	5.01
Fruits median	4.95
Leaves sum	104.84
Tubers sum protein	59.95
Seeds sum protein	80.03
Nuts sum protein	38.55
Fruits sum protein	20.05
Total Dietary Fiber avg med sum	
Leaves average	41.38
Leaves median	44.29
Tubers average	19.93
Tubers median	6.72
Seeds average	15.45
Seeds median	19.60
Nuts average	12.74
Nuts median	11.04
Fruits average	31.97
Fruits median	31.98
Leaves sum	206.90
Tubers sum TDF	295.22
Seeds sum TDF	46.35

Nuts sum TDF	38.23
Fruits sum TDF	127.89
Carbs avg med sum	
Leaves average	26.1
Leaves median	21.6
Tubers average	70.54
Tubers median	79.8
Seeds average	18
Seeds median	9.1
Nuts average	12.23
Nuts median	11
Fruits average	57
Fruits median	58.9
Leaves sum carbs	130.5
Tubers sum carbs	705.4
Seeds sum carbs	36
Nuts sum carbs	36.7
Fruits sum carbs	228
kcal/100 gr dry weight avg med sum	
Leaves average	375.054
Leaves median	323.74
Tubers average	377.79
Tubers median	391.45
Seeds average	603.55
Seeds median	604.53
Nuts average	685.25
Nuts median	718.27
Fruits average	366.16
Fruits median	364.05
Leaves sum kcal	1875.27
Tubers sum kcal	3777.92
Seeds sum kcal	1810.65
Nuts sum kcal	2055.74
Fruits sum kcal	1464.62

B. 2. Raw Data for Fat Measurements

Grams of extracted fat R2	Standard Deviation (n=2)	M0 Initial mass of sample (in grams) R1	M0 Initial mass of sample (in grams) R2	M1 Empty flask weight with boiling stones (in gram) R1	M1 Empty flask weight with boiling stones (in gram) R2	M2 Flask weight with fat contents (in grams) R1	M2 Flask weight with fat contents (in grams) R2	Total fat content (percentage) R1	Total fat content (percentage) R2	Sum of extracted fat (in grams) R1 +R2	Sum of total sample weight (in grams) R1 + R2	Average percentage of fat in sample OR estimated grams of fat in 100 grams of sample mass
0.0668	0.007	3.0724	3.0636	107.0241	102.9979	107.1008	103.0647	2.490	2.180	0.1435	6.136	2.339
0.0385	0.004	3.0536	3.0511	103.167	102.4878	103.2	102.5263	1.081	1.262	0.0715	6.1047	1.171
0.0888	0.010	3.1192	3.1325	106.906	106.3381	106.9812	106.4269	2.411	2.835	0.164	6.2517	2.623
1.5262	0.030	3.1805	3.1802	102.9651	107.0191	104.5338	108.5453	49.32	47.99	3.0949	6.3607	48.657
0.099	0.007	3.0815	3.1341	106.6733	106.7226	106.7624	106.8216	2.891	3.159	0.1881	6.2156	3.026
0.2476	0.003	3.0005	3.002	106.5004	102.4818	106.7432	102.7294	8.092	8.248	0.4904	6.0025	8.170
1.3127	0.048	3.0505	3.0675	108.631	106.4588	110.0116	107.7715	45.26	42.79	2.6933	6.118	44.023
2.5077	0.234	3.0743	3.0747	107.95	107.9122	110.1273	110.4199	70.82	81.56	4.685	6.149	76.191
2.0133	0.043	3.108	3.0629	108.6565	95.4873	110.73	97.5006	66.71	65.73	4.0868	6.1709	66.227

0.1737	0.000	3.0158	3.0142	113.1713	105.8852	113.3444	106.0589	5.740	5.763	0.3468	6.03	5.751
0.0309	0.001	2.0284	2.0281	107.4417	98.1938	107.4714	98.2247	1.464	1.524	0.0606	4.0565	1.494
0.271	0.001	3.1383	3.1393	108.0957	106.2652	108.3687	106.5362	8.699	8.632	0.544	6.2776	8.666
0.0173	0.005	3.0411	3.0357	107.1983	112.7944	107.2091	112.8117	0.355	0.570	0.0281	6.0768	0.462
0.0447	0.001	3.0044	3.0033	108.1352	95.8806	108.1817	95.9253	1.548	1.488	0.0912	6.0077	1.518
0.0263	0.005	3.0094	3.0041	97.9198	107.6336	97.9387	107.6599	0.628	0.875	0.0452	6.0135	0.752
0.0769	0.127	1.0213	1.0207	107.4895	107.2493	107.7458	107.3262	25.10	7.534	0.3332	2.042	16.317
0.0065	0.000	3.1555	3.1741	105.7637	107.6614	105.7707	107.6679	0.222	0.205	0.0135	6.3296	0.213
0.0805	0.088	1.0009	1.0056	106.4795	106.7028	106.684	106.7833	20.43	8.005	0.285	2.0065	14.204
0.0116	0.002	3.0549	3.0534	106.3271	102.71	106.3358	102.7216	0.285	0.380	0.0203	6.1083	0.332
0.0109	0.001	3.0192	3.1275	102.7732	107.3934	102.782	107.4043	0.291	0.349	0.0197	6.1467	0.320
0.0076	0.004	3.0152	3.0259	113.1374	108.0135	113.15	108.0211	0.418	0.251	0.0202	6.0411	0.334
0.0129	0.019	3.0008	3.0085	97.8602	102.4109	97.8995	102.4238	1.310	0.429	0.0522	6.0093	0.869
0.0111	0.003	3.0005	3.0045	107.7859	105.4839	107.8006	105.495	0.490	0.369	0.0258	6.005	0.430

					103.760	109.184	104.652					
0.8925	0.025	2.1078	2.1074	108.257	1	7	6	44.01	42.35	1.8202	4.2152	43.182
				106.076	109.520	106.084	109.548					
0.0277	0.014	3.0061	3.0031	3	4	2	1	0.263	0.922	0.0356	6.0092	0.592
				107.787	106.778	108.584	107.595					
0.817	0.014	1.5586	1.5798	4	2	7	2	51.15	51.72	1.6143	3.1384	51.437
				106.239	105.964	106.295	106.069					
0.1048	0.035	3.0519	3.0586	4	9	2	7	1.828	3.426	0.1606	6.1105	2.628
				105.925	107.562							
0.7255	0.046	1.4471	1.5613	1	5	106.585	108.288	45.60	46.47	1.3854	3.0084	46.051
					106.247	112.694	107.260					
1.0127	0.093	1.4295	1.4191	111.55	9	3	6	80.05	71.36	2.157	2.8486	75.721
				107.837	107.110	107.861	107.137					
0.0265	0.002	3.0432	3.0766	5	7	6	2	0.792	0.861	0.0506	6.1198	0.827

B. 3. Raw Protein Measurements Dumas

Sample code	File name	Corrected % Protein	Protein in mg	Standard Deviation (n=2)	Weight (mg)	Conv. Factor	% N	% Protein
ELO 001 (1) **Thijs & Anastacia**	elo001(1)	5.81224444	0.836759771	0.005	14.3965	6.25	1.03110361	6.444397449
ELO 001 (2)	elo001(2)	5.78865591	0.830353746		14.3445	6.25	1.02732944	6.420808792
ELO 006 (1)	elo006(1)	7.22350809	0.940753576	0.002	13.0235	6.25	1.25690579	7.855661392
ELO 006 (2)	elo006(2)	7.21960622	0.943313749		13.066	6.25	1.2562815	7.851759434
KUN 002 (1)	kun002(1)	5.17152692	0.712015827	0.001	13.768	6.25	0.92858881	5.803679943
KUN 002 (2)	kun002(2)	5.28181787	0.713019003		13.4995	6.25	0.94623536	5.913970947
ELO 002 (1)	elo002(1)	9.53951213	1.285640049	0.009	13.477	6.25	1.62746644	10.17166519
ELO 002 (2)	elo002(2)	9.59814149	1.298340599		13.527	6.25	1.63684714	10.23029423
ELO 004 (1)	elo004(1)	6.7233223	0.945769748	0.008	14.067	6.25	1.17687607	7.355475426
ELO 004 (2)	elo004(2)	6.74458104	0.957393279		14.195	6.25	1.18027747	7.376734257
blank 1 cellulose Thijs & Anastasia	blank1thijs&anastacia	0	0		8.836	1	0.1011445	0.1011445
Herh. ELO 003	herh003	5.71614094	0.734324046	0.019	12.8465	6.25	0.91458255	5.716140747
Herh. ELO 003 II	hh003ii	5.71995638	0.707301207		12.3655	6.25	0.91519302	5.719956398
BOS 018 (1)	bos018(1)	6.54806816	0.951630746	0.002	14.533	6.25	1.12437403	7.027337551
BOS 018 (2)	bos018(2)	6.68156021	0.948881774		14.2015	6.25	1.14573276	7.160829544
ELO 008 (1)	elo008(1)	7.41085135	1.087653599	0.026	14.6765	6.25	1.26241934	7.890120983
Herh. ELO 008	herh008	7.65807778	1.051339208		13.7285	6.25	1.22529244	7.658077717
ELO 005 (2)	elo0052	5.83240041	0.874043525	0.009	14.986	6.25	1.00986719	6.311669827
Herh. ELO 005	herh005	6.36640638	0.886808577		13.9295	6.25	1.01862502	6.366406441
BOS 011 (1)	bos0111	4.44738925	0.632040724	0.000	14.2115	6.25	0.78826541	4.92665863
BOS 011(2)	bos0112	4.377587	0.632670761		14.4525	6.25	0.77709705	4.856856346
blank 2 cellulose Thijs & Anastasia	blank2thijs	0	0		8.324	1	0.07668313	0.076683126
BIZ 003 91)	biz0031	7.76026547	1.046394196	0.014	13.484	6.25	1.33563328	8.347707748
BIZ 003 (2)	biz0032	7.99189135	1.06659782		13.346	6.25	1.37269342	8.579334259

KUN 001 (1)	kun0011	6.85056299	0.951269177	0.001	13.886	6.25	1.19008088	7.438005447
KUN 001 (2)	kun0012	6.88098818	0.950264468		13.81	6.25	1.19494891	7.468430519
BOS 004 edible (1)	bos004ed1	19.4977716	2.697224229	0.003	13.8335	6.25	3.21363425	20.08521461
BOS 004 edible (2)	bos004ed2	19.9779615	2.701519841		13.5225	6.25	3.29046464	20.56540489
BOS 004 inedible (1)	bos004in1	13.7666941	1.868278052	0.032	13.571	6.25	2.29666185	14.35413647
BOS 004 inedible (2)	bos004in2	13.6286676	1.823720154		13.3815	6.25	2.27457762	14.21611023
Herh. BOS 010	herhbos010	39.8513287	4.866046495	0.107	12.2105	6.25	6.3762126	39.8513298
Herh. BOS 010 II	hhbos010ii	39.511022	5.01710957		12.698	6.25	6.32176352	39.51102066
blank 3 cellulose Thijs & Anastasia	blank3thijs	0	0	0.000	8.1695	1	0.0939908	0.093990803
check methionine 3	checkmeth3	0	0	0.000	5.9165	1	9.27669621	9.276696205
BOS 005 (1)	bos0051	5.68745201	0.763483558	0.029	13.424	6.25	1.00407517	6.27546978
BOS 005 (2)	bos0052	5.42219086	0.722208711		13.3195	6.25	0.96163338	6.010208607
BOS 017 (1)	bos0171	3.68277347	0.497910973	0.005	13.52	6.25	0.6833266	4.270791054
BOS 017 (2)	bos0172	3.78412148	0.505445106		13.357	6.25	0.69954228	4.372139454
ELO 007 (1)	elo0071	21.8129708	3.043781951	0.064	13.954	6.25	3.58415818	22.40098953
ELO 007 (2)	elo0072	21.8081369	2.952930778		13.5405	6.25	3.58338475	22.3961544
BOS 003 (1)	bos0031	28.1810015	3.781608585	0.054	13.419	6.25	4.60304308	28.76902008
BOS 003 (2)	bos0032	28.0577003	3.70544019		13.2065	6.25	4.5833149	28.64571762
BOS 015 (1)	bos0151	5.62408632	0.825784594	0.027	14.683	6.25	0.99393666	6.212104321
BOS 015 (2)	bos0152	5.31026563	0.786954815		14.8195	6.25	0.94372535	5.898283482
blank 4 cellulose Thijs & Anastasia	blank4thijs	0	0		8.0955	1	0.09408285	0.094082847
BOS 001 (1)	bos0011	6.5363666	0.905940411	0.031	13.86	6.25	1.13817179	7.113573551
BOS 001 (2)	bos0012	6.33962881	0.862601594		13.6065	6.25	1.10669374	6.916835785
BOS 016 (1)	bos0161	3.88373788	0.523430773	0.013	13.4775	6.25	0.7137512	4.460945129
BOS 016 (2)	bos0162	4.17032782	0.542142617		13	6.25	0.75960559	4.747534752
ELO 004 (1)	elo0041	6.69911336	0.899154995	0.004	13.422	6.25	1.16421127	7.276320457
ELO 004 (2)	elo0042	6.64957073	0.894167776		13.447	6.25	1.15628445	7.22677803

BOS 007 (1)	bos0071	28.286819	3.5596133	0.015	12.584	6.25	4.61824417	28.86402512
BOS 007 (2)	bos0072	29.5486851	3.580709656		12.118	6.25	4.82014275	30.12589264
BOS 007 (1)	bos0071ii	28.3542527	3.872907375	0.014	13.659	6.25	4.62903357	28.93145943
BOS 007 (2)	bos0072ii	28.4943087	3.852430537		13.52	6.25	4.65144253	29.07151604
blank 5 cellulose thijs & anastasia	blank5thijs	0	0		8.1299	1	0.09235314	0.092353135
KUN 003 (1)	kun0031	28.1657882	3.54663605	0.031	12.592	6.25	4.76372147	29.77325821
KUN 003 (2)	kun0032	28.6599852	3.502250193		12.22	6.25	4.8161974	30.10123444
BIZ 001 (1)	biz0011	22.8781335	3.300971493	0.238	14.4285	6.25	3.89110112	24.31938171
BIZ 001 (2)	biz0012	22.5700934	2.964081433		12.272	6.25	3.72283077	23.26769257
BOS 013 (1)	bos0131	27.7907141	3.392829325	0.027	12.2085	6.25	4.67711401	29.2319622
BOS 013 (2)	bos0132	27.9943265	3.354000258		11.981	6.25	4.709692	29.43557549
BOS 015 (1)	bos0151	4.0720176	0.565277483	0.029	13.882	6.25	0.88212258	5.513266087
BOS 015 (2)	bos0152	4.61797235	0.606639937		13.1365	6.25	0.8504914	5.315571308
BOS 012 (1)	bos0121	10.7929897	1.358826611	0.047	12.5899	6.25	1.83849418	11.49058819
BOS 012 (2)	bos0122	10.3853535	1.292664955		12.447	6.25	1.7732724	11.0829525
blank 6 cellulose thijs & anastasia	blank6thijs	0	0		8.389	1	0.11161583	0.111615829
BOS 002 (1)	bos0021	4.35346663	0.646185052	0.004	14.843	6.25	0.69655466	4.353466511
BOS 002 (2)	bos0022	4.41844501	0.641050094		14.5085	6.25	0.7069512	4.41844511
BIZ 002 (1)	biz0021	8.21315199	1.089228217	0.000	13.262	6.25	1.31410432	8.213151932
BIZ 002 (2)	biz0022	8.10999721	1.088929326		13.427	6.25	1.29759955	8.109996796
BOS 006 (1)	bos0061	7.36846402	0.936163354	0.026	12.705	6.25	1.17895424	7.368463993
BOS 006 (2)	bos0062	7.3565051	0.898964923		12.22	6.25	1.17704082	7.356504917

%N - Blank N	Sum protein in mg R1+R2	Sum weight of sample in mg	Average percentage protein in sample	Date	Time	Type of sample
0.92995911	1.66711352	28.741	5.8005	22/07/2022	09:23	UNK
0.92618494				22/07/2022	09:27	UNK
1.15576129	1.88406732	26.0895	7.2216	22/07/2022	09:31	UNK
1.155137				22/07/2022	09:36	UNK
0.82744431	1.42503483	27.2675	5.2261	22/07/2022	09:40	UNK
0.84509086				22/07/2022	09:44	UNK
1.52632194	2.58398065	27.004	9.5689	22/07/2022	09:48	UNK
1.53570264				22/07/2022	09:53	UNK
1.07573157	1.90316303	28.262	6.7340	22/07/2022	09:57	UNK
1.07913297				22/07/2022	10:01	UNK
0				22/07/2022	10:05	UNK
0.91458255	1.44162525	25.212	5.7180	22/07/2022	10:10	UNK
0.91519302				22/07/2022	10:14	UNK
1.04769091	1.90051252	28.7345	6.6140	22/07/2022	10:18	UNK
1.06904963				22/07/2022	10:22	UNK
1.18573622	2.13899281	28.405	7.5303	22/07/2022	10:27	UNK
1.22529244				22/07/2022	15:15	UNK
0.93318406	1.7608521	34.986	5.0330	22/07/2022	11:15	UNK
1.01862502				22/07/2022	15:15	UNK
0.71158228	1.26471149	28.664	4.4122	22/07/2022	11:24	UNK
0.70041392				22/07/2022	11:28	UNK
0				22/07/2022	11:32	UNK
1.24164248	2.11299202	26.83	7.8755	22/07/2022	11:36	UNK

1.27870262				22/07/2022	11:41	UNK
1.09609008	1.90153364	27.696	6.8657	22/07/2022	11:45	UNK
1.10095811				22/07/2022	11:49	UNK
3.11964345	5.39874407	27.356	19.7351	22/07/2022	11:53	UNK
3.19647384				22/07/2022	11:58	UNK
2.20267105	3.69199821	26.9525	13.6982	22/07/2022	12:02	UNK
2.18058681				22/07/2022	12:06	UNK
6.3762126	9.88315606	24.9085	39.6778	22/07/2022	15:32	UNK
6.32176352				22/07/2022	15:36	UNK
0				22/07/2022	12:19	UNK
0				22/07/2022	12:23	UNK
0.90999232	1.48569227	26.7435	5.5553	22/07/2022	12:27	UNK
0.86755054				22/07/2022	12:31	UNK
0.58924375	1.00335608	26.877	3.7331	22/07/2022	12:36	UNK
0.60545944				22/07/2022	12:40	UNK
3.49007533	5.99671273	27.4945	21.8106	22/07/2022	12:44	UNK
3.48930191				22/07/2022	12:48	UNK
4.50896023	7.48704878	26.6255	28.1198	22/07/2022	12:53	UNK
4.48923205				22/07/2022	12:57	UNK
0.89985381	1.61273941	29.5025	5.4664	22/07/2022	13:01	UNK
0.8496425				22/07/2022	13:05	UNK
0				22/07/2022	13:10	UNK
1.04581866	1.76854201	27.4665	6.4389	22/07/2022	13:14	UNK
1.01434061				22/07/2022	13:18	UNK
0.62139806	1.06557339	26.4775	4.0244	22/07/2022	13:23	UNK
0.66725245				22/07/2022	13:28	UNK
1.07185814	1.79332277	26.869	6.6743	22/07/2022	13:32	UNK

1.06393132				22/07/2022	13:37	UNK
4.52589104	7.14032296	24.702	28.9058	22/07/2022	13:41	UNK
4.72778961				22/07/2022	13:45	UNK
4.53668043	7.72533791	27.179	28.4239	22/07/2022	13:49	UNK
4.55908939				22/07/2022	13:54	UNK
0				22/07/2022	13:58	UNK
4.65210564	7.04888624	24.812	28.4092	22/07/2022	14:02	UNK
4.58559763				22/07/2022	14:07	UNK
3.66050136	6.26505293	26.7005	23.4642	22/07/2022	14:11	UNK
3.61121494				22/07/2022	14:15	UNK
4.44651425	6.74682958	24.1895	27.8916	22/07/2022	14:20	UNK
4.47909224				22/07/2022	14:24	UNK
0.65152282	1.17191742	27.0185	4.3375	04/08/2022	14:28	UNK
0.73887558				04/08/2022	14:32	UNK
1.72687835	2.65149157	25.0369	10.5903	22/07/2022	14:37	UNK
1.66165657				22/07/2022	14:41	UNK
0				22/07/2022	14:45	UNK
0.69655466	1.28723515	29.3515	4.3856	22/07/2022	14:50	UNK
0.7069512				22/07/2022	14:54	UNK
1.31410432	2.17815754	26.689	8.1613	22/07/2022	14:58	UNK
1.29759955				22/07/2022	15:02	UNK
1.17895424	1.83512828	24.925	7.3626	22/07/2022	15:07	UNK
1.17704082				22/07/2022	15:11	UNK

B. 4. Raw Data for TDF Analysis

Sample ID	Residue Weight R1 (g)	Residue Weight R2 (g)	Standard Deviation (n=2)
BOS 004 (inedible)	0.5379	0.5295	0.006
BOS 004 (edible)	0.6071	0.4123	0.138
BOS 010	0.551	0.5393	0.008
BOS 012	0.7347	0.733	0.001
ELO 007	0.5929	0.6021	0.007
BOS 005	0.2124	0.2609	0.034
BOS 013	0.4433	0.4258	0.012
KUN 001	0.551	0.5215	0.021
BIZ 002	0.23	0.1915	0.027
BOS 001	0.639	0.5971	0.030
BOS 002	0.444	0.4782	0.024
BOS 015	0.2748	0.3196	0.032
BOS 006	0.1561	0.0695	0.061
BOS 011	0.8103	0.8194	0.006
BOS 016	0.7722	0.7822	0.007
KUN 002	0.1323	0.147	0.010
ELO 001	0.0806	0.0718	0.006
ELO 002	0.0744	0.0481	0.019
ELO 003	0.0865	0.0626	0.017
ELO 004	0.042	0.0653	0.016
ELO 005	0.0884	0.0881	0.000
ELO 006	0.0833	0.0868	0.002

ELO 008	0.1476	0.1684	0.015
BIZ 001	0.7342	0.7003	0.024
BOS 018	0.8858	0.9101	0.017
BOS 007	0.3314	0.2835	0.034
BOS 003	0.5717	0.4852	0.061
KUN 003	0.5886	0.563	0.018
BIZ 003	0.5955	0.6117	0.011
BOS 017	0.8191	0.7923	0.019

Sample	Weight crucible after overnight 105 degrees (grams)	Weight of residue within crucible (grams)	Weight fiber residue (grams)	Weight of Ash residue within Crucible (grams)	Weight ash residue (grams)	Sample weight in grams
Blank	51.45	51	0.0311	51.4415	-0.0038	0
BOS 004 inedible (1)	51.48	52	0.5379			
BOS 004 edible (2)	49.76	50	0.4123			1
BOS 004 inedible (2)	51.07	52	0.5295	51.1162	0.0507	
BOS 004 edible (1)	50.08	51	0.6071	50.245	0.1628	1
BOS 005 (2)	50.78	51	0.2609	50.6347	0.0346	
BOS 005 (1)	51.41	52	0.2124			1
BOS 011 (1)	51.33	52	0.8103	51.3586	0.0249	
BOS 010 (1)	50.73	51	0.551			1
Blank	50.99	51	0.0265	50.9837	-0.0058	
BOS 012 (2)	51.11	52	0.733			1
BOS 011 (2)	51.55	52	0.8194			
BOS 012 (1)	50.78	52	0.7347	50.8138	0.0374	1
BOS 010 (2)	51.18	52	0.5393	51.2271	0.05	
ELO 006 (2)	50.08	50	0.0868	50.0853	0.0031	
ELO 008 (2)	50.36	51	0.1684	50.3759	0.0128	1

ELO 001 (1)	50.78	51	0.0806			1
ELO 001 (2)	52.15	52	0.0718	52.1362	-0.0105	1
Blank (2)	51.5	52	0.006			0
KUN 002 (2)	50.79	51	0.147			1
Blank (1)	51.08	51	0.0113	51.0673	-0.0102	0
ELO 007 (2)	50.72	51	0.6021	50.7476	0.0247	1
BIZ 001 (2)	51.28	52	0.7003			1
ELO 005 (2)	51.38	51	0.0881			1
ELO 007 (1)	51.33	52	0.5929			1
ELO 006 (1)	51.47	52	0.0833			1
ELO 005 (1)	51.06	51	0.0884	51.794	0.739	1
BIZ 001 (1)	51.06	52	0.7342	51.1971	0.1373	1
ELO 008 (1)	51.44	52	0.1476			1
BOS 16 (1)	51.33	52	0.7722	51.3314	0.0045	
BOS 16 (2)	50.35	51	0.7822			1
ELO 002 (1)	51.19	51	0.0744	51.1811	-0.0097	1
ELO 002 (2)	51.49	52	0.0481			1
KUN 002 (1)	51.15	51	0.1323	51.1305	-0.0172	1
BOS 013 (1)	51.33	52	0.4433	51.3425	0.016	1
BOS 013 (2)	51.72	52	0.4258			1
ELO 003 (1)	49.84	50	0.0865	49.8474	0.0104	1
ELO 003 (2)	51.69	52	0.0626			1
BOS 018 (1)	51.38	52	0.8858			1
BOS 018 (2)	51.48	52	0.9101	51.4444	-0.0339	1

KUN 003 (1)	51.81	52	0.5886			1
KUN 003 (2)	50.94	52	0.563	50.9335	-0.0035	1
ELO 004 (1)	51.24	51	0.042			1
ELO 004 (2)	51.74	52	0.0653	51.693	-0.05	1
Blank 1	50.69	51	-0.0182			0
Blank 2	51.66	52	-0.0099	51.5991	-0.0569	0
BOS 007 (2)	50.74	51	0.2835	50.778	0.0407	1
BOS 007 (1)	51.28	52	0.3314			1
BOS 017 (1)	51.36	52	0.8191			1
BOS 017 (2)	51.51	52	0.7923	51.5248	0.0173	1
BOS 001 (1)	51.71	52	0.639			1
BOS 001 (2)	51.89	52	0.5971	51.9536	0.0654	1
BOS 003 (1)	52.15	53	0.5717			1
BOS 003 (2)	51.55	52	0.4852	51.488	-0.0577	1
BOS 006 (1)	51.1	51	0.1561			1
BOS 006 (2)	51.67	52	0.0695	51.6274	-0.0455	1
Blank 1	51.44	51	0.003			0
Blank 2	50.37	50	0.001	50.3382	-0.0338	0
BOS 015 (1)	51.64	52	0.2748			1
Bos 015 (2)	50.86	51	0.3196	50.8677	0.0052	1
KUN 001 (1)	51.39	52	0.551			1
KUN 001 (2)	51.11	52	0.5215	51.1421	0.0361	1
BOS 002 (1)	50.72	51	0.444			1
BOS 002 (2)	51.08	52	0.4782	51.0887	0.0132	1

Blank 1	50.08	50	0.0009	50.0682	-0.0149	0
Blank 2	50.39	50	0.002			0
BIZ 003 (2)	51.49	52	0.6117			1
BIZ 003 (1)	50.93	52	0.5955	50.9494	0.0239	1
BIZ 002 (1)	50.64	51	0.23	50.6399	0.0025	1
BIZ 002 (2)	51.62	52	0.1915			1
Blank 1	50.4	50	0.0128			
Blank 2	51.22	51	0.0066	51.1985	-0.0217	
KUN 003 (1) rerun	51.18	52	0.6009			
ELO 005 (1) rerun	51.36	51	0.064			
ELO 005 (2) rerun	51.53	52	0.0945	51.5204	-0.0047	
KUN 003 (2) rerun	51.43	52	0.6386	51.4947	0.0613	

Amount of protein in g	Weight of protein sample in mg	Amount of protein in mg	Percentage of protein in sample
0.000435587	12.512	0.435587281	3.481356144
0.00067012	13.107	0.6701199	5.112687111
0.000949684	13.366	0.949683811	7.105220795
0.000983756	13.775	0.983756272	7.141606331
0.001724475	12.282	1.724474669	14.04066658
0.002135422	13.217	2.135422485	16.15663528
0.00058657	12.907	0.586570292	4.544590473
0.000704382	12.767	0.704382141	5.51720953
0.000198455	14.2215	0.198455481	1.395460963
0.001047787	13.787	1.047786808	7.599817276
0.002847114	11.4575	2.847114055	24.84934807
0.000559605	13.3	0.559605108	4.207557201

0.001231609	14.787	1.231609159	8.328999519
0.000882208	13.689	0.882207966	6.444648743
0.000571059	11.676	0.571058994	4.890878677
0.000510005	11.576	0.510005401	4.405713558
0.000960109	13.8375	0.960108725	6.938455105
0.005060053	12.365	5.060052578	40.92238235
0.000588032	14.919	0.588032199	3.941498756
0.000396761	13.3185	0.396760541	2.979018213
0.005461412	11.995	5.461411753	45.53073575
0.000697104	11.7925	0.697104067	5.911418842
0.000150588	14.423	0.150587589	1.044079522

0.003651313	12.8765	3.651312924	28.35640837
0.000461198	13.5045	0.461197556	3.415139811
0.001041347	11.057	1.041347205	9.417990455
0.00112214	12.043	1.122139562	9.317774326
0.001631727	12.043	1.631726806	13.54917218
0.00047974	12.47	0.479740301	3.84715558
0.000759588	12.1275	0.759588313	6.263354467
0.001003796	12.398	1.003795915	8.096434223
0.000687885	11.5385	0.687885383	5.961653451
0.000407404	13.505	0.40740429	3.01669226
0.001711378	12.979	1.711378081	13.18574683
0.001821835	13.681	1.821834653	13.31653134
	13.3090	0.122491414	0.920365274

14.0545	5.664020223	40.30040359
12.0795	1.368986922	11.33314228

	Sample identifier	Sample weights			Residue weights		Protein	Ash	Dietary Fibre
			m_1 (g)	m_2 (g)	R_1 (sample) or BR_1 (blank) (g)	R_2 (sample) or BR_2 (blank) (g)	P (sample) or BP (blank) (g)	A (sample) or BA (blank) (g)	% w/w
1	BOS 004 inedible	**Sample**	1.0000	1.0000	0.5379	0.5295	0.0007	0.0507	44.8165
		Blank			0.0265	0.0311	0.0004	-0.0058	
2	BOS 004 edible	**Sample**	1.0003	1.0003	0.6071	0.4123	0.0009	0.1628	31.1658
		Blank			0.0265	0.0311	0.0004	-0.0058	
3	BOS 005	**Sample**	1.0000	1.0000	0.2124	0.2609	0.0010	0.0346	16.6867
		Blank			0.0265	0.0311	0.0004	-0.0058	
4	BOS 011	**Sample**	1.0007	1.0006	0.8103	0.8194	0.0006	0.0249	75.4673
		Blank			0.0265	0.0311	0.0004	-0.0058	
5	BOS 012	**Sample**	1.0001	1.0001	0.7347	0.7330	0.0021	0.0374	66.0049
		Blank			0.0265	0.0311	0.0004	-0.0058	
6	BOS 010	**Sample**	1.0007	1.0007	0.5510	0.5393	0.0017	0.0500	45.8904
		Blank			0.0265	0.0311	0.0004	-0.0058	
7	ELO 006	**Sample**	0.9999	1.0000	0.0833	0.0868	0.0009	0.0031	6.2411
		Blank			0.0113	0.0060	0.0002	-0.0102	
8	ELO 008	**Sample**	1.0001	1.0000	0.1476	0.1684	0.0006	0.0128	12.5973
		Blank			0.0113	0.0060	0.0002	-0.0102	
9	ELO 001	**Sample**	0.9999	1.0001	0.0806	0.0718	0.0007	-0.0105	6.7346

		Blank			0.0113	0.0060	0.0002	-0.0102	
10	KUN 002	**Sample**	1.0007	1.0006	0.1323	0.1470	0.0010	-0.0172	13.7071
		Blank			0.0113	0.0060	0.0002	-0.0102	

	Sample identifier	**Sample weights**			**Residue weights**		**Protein**	**Ash**	**Dietary Fibre**
			m_1 (g)	**m_2 (g)**	**R_1 (sample) or BR_1 (blank) (g)**	**R_2 (sample) or BR_2 (blank) (g)**	**P (sample) or BP (blank) (g)**	**A (sample) or BA (blank) (g)**	**% w/w**
1	ELO 007	**Sample**	1.0003	1.0004	0.5929	0.6021	0.0012	0.0247	55.2726
		Blank			0.0113	0.0060	0.0002	-0.0102	
2	BIZ 001	**Sample**	1.0004	1.0003	0.7342	0.7003	0.0028	0.1373	55.8265
		Blank			0.0113	0.0060	0.0002	-0.0102	
3	ELO 005	**Sample**	0.9999	0.9999	0.0884	0.0881	0.0006	0.7390	-67.0026
		Blank			0.0113	0.0060	0.0002	-0.0102	
4	BOS 016	**Sample**	1.0000	0.9998	0.7722	0.7822	0.0005	0.0045	72.9573
		Blank			-0.0182	-0.0099	0.0002	-0.0569	
5	ELO 002	**Sample**	0.9999	0.9998	0.0744	0.0481	0.0010	-0.0097	2.7344
		Blank			-0.0182	-0.0099	0.0002	-0.0569	
6	BOS 013	**Sample**	1.0000	0.9999	0.4433	0.4258	0.0051	0.0160	37.0859
		Blank			-0.0182	-0.0099	0.0002	-0.0569	

7	ELO 003	**Sample**	1.0003	1.0002	0.0865	0.0626	0.0006	0.0104	2.0907
		Blank			-0.0182	-0.0099	0.0002	-0.0569	
8	BOS 018	**Sample**	1.0000	0.9998	0.8858	0.9190	0.0004	-0.0339	89.3354
		Blank			-0.0182	-0.0099	0.0002	-0.0569	
9	KUN 003	**Sample**	1.0003	1.0003	0.5886	0.5630	0.0055	-0.0035	53.1031
		Blank			-0.0182	-0.0099	0.0002	-0.0569	
10	ELO 004	**Sample**	0.9999	1.0003	0.0420	0.0653	0.0002	-0.0500	6.0843
		Blank			-0.0182	-0.0099	0.0002	-0.0569	

	Sample identifier	**Sample weights**			**Residue weights**		**Protein**	**Ash**	**Dietary Fibre**
			m_1 (g)	**m_2 (g)**	**R_1 (sample) or BR_1 (blank) (g)**	**R_2 (sample) or BR_2 (blank) (g)**	**P (sample) or BP (blank) (g)**	**A (sample) or BA (blank) (g)**	**% w/w**
1	BOS 007	**Sample**	1.0006	1.0008	0.3314	0.2835	0.0037	0.0407	22.7620
		Blank			0.0030	0.0010	0.0005	-0.0338	
2	BOS 017	**Sample**	1.0001	1.0004	0.8191	0.7923	0.0005	0.0173	75.2451
		Blank			0.0030	0.0010	0.0005	-0.0338	
3	BOS 001	**Sample**	1.0009	1.0008	0.6390	0.5971	0.0010	0.0654	51.5872
		Blank			0.0030	0.0010	0.0005	-0.0338	
4	BOS 003	**Sample**	1.0006	1.0002	0.5717	0.4852	0.0011	-0.0577	54.9508

		Blank			0.0030	0.0010	0.0005	-0.0338	
5	BOS 006	**Sample**	1.0004	1.0005	0.1561	0.0695	0.0016	-0.0455	12.1314
		Blank			0.0030	0.0010	0.0005	-0.0338	
6	BOS 015	**Sample**	1.0003	1.0001	0.2748	0.3196	0.0008	0.0052	25.5890
		Blank			0.0030	0.0010	0.0005	-0.0338	
7	KUN 001	**Sample**	1.0004	0.9999	0.5510	0.5215	0.0010	0.0361	46.3777
		Blank			0.0030	0.0010	0.0005	-0.0338	
8	BOS 002	**Sample**	0.9999	1.0001	0.4440	0.4782	0.0007	0.0132	41.1913
		Blank			0.0030	0.0010	0.0005	-0.0338	
9	BIZ 003	**Sample**	0.9999	0.9999	0.5955	0.6117	0.0017	0.0239	56.2102
		Blank			0.0009	0.0020	0.0004	-0.0149	
10	BIZ 002	**Sample**	1.0004	1.0005	0.2300	0.1915	0.0018	0.0025	19.0393
		Blank			0.0009	0.0020	0.0004	-0.0149	

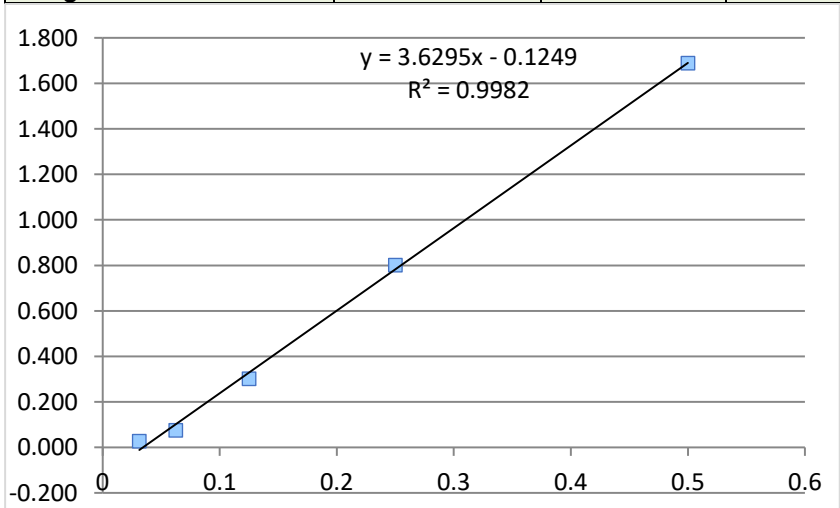
	Sample identifier	**Sample weights**			**Residue weights**		**Protein**	**Ash**	**Dietary Fibre**
			m_1 (g)	**m_2 (g)**	**R_1 (sample) or BR_1 (blank) (g)**	**R_2 (sample) or BR_2 (blank) (g)**	**P (sample) or BP (blank) (g)**	**A (sample) or BA (blank) (g)**	**% w/w**
1	KUN 003 rerun	**Sample**	1.0000	1.0000	0.6009	0.6386	0.0056	0.0613	52.1550
		Blank			0.0128	0.0066	0.0001	-0.0217	

2	ELO 005 rerun	**Sample**	1.0002	1.0000	0.0640	0.0945	0.0014	-0.0047	5.1285
		Blank			0.0128	0.0066	0.0001	-0.0217	

B. 5. Raw Data Used for Total Phenol Analysis

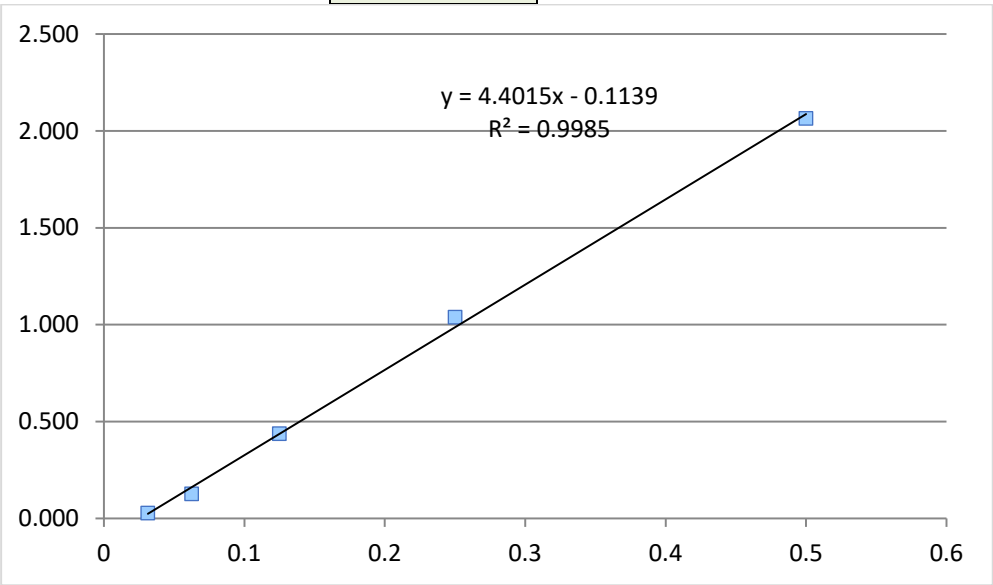
Sample				ABS	ABS - Average of 2 reads		
				blank substr	Average	Stdev	RSD%
Blank 1/2				-	0.004	0.000	5.2
GA Standard 0.5000 1/2			0.5	1.689	1.693	0.007	0.4
GA Standard 0.2500 1/2			0.25	0.801	0.805	0.000	0.0
GA Standard 0.1250 1/2			0.125	0.301	0.305	0.000	0.0
GA Standard 0.0625 1/2			0.0625	0.074	0.078	0.002	2.4
GA Standard 0.0313 1/2			0.0313	0.027	0.031	0.000	0.2
	mg / g dry weight	**mg dry weight**	mg GAE				
BOS 004 edible 1 1/2	**6.81**	537.5	**0.3663**	1.205	1.209	0.002	0.2
BOS 004 edible 2 1/2	**6.62**	553.0	**0.3658**	1.203	1.207	0.004	0.3
BOS 004 inedible 1 1/2	**5.67**	507.1	**0.2877**	0.920	0.924	0.007	0.8
BOS 004 inedible 2 1/2	**3.82**	503.6	**0.1923**	0.573	0.577	0.001	0.3
BOS 010 1 1/2	**3.91**	515.9	**0.2020**	0.608	0.612	0.001	0.1
BOS 010 2 1/2	**3.90**	519.9	**0.2028**	0.611	0.615	0.001	0.2
BOS 011 1 1/2	**7.63**	523.4	**0.3992**	1.324	1.328	0.013	1.0
BOS 011 2 1/2	**7.90**	529.5	**0.4183**	1.393	1.398	0.001	0.1
BOS 012 1 1/2	**9.16**	511.4	**0.4685**	1.576	1.580	0.002	0.1
BOS 012 2 1/2	**8.54**	509.1	**0.4348**	1.453	1.457	0.011	0.8

BOS 013 1 1/2	**8.19**	587.2	**0.4811**	1.621	1.625	0.012	0.7
BOS 013 2 1/2	**9.72**	508.9	**0.4944**	1.670	1.674	0.009	0.5
KUN 002 1 1/2	**4.69**	518.9	**0.2434**	0.759	0.763	0.001	0.1
KUN 002 2 1/2	**3.74**	510.1	**0.1906**	0.567	0.571	0.002	0.4
ELO 001 1 1/2	**2.86**	501.6	**0.1437**	0.397	0.401	0.000	0.0
ELO 001 2 1/2	**3.71**	508.9	**0.1889**	0.561	0.565	0.001	0.3
ELO 002 1 1/2	**1.36**	505.6	**0.0686**	0.124	0.128	0.000	0.3
ELO 002 2 1/2	**1.76**	503.7	**0.0886**	0.197	0.201	0.000	0.1
ELO 003 1 1/2	**1.99**	504.8	**0.1005**	0.240	0.244	0.005	2.2
ELO 003 2 1/2	**1.44**	503.5	**0.0726**	0.139	0.143	0.000	0.3
ELO 004 1 1/2	**1.69**	501.0	**0.0848**	0.183	0.187	0.000	0.0
ELO 004 2 1/2	**1.78**	503.0	**0.0894**	0.200	0.204	0.002	0.8
Average mg /g dry weight							



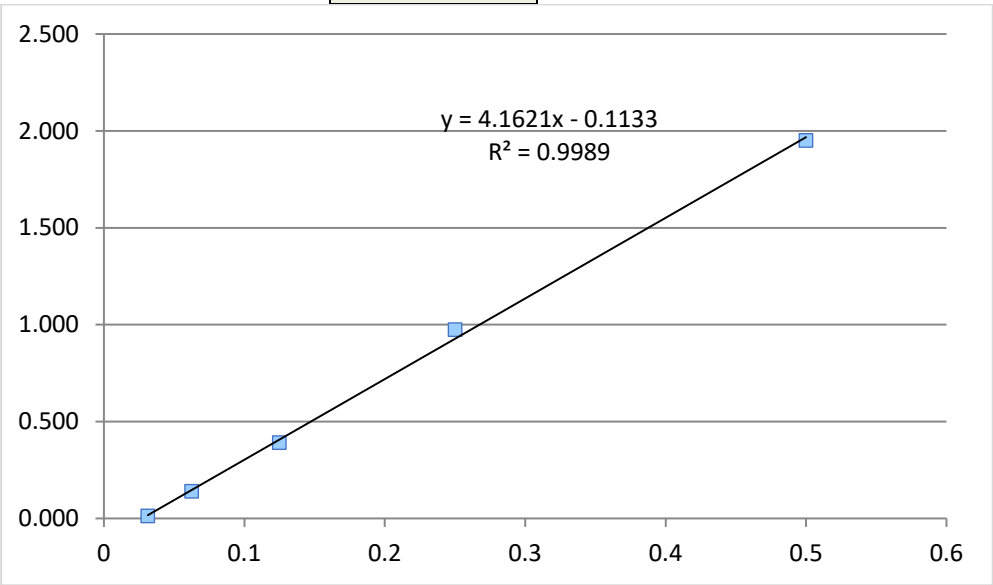
Sample				ABS	ABS - Average of 2 reads			
				blank substr	Average	Stdev	RSD%	
Blank 1/2				-	-0.010	0.000	-3.0	
GA Standard 0.5000			0.5	2.065	2.055			
GA Standard 0.2500			0.25	1.038	1.029			
GA Standard 0.1250			0.125	0.437	0.427			
GA Standard 0.0625			0.0625	0.127	0.117			
GA Standard 0.0313			0.0313	0.028	0.019			
	mg / g dry weight	**mg dry weight**	mg GAE					Average of duplicates
BIZ 001 1 1/2	**6.98**	546.3	**0.4**	1.565	1.555	0.027	1.8	1.5237
BIZ 001 2 1/2	**6.74**	544.4	**0.4**	1.502	1.492	0.006	0.4	
KUN 003 1 1/2	**6.06**	508.2	**0.3**	1.242	1.232	0.028	2.3	1.0841
KUN 003 2 1/2	**4.74**	507.4	**0.2**	0.946	0.936	0.019	2.0	
ELO 001 1 1/2	**2.95**	501.7	**0.1**	0.538	0.529	0.005	1.0	0.5528
ELO 001 2 1/2	**3.17**	502.3	**0.2**	0.586	0.577	0.001	0.1	
ELO 005 1:2 1 1/2	**2.32**	501.1	**0.1**	0.142	0.132	0.006	4.8	0.1272
ELO 005 1:2 2 1/2	**2.23**	501.4	**0.1**	0.132	0.122	0.002	1.5	
KUN 002 1 1/2	**4.00**	502.7	**0.2**	0.770	0.761	0.005	0.6	0.7594
KUN 002 2 1/2	**3.99**	502.4	**0.2**	0.768	0.758	0.032	4.2	

Average mg /g dry weight								
BIZ 001	**6.86**							
KUN 003	**5.40**							
ELO 001	**3.06**							
ELO 005	**2.27**							
KUN 002	**3.99**							
% in grams								
BIZ 001	**0.69**							
KUN 003	**0.54**							
ELO 001	**0.31**							
ELO 005	**0.23**							
KUN 002	**0.40**							



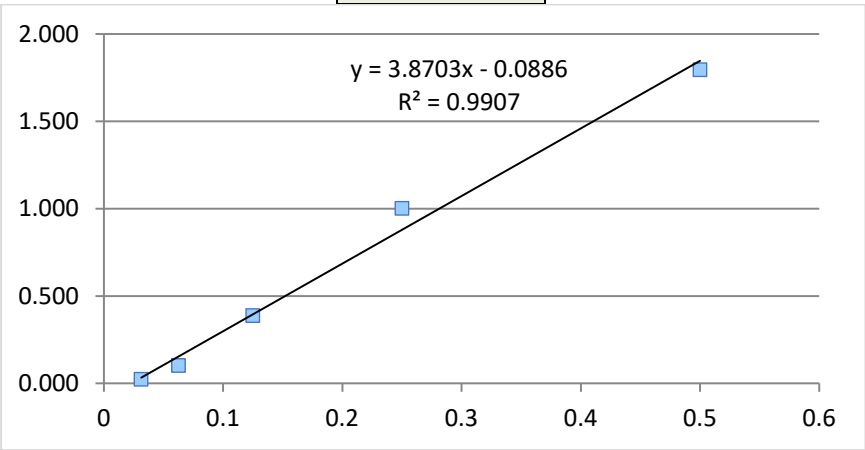
Sample				ABS	ABS - Average of 2 reads		
				blank substr	Average	Stdev	RSD%
Blank 1/2				-	0.001	0.004	683.5
GA Standard 0.5000			0.5	1.950	1.950		
GA Standard 0.2500			0.25	0.974	0.975		
GA Standard 0.1250			0.125	0.390	0.391		
GA Standard 0.0625			0.0625	0.140	0.140		
GA Standard 0.0313			0.0313	0.012	0.013		
	mg / g dry weight	**mg dry weight**	mg GAE				
BOS 012 1 1/2	**8.72**	536.8	**0.5**	1.834	1.835	0.001	0.1
BOS 012 2 1/2	**9.17**	534.6	**0.5**	1.926	1.927	0.011	0.6
BOS 018 1 1/2	**1.05**	501.7	**0.1**	0.107	0.107	0.001	1.1
BOS 018 2 1/2	**1.17**	501.3	**0.1**	0.132	0.132	0.001	0.4
BOS 004 inedible 1 1/2	**4.52**	501.8	**0.2**	0.830	0.831	0.015	1.8
BOS 004 inedible 2 1/2	**3.52**	502.4	**0.2**	0.623	0.624	0.002	0.3
BOS 005 1:10 1 1/2	**19.15**	505.4	**0.1**	0.290	0.290	0.002	0.5
BOS 005 1:10 2 1/2	**18.33**	506.6	**0.1**	0.273	0.274	0.002	0.6
ELO 005 1:5 1 1/2	**4.59**	505.2	**0.0**	0.080	0.080	0.004	5.1
ELO 005 1:5 2 1/2	**3.09**	506.3	**0.0**	0.017	0.018	0.004	23.4

Average mg /g dry weight							
BOS 012	**8.94**						
BOS 018	**1.11**						
BOS 004 inedible	**4.02**						
BOS 005	**18.74**						
ELO 005	**3.84**						
% in grams							
BOS 012	**0.89**						
BOS 018	**0.11**						
BOS 004 inedible	**0.40**						
BOS 005	**1.87**						
ELO 005	**0.38**						



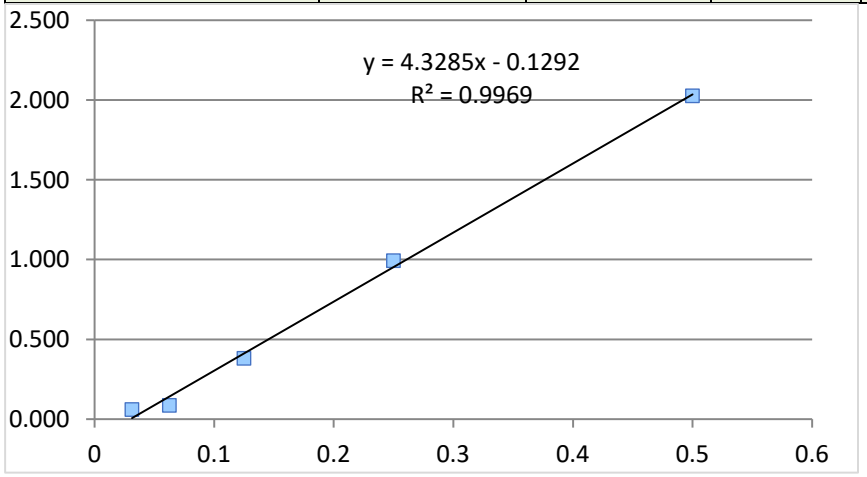
Sample				ABS	ABS - Average of 2 reads		
				blank substr	Average	Stdev	RSD%
Blank 1/2				-	-0.001	0.002	-183.3
GA Standard 0.5000			0.5	1.795	1.793		
GA Standard 0.2500			0.25	1.001	1.000		
GA Standard 0.1250			0.125	0.387	0.385		
GA Standard 0.0625			0.0625	0.102	0.100		
GA Standard 0.0313			0.0313	0.022	0.021		
	mg / g dry weight	**mg dry weight**	mg GAE				
ELO 002 1 1/2	**4.22**	505.6	**0.2**	0.737	0.736	0.012	1.6
ELO 002 2 1/2	**4.22**	508.9	**0.2**	0.742	0.741	0.002	0.2
BIZ 003 1 1/2	**7.95**	502.2	**0.4**	1.456	1.455	0.006	0.4
BIZ 003 2 1/2	**6.55**	526.1	**0.3**	1.245	1.244	0.006	0.5
BOS 006 1 1/2	**3.95**	503.5	**0.2**	0.680	0.679	0.011	1.6
BOS 006 2 1/2	**2.88**	511.3	**0.1**	0.481	0.480	0.004	0.8
BOS 002 1:10 1 1/2	**59.33**	503.1	**0.3**	1.067	1.065	0.006	0.5
BOS 002 1:10 2 1/2	**62.02**	503.3	**0.3**	1.119	1.118	0.002	0.2
BOS 015 1:10 1 1/2	**11.39**	514.1	**0.1**	0.138	0.137	0.003	2.1
BOS 015 1:10 2 1/2	**11.26**	509.8	**0.1**	0.133	0.132	0.007	5.4

Average mg / g dry weight							
ELO 002	**4.22**						
BIZ 003	**7.25**						
BOS 006	**3.41**						
BOS 002	**60.67**						
BOS 015	**11.32**						
% grams							
ELO 002	**0.42**						
BIZ 003	**0.72**						
BOS 006	**0.34**						
BOS 002	**6.07**						
BOS 015	**1.13**						

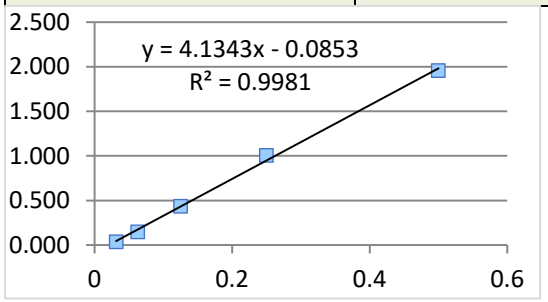


Sample				ABS	ABS - Average of 2 reads		
				blank substr	Average	Stdev	RSD%
Blank 1/2				-	-0.042	0.001	-1.7
GA Standard 0.5000 1/2			0.5	2.026	1.984		
GA Standard 0.2500 1/2			0.25	0.992	0.950		
GA Standard 0.1250 1/2			0.125	0.382	0.340		
GA Standard 0.0625 1/2			0.0625	0.087	0.045		
GA Standard 0.0313 1/2			0.0313	0.060	0.018		
	mg / g dry weight	**mg dry weight**	mg GAE				
KUN 001 1 1/2	**5.39**	508.5	**0.3**	1.057	1.015	0.008	0.8
KUN 001 2 1/2	**6.47**	505.6	**0.3**	1.287	1.245	0.209	16.8
BIZ 002 1 1/2	**7.76**	513.7	**0.4**	1.595	1.553	0.011	0.7
BIZ 002 2 1/2	**7.68**	515.5	**0.4**	1.584	1.542	0.016	1.0
BOS 001 1 1/2	**5.88**	501.9	**0.3**	1.149	1.107	0.002	0.1
BOS 001 2 1/2	**5.62**	504.0	**0.3**	1.098	1.056	0.003	0.3
BOS 003 1 1/2	**6.49**	515.0	**0.3**	1.316	1.274	0.017	1.4

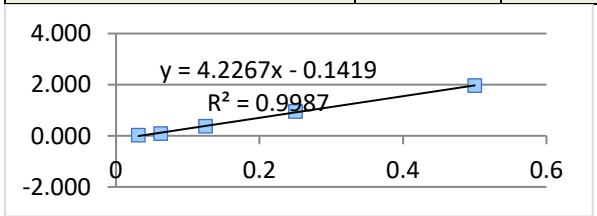
BOS 003 2 1/2	**6.25**	521.5	**0.3**	1.282	1.240	0.003	0.2
Average mg /g dr weight							
KUN 001	**5.93**						
BIZ 002	**7.72**						
BOS 001	**5.75**						
BOS 003	**6.37**						
Average % grams							
KUN 001	**0.59**						
BIZ 002	**0.77**						
BOS 001	**0.58**						
BOS 003	**0.64**						



Sample				ABS	ABS - Average of 2 reads		
				blank substr	Average	Stdev	RSD%
Blank 1/2				-	-0.015	0.001	-9.3
GA Standard 0.5000 1/2			0.5	1.956	1.941	0.005	0.2
GA Standard 0.2500 1/2			0.25	1.006	0.991	0.001	0.1
GA Standard 0.1250 1/2			0.125	0.434	0.419	0.002	0.5
GA Standard 0.0625 1/2			0.0625	0.146	0.131	0.001	0.6
GA Standard 0.0313 1/2			0.0313	0.036	0.021	0.001	3.7
	mg / g dry weight	**mg dry weight**	mg GAE				
BOS 007 1 1/2	**8.55**	508.1	**0.4**	1.710	1.695	0.002	0.1
BOS 007 2 1/2	**8.47**	508.9	**0.4**	1.696	1.681	0.007	0.4



Sample				ABS	ABS - Average of 2 reads		
				blank substr	Average	Stdev	RSD%
Blank 1/2				-	0.011	0.000	0.0
GA Standard 0.5000			0.5	1.962	1.973		
GA Standard 0.2500			0.25	0.947	0.958		
GA Standard 0.1250			0.125	0.370	0.381		
GA Standard 0.0625			0.0625	0.088	0.099		
GA Standard 0.0313			0.0313	0.019	0.030		
BOS 017 1:10 1 1/2	**93.12**	507.7	**0.5**	1.856	1.868	0.022	1.2
BOS 017 1:10 2 1/2	**57.90**	501.8	**0.3**	1.086	1.097	0.002	0.2
BOS 011 1:10 1 1/2	**11.98**	523.4	**0.1**	0.123	0.134	0.003	2.1
BOS 011 1:10 2 1/2	**11.02**	529.5	**0.1**	0.105	0.116	0.000	0.3
BOS 016 1:10 1 1/2	**31.92**	507.1	**0.2**	0.542	0.553	0.000	0.1
BOS 016 1:10 2 1/2	**33.85**	506.4	**0.2**	0.583	0.594	0.001	0.2



Sample				ABS	ABS - Average of 2 reads		
				blank substr	Average	Stdev	RSD%
Blank 1/2				-	0.012	0.001	7.4
GA Standard 0.5000 1/2			0.5	1.887	1.900		
GA Standard 0.2500 1/2			0.25	0.930	0.943		
GA Standard 0.1250 1/2			0.125	0.360	0.373		
GA Standard 0.0625 1/2			0.0625	0.089	0.102		
GA Standard 0.0313 1/2			0.0313	0.007	0.019		
	mg / g dry weight	**mg dry weight**	mg GAE				
ELO 007 1 1/2	**8.34**	505.4	**0.4**	1.584	1.596	0.005	0.3
ELO 007 2 1/2	**8.02**	507.0	**0.4**	1.522	1.535	0.001	0.1
ELO 008 1 1/2	**1.90**	501.3	**0.1**	0.252	0.265	0.003	1.3
ELO 008 2 1/2	**1.55**	503.6	**0.1**	0.183	0.195	0.000	0.1
ELO 006 2 1/2	**3.73**	508.5	**0.2**	0.638	0.650	0.001	0.2
ELO 006 1 1/2	**11.05**	504.1	**0.6**	2.136	2.148	0.007	0.3